

# PROCEEDINGS OF PAKISTAN CONGRESS OF ZOOLOGY

(Proc. Pakistan Congr. Zool.)

Volume 32

2012

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

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

## **ACKNOWLEDGMENTS**

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

Grants were received from Higher Education Commission, Islamabad, COMSTECH, Islamabad, World Wildlife Fund-Pakistan, Chemical House, 6-C, Sikandar Malhi Road, Canal Park, Gulberg-II, Lahore, Welcare Chemical Corporation Limited, Sahiwal and Hamdard Foundation, Pakistan.

**32<sup>nd</sup> PAKISTAN CONGRESS OF ZOOLOGY (INTERNATIONAL)**

**GOVERNMENT COLLEGE UNIVERSITY, LAHORE,  
PAKISTAN**

**March 6 – 8, 2012**

**TUESDAY, March 6, 2012**

- 08:30: REGISTRATION  
09:30 Inauguration: Recitation from the Holy Quran  
09:35 Welcome Address  
09:45 Address by Secretary General, Zoological Society of Pakistan  
10:00 Address by the President, Zoological Society of Pakistan  
10:20 Distribution of Medals and Awards  
10:50 Address by the Chief Guest  
11:15 Vote of Thanks  
11:25 Refreshment

**JOINT SESSION I: Plenary Lectures**

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

**Co-chairperson:** Prof. Dr. M. Afzal Kazmi

- Speakers: 1. **Prof. Dr. Javed Akram**  
*Principal, Allama Iqbal Medical College, Lahore*  
**Management of critically ill adult patients of dengue shock syndrome.**
2. **Prof. Dr. Nusrat Jahan**  
*Chairperson, Department of Zoology, GC University, Lahore.*  
**Prevention and Control of Dengue Fever**

01:00 PM Lunch and Prayer

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

	Chairperson:	Prof. Dr. Mazhar Qayyum
	Co-chairperson:	Dr. Nazish Mazhar Ali
02:00 AM	Paper reading	
04:30 PM	Tea Time	

**SESSION II**

	Chairperson:	Prof. Dr. Musarrarat Yousaf
	Co-chairperson:	Dr. Faiza Saleem
05:00 PM	Paper reading	
06:30 PM	Prayer	

**SESSION III**

	Chairperson:	Prof. Dr. Shamsuddin Shaikh
	Co-chairperson:	Dr. Bushra Muneer
06:45 AM	Paper reading	
08:00 PM	Dinner	

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

	Chairperson:	Dr. Syed Azhar Hasan
	Co-chairperson:	Dr. Zahoor Salihah
02:00 PM	Paper reading	
04:30 PM	Tea Time	

### **SESSION II**

05:00 PM Chairperson: Prof. Dr. Imtiaz Ahmad  
06:30 PM Co-chairperson: Dr. Abid Farid  
Paper reading  
Prayer

### **SESSION III**

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

### **HALL – 3**

#### **SECTION IV: PARASITOLOGY**

##### **SESSION I**

02:00 AM Chairperson: Prof. Dr. F.M. Bilqees  
04:30 PM Co-chairperson: Dr. Asmattullah Kakar  
Paper reading  
Tea Time

##### **SESSION II**

05:00 PM Chairperson: Prof. Dr. Akram Shah  
06:30 PM Co-chairperson: Dr. Fiaz Qammar  
Paper reading  
Prayer

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

##### **SESSION I**

06:45 AM Chairperson: Prof. Dr. Pirzada Jamal Siddiqui  
08:00 PM Co-chairperson: Dr. Khalil Ahmad  
Paper reading  
Dinner

**WEDNESDAY, MARCH 7, 2012**

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

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

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

- 09:00 AM 1. **Prof. Dr. Syed Akram Shah,**  
*Department of Zoology, University of Peshawar, Peshawar*  
**Cutaneous Leishmaniasis in KPK: Special reference to the use of molecular approaches for a better understanding of its epidemiology**
2. **Prof. Dr. Daniel Hoessli,**  
*Department of Pathology, CMU, University of Geneva, Geneva, Switzerland*  
**After the genes, the proteins or the tools for research in the post genomic era.**
3. **Prof. Dr. A.R. Shakoori,**  
*Distinguish National Professor & Director, School of Biological Sciences, University of the Punjab, Lahore*  
*Ex-DG Wildlife*  
**Realm of a Cell**

**HALL – 1**

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

**SESSION IV**

	Chairperson:	Dr. M. Afzal Ghauri
	Co-chairperson:	Dr. Abdul Rehman
10:30 AM	Paper reading	
11:30 PM	Tea Break	

### **SESSION V**

Chairperson: Prof. Dr. Javaid Iqbal Qazi  
Co-chairperson: Dr. Shagufta Naz  
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

### **SESSION VII**

Chairperson: Dr. Shahid Nadeem  
Co-chairperson: Dr. Irfan Zia Qureshi  
05:00 PM Paper reading  
05:00 PM Executive Council Meeting  
08:00 PM Dinner

### **HALL – 2**

### **SECTION III: PEST AND PEST CONTROL**

#### **SESSION IV**

Chairperson: Mr. Abdul Aziz  
Co-chairperson: Dr. Farzana Perveen  
10:30 AM Paper reading  
11:00 PM Tea Break

#### **SESSION V**

Chairperson: Prof. Dr. Iftikhar Hussain  
Co-chairperson: Dr. Abida Butt  
11:30 AM Paper reading  
01:00 PM Lunch and Prayer

### SECTION III: ENTOMOLOGY

#### SESSION I

	Chairperson:	Prof. Dr. Nusrat Jahan
	Co-chairperson:	Dr. Tasneem Farasat
02:00 PM	Paper reading	
04:30 PM	Tea Break	

#### SESSION II

	Chairperson:	Prof. Dr. Shahnaz A. Rana
	Co-chairperson:	Dr. Syeda Azra Qamar
05:00 PM	Paper reading	
05:00 PM	Executive Council Meeting	
08:00 PM	Dinner	

### HALL – 3

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

#### SESSION II

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

#### SESSION III

	Chairperson:	Prof. Dr. M. Naeem Khan
	Co-chairperson:	Dr. M. Ashraf
11:30 AM	Paper reading	
01:00 PM	Lunch Break and Prayer Break (Zuhar)	

#### SESSION IV

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

## SESSION V

	Chairperson:	Prof. Dr. Wazir Ali Baloch
	Co-chairperson:	Dr. A.Q. Khan Sulehria
05:00 PM		Paper reading
05:00 PM		Executive Council Meeting
06:30 PM		Prayer Break (Maghrib)
08:00 PM		Dinner

**THURSDAY, MARCH 8, 2012**

### JOINT SESSION III: (Plenary Lectures)

**Chairman:** Prof. Dr. Fatima Mujib Bilqees  
**Co-chairman:** Prof. Dr. Shamsuddin Shaikh

- 09:00 AM 1. **Prof. Dr. Javaid Iqbal Qazi,**  
*Department of Zoology, University of the Punjab, Lahore.*  
**Comprehensive bioprocessing of low/no cost substrates for bioenergy – Prospects in Pakistan**
2. **Dr. Muhammad Irshad,**  
*National Agricultural Research Center, Islamabad*  
**Pollinators – The silent, forgotten friends of humanity – a Pakistan case**
3. **Prof. Dr. Pirzada Jamal Siddiqui,**  
*Director, Center of Excellence in Marine Biology, University of Karachi*  
**Marine biodiversity – A case study for Pakistan**

## HALL – 1

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

## SESSION VIII

Chairperson: Dr. Idrees Khan  
Co-chairperson: Dr. Dil Ara Abbas Bukhar

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

### **SESSION IX**

Chairperson: Prof. Dr. Rubina Mushtaq  
Co-chairperson: Dr. Zulfiqar Ali Saqib  
11:30 AM Paper reading  
01:00 PM Lunch Break

### **SESSION X**

Chairperson: Prof. Dr. Samina Jalali  
Co-chairperson: Dr. Nabila Roohi  
02:00 PM Paper reading  
03:00 PM General Body Meeting

### **HALL – 2**

### **SECTION III: ENTOMOLOGY**

#### **SESSION III**

Chairperson: Prof. Dr. Muhammad Afzal  
Co-chairperson: Dr. Amjad Farooq  
10:30 AM Paper reading  
11:00 AM Tea Break

#### **SESSION IV**

Chairperson: Prof. Dr. M.S. Wagan  
Co-chairperson: Dr. Nasreen Muzaffar  
11:00 AM Paper reading  
11:30 AM Tea Break

#### **SESSION V**

Chairperson: Dr. Amanullah Khan  
Co-chairperson: Dr. A. Aihetsham

02:00 PM Paper reading  
03:30 PM General Body Meeting

### **HALL – 3**

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

##### **SESSION VI**

Chairperson: Prof. Dr. N.T. Narejo  
Co-chairperson: Dr. Zafar Iqbal  
11:00 AM Paper reading  
11:30 AM Tea Break

##### **SESSION VII**

Chairperson: Prof. Dr. Shahid M. Rana  
Co-chairperson: Dr. Ali Muhammad Yousafzai  
11:30 AM Paper reading

##### **SESSION VIII**

Chairperson: Dr. Zahida Tasawar  
Co-chairperson: Dr. Atif Yaqoob  
02:00 PM Paper reading  
03:00 PM General Body Meeting  
04:00 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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Bhatti, R.Z.	Mustafa, K.
Butt, R.K.	Nadeem, M.S. (Dr.)
Faheem, B.	Naqvi, S.R.
Farooq, M.	Nawaz, M.
Fatima, H.	Nazir, K.
Gilani, N.	Nazir, S.A.
Goursi, U.H.	Nosheen, S.
Haider, J.	Pasha, S.
Hameed, K.	Qayyum, M. (Dr.)
Hanif, S.	Rais, U.
Hussain S.	Rashid, N.
Hussain, A.	Rashid, T.
Hussain, I. (Dr.)	Rauf, S.
Hussain, R.	Rehman, F.
Hussain, R.I.	Riaz, M.
Ibrahim	Safdar, A.
Iqbal, S.	Shafiq, A.
Irshad, M.	Shah, S.I.
Irshad, N.	Shahbaz, M.
Jabbar, M.	Shahid, I.
Jilani, M.J.	Shahinshah
Kabeer, B.	Shaikh, U.A.A.

Shehnaz, L.  
Tabassum, T.  
Taher, S.  
Tasleem, N.  
Turab, S.  
Yasmin, S.  
Zafar, S.  
Zeenat

**SAHIWAL**

Gul, Y.

**SARGODHA**

Nawaz, S.K. (Dr.)  
Riaz, M.A. (Dr.)

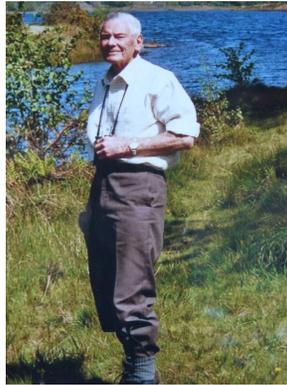
**TALAGANG**

Ahmed, S.

**TANDOJAM**

Bukero, A.  
Khan, M.H.  
Memon, R.M.  
Rashdi, S.M.M.S.  
Rauf, I.  
Suleman, N. (Dr.)  
Talpur, M.A.



**CITATIONS****RECIPIENT OF  
LIFE TIME ACHIEVEMENT AWARD 2012**

**Dr. Thomas Jones Roberts, *SI*  
(1924-2013)**

Dr. Thomas Jones Roberts son of Sir William Roberts, was born on 2<sup>nd</sup> September, 1924 at Bangor, North Wales, U.K.

Dr. T.J. Roberts is an internationally recognized, distinguished and unmatched authority on the wildlife of Pakistan. His life long passion has been to study the natural history of mammals, birds, reptiles, including his love for butterflies and flowers. He has produced knowledge-based works in the highest scientific tradition of an amateur naturalist.

He came to Pakistan in 1946 along with elder Roberts. He received his Ph.D. from Cambridge University, U.K. Earlier to it he got M.S.A. from British Columbia University Canada.

He produced comprehensive accounts of the mammals and birds of Pakistan in the shape of two books – *The mammals of Pakistan* (1977, 1997) and *Birds of Pakistan* (two volumes, 1991). These are the only definitive reference books – a great value to the mammalogists, ornithologist, conservationists, researchers and university students. Authored by Thomas Roberts, Oxford University Press published two Field Guides on Large and

Small Mammals of Pakistan in 2005. In addition to this, Thomas Roberts authored or co-authored three 'Field Guides on Birds' of Indo-Pakistan, together with those of Bangladesh, Nepal, Sikkim, Bhutan and Sri-Lanka. Thomas Roberts acted as editor of the Mammal Section of Encyclopedia of Indian Natural History (1986). He also edited FAO/UNDP "Handbook of Vertebrate Pest Control in Pakistan" in 1981. His love for flowers and butterflies came out in the shape of two books-Wild Flowers of Pakistan (editor) and Butterflies of Pakistan in 2001.

In appreciation of his work in the natural history of Pakistan, he was decorated by the President of Pakistan with Sitare-e-Imtiaz on 23<sup>rd</sup> March, 1994. He was a founder Governor of World Wide Fund-Pakistan and received World Wildlife Fund (International) Award for conservation. In 2002, he received Stanford Raffles Award, thrice. Thomas Roberts was honorary fellow of the Zoological Society of Pakistan.

Since Dr. T.J. Roberts, who is spending his retired life in Great Britain and has not been able to make it to Pakistan to receive the award, the Society will make arrangements to have the award delivered at his address in the U.K.

**RECIPIENT OF  
LIFE TIME ACHIEVEMENT AWARD 2012**



**Prof. Dr. Bilqees Fatima Mujib**

Prof. Dr. Bilqees F. Mujib was born on February 10, 1936 at Hyderabad Daccan (British India). She received M.Sc. degree in Zoology with specialization in Parasitology in 1958 from the University of Karachi, and Ph.D. degree from University of Toronto, Canada in 1966. After that she received NRC-CIDA Research Fellowship and worked in McGill University, Quebee, Canada in the year 1975, 1977, 1978 for three months each year in summer. She also received training in electorn microscopy in British Museum, London and worked in University College Cardiff, Wales under the sponsorship of Pakistan Academy of Sciences and British Museum

Dr. Bilqees has more than 550 publications to her credit in international journals, including 69 in foreign journals, 34 books, 28 published in Germany, two scientific dictionaries and 78 scientific articles in Urdu. She was awarded D.Sc. from University of Karachi in 1976 for her outstanding contributions in the field of Parasitology. She is the founder editor of the “Proceedings of Parasitology” and Biological Research Journal. She is also on the editorial boards of *Biologia* published from Lahore and *Journal of Baqai Medical University*. She has been the member of Editorial and Advisory Board of *Pakistan Journal of Zoology*, *Zoologica Pakistan*, *Pakistan J. Nematology and Fauna and Flora*, India.

Dr. Bilqees was declared Zoologist of the Year in 1997. She has been the Vice President (south) of the Zoological Society of Pakistan and successfully organized Fourteenth Pakistan Congress of Zoology. She has supervised 53 M.Sc., 14 M.Phil and 15 Ph.D. projects on which degrees were awarded by the University of Karachi, Jinnah University for Women, University of Balochistan, University of Sindh and Urdu University Karachi. She has successfully completed several PSF, PARC and HEC funded research projects.

**RECIPIENT OF  
ZOOLOGIST OF THE YEAR AWARD 2012\***



**Dr. Javed Iqbal Qazi**

*Professor of Zoology, University of the Punjab, Lahore.*

Prof. Dr. Javed Iqbal Qazi was born in 1963. He obtained his M.Sc. and Ph.D. degrees from University of the Punjab, Lahore. In 2008 he availed of a HEC sponsored post-doctoral fellowship at University of Bangor, U.K. He has 25 years' teaching and research experience and his areas of research interests include Biotechnology, Environmental Microbiology, Skeletal Muscle Transplantation and Public Health. 11 Ph.D., 7 M.Phil. and 49 M.Sc./B.S. scholars have completed their research theses under his supervision. Prof. Qazi has published 88 research papers in National and International Research Journals of high repute, authored 3 Books and contributed chapters in two books. He has completed five Research Projects. He has participated and presented research work and delivered seminars on important issues in more than 60 National/International conferences and workshops.

Dr. Qazi is member of Biotechnology Society and Life fellow of Zoological Society of Pakistan, Biological Society of Pakistan, Fisheries Society of Pakistan, Myco-Pathological Society of Pakistan and Medical Research Society of Pakistan. He was on the Advisory Board of Pakistan Journal of Zoology and is continuing as Associate Editor of Punjab University Journal of Zoology. He has established a repository of highly promising bacterial isolates in his laboratory; including bacteria producing bioethanol and H<sub>2</sub> fuel and those capable of rehabilitating the deteriorating environments.

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\*Other nominee of this award were Dr. Tariq Mukhtar and Dr. Shamim Akhter.

**RECIPIENT OF  
PROF. DR. MIRZA AZHAR BEG GOLD MEDAL 2012\***



**Dr. Muhammad Sajid Nadeem**

*Assistant Professor*

*Department of Zoology, Arid Agriculture University, Rawalpindi*

Dr. Nadeem's area of interest is wildlife ecology, specifically the food preferences, habitat, breeding and population studies as well as conservation of mammals and birds. He has supervised 11 M.Phil students and presently 06 Ph.D. students are working under his supervision. He has published 21 articles in international and 16 in national journals (25 are HEC recognized); out of which 14 are in Impact Factor journals.

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\*Other applicant for this award were

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

- 1. Muzaffar Ahmad Gold Medal 2012**  
Sixteenth Muzaffar Ahmad Gold Medal 2012 was received by Miss Shabnam Shehzadi for obtaining first position in the M.Sc. Zoology examination of the University of the Punjab.
- 2. Afsar Mian Gold Medal 2012**  
Third Afsar Mian Gold Medal 2012 was given to Ms. Kiren Mustafa who obtained first position in the M.Sc. Biology/Zoology examination of the Arid Agriculture University, Rawalpindi.
- 3. Muhammad Afzal Hussain Memorial Gold 2012**  
Thirteenth Muhammad Afzal Hussain Memorial Gold 2012 was given to Ms. Sundus Iqbal for obtaining first position in Parasitology for her M.Sc. Zoology examination of the University of Karachi.
- 4. Mujib Memorial Gold Medal 2012**  
Sixteenth Mujib Memorial Gold Medal 2012 was given to Miss Shumaila Naz who obtained first position in the M.Sc. Zoology examination of the University of Sindh, Jamshoro.

## **QUALITY ASSESSMENT OF SOME BRANDED HONEY SAMPLES MARKETED IN LAHORE**

ANAM JAVED, NASREEN MUZAFFAR AND JAVED IQBAL QAZI\*  
*Microbial Biotechnology Lab., Department of Zoology, University of the Punjab,  
Lahore*

**Abstract.-** In the present study 11 honey samples including of local origin as well as exported ones were analyzed for various physicochemical, bacteriological and palynological parameters. Results of this study showed that none of the samples contained insoluble matter. While the local and the imported honey samples showed pH ranging from 4.1 to 7.47 and 2.9 to 5.28, respectively. Moisture content ranged from 15.51% to 21.11% and 16.53% to 25.70% for local and imported samples, respectively. Moisture contents above to 25% (permissible limit) indicate lesser shelf life of the produce. Out of 11 samples, 81.82% showed the presence of coliform bacteria ranging from 1944 to 6024 and 1968 to 5088 C.F.U./ml for the local and imported honey samples, respectively. Whereas two of the latter category of the honey samples were found free of coliform contamination. Due to its particular composition, honey is able to inhibit and destroy microorganisms. However, it appears that improper washing of the containers and handling of the honey during its processing were probably not of established standards. Regarding the palynological observations, two of the Pakistani samples: Swat Honey (Pure) and Marhaba Honey were monofloral, while the remaining ones were multifloral. Results of the present study strongly recommend strict appliance of physicochemical and microbiological standards of honey for assuring quality of the product before merchandising.

**Key words:** Honey adulteration, Coliform bacteria in honey, Honey palynology.

### **INTRODUCTION**

Honey, produced by honeybee from the nectar of living parts of plants is a strong concentrated solution of invert sugar and other carbohydrates like glucose and fructose, amino acids, minerals, aromatic substances, pigments, waxes and pollen grains (Bogdanov *et al.*, 1998; Qiu *et al.*, 1999; Iftikhar, 2010).

Variations in physicochemical properties of honey samples, such as ash contents, the spectrum of saccharides, the activity of enzymes, hydroxymethylfurfural (HMF), electrical conductivity, pH and optical rotation represent regional and floral differences (Sabatini *et al.*, 1995; Serra and Ventura,

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1995; Singh and Bath, 1997; Golob and Plestenjak, 1999; Terrab *et al.*, 2003; Iftikhar, 2010). Contents of honey depend on biotic and abiotic factors around the beehives. Correlation between the elemental content of honey and the nature of the environment has been documented. Even presence of heavy metals in honey has been reported and related to its geographical and botanical origin (Kump *et al.*, 1996; Caroli *et al.*, 1999, Iftikhar, 2010).

Presence of enzymes such as glucose oxidase produces acids like gluconic acid. Lower pH, presence of oxidizing agents such as hydrogen peroxide and hyperosmotic nature of honey serve as antimicrobials. Since ancient times, natural unprocessed honey was used to prevent microbial infections and aid wound healing (Gulfraz *et al.*, 2010). However, being a natural product, quality of honey collected from different locations varies greatly. Conditions of containers employed for collecting the honey, general cleanliness, homogeneity of the batch, organoleptic individuality (taste and aroma), colour, moisture content, degradation level of honey assessed by diastase and HMF contents, composition of principal sugars and microscopic evaluation encompassing botanical and geographical source are the major parameters taken into account while certifying a honey sample/ lot by many enterprises (Clément *et al.*, 2002; Nombé *et al.*, 2010).

Honey is in general evaluated by the physico-chemical analysis of its constituents. Quite a few of such constituents are of great importance to the honey industry as they persuade the storage quality, granulation, texture, flavor and the nutritional and medicinal quality of the honey (Iftikhar, 2010). They may also indicate honey adulteration, which is a major menace to consumers and eventually the farmers (Iftikhar, 2010; Muthui, 2013). For most simple adulterations of honey, authorized quality standards include high sucrose content (> 8%) indicative of simple cane or beet sugars addition (White, 1980).

Quality control of honey has two principle purposes. That is to verify its genuineness for adulteration and secondly to determine its quality. In many countries laws and regulations exist to which one must refer for marketing of the product. Unfortunately such laws and especially their executions are at their infancy in Pakistan. Whereas food quality assurance in the present anthropogenically polluted environment is a serious concern and authenticity in this regard is demanded by the consumers. Due to beneficial characteristics of honey like its high nutritional value, fast absorption of its carbohydrates and being religiously recommended remedial item, availability of both imported and

local products is common in the local market. While due to non existence of effective food quality assurance legislation lot of adulteration is done before packaging and marketing of honey. Remedial importance of the honey has also been cited in the Holy book of Muslims (Quran; Section 16 Verses 68-69). Accordingly the product has higher demands by the Muslims. Whereas the faith and medicinal importance of the honey is exploited by various suppliers in the form of adulterations. The purpose of the present study was to determine moisture, insoluble solids, electrical conductivity, pH, free acidity, pollen spectrum, reducing sugars, sucrose, and coliform bacterial content of natural and marketed honey samples. Such information are likely to pave for establishing quality assurance of honey products in Pakistan at par with international standards to provide quality product locally and promote the export that will contribute wards economy of the country.

## MATERIALS AND METHODS

### *Collection of honey samples*

Six national and five international honey brands were purchased from local market during October, 2012. Relevant details and labeling codes of the samples are given in Table I.

TABLE I.- BRANDS OF HONEY SAMPLES ALONGWITH COUNTRIES OF ORIGIN EMPLOYED IN THE PRESENT STUDY.

Sample code	Name of brand	Country
A	Ummul Shifa (Kalonji) Honey	Pakistan
B	Young's Natural	Pakistan
C	Langnese	Germany
D	Alshifa	Saudi Arabia
E	Swat Honey(Pure)	Pakistan
F	Marhaba Honey	Pakistan
G	Salman's Pak Honey	Pakistan
H	Billy Bee	Canada
I	Punjab University Honey (Beri)	Pakistan
J	Honsen Pure Honey	China
K	Dates Honey	Abu Dhabi

The procured honey samples were stored at room temperature in Microbiology laboratory of Department of Zoology, University of the Punjab,

Lahore, till further use. The analyzed parameters of the honey samples were compared with FAO and Food Standards Agency (Crane, 1990; Food Standards Agency, 2007).

#### *Analyses of the honey samples*

Moisture, insoluble solids, electrical conductivity, pH, free acidity, pollen spectrum, reducing sugars, sucrose, and coliform content of all the honey samples were determined.

For manual measurement of amount of moisture in each sample, Milwaukee refractometer was used. After calibration with distilled water, the honey smear was formed on prism of refractometer and it was covered with lid to note reading. This procedure was repeated thrice for each sample to calculate the percentage of moisture contents. Further dilution(s) were made if required (I.S.I. Handbook of Food Analysis (Part II) 1984).

The pH, free acidity and electrical conductivity were determined as described in Codex Alimentarius Commission (1969) with the help of digital pH meter (WTW). Amount of insoluble matter of the honey samples was determined according to the methods of Codex Alimentarius Commission (1969) and Lord *et al.* (1988).

For estimation of coliform contents, 2.5g of a honey was initially diluted up to 10 ml with sterile distilled water. Further dilution(s) were made if required. Known volume of the diluted honey samples was spread on EMB agar plates which were then incubated at 37°C for 24 hrs. C.F.U. and colonial morphologies of the coliforms were then recorded. Morphological characteristics permitted presumptive identification of the colonies following the guidance provided in Merck (1996/97). Only those Petri plates were considered valid for data recording which yielded C.F.U. in the range of 30-300.

The pollen spectrum of the honey samples was identified as described by Barth (1989). Sucrose contents of the honey samples were assessed with the help of Milwaukee refractometer. Whereas reducing sugars of the samples were determined according to Miller (1959).

## RESULTS AND DISCUSSION

Honey is a natural and very complex matrix and thus needs to be characterized by a set of physicochemical parameters for various purposes. The objectives might include nutritional and medicinal comparisons, identification of a desired product and to verify the authenticity of the material. The latter notion is of prime importance in this country. As being a Muslim country, the religious importance of honey is well established amongst the population; whereas due to some social reasons adulteration of food items is not uncommon here. Absence of food processing and packaging quality assurance; especially for small scale operating units, further add to the intensity of the problem. Thus it is very imperative to measure different physicochemical properties of local as well as imported honey samples. The latter category might be influenced by local packaging and environmental insults. The information obtained during the course of the present study is highly relevant in giving inputs for quality assurance of the natural product.

TABLE II.- DETERMINATION OF SOME PHYSICOCHEMICAL PARAMETERS OF THE HONEY SAMPLES

Sample code	% Moisture content	pH	Free acidity (mmol)	Electrical conductivity (mS/cm)	Reducing sugars (%)	Sucrose (%)
A	17.70±0.33	4.10±0.14	13.33±3.4	164.80±5.0	1.12± 0.03	6.0 ± 0.00
B	21.30±0.67	4.10±0.11	21.70±1.68	167.03±3.50	0.74±0.09	5.0 ± 0.00
C	20.70±0.33	2.90±0.09	23.33±4.42	134.30±3.43	1.97 ± 0.02	7.3 ± 0.15
D	20.00±0.00	3.90±0.140	21.70±1.67	159.47±5.0	1.23 ± 0.09	6.3 ± 0.03
E	18.30±0.33	4.20±0.101	13.33±3.40	161.23±7.0	1.25 ± 0.18	8.0 ± 0.12
F	22.70±0.33	4.08±0.13	20.0±0.00	165.60±5.08	1.21 ± 0.02	5.5 ± 0.17
G	18.30±0.33	4.27±0.01	15.0±5.03	156.13±9.80	1.38 ± 0.07	7.7 ± 0.18
H	18.70±0.67	4.51±0.06	16.70±1.67	143.3±14.20	1.16 ± 0.07	8.07±0.07
I	21.70±0.67	7.47±0.11	0.33±1.67	-18 ±8.02	1.34 ± 0.05	6.70±0.07
J	19.70±0.88	4.53±0.08	18.33±6.02	141.7±7.21	1.04 ± 0.04	6.50±0.07
K	25.00±0.58	5.28±0.15	61.7±3.40	58.40±45.25	1.24 ± 0.12	5.90±0.07

Values are means of triplicates ± S.E.M.

Moisture content of the honey samples analyzed in this study ranged from 17.70% to 25.00% (Table II). Recommended range for moisture contents of good quality honey is 20%. Thus the samples No. B, C, F, I and K had 1.3%, 0.7%, 2.7%, 1.7% and 5% higher than the permissible limit of moisture contents, respectively. Measuring moisture contents with the help of refractometer is a

recommended method and is less time consuming (White, 1969; AOAC, 1995). Variation of moisture content of honey is correlated with climatic conditions and level of maturity of the product. A moisture content of 20% is maximum prescribed limit (Codex Alimentarius Commission Standards, 2001). In the present study only samples, F and K had considerably higher moisture contents with values around 23% and 25%, respectively. These two samples had their origin from Pakistan (Swat) and Abu Dhabi, respectively. It is known that moisture content in honey increases due to its hygroscopic nature and moisture from surrounding environment may be absorbed (Bibi *et al.*, 2008). In this regard it is very important to consider, final packaging of honey, especially keeping in view the shelf life of the product in specific region(s). Proper packaging should stop the moisture intrusion from the environment.

pH of the honey samples ranged from 2.9 to 7.47 (Table II). This parameter determines the sour or otherwise taste of honey. The slightly alkaline pH was recorded for the honey sample of Punjab University Bee Farming Centre. It is well known that honey is generally acidic in nature (Saxena *et al.*, 2010). The recommended range of this parameter is 3.7 to 4.5 as per standard being followed in this study. While it is also known that pH of honey might not directly be related to the free acidity because of the buffering action of various amino acids and minerals present (Abu-Tabroush, 1993). Therefore, in various protocols for characterizing the honeys in addition to determination of pH, free acidity is also recommended. Free acidity of the honey samples ranged from 0.33 mmol/1000g to 61.7mmol/1000g (Table II). The sample K had a value of the parameter 61.7 mmol/1000g which is higher than the Honey commission's recommended level of free acidity which is 50mmol/1000g.

Regarding electrical conductivity, values of the parameter ranged from -18 to 167mScm<sup>-1</sup> (Table II). Food Standards Agency, European Honey commission and FAO recommended range describes a maximum value of 0.8 mScm<sup>-1</sup> for good quality honey. The electrical conductivity of honey is closely related to concentration of mineral salts, organic acids, proteins and in short the ash content (Bogdanov *et al.*, 2000).

According to the European standards, FAO and Food Standards Agency (Crane, 1990; Food Standards Agency, 2007) the standard amount of reducing sugars in honey is 60%. Whereas in the present study amount of reducing sugars ranged from 0.7365 to 1.3751% (Table II). The much lesser values of reducing sugars might had been due to improper storage of the produce. Sucrose contents

of the honey samples ranged from 5% to 8.07% (Table II). Being 5% as the standard value recommended by Food Standards Agency, European Honey commission and FAO, except honey sample B remaining all the samples had higher percentages of sucrose than the permissible limit.

Pollen analysis of the honey samples revealed higher frequency for *Eucalyptus*, as its pollens were observed in A, I and J samples. The palynological analysis of the honey samples showed that all possessed pollens of various taxa, except the samples designated as F and G each of which depicted only one species. These two samples had been processed and packaged by the Pakistani food industries the Marhaba (F) and Salman (G) (Table III). Diversity of pollen in a honey sample obviously indicates the diversity of plants foraged by the bees while collecting and preparing the honey. For example, Stawiarz (2008) showed that rapeseed honeys were characterized by a variety of colors and that their aroma resembled that of rapeseed flowers. Regarding the results of the present study, the two monofloral and the remaining multifloral honey samples may easily be connected to their source of nectar and thus probable properties.

TABLE III.- POLLEN SPECTRA OF THE HONEY SAMPLES

Sample code	Pollens of plant species
A	<i>Areca catechu</i> , <i>Corylus avellana</i> , <i>Eucalyptus fibrosa</i> , <i>Dodonea viscosa</i> , <i>Broussonetia papyrifera</i>
B	<i>Tamarix indica</i> , <i>Vicia cracca</i> , <i>Cannabis sativa</i>
C	<i>Trifolium hybridum</i> , <i>Trifolium hybridum</i> , <i>Tamarix dioca</i>
D	<i>Melilotus spp.</i> , <i>Corylus avellana</i> , <i>Dodonea viscosa</i> , Members of Euphorbiaceae
E	Members of Astreaceae,
F	<i>Eucalyptus fibrosa</i> ,
G	<i>Cannabis sativa</i> , <i>Juniperus coahuilensis</i> , <i>Syzygium commenis</i>
H	<i>Cannabis sativa</i> , <i>Cannabis sativa</i> , <i>Eucalyptus fibrosa</i> , <i>Albizia julibrissim</i>
I	<i>Eucalyptus fibrosa</i> , <i>Dodonea viscosa</i> , <i>Eucalyptus fibrosa</i> , <i>Broussonetia papyrifera</i> , <i>Broussonetia papyrifera</i>
J	<i>Caesalpinia spp.</i> , <i>Tamarix alii</i> , <i>Eucalyptus fibrosa</i> , <i>Eucalyptus fibrosa</i> , <i>Trifolium hybridum</i>
K	<i>Cannabis sativa</i> , <i>Dodonea viscosa</i> , <i>Melilotus spp.</i> ,

Inasmuch as the coliform bacterial content of the honey samples is concerned, except for the samples J and K, all the other 9 samples did contain varying levels of the fecal pollution indicating microorganisms. Colony forming units/ml of the honey samples ranged from 1944 to 6024 (Table IV). Colonial morphologies of the C.F.U. on E.M.B. agar medium permitted presumptive

TABLE IV.- C.F.U. AND COLONY MORPHOLOGIES OF COLIFORM BACTERIAL CONTENT OF THE HONEY SAMPLES <sup>A</sup>.

Sample code	C.F.U /g	Colony features				Diameter (mm) Mean $\pm$ S.E.M.	Identification
		Configuration	Elevation	Margins			
<b>A</b>	6024	Fried egg	Umbonate	Rounded	5.6 $\pm$ 3.8	<i>K. pneumonia</i> & <i>E. cloacae</i>	
		Irregular & spreading	Hilly	Irregular	6.4 $\pm$ 3.41		
<b>B</b>	5088	Rounded with raised margins	Umbonate	Wavy	8.0 $\pm$ 3.7	<i>K. pneumonia</i> , <i>E. coli</i> & <i>E. cloacae</i>	
		Fried egg	Crateriform	Rounded	6.4 $\pm$ 3.41		
<b>C</b>	3024	Wrinkled	Hilly	Irregular	5.0 $\pm$ 3.3	<i>K. pneumonia</i> , <i>E. coli</i> & <i>E. cloacae</i>	
		Fried egg	Umbonate	Rounded	4.8 $\pm$ 3.8		
		Rounded with raised margins	Crateriform	Wavy	4.4 $\pm$ 1.85		
		Wrinkled	Hilly	Irregular	7.2 $\pm$ 1.25		
<b>D</b>	2580	Filamentous	Hilly	Branching	5.8 $\pm$ 3.7	<i>K. pneumonia</i> , <i>E. coli</i> , <i>B. cereus</i> & <i>E. cloacae</i>	
		Fried egg	Umbonate	Rounded	4.6 $\pm$ 3.8		
<b>E</b>	3768	Wrinkled	Hilly	Irregular	2.8 $\pm$ 2.31	<i>K. pneumonia</i> , <i>E. coli</i> , <i>B. cereus</i> & <i>E. cloacae</i>	
		Irregular & spreading	Hilly	Irregular	8.0 $\pm$ 0.49		
<b>F</b>	1944	Fried egg	Umbonate	Rounded	3.8 $\pm$ 3.41	<i>K. pneumonia</i> , <i>E. coli</i> , <i>B. cereus</i> & <i>E. cloacae</i>	
		Irregular & spreading	Hilly	Irregular	6.0 $\pm$ 1.8		
<b>G</b>	2088	Wrinkled	Hilly	Irregular	6.4 $\pm$ 0.32	<i>K. pneumonia</i> , <i>E. coli</i> , <i>B. cereus</i> & <i>E. cloacae</i>	
		Fried egg	Umbonate	Rounded	4.6 $\pm$ 1.31		
<b>H</b>	1968	Rounded	Drop like	Smooth	4.8 $\pm$ 3.8	<i>K. pneumonia</i> , <i>E. coli</i> , <i>B. cereus</i> & <i>E. cloacae</i>	
		Raised margin	Flat	Smooth	3.4 $\pm$ 0.52		
<b>I</b>	2232	Irregular	Crateriform	Wavy	2.6 $\pm$ 2.72	<i>K. pneumonia</i> , <i>B. cereus</i> & <i>E. cloacae</i>	
		Rounded	Drop like	Smooth	6.4 $\pm$ 0.32		
		Wrinkled	Convex	Smooth	3.4 $\pm$ 0.5	<i>B. cereus</i> & <i>E. coli</i>	
		Fried egg	Hilly	Irregular	4.2 $\pm$ 0.5		
		Rounded with raised margins	Umbonate	Rounded	3.6 $\pm$ 0.52		
			Crateriform	Wavy	2.8 $\pm$ 0.31		

<sup>A</sup>. Coliforms were not detected in the samples J and K. Values of colony diameters represent means of triplicates.

identification of the bacteria following the guidance provided in Merck (1996/97). The samples J and K, which were devoid of any culturable coliform content, had their origin from China and Abu- Dhabi, respectively. It is probable that these two samples might have been packaged in the respective countries of origin. While all Pakistani and other samples had been packaged into the marketed containers here in Pakistan or anywhere else where strict quality assurance legislation and aseptic handling is not followed. The presence / absence of fecal coliforms is an established indicator of sanitary quality and safety of honey (Estevinho *et al.*, 2012). According to the Food Standards Agency, European Honey commission and FAO honey of quality should be ideally free of coliform content.

Conclusively, the physicochemical, microbiological and palynological analyses of the honey samples are mandatory both for declaring authenticity of the natural product as well as to determine the nutritional, medicinal and safety status. The analyses are also now mandatory for commercial quality assessment of honeys. The techniques established in this laboratory will be implemented in future to explore the proper ways of processing and storage of the natural product as to warrant it long shelf life and render the product quality of international standards.

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TABLE IV.- C.F.U. AND COLONY MORPHOLOGIES OF COLIFORM BACTERIAL CONTENT OF THE HONEY SAMPLES <sup>A</sup>.

Sample code	C.F.U /g	Colony features			Diameter (mm) Mean $\pm$ S.E.M.	Identification
		Configuration	Elevation	Margins		
A	6024	Fried egg	Umbonate	Rounded	5.6 $\pm$ 3.8	<i>K. pneumonia</i> & <i>E. cloacae</i>
		Irregular & spreading	Hilly	Irregular	6.4 $\pm$ 3.41	
B	5088	Rounded with raised margins	Umbonate	Wavy	8.0 $\pm$ 3.7	<i>K. pneumonia</i> , <i>E. coli</i> & <i>E. cloacae</i>
		Fried egg	Crateriform	Rounded	6.4 $\pm$ 3.41	
		Wrinkled	Hilly	Irregular	5.0 $\pm$ 3.3	<i>K. pneumonia</i> , <i>E. coli</i> & <i>E. cloacae</i>
C	3024	Fried egg	Umbonate	Rounded	4.8 $\pm$ 3.8	
		Rounded with raised margins	Crateriform	Wavy	4.4 $\pm$ 1.85	
		Wrinkled	Hilly	Irregular	7.2 $\pm$ 1.25	
		Filamentous	Hilly	Branching	5.8 $\pm$ 3.7	<i>K. pneumonia</i> , <i>E. coli</i> , <i>B. cereus</i> & <i>E. cloacae</i>
D	2580	Fried egg	Umbonate	Rounded	4.6 $\pm$ 3.8	
		Wrinkled	Hilly	Irregular	2.8 $\pm$ 2.31	<i>K. pneumonia</i> , <i>E. coli</i> , <i>B. cereus</i> & <i>E. cloacae</i>
E	3768	Irregular & spreading	Hilly	Irregular	8.0 $\pm$ 0.49	
		Fried egg	Umbonate	Rounded	3.8 $\pm$ 3.41	<i>K. pneumonia</i> , <i>E. coli</i> , <i>B. cereus</i> & <i>E. cloacae</i>
F	1944	Irregular & spreading	Hilly	Irregular	6.0 $\pm$ 1.8	
		Wrinkled	Hilly	Irregular	6.4 $\pm$ 0.32	<i>K. pneumonia</i> , <i>E. coli</i> , <i>B. cereus</i> & <i>E. cloacae</i>
		Fried egg	Umbonate	Rounded	4.6 $\pm$ 1.31	
G	2088	Rounded	Drop like	Smooth	4.8 $\pm$ 3.8	<i>K. pneumonia</i> , <i>E. coli</i> , <i>B. cereus</i> & <i>E. cloacae</i>
		Rounded	Flat	Smooth	3.4 $\pm$ 0.52	
H	1968	Raised margin	Crateriform	Wavy	2.6 $\pm$ 2.72	<i>K. pneumonia</i> , <i>B. cereus</i> & <i>E. cloacae</i>
		Irregular	Drop like	Smooth	6.4 $\pm$ 0.32	
		Rounded	Convex	Smooth	3.4 $\pm$ 0.5	<i>B. cereus</i> & <i>E. coli</i>
I	2232	Wrinkled	Hilly	Irregular	4.2 $\pm$ 0.5	
		Fried egg	Umbonate	Rounded	3.6 $\pm$ 0.52	
		Rounded with raised margins	Crateriform	Wavy	2.8 $\pm$ 0.31	

A. Coliforms were not detected in the samples J and K. Values of colony diameters represent means of triplicates.

## HONEY QUALITY ASSESSMENT

## PROFILE OF METALS' RESISTANT DENITRIFYING BACTERIA AT DIFFERENT DEPTHS OF TANNERIES' EFFLUENTS EFFECTED SOIL

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**Abstract.** The present report refers to screening of denitrifying bacteria from a land area which had been influenced by chronic exposure of chromium laden tanneries' effluents in the city Kasur. Twenty denitrifying bacterial strains were isolated from different depths (20 to 60 cm) of the soil on selective media. The deepest soil location studied (60cm) indicated the preferred zone for the presence of denitrifiers. The bacterial isolates were found resistant against different heavy metals with MICs ranging 1050-1250 for Pb, 40-70 for Hg and 650-1050 ppm for Cu. Considerable fraction of denitrifiers (40%) showed complete reduction of 50 µg/ml of Cr(VI) within 48 hrs of incubation at 37°C. However, this concentration of the metal inhibited denitrification potential of 70% of the community. The isolates ASDT1A ASDF3a, ASDF3b and ASDS9 were found to be the most potent denitrifiers which sustained their denitrification ability even in the presence of 50µg/ml of Cr(VI). Generic screening of the bacterial isolates, based on phenotypic and biochemical characterization, showed that the denitrifiers belonged to three genera *Bacillus*, *Corynebacterium* and *Pseudomonas* whereas the community was dominated by the genus *Bacillus*. These denitrifying bacteria may be inflicted for bioremediation of nitrates and heavy metals' polluted soils and waters.

**Key words:** Denitrifiers, bacterial metal resistance, *Bacillus*.

## INTRODUCTION

The process of denitrification, completes the N-cycle by removing nitrogen contents from terrestrial and aquatic ecosystems. Biological denitrification is a dissimilatory process where oxidized nitrogen compounds ( $\text{NO}_3^-$  and  $\text{NO}_2^-$ ) are used as alternative electron acceptors for energy production. It is a ubiquitous phenomenon in soils and sequentially removes nitrogen oxides by reduction mechanism passing through various oxidized forms of nitrite, nitric oxide, nitrous oxide and finally to nitrogen involving different enzymes for each particular reduction step (Zumft, 1997). In marine coastal sediments, 40 to 50% of external inputs of dissolved inorganic nitrogen are

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removed by denitrification (Seitzinger, 1990) This dissimilatory reduction is a facultative trait of bacteria (Tiedje, 1988; Zumft, 1992). Any stress on either part of N cycle would disturb all components involved in its cycling.

Since nitrate is a terminal electron acceptor for anaerobic respiration, a competitive phenomenon in the presence of other electron acceptors could change the rate of denitrification (Viamajala *et al.*, 2002; Middleton *et al.*, 2003) Certain bacteria also respire Cr (VI) as an electron acceptor under anaerobic conditions. Thus denitrification could be affected in the areas contaminated with industrial effluents loaded with chromium. Different attributes of nitrate and chromium when both are present as co-contaminant have been reported. For denitrifying bacteria, Cr (VI) could either exert toxic effects on nitrate reduction or it may interfere with its reduction. Middleton *et al.* (2003) reported inhibitory relationship between Cr(VI) and nitrate reduction due to competitive selection of one electron acceptor over the other by *Shewanella oneidensis* MR-1. Chromium has previously been reported to enhance denitrification in sediments (Slater and Capone, 1984). In another study nitrate stimulated the chromium reduction in denitrifying consortia when the medium was supplied with molasses as organic carbon source (Oliver *et al.*, 2003). However, these interactions are concentration dependent, as at low levels of Cr (VI) and nitrate both the electron acceptors might be reduced simultaneously (Konovalova *et al.*, 2008). Removal of nitrogen is very imperative for waste discharges containing nitrogenous compounds due to the issue of eutrophication. Since the soil microbiota provide necessary machinery to regulate biogeochemical cycles, thus studying potential activities of bacteria from chronically metals contaminated soils would generate important information regarding soil health status, bioremediating such soils and maintaining nutrient balance therein. Thus efficient chromium resistant denitrifiers worked out during the course of this study can be exploited for treatments of chromium and nitrate contaminated soils/wastewaters.

## MATERIALS AND METHODS

Soil samples were collected from a locality called Deen Ghar, Kasur. This land area is situated in southern east of Kasur, a city of Punjab province of Pakistan with an area of 3,995 km<sup>2</sup>, elevation 218 m, located at 31.12° North latitude, 74.45° East longitude and a population density of 595/km<sup>2</sup> (Sheik *et al.*, 2012). The study location had been receiving effluents of the tanneries for few decades and a decade before the whole area resumed the look of shallow ponds withholding pinkish effluents. Thereafter, the tanneries effluents' drainage was improved and the area resumed an open field look.

Soils were sampled from 20, 40 and 60 cm depths from the surface. From a described depth soil was sampled from vertical surface of freshly digged out pit by pressing the sterile open mouthed glass bottle directly against the freshly exposed vertical surface to avoid cross contamination.

#### *Isolation of denitrifying bacteria*

For enrichment of denitrifying bacteria, 1 gram of soil from each of the different depths *i.e.*, 20, 40 and 60cm was inoculated into 10ml Trypton Soy Broth (TSB) in screw capped test tubes. One inch column of sterilized paraffin oil was added over the inoculated broths to provide anaerobic conditions and placed at room temperature ( $30\pm 1^\circ\text{C}$ ) for one week. Then submerged agar medium method was used for the isolation of denitrifying bacteria from the enriched culture (Archana *et al.*, 2004). For this purpose 0.1ml of enriched TSB culture of each respective soil sample was mixed with 15mL molten sterilized Yeast Trypton Soy Nutrient (YTSN) agar medium and poured into sterilized Petri plates. Thus cultured medium was allowed to solidify at room temperature. Then a layer of molten wax was applied over the plates. The inoculated plates were placed at room temperature ( $30\pm 1^\circ\text{C}$ ) for 20 days. Well separated embedded bacterial colonies in the agar medium were cut with the help of sterilized scalpel and forceps, pure cultured and preserved in slants under paraffin oil layer.

#### *Determination of minimum inhibitory concentration (MIC) of different metals for bacterial growth*

For finding MICs of Cr, Cu, Pb and Hg for the bacterial isolates, microdilutions of double strength nutrient broth according to the required metal concentrations were prepared. The metal salts used were  $\text{K}_2\text{CrO}_4$ ,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ,  $\text{Pb}(\text{NO}_3)_2$  and  $\text{HgCl}_2$ . Two percent of fresh bacterial growth was inoculated into metal amended media and incubated at  $37^\circ\text{C}$  for 48 h. Optical density of the growth was then recorded at 600 nm in UV spectrophotometer. Lowest concentration of metal which completely inhibited the bacterial growth in nutrient broth was considered minimum inhibitory concentration (MIC).

#### *Chromium reduction potential*

For finding Cr(VI) reduction potential of the bacterial isolates, 20 ml of nutrient broth spiked with  $50 \mu\text{g/ml}$  of Cr(VI) as  $\text{K}_2\text{CrO}_4$  was prepared in 100 ml culture bottles and inoculated with 10% of fresh (overnight incubated) bacterial

culture. Inoculated bottles were incubated at 37°C in shaker at 120 rpm. Uninoculated broth processed similarly served as control. Following 48 h of incubation 1.5 ml of a bacterial culture was taken and centrifuged at 10000 rpm for 5 min. Supernatant was analyzed for residual Cr(VI) in the media by colorimetric method using diphenylcarbazide (DPC) reagent (Rehman *et al.*, 2008). Percent Cr(VI) reduction was determined by the following formula:

$$\% \text{ Cr (VI) reduction} = \frac{\text{Final Cr (VI) Conc. After 48 h} - \text{Initial Cr (VI) Conc. at h 0}}{\text{Initial Cr (VI) Conc. at h 0}} \times 100$$

#### *Qualitative analysis of nitrate and nitrite reduction*

To determine the isolates' potential of nitrate reduction, nitrate broth was prepared (g/L; peptone 20, potassium nitrate 2, pH 7), dispensed in an amount of 4ml/test tube and autoclaved routinely. The broth was inoculated with the isolates' growth and covered with 1cm layer of autoclaved paraffin oil and cotton plugged. The test tubes were then incubated for 48 h at 37°C. After the incubation, oil was removed and 0.25ml of reagent I (8g of sulphanilic acid in 1 liter of 5N glacial acetic acid) was added soon after which 0.25ml of reagent II (5g Dimethyl- $\alpha$ -naphthylamine in 1 liter of 5N glacial acetic acid) was added. The presence of nitrite was evidenced by the development of red color within 1-2 minutes. The cultures which showed no red color, were considered for two possibilities that is either nitrate had reduced to nitrite or the nitrate was reduced completely into gaseous forms (N<sub>2</sub>). Therefore, to the negative tubes a small quantity of zinc dust was added. A negative test was assessed for its level of denitrification as when a red color appeared due to nitrate formed due to oxidation of nitrite by the zinc. Whereas complete denitrification was considered for the culture which showed no color even after zinc dust addition (Weyant *et al.*, 1995).

#### *Physicobiochemical characterization for rapid generic diversity assessment of the bacterial isolates*

Bacterial isolates of the present study were characterized morphologically through Gram and endospore stainings and motility test in addition to the consideration of colony morphology. While they were characterized biochemically through catalase, oxidase, MR-VP, casein hydrolysis, starch hydrolysis, gelatin liquefaction, sulfide production, indole production, citrate utilization, nitrate reduction and sugar fermentation tests as described by Benson

(1994). Genus level identification was then assessed for all the bacterial isolates after the Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994) for having a general look upon the vertical distribution patterns of the different bacterial genera along the soils' different depths.

## RESULTS AND DISCUSSION

### *Isolation of denitrifying bacteria*

The present study reports isolation of 20 denitrifying bacterial isolates from different depths of soils of the study area with maximum score from the deepest location (60cm) studied. Highest diversity of the bacteria was also found at the deepest depth. Distribution of denitrifying bacterial isolates among different soil depths enumerated as six, three and eleven at 20, 40 and 60cm depths, respectively. Cloete and Muyima (1997) reported predominance of denitrifying bacteria in anoxic zones where limited oxygen conditions favor proliferation of nitrate reducing microbes. Presence of denitrifying microorganisms in contaminated environments has been reported in many studies from different geographical locations (Fries *et al.* 1994; Leta *et al.*, 2004). Denitrifying bacterial community depends upon the source of nitrate as well as other environmental factors for its structural development. Negative factors may include heavy metals in agricultural fields with potential stressful effects on the process of denitrification (Enwall *et al.*, 2005).

Denitrifying bacteria could be utilized for decontaminating diverse environments. Frequency of the isolation of denitrifying bacteria for different soil depths indicated effects of nutrients and oxygen availability etc. A long term exposure to a heavy metal may allow presence, selection and/ or proliferation of microorganisms resistant to that metal and capable of performing their primary roles in the altered environment.

### *Minimum inhibitory concentration (MIC) against different metals*

MIC levels of chromium for denitrifying bacteria ranged from 1400 to 1650ppm. Whereas for lead the MIC values fluctuated from 1050-1250ppm. For copper and mercury the MIC ranges were 650 to 850 and 40 to 70 ppm, respectively. The maximum chromium MIC (1650ppm) was expressed by strain (ASDT1a) isolated from 20cm and another (ASDS9) from 60cm soil depth (Table I).

TABLES I.- MINIMUM INHIBITORY CONCENTRATIONS (ppm) OF DIFFERENT METALS FOLLOWING CULTIVATION IN NUTRIENT BROTH FOR 48HRS AND DENITRIFYING POTENTIAL OF THE BACTERIAL ISOLATES IN NITRATE BROTH MEDIUM

Soil depth (cm)	Isolate code	Concentration (ppm)				Denitrification	
		Cr <sup>6+</sup>	Pb <sup>2+</sup>	Hg <sup>2+</sup>	Cu <sup>2+</sup>	Without (Cr <sup>6+</sup> )	Cr <sup>6+</sup> : (50µg/ml)
20	ASDT1a	1650	1050	70	850	+++	+
	ASDT1b	1600	1200	40	850	+++	+
	ASDT2	1600	1200	40	850	+	+
	ASDT5	1600	1200	40	650	+++	++
	ASDT7a	1600	1200	40	850	+	+
	ASDT7b	1600	1200	55	800	+++	+++
40	ASDF3a	1600	1200	40	800	+++	+++
	ASDF3b	1600	1200	40	800	+++	++
	ASDF3c	1550	1200	40	800	+++	+
	ASDS1a	1600	1200	40	650	+++	+
60	ASDS1b	1600	1200	40	850	+	+
	ASDS1c	1550	1150	40	850	+++	++
	ASDS2	1600	1200	50	850	+++	++
	ASDS3b	1600	1200	40	850	+++	++
	ASDS4	1500	1200	40	750	+++	++
	ASDS5a	1600	1150	50	650	+++	++
	ASDS5b	1550	1150	40	800	+++	++
	ASDS8b	1600	1150	50	800	+++	+++
	ASDS9	1650	1150	50	800	+++	++
	ASDS10a	1550	1150	40	800	+++ <sup>a</sup>	+++

+++ , bubble formation observed with complete denitrification;

++ , complete denitrification; + , only nitrate reduction.

The higher MIC for chromium suggests selective proliferation of bacterial community in the tanneries effluents' effected soils which resulted in isolation of narrow range of the bacteria in terms of their heavy metals' tolerances. A variety of multiple metal resistant bacteria has previously been isolated from contaminated sites. Resistance mechanisms are either plasmid linked which encode membrane transporters to efflux metals, whereas chromosome linked resistance is related to detoxification mechanisms involving reduction (Ramirez-Diaz *et al.*, 2007). Detailed analysis in this regard is required for the present isolates as the information of metals' reduction mechanisms is pivotal for practical applications and concerned predictions. It has been reported previously that most Cr (VI) resistant microorganisms are tolerant up to 4500 mg L<sup>-1</sup> of Cr(VI) (Shakoori *et al.*, 1999).



TABLE II.- PHENOTYPIC AND BIOCHEMICAL CHARACTERIZATION OF THE DENITRIFYING BACTERIA ISOLATED FROM DIFFERENT DEPTHS OF THE SOIL.

IC	Mp	GS	M	ES	Gr in NaCl (6.5%)	Biochemical tests			Carbohydrate utilization						Hydrolysis of			Genus		
						Cat	Oxi	VP	Gl	Ga	La	Xy	Ar	St	Ge	Ca				
ASDT1a	Rods (single)	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	Bacillus
ASDT1b	Rods	-	+	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	Pseudomonas
ASDT2	Rods	-	+	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	Pseudomonas
ASDT5	Rods(single)	-	+	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	Pseudomonas
ASDT7a	Rods(single)	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	Bacillus
ASDT7b	Rods(chains)	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	Bacillus
ASDF3a	Rods(single)	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	Bacillus
ASDF3b	Rods(chains)	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	Bacillus
ASDF3c	Rods(single)	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	Bacillus
ASDS1a	Clumps of rods	+	-	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	Bacillus
ASDS3b	Rods	-	+	ve	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	Pseudomonas
ASDS1c	Rods(single)	-	+	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	Pseudomonas
ASDS2	Long chains of rods	+	-	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	Bacillus
ASDS3b	Short rods (single)	-	+	Ve	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	Pseudomonas
ASDS4	Rods(single)	+	-	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	Bacillus
ASDS5a	Rods(single)	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	Bacillus
ASDS5b	Rods(single)	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	Bacillus
ASDS8b	Rods(chains)	+	-	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	Bacillus
ASDS9	Rods(chains)	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	Bacillus
ASDS10a	Rods(single)	+	+	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	Corynebacterium

Abbreviations used: SD, Soil depth; IC, Isolation code; Mp, Morphology; GS, Gram staining; M, Motility; ES, Endospore staining; Gr, Growth; Cat, Catalase; Oxi, Oxidase; VP, Voges-Proskauer test; Gl, Glucose; Ga, Galactose; La, Lactose; Xy, Xylose; Ar, Arabinose; St, Starch; Ge, Gelatin; Ca, Casein; -, negative; +, positive.

in 15% of the denitrifying community. Seventy percent of the bacterial isolates experienced inhibitory effects of Cr(VI) on denitrification. Severe inhibitory effects were seen in four isolates; ASDT1b, ASDT2, ASDS1a and ASDS1b where complete denitrification was restricted to nitrate reduction level. Only four isolates, one from 20cm depth (ASDT1a), two from the 40 cm soil depth (ASDF3a and ASDF3b) and one from 60s cm soil depth(ASDS9) showed denitrification in the presence of chromium comparable to the respective cultivation in absence of the metal (Table I).

*Physicobiochemical characterization for rapid generic diversity assessment of the bacterial isolates*

Chromium resistant bacterial community in the present study, in general, belonged to three major genera, i.e., *Bacillus*, *Pseudomonas* and *Corynebacterium* and represented 65, 30 and 5% of the total bacterial isolates, respectively (Table II). Roane and Kellogg (1996) justified *Bacillus*, as a dominant genus in heavy metal polluted soils as a function of their spore forming trait, while unique characteristic of lipid membranes of *Corynebacterium* helps them to combat heavy metal stress. Denitrification is well reported in Gram positive bacteria and *Bacillus sp.* represent one of the most abundant denitrifying bacterial group (Garcia, 1977; Flores-Mireles *et al.*, 2007; Verbaendert *et al.*, 2011).

The present study concludes that the denitrifying bacteria are not only chromium resistant but they are also capable of expressing varying levels of the metal reduction. The Cr (VI) reducing potential as well as sustenance of denitrifying role under the metal stress of these denitrifying bacteria might find increasing applications for concomitant removal of nitrates and the heavy metals from contaminated waters. Appropriate amounts of nitrates and Cr (VI) contaminations from different industrial sources might be adjusted for efficient removal of the pollutants. Agricultural land run-off characterized with higher nitrates and/or metal contents will also be addressable by the application of the bacterial isolates reported in this communication.

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## CULTIVATION OF *BACILLUS SUBTILIS*-A4, A FISH GROWTH ESCALATING PROBIOTIC IN SUGARCANE BAGASSE

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**Abstract.-** The present study provides a cost-effective solid state cultivation method of a fish probiotic for growing aquaculture industry in this country. *Bacillus subtilis* A-4, a fish growth promoting probiotic was rejuvenated in nutrient broth from stock culture of Microbial Biotechnology Laboratory, University of the Punjab, Lahore, Pakistan. The bacterial cells were then cultured in aqueous extract of 2% sugarcane bagasse. The bacterium grew well when incubated at 45°C, pH 7 and under aerated conditions with 5% inoculum size. Following 48 hours of incubation, the bacterium yielded sufficient cellulase activity which hydrolyzed the sugarcane bagasse cellulose into monomeric sugars which could then be utilized for its growth. The units of cellulolytic activity were measured upto 378.296 U/ml/h. The solid state cultivation (SSC) experiment employed 9.09% of the pulverized sugarcane bagasse and expressed progressive increase in growth of *Bacillus subtilis*-A4 till 3<sup>rd</sup> day of the fermentation. The SSC mass was dried at room temperature and then processed to check the dissociation/adherence potential of the bacterium with the unutilized substrate. The dried fermented matter was exposed to different conditions and it was found that maximum dissociation occurred at pH 8 while least at pH 7 following 15 minutes of exposure at room temperature. These information are relevant in designing modes of administration of probiotics in aquaculture.

**Key words:** Aquaculture, Solid state cultivation, Probiotic.

### INTRODUCTION

There is a significant role of fishery and fishing industry in the national economy of Pakistan. The country is bestowed with large natural freshwater resources as well as diversity of fish fauna. The freshwater fish fauna of Pakistan is represented by a minimum of 193 fish species (Rafique, 2007; Rafique and Khan, 2012). Among the total fish fauna of Pakistan, on the basis of economic importance and rarity, 86 species have been identified as “species of special importance” (Rafique and Khan, 2012).

During the last two decades Asian aquaculture has evolved to a science

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based activity from a traditional practice and it is now becoming an important food production sector. Aquaculture (or fish farming) is new in Pakistan and there is vast potential for development of this sector. Besides the rich fish fauna only seven warm species and two cold water species are cultivated on commercial scale in Pakistan. Intensive aquaculture has not yet been developed because of limited expertise and non-availability of low cost feed. High quality fishmeal albeit provides a balanced amount of all essential amino acids, phospholipids, and fatty acids for optimum development, growth, and reproduction, especially of larvae and brood stock. But increasing cost of fishmeal and doubt concerning its long-term availability dictated for research addressing the use of plant and animal by-products to substitute the fishmeal (Gallagher, 1994; Kaushik *et al.*, 1995; Stickney *et al.*, 1996; Brunson *et al.*, 1997; Hasan and Amin, 1997). However, provision of balance feed is not the sole requirement for efficient aquaculture systems. Rather it is the feed conversion efficiency which is a directly measure of fish growth. This aspect depends upon a number of factors such as age of fish, its intestinal health and various physicochemical parameters of the water body. Relatively recently role of beneficial bacteria in the fish gut has been appreciated for its better growth and health. Cultivations of such beneficial microorganisms and their administrations to enhance growth of the fish are now being documented all over the world.

The modern concept of probiotics was introduced first time 40 years ago by Parker (1974), who originally referred organisms and substances which contribute to intestinal microbial balance as probiotics. Probiotics help in gaining live weight by increasing feed conversion efficiency (Al-Dohail *et al.*, 2009; Saenz de Rodriguez *et al.*, 2009). There are several ways by which probiotics can be given to the host or added to its aquatic environment *i.e.*, by addition in food (Gomez-Gill *et al.*, 1998); through bathing (Austin *et al.*, 1995; Gram *et al.*, 1999); addition in culture water (Moriarty, 1998; Spanggaard *et al.*, 2001) and supplementation of artificial diet (Rengpipat *et al.*, 2000).

In aquaculture use of microbial probiotics use is now widely accepted (Vine *et al.*, 2006; Wang and Xu, 2006; Wang, 2007). For aquaculture practices several probiotics' preparation are commercially available (Decamp and Moriarty, 2006; Ghosh *et al.*, 2007).

Use of *Bacillus* species as probiotics is relatively recent and three principle reviews document the progress in this regard. (Mazza, 1994; Sanders *et al.*, 2003; Hong *et al.*, 2005). Bairagi *et al.* (2004) documented benefit of adding *B. subtilis*

and *B. circulans* to the diet of rohu (*Labeo rohita*) in terms of increased performance as judged by a number of factors including growth, feed conversion ratio, and protein efficiency ratio. They attributed beneficial effects of the probiotics to their ability of extracellular cellulolytic and amylolytic enzymes' productions.

The present study was aimed at cultivation of a known fish growth promoting probiotic *Bacillus subtilis*-A4 in sugarcane bagasse for its low cost provision to the aquaculture industry. The probiotic was also cultivated in solid state employing 9.09% sugarcane bagasse. The SSC dried matter containing the cells of probiotics can be applied for cost effective and controlled delivery of the probiotic for aquaculture practices.

## MATERIALS AND METHODS

### *Bacterial source*

*Bacillus subtilis*-A4, a known fish growth promoting probiotic (Choudhry, 2007) was rejuvenated in nutrient broth from glycerol stock culture of Microbial Biotechnology Laboratory, University of the Punjab, Quaid-e-Azam campus, Lahore, Pakistan.

### *Growth optimization in sugarcane bagasse broth (SCB)*

Two grams of ground sugarcane bagasse was suspended in 100ml of distilled water and routinely autoclaved for 15 minutes. The autoclaved material was filtered through Whatman filter paper No. 1 and pH of the filtrate was adjusted at 7. The medium was again autoclaved and finally poured into culturing vials under sterilized conditions.

The bacterium *B. subtilis*-A4 was cultured by transferring its 0.1ml of overnight grown culture to 10 ml of the 2% aqueous extract of SCB and the vials were incubated at 30°C, 37°C, 45°C and 50°C for 2 days. To assess suitable inoculum size medium having pH 7 was inoculated with 1%, 5% and 10% freshly grown culture and incubated at 45°C for 2 days. For the determination of the requirement of oxygen, the bacterium was inoculated at its determined conditions for 2 days in a shaking water bath and without aeration. For all the optimization experiments, growth of the cultures was assessed by recording O.D. at 600nm after 24 and 48 hours of incubation.

*Solid state bacterial cultivation (SSC)*

For solid state cultivation, 250ml Erlenmeyer flasks containing 55ml of the medium were employed. The medium was prepared by mixing 5 g of the ground sugarcane bagasse in 52.25 ml of distilled water and its pH was then adjusted to 7. The cotton-plugged flasks were autoclaved at 15-lbs/inch<sup>2</sup> (121°C) for at least 20 minutes. The production medium was then cooled at room temperature and inoculated with 2.75 ml of 24 h old bacterial culture that was raised in nutrient broth. All the flasks containing the inoculated sugarcane bagasse were weighed by using electric balance and then incubated in shaking water bath at 45°C.

The solid state fermentation was carried out for 6 days and growth of the bacterium was monitored after every 24 hours. Amount of autoclaved water was added daily to compensate the water loss through evaporation. To assess growth, 0.1 ml sample was taken from a culture flask with the help of micropipette fitted with an autoclaved tip which was made wide enough by cutting the tapering end to allow sampling of solid medium. The sample was diluted 20 times with distilled water and allowed to remain at room temperature. After 10 minutes the sample was vortexed for 1 minute and kept without disturbing for 5 minutes. Growth was recorded by taking O.D. of the fluid portion at 600 nm with the help of a spectrophotometer. Cellulase activity of the culture fluid was also assessed after Lai *et al.* (2006). One unit of cellulase corresponds to the liberation of 1.0  $\mu$  mol of glucose from cellulose under the assay conditions. For this purpose measured amount of cell free culture fluid was mixed with cellulose and glucose contents were then determined by *o*-toluidine method as described by Hartel *et al.* (1969).

*Substrate dissociation/adherence potential of Bacillus subtilis-A4, following exposure to different pH*

Following 10 days of solid state fermentation SCB was dried in sterilized Petri plates kept in laminar flow for 3 hours. Then 0.1 g of the dried mass was introduced in small sterilized vials under aseptic conditions. Then to different sets, each containing 3 vials, 3 ml autoclaved water having pH 6, 7 and 8 was added. After thorough vortexing, the mixtures were allowed to stand at room temperature for 1 h. One ml of the fluid was sampled after every 15 minutes and diluted with 2 ml of water. O.D. of the diluted sample was then measured at 600nm to assess dissociation / adherence potential of the bacterial cells to the solid substrate.

## RESULTS

The bacterium *Bacillus subtilis*-A4, showed best growth at 45°C with O.D. of  $0.41 \pm 0.08$  following 48 hours of incubation with initial pH of 7. The bacterium revealed best growth when the culture was started with 5% inoculum and O.D. reached up to  $0.53 \pm 0.03$  following 48 hours of incubation. Under the above mentioned optima *Bacillus subtilis*-A4, showed best growth in aerated condition (Fig. 1).

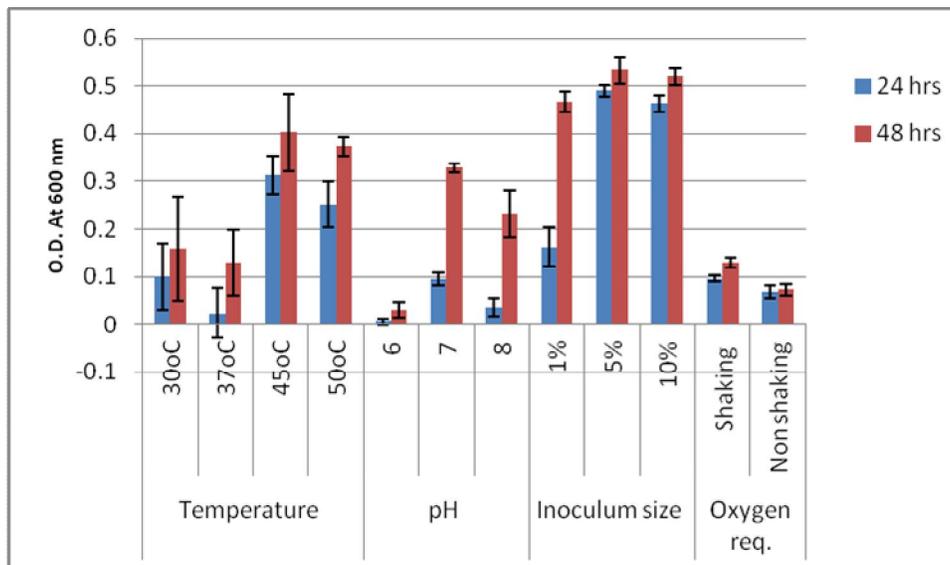


Fig. 1. Optimization of growth conditions following cultivation of *Bacillus subtilis*-A4 in aqueous extract of 2% sugarcane bagasse.

### *Solid state cultivation*

At first day of incubation the growth reached up to  $4.15 \pm 0.04$  O.D., while the *Bacillus subtilis*-A4 showed maximum growth up to  $4.91 \pm 0.28$  O.D. at 3<sup>rd</sup> day of incubation in 9.09% SCB. Appreciable amount of growth in the medium comprising of SCB as sole source of nutrient clearly demonstrated the cellulolytic potential of the bacterium. The cellulose production was measured up to 378.3 U/ml/h. After third sampling point gradual decrease in the growth was observed followed by a rapid decline between the last two sampling points (Fig.2).

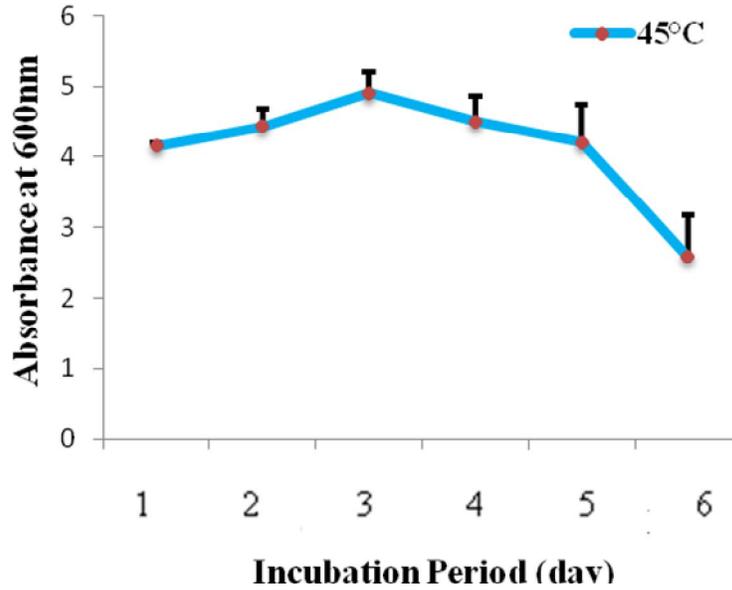


Fig. 2. Growth curve of *Bacillus subtilis*-A4 following its solid state cultivation at optimum incubation conditions.

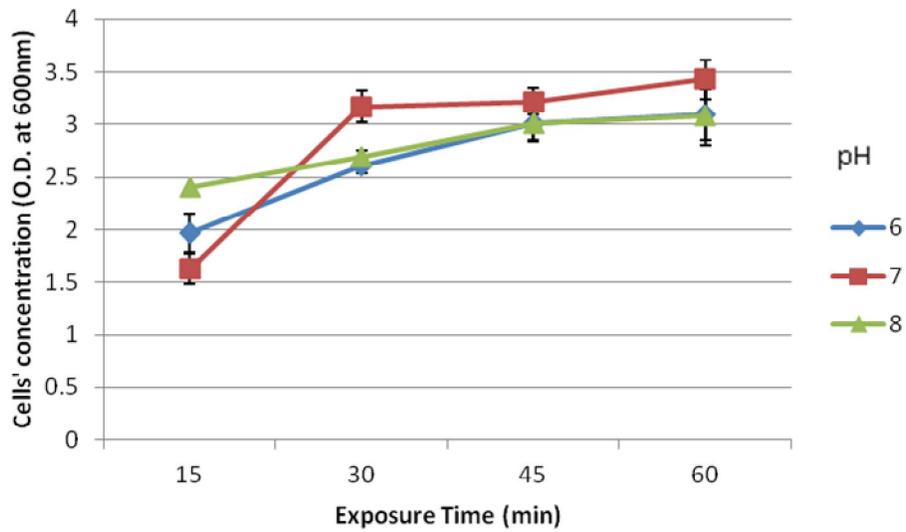


Fig. 3. Cells' concentrations (O.D. at 600nm) of *Bacillus subtilis*-A4, following exposure of dried solid state cultivated and SCB at different pH for varying time intervals.

*Substrate dissociation / adherence potential of B. subtilis-A4*

The substrate dissociation / adherence potential of *B. subtilis*-A4 for un-consumed SCB appeared primarily dependent upon the time and exposure's pH, as after 15 minutes of exposure, maximum bacterial adherence to the substrate was found with pH 7 and least with pH 8. Whereas after 30 minutes of exposure maximum adherence was found in case of pH 6 and 8. In case of 45 and 60 minutes' exposure the bacterial adherence to the substrate did not differ, in general, with respect to variations in the pH (Fig. 3). It is to be noted that substrate adherence potential can be translated inversely to its dissociation ability. Such information are relevant while recommending / procuring the route of administration of probiotics in aquaculture.

**DISCUSSION**

Fish make a dynamic contribution for the health and survival of a substantial portion of the world's population. Fish is a rich source of high quality proteins and fats (macronutrients), vitamins and minerals (micronutrients). For aquaculture development of the various aspects studied, importance of fish gut microflora for the fish growth and its related phenomena has been established relatively recently. It is generally believed that there is a possible symbiotic relationship between gut microflora and the fish (Verschuere *et al.*, 2000). It has now been elaborated that under stress conditions the microbial community of fish gut get disturbed and might consume all the food themselves or allow less absorption of the digested food of the intestinal mucosa. Therefore, the disturbed fish gut microbial flora may limit host's digestive potential. These information are suggestive for practicing of artificial feeds inoculated with microorganisms capable of improving the feed conversion efficiency and exerting antagonistic effects to the undesirable microorganisms with in the gut. The present study focused on the solid state cultivation of *B. subtilis*-A4, a fish growth escalating probiotic, in sugarcane bagasse. The substrate is a waste product of sugar industry in this country and thus represents a no-cost feedstock.

The bacterium *B. subtilis*-A4 showed appreciable growth at 45°C within two days of incubation while employing SCB as sole nutritive medium. It is known that with change of medium composition optimum growth temperature of bacteria may vary. Choudhry (2007) had found the optimum growth temperature as 37°C when this bacterium was cultivated in nutrient broth. Thermophilic microorganisms are the important source of interesting enzymes that are both

thermoactive and thermostable (Niehaus *et al.*, 1999). Further metabolic activities occur at relatively faster rates at elevated temperature. Microbial degradation of biological wastes is a natural process and during controlled fermentation process microorganisms might utilize wastes as source of energy as well as metabolites to synthesis very useful products such as enzymes and cost of the produce depends on the nature of agricultural biomass employed as carbon source (Hayat *et al.*, 2001; Tengerdy and Szakacs, 2003). For the production of various extracellular enzymes different sources of lignocellulosic wastes such as wheat bran, rice husk and sugarcane bagasse have been utilized (Pessoa *et al.*, 1997; Parajo *et al.*, 1998). Sugarcane bagasse is one of the low-value, high-volume agri-byproducts and therefore for economical fermentation process, it is a reasonable growth substrate (Pandey *et al.*, 2000). Successful cultivation of *B. subtilis*-A4 in sugarcane bagasse in the present study indicates a useful application of agri-waste. Another advantage of endospore formers is their potential of heat stability and thus they have a number of benefits over other non-spore formers such as *Lactobacillus* sp. Products containing *Bacillus* probiotics thus can be stored at room temperature in a desiccated form without any lethal effect on viability. A second advantage is that in the low pH of the gastric barrier, the spores have capability to survive (Spinosa *et al.*, 2000; Barbosa *et al.*, 2005).

In present study cellulase production by *Bacillus subtilis*-A4 measured up to 378.30 U/ml/h. Cellulose is the most lavish biomass on earth but its mobilization both in nature and anthropogenically designed processes is dependent upon the availability of potent cellulases (Tomme *et al.*, 1995). Cellulase enzymes which commonly cause the degradation of cellulose are produced by several microorganisms, commonly by bacteria and fungi (Bahkali, 1996; Magnelli and Forchiassin, 1999; Shin *et al.*, 2000; Immanuel *et al.*, 2006).

Besides the appreciable amount of cost-effective growth of the probiotic in SCB, another ability of the *B. subtilis*-A4 is its differential pattern of adherence / dissociation to the substrate at varying pH. Bacterial adhesion is considered to be one of the most preferable characteristics of probiotic strains. The adherence ability of probiotic bacteria to mucus and/or intestinal epithelial cells is one of the mechanisms that protect the host organisms from pathogenic invasion or adhesion. The effect is observed even if the bacterial adhesion is transient and does not lead to permanent intestinal colonization (Isolauri *et al.*, 1995; Candela *et al.*, 2005). *Bacillus* strains have ability to adhere to abiotic surfaces which helps them to remain for several days in the bacterial flora of gut and to be active during the intestinal transit and participate in the digestive processes, and thus

help in the elimination of potential pathogens and finally create a healthy environment for the host (Larpen *et al.*, 1994). In the present study, the substrate adherence/ dissociation pattern of *B. subtilis*-A4 cultivated in SCB showed maximum adherence at pH 7 following 15 minutes of exposure, while following one hour's exposure maximum dissociation was achieved for all the pH tried. The differential pattern of SCB adherence or dissociation of the probiotic under varying conditions of pH and exposure times are relevant for its specific applications. For example, in case of oral administration, the *B. subtilis*-A4 laden dried SCB can be introduced as such so that within 15-minutes its ingestion by fish might ensure introduction to gastrointestinal tract. Alternatively, if the probiotic is to be introduced into water body, the fermented substrate can be exposed first to the specific pH for desirable time which will render the bacterial dissociation from the cultivated SCB. The present results suggest application of the SSC for intended route of delivery of the probiotics to aquaculture practices. The promising results indeed need validation at laboratory as well as at fish pond level.

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## **Some Abstracts**

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**SECTION - I****CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY,  
TOXICOLOGY****LEAD LECTURE****MANAGEMENT OF CRITICALLY ILL ADULT PATIENTS OF DENGUE SHOCK  
SYNDROME**

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Dengue is one of the most challenging clinical illnesses and once the patient enters the critical phase of the dengue shock syndrome then the proper management is mandatory for the smooth recovery of the patient using the various standard treatment guidelines whereby it is very rightly believed that no patient of dengue shock syndrome should die and all walked in patients of this disease must walk out of the hospital. The identification of the patients of dengue hemorrhagic shock remains of paramount importance before they develop any organ damage and accordingly the fluid management enables the treating physicians to steer their patients to smooth recovery. In patients not responding to the adequate management in the intensive care unit one must consider urgent correction of acidosis, bleeding, calcium and sugar(ABCS). Treatment of the patients of dengue shock syndrome remains extremely rewarding as once these patients recover then generally very rapidly they go on to their usual quality of life without any chronicity. Since Pakistani physicians are now faced with DSS patients very often in our hospitals thus to save these precious lives it is mandatory that we all equip ourselves with the art of managing these patients to achieve desirable case fatality rates.

**LEAD LECTURE****ISOLATION, CULTURE AND INFECTION KINETICS OF *LEISHMANIA TROPICA*  
ISOLATES KTH<sub>16</sub> & KWH<sub>23</sub> FROM KPK: WITH SPECIAL REFERENCE TO THE USE  
OF HUMAN LEUKEMIA MONOCYTE CELL LINE THP-1 AS AN *IN VITRO* MODEL  
FOR SCREENING ANTILEISHMANIAL DRUGS**AKRAM SHAH<sup>1</sup>, NAZMA HABIB<sup>2</sup>, COLIN SUTHERLAND<sup>2</sup>, VANESSA YARDLEY<sup>2</sup>,  
QAISAR JAMAL<sup>1</sup>, AKHTAR MUNEER<sup>3</sup>, INAMULLAH KHAN<sup>4</sup>, WASEEM AHMAD<sup>5</sup><sup>1</sup>*Department of Zoology, University of Peshawar, Pakistan,*<sup>2</sup>*London School of Hygiene and Tropical Medicine, London,*<sup>3</sup>*Kuwait Teaching Hospital, Peshawar, Pakistan,*<sup>4</sup>*Khyber Teaching Hospital, Peshawar, Pakistan,*<sup>5</sup>*BBS District Teaching Hospital, Abbottabad, Pakistan*

Leishmaniasis is a cause of severe mortality and morbidity in at least 88 countries worldwide including Pakistan. Current anti-leishmanial drugs are highly toxic and resistance is developing at an alarming rate, therefore, new and effective treatments are urgently needed. In this study we were able to isolate and culture successfully the *Leishmania tropica* isolates KTH<sub>16</sub> &

KWH<sub>23</sub> isolated from patients attending the Khyber teaching hospital, Peshawar and Kuwait teaching hospital in Peshawar. KTH<sub>16</sub> was a male 5 years old, belonging to Jamrud, Khyber Agency (Pakistan) with nose lesion. The exudates were obtained by Miss Nazma Habib (London School of Hygiene and Tropical Medicine) on September 20, 2010 from the OPD of Khyber Teaching Hospital in Peshawar. The sample was PCR positive. KWH<sub>23</sub> was also a male 8 years old, belonging to Jamrud, Khyber Agency (Pakistan) with lesion on right arm from the OPD of Kuwait Teaching Hospital in Peshawar. The exudate was obtained by Miss Nazma Habib on September 17, 2010, from the OPD of Kuwait Teaching Hospital in Peshawar. This sample was also proved positive when tested on PCR in London School of Hygiene and Tropical Medicine. THP-1 cells were successfully infected with KTH<sub>16</sub> & KWH<sub>23</sub> first time in our lab instead of routine use of J774 macrophages. The optimum PMA concentration, which induced differentiation of THP-1 cells to macrophages after 48 hours incubation, was 20ng/mL. Ability of metacyclic promastigotes to infect THP-1 cells was compared. THP-1 cells were incubated with parasites for 24, 48, 72 and 120 h (20:1 and 10:1 parasites: THP-1 ratio). In consistent with the changing growth curve for KWH<sub>23</sub> infection rate was consistently higher than that found using KTH<sub>16</sub> regardless of the infection ratio employed. In a typical experiment KWH<sub>23</sub> infected 2-fold more macrophages than KTH<sub>16</sub>, 91±5.6% versus 51±3.4% infected macrophages, respectively. Likewise the number of amastigotes per infected THP-1 cell was higher when KWH<sub>23</sub> rather than KTH<sub>16</sub> were used (7.8±2.9 versus 4.1±1.8 per cell respectively). Anti-leishmanial properties of Amphotericin B, Miltefosin, Pentostam and Meglumine antimonate on *Leishmania tropica* KTH<sub>16</sub> and KWH<sub>24</sub> strains were tested with some encouraging results.

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#### PLENARY LECTURE

#### COMPREHENSIVE BIOPROCESSING OF LOW/NO COST SUBSTRATES FOR BIOENERGY – PROSPECTS IN PAKISTAN

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Every increasing human population accompanied with the developing life standards, necessitating more energy per capita, are very rapidly depleting the known reserves of fossil fuels. With a predicted global energy increase of 71% by 2030, humans will be more hungry for ever greater power and transport energy. Looking for the alternatives, scientists have responded this situation keeping in view economics, food security and environmental concerns for provision of sustainable bioenergy from renewable sources. Main source of renewable material available in the USA for fermentation was starch extract from maize. In this regard impact of rapid biofuel development on food security must be considered seriously. Rush to convert food such as maize, wheat, sugar and palm oil into fuel is creating a battle between food and fuel that will leave the poor and hungry in developing countries at the mercy of rising prices of food, land and water. In this context, presently cellulose is the substrate of choice. Cellulose is the largest polymer synthesized and metabolized on the globe each year. Plant biomass indeed converts and stores solar energy by incorporating simple molecules into cellulosic polymer. The biomass represents energetically a stable gathering of the carbohydrate moiety. Abundant availability of cellulosic material in all habitats of the biosphere has made it a very attractive substrate to scientists involved in sustainable provision of biofuels. Transport fuels must qualify high energy and low volume

parameter. Production and use of ethanol for fuel is well known and is used in United States as an oxygenate to reduce automotive emissions. Ethanol has been verified as compatible with conventional automobile engines in blends containing 10-22% ethanol with gasoline. While in “flexible fuel” vehicles like E85 in the United States and “hydrous” in Brazil higher blends of ethanol are used. Biohydrogen has been advocated as another energy rich and totally pollutant free fuel. Microorganisms capable of producing hydrogen as a byproduct of their metabolism have well and rapidly been documented. Many photoauto/heterotrophs produce H<sub>2</sub>. Several photoheterotrophs appear promising to yield H<sub>2</sub> while consuming agro/food industrial wastes as carbon and nitrogen sources. Future of biofuels will depend at least partly on exploitation of agro/food industrial wastes with food security together with reducing the allied environmental pollution. The term biofuels includes biogas (methane 50-75%) and biodiesel. However, this talk is restricted to bioethanol and biohydrogen production and addresses the issues according to the following plans for each of the biofuel.

**Source of fermentation substrates:** Sustainability, Economics and food conflict. Exploitation of agro/food industrial wastes.

**Substrates' Pretreatment:** Chemical versus Biological.

**Saccharification of substrates:** Environmental issues and economics of the process.

**Fermentations:** Technoeconomics aspects; Employing extremophiles.

**Product Recovery**

**Ferment Residue:** Economic Utilization

It is hoped that God gifted habitat diversity ranging from sea level to the second highest mountainous range in the world, translated into great biodiversity of Pakistan will provide the future supplies of biofuels. Diversity of both substrates of varying economic status and the microbial agents together with the availability of almost throughout the year of sun shine for majority of the countryside areas and availability of manpower at highly affordable levels provide excellent platforms for the investors in this country for provision of sustainable supplies of biofuels at economically attractive level.

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## ISOLATION AND CHARACTERIZATION OF CHITIN DEGRADING BACTERIA

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Chitinase is an enzyme which is of significant importance in paper and textile production, bioremediation of heavy metal contamination, as additive in animal feed and as consumer products such as cosmetics. In this study 12 bacterial isolates were screened for chitinase activity and 3 were further selected based on enzyme activity (%). All bacterial isolates showed optimum growth at 37°C and at pH 7. The two bacterial isolates on the basis of molecular and biochemical tests were identified as *Stenotrophomonas maltophilia* and one was identified as *Bacillus licheniformis*. Growth curves in the presence of chitin were also determined. Enzyme was characterized for its optimum temperature and pH activity. Enzyme activity in the presence of various metal ions was also determined.

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**USE OF POWDER EGG YOLK IN EXTENDER FOR CRYOPRESERVATION OF BUFFALO BULL SPERMATOZOA**

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Fresh egg yolk is routinely used as cryoprotectant in semen extenders for buffalo bull semen. However, the hygienic risks associated with the use of fresh egg yolk necessitate the evaluation of a substitute of fresh egg yolk. It was hypothesized that powdered egg yolk that is pasteurized during processing may be a substitute of fresh egg yolk in semen extenders for buffalo semen. This study was conducted to compare the extenders containing either fresh and/or powder egg yolk for the cryopreservation of buffalo bull spermatozoa. Semen was collected from three Nili-Ravi buffalo (*Bubalus bubalis*) bulls of similar age group with artificial vagina (42°C) for three weeks (replicate). Qualifying semen ejaculates were split into two aliquots and diluted (37°C having approximately  $50 \times 10^6$  spermatozoa ml<sup>-1</sup>) in *tris*-citric acid extender containing either 20% fresh or powder egg yolk. Diluted semen was cooled to 4°C in 2 hours at the rate of 0.275°C min<sup>-1</sup> and equilibrated for 4 hours at 4°C. Cooled semen was then filled in 0.5 ml French straws at 4°C, kept on liquid nitrogen vapors for 10 min and plunged in the liquid nitrogen. Semen was thawed after 24 hours at 37°C for 30 seconds for the assessment post thawed quality. Straws were then plunged and stored in liquid nitrogen (-180°C). Semen quality assays viz; motility (%), viability (%), plasma membrane integrity (%) and acrosomal integrity (%) were performed after dilution, after equilibration and at post thaw. Data were analyzed by t- test and are presented as mean ( $\pm$  SD). A 5% ( $P < 0.05$ ) level was used to determine statistical significance. The percentage motility, viability, plasma membrane integrity and normal apical ridge of buffalo bull spermatozoa remained similar ( $P > 0.05$ ) in fresh egg yolk compared to powder egg yolk extenders after dilution, after equilibration and at post thaw stage. It is suggested that powder egg yolk can be used to replace fresh egg yolk in *Tris*-citric extender for cryopreservation of buffalo bull spermatozoa.

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**ALTERATIONS IN SERUM BIOCHEMICAL PARAMETERS IN NORFLOXACIN TREATED BROILER CHICKENS**

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Norfloxacin is a broad spectrum synthetic flouroquinolone antibiotic which is used as a routine treatment of mycoplasmosis, colibacillosis and pasteurellosis in chickens. In the current study, we investigated the effects of overdose of norfloxacin on serum biochemical parameters in broiler chickens. A total of twenty four healthy male broiler chickens aged 30-40 days, weighing between 1.2-2.3 kg were divided into four equal groups, each containing six birds. Groups II, III and IV received oral doses of norfloxacin at the concentrations of 12, 60 and 120 mg /kg b.w. respectively, daily for fifteen days. Group I was control that received 0.9% saline solution orally. Blood samples were collected at day 0, 8 and 15 through brachial vein puncture. The serum samples were analyzed for total protein, albumin, globulin, total cholesterol, triglycerides, calcium, potassium, creatinine, uric acid and for the enzyme activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). Results demonstrated that the

levels of creatinine ( $p<0.0001$ ), uric acid ( $p<0.008$ ) and potassium ( $p<0.036$ ) were significantly elevated in the high dose groups, with increasing time duration, as compared to the control and pre-dose group. The concentrations of ALP, triglycerides and globulins were significantly decreased but were normalized afterwards 15<sup>th</sup> day. Serum total protein showed significant variation from pre-dose but did not follow any consistent pattern throughout the experiment. The levels of calcium and albumin were reduced significantly in the treated groups. Serum ALT and ALP levels in contrast, did not show any significant variation. In addition, a negative correlation was found between potassium and creatinine levels ( $r\text{-value} = -0.926$ ) in the treated groups, at different time intervals. The present study suggests that norfloxacin overdose may lead to hypoalbuminaemia, hypocalcemia, hyperkalemia and impaired kidney function in the chickens. Careful use of the antibiotic is therefore recommended as regards dosage and duration of administration.

**PREVALENCE OF DIFFERENT REPRODUCTIVE DISORDERS ENDOMETRITIS,  
ANOESTRUS, OVARIAN CYSTS IN NILI RAVI BUFFALO IN AND AROUND  
FAISALABAD**

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Data of 10564 buffaloes brought to the clinic of Animal Reproduction Department, University of Agriculture, and Faisalabad for the treatment of Endometritis, Anoestrus and Ovarian cysts in Nili Ravi buffalo were analysed to evaluate the relative occurrence and temporal distribution. Endometritis was found to be 42.56% among all reproductive disorders. The highest occurrence of the problem was observed in 2<sup>nd</sup> lactation (24.05%) followed by 3<sup>rd</sup> (21.20%) 4<sup>th</sup> (17.09%), 1<sup>st</sup> (16.26%), 5<sup>th</sup> and above (12.85%) and heifers (8.56%). Distribution of Endometritis in the Summer, Winter, Autumn and Spring was 35.59, 25.16, 21.68, and 17.57 % respectively. The analysis of variance indicated a highly significant ( $P<0.05$ ) affect of lactation and season on the prevalence of Endometritis. The prevalence of Anoestrus observed to be 53.99 % of all reproductive disorders. Further classification of anoestrus revealed that 60.16, 30.37 and 9.47% were due to inactive ovaries, silent oestrus and heifers with infertile genitalia respectively. The problem was more prevalent during 3<sup>rd</sup> lactation, followed by heifers (21.29%), 2<sup>nd</sup> lactation (19.88%), 1<sup>st</sup> lactation (16.97%), 4<sup>th</sup> lactation (13.07%) and 5<sup>th</sup> & above lactation (8.43%) respectively. A higher incidence of Anoestrus was observed during Summer (32.34%), lower in Autumn (24.05%) and Winter (24.53%) respectively. It was further revealed that a total 7.12 percent buffaloes were found to have ovarian cysts 55.17 percent on right ovary, 34.48 percent on left and 10.35 percent on both the ovaries. A higher incidence was recorded in 3<sup>rd</sup> lactation (27.58%) and during winter season (34.48%).

**MITOCHONDRIAL DIVERSITY AND PHYLOGENETIC STRUCTURE OF  
YELLOWCHEEK CARP *ELOPICHTHYS BAMBUSA* AS INFERRED FROM  
CYTOCHROME B GENE SEQUENCES**

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The hydrological alterations have imperiled ichthyofauna of the Yangtze River, China at various scales. The cyprinid fish *Elopichthys bambusa* is the only species of genus *Elopichthys*. Moreover, the declining populations of *E. bambusa* in the Yangtze riparian ecosystem have raised concern over its genetic integrity. In this study, the population structure and molecular phylogeny of the species was examined from five populations in the middle and lower reaches of Yangtze River basin. Total 73 samples of this highly vulnerable species were collected from geographically distinct localities. The complete mitochondrial Cytochrome *b* gene of each specimen was cloned and sequenced. The rigorous analyses of gene sequences revealed three distinct mtDNA lineages of *E. bambusa* showing high nucleotide divergence and haplotypes diversity. The sequence analysis showed 39 haplotypes with haplotype diversity ranging from 0.8727 to 0.9777. The results of divergence, phylogenetic tree and AMOVA indicated a historically restricted gene flow among the lineages. The populations of Poyang Lake, Dongting Lake and Wuhan gathered into one lineage whereas the samples from Taihu Lake and Danjiang Reservoir appeared as independent lineages. Added to the old geological events, the hydrological alterations in the Yangtze River basin during the last century have contributed to the evolutionary process. The results point out an underestimated species diversity of the *Elopichthys* and that each of the mtDNA lineages may correspond to three distinct types. More robust studies are suggested to resolve the genetic and taxonomic status of this vulnerable species.

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**RELATIONSHIP OF TNF-  $\alpha$  WITH INSULIN RESISTANCE AND OTHER  
PARAMETERS OF METABOLIC SYNDROME AMONG TYPE 2 DIABETIC  
PAKISTANI SUBJECTS**

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Diabetes mellitus is a multifactorial disorder. Cytokines secreted by the monocyte-macrophage system play a key role in the pathogenesis of type 2 diabetes. Pro-inflammatory cytokine TNF- $\alpha$  is believed to have an important role in the progression of diabetes. The present study was conducted to assess the level of TNF- $\alpha$  and to find its relationship with insulin sensitivity and other indicators of metabolic syndrome among newly diagnosed type 2 diabetic subjects. 100 diabetic subjects (age; 48.63 $\pm$ 0.92, BMI; 28.51 $\pm$ 0.52) were included in the study. Four groups were made on the basis of BMI as normal weight, overweight, moderately obese, severely obese subjects. Different demographic parameters as age, BMI, B.P, personal history and socioeconomic status were recorded. Fasting glucose, random glucose, HbA1c and lipid profile were analyzed by chemistry analyzer. Serum insulin and plasma TNF- $\alpha$  levels were assessed by ELISA. Insulin

sensitivity and insulin resistance was calculated. On correlation analysis, TNF- $\alpha$  was significantly correlated with BMI, WHR and TG ( $p < 0.01$ ). Severely obese subjects had higher insulin level and a strong correlation was observed between plasma TNF- $\alpha$  and serum insulin ( $p < 0.01$ ). TNF- $\alpha$  was significantly correlated with insulin sensitivity ( $p < 0.05$ ). TNF- $\alpha$  was significantly associated with insulin resistance ( $p < 0.01$ ). TNF- $\alpha$  was also negatively correlated with HDL-cholesterol ( $p < 0.05$ ) while non-significant relationship was observed between TNF- $\alpha$  and HbA1c, systolic B.P, diastolic B.P, cholesterol and LDL-cholesterol ( $p > 0.05$ ).

**CYTOCHROME C OXIDASE SUBUNIT I (COI) GENE ISOLATION FROM *TOR PUTITORA* AND ITS PHYLOGENETIC ANALYSIS**

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*Tor putitora* belongs to genus *Tor*, which includes the fresh water fish which are distributed throughout South and South East Asia. The genus comprises of approximately 30 species. The extreme inter species similarities resulted in confusing morphological features causing problem to identify individual species. So to clear this ambiguity scientist used many molecular markers including SSR, RAPD, RFLP and AFLP etc. However, due to problem of reproducibility and result interpretation with these markers scientists switched to new dimension of DNA barcoding using cytochrome oxidase C subunit 1 (COI), a mitochondrial gene. Samples of *Tor putitora* were collected from different rivers of Northern Areas of Pakistan and Azad Jammu Kashmir. The mitochondrial COI gene was amplified using gene specific primers. The amplified COI gene was confirmed through sequencing. The gene sequence was used to construct phylogenetic relationship with other Mahseer. The phylogenetic trees and the topologies test supported the monophyly of *Naziritor* and *Tor*. *Neolissochilus* as a sister group to *Naziritor* and *Tor*. Sequence comparison revealed A and G transitions at different position in of COI gene among species of all three genera.

**ANALYSIS OF SODIUM CHANNEL SUBUNIT BETA-1 (*SCN1B*) MUTATIONS INVOLVED IN GENERALIZED EPILEPSY WITH FEBRILE SEIZURES PLUS (GEFS+) PATIENTS IN PUNJAB**

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Generalized epilepsy with febrile seizures plus (GEFS+; MIM#604233) is a familial epilepsy syndrome characterized by phenotypic and genetic heterogeneity. It was associated with the mutations in the neuronal voltage gated sodium channel subunit gene. Febrile seizures represent the most common form of childhood seizures. FSs are not thought as the true epileptic disease but rather as a special syndrome characterized by its provoking factor (fever) and a typical range of 3 months to 6 years. Although specific genes affecting the majority of FS cases have not been identified yet, several genetic loci have been reported recently. The aim of this report is to search *SCN1B* gene mutation in the local population. PCR amplification was performed to screen mutation in *SCN1B* gene. Exon 1, 3, 4 and 5 were amplified and mutation analysis was performed by direct sequencing. The objective was to investigate whether there is mutation in *SCN1B* gene as well as

to describe the association of *SCN1B* gene mutations to generalized epilepsy with febrile seizure plus (GEFS+) in local population of Punjab, Pakistan. We investigated a novel heterozygous IVS2-1G>T transversion mutation in splice acceptor site of exon 3 of the voltage gated sodium ion channel gene. Out of 14 only one patient was carrier for this mutation. The present study findings showed that exonic *SCN1B* mutations in our population are less common.

**COMPARATIVE EVALUATION OF *PER OS* AND PARENTERAL ADMINISTRATION OF TRIBRISSEN<sup>TM</sup> IN EQUINES SUFFERING FROM BACTERIAL INFECTIONS**

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Equines including horses, donkeys and mules are very important for the economy of Pakistan. Contaminated injury wounds and contagious respiratory infections result in an adverse effect on health and performance of these species. Tribriessen<sup>TM</sup> is an antibacterial agent effective against a wide spectrum of bacterial pathogens. The present study was planned to determine the comparative efficacy of *per os* and parenteral administration of Tribriessen<sup>TM</sup> in equines suffering from bacterial infections. All the three species were randomly divided into 2 equal groups, A and B. Tribriessen<sup>TM</sup> 48% at 1ml/30 Kg body weight was given parenterally to group A and *per os* to group B. Clinical severity scores before and after five days of treatment were noted. Group A&B were having severity scores of 3.15±0.182 and 2.8±0.213 respectively before the start of treatment with Tribriessen<sup>TM</sup>. After the treatment of 5 days, the severity scores of group A and B were 0.90±0.25 and 0.95±0.276, respectively. All the forty animals showed highly significant decrease (P<0.01) in their severity scores after five days of the treatment with Tribriessen<sup>TM</sup>. All the three species exhibited significant decrease in their severity scores post treatment with Tribriessen<sup>TM</sup> irrespective of the route. Group A revealed a highly significant decrease (P<0.01) in severity scores in horses and donkeys after the treatment, whereas, mules had shown significant decrease in severity scores after the treatment. Severity scores were significantly low by *per os* treatment. It was noted that both the routes were equally effective for the treatment with Tribriessen<sup>TM</sup> in eqines. Comparison of routes of individual species (horse, donkey, mules) has shown non-significant differences in the route of treatment.

**HISTOPATHOLOGICAL AND BIOCHEMICAL EFFECTS OF INDUCED TOXICITY OF HEAVY METALS COPPER AND COBALT IN COMMON CARP (*CYPRINUS CARPIO*)**

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Copper and cobalt are those heavy metal elements that also act as micronutrients and are therefore physiologically relevant in fishes. However, greater than normal concentrations of heavy metals in water bodies as a result of anthropogenic activities can be harmful. This also makes them good bioindicator of toxicity. The present study was conducted to evaluate impact on biochemical and histopathological aspects of body tissues following 96 hrs exposure to copper (0.25 mg/l as

copper sulphate) and cobalt (250 mg/l as cobalt chloride) in combined form and as alone treatments. Liver enzymes, serum proteins, plasma glucose and muscle glycogen, red cell morphology and histomorphology of gills, kidney, muscle and skin were the parameters taken into consideration. Comparison among groups was done with one-way ANOVA.  $P < 0.05$  was considered significant difference. Results demonstrated decreased body weight in all experimental groups. ALT levels were elevated in all treatment groups. AST levels were increased ( $p < 0.028$ ) in the combined and copper alone ( $p < 0.0001$ ) treatment groups. Serum total protein was decreased in all treatment groups. Between treatment comparison showed maximum decrease in the combined ( $p < 0.001$ ) treatment group. Plasma glucose concentration was increased in the combined ( $p < 0.01$ ) and copper alone ( $p < 0.001$ ) treatment groups. Glycogen concentration was increased in the copper ( $p < 0.02$ ) and cobalt ( $p < 0.01$ ) alone treatment group. Red cell membranes were ruptured and cells were swollen with pyknotic nuclei and irregular notches. Histomorphology of tissues showed damage to the gills, liver, muscle and skin tissues. The study concludes that copper is relatively more toxic for this fish species in the alone and combined treatment state. On the other hand, cobalt appears to have countered the copper toxicity. The study suggests that cobalt can be used as antidote toward copper toxicity.

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**EFFECT OF POLLUTED WATER ON MICRO- AND MACROELEMENT  
CONCENTRATION AND HISTOMORPHOLOGICAL DAMAGE TO SOME VITAL  
ORGANS OF A FISH *CHANNA PUNCTATA***

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In Pakistan the increased population growth, urbanization, and discharge of processed or unprocessed waste materials from tanneries have all resulted in excessive contamination of water bodies with heavy metals. The current study was designed to assess the damage caused by the industrial effluents to local fish population. Bottom dweller carnivorous fish *Channa punctata* were collected from Wazirabad's nullahs. Control samples from non polluted sites were also collected for comparison. The concentration of both the macronutrients (Na, K, Mg, Ca) and heavy metals (Fe, Cr, Cu, Co, Mn, Ni, Zn, Pb, Cd) were determined in tissues through atomic absorption spectrophotometry. Gills and heart were assessed for histomorphological damage. Heavy metals concentrations were compared using the Student's t-test. Results indicated increased concentration of macro- and microelements accumulation in gills, skin, heart, brain, spleen in polluted sites fish. In the gills and skin, Ni, Cr, Co, Cd, Na, and K were significantly increased ( $p < 0.05$ ). In the heart, Ni, Co, Pb, Zn, Fe, Na, Ca ( $p < 0.05$ ) and K were increased ( $p < 0.001$ ). In the brain significant differences were found for some micro and macro elements such as Cr, Co, Cd ( $p < 0.05$ ) and Fe ( $p < 0.001$ ), and K ( $p < 0.05$ ) were elevated. In spleen tissue, Zn, Na, K ( $p < 0.05$ ) and Mg ( $p < 0.001$ ) were found elevated. Gills of the polluted water fish showed degeneration of pillar cells and a thickening of the primary lamellar epithelium, lamellar disorganization, hypertrophy of the lamellar epithelium and epithelium rupture with excessive hemorrhage. Heart muscles showed atrophy and fragmentation. The present study provided ample evidence that industrial effluents are potentially dangerous to the ecosystem and can readily pass to human bodies via the food chains.

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**EFFECT OF DIFFERENT CONCENTRATIONS OF METALS ON *BACILLUS* AND *PSEUDOMONAS* SPP. ISOLATED FROM INDUSTRIAL EFFLUENTS OF FAISALABAD**

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With the intention to determine effects of different metals on growth of bacteria that were previously identified as heavy metal resistant, total six bacterial samples were selected, repurified and reidentified from the bacterial isolates. Different morphological and biochemical tests were performed that differentiated bacteria into two genera *i.e.* *Bacillus* sp. and *Pseudomonas* sp. Bacteria was grown at pH 7 and temp of 37°C up to incubation time of 96 hours. Three metals (Na<sup>+</sup>, Ca<sup>2+</sup>, Zn<sup>2+</sup>) were used with concentrations ranged between 25-200 mg/ml. It was found that as the concentrations of metals increased, growth of *Bacillus* and *Pseudomonas* spp. firstly increased and then decreased in case of all selected metals. Lowest growth occurred at 200 mg/ml of metal concentration. Optical density at 660 nm was considered as a measure of growth of bacteria. As growth increased, optical density increased and vice versa. Na<sup>+</sup> was found to have a more negative effect on growth of bacterial isolates as compared to other two metals *i.e.* Ca<sup>2+</sup>, Zn<sup>2+</sup>. *Bacillus* sp. under investigation was found to be more resistant to these metals as compared to *Pseudomonas* sp. Both bacterial isolates showed their maximum growth after 72 hours of incubation in most of the cases, afterwards growth decreased. Decreasing resistance pattern for both of bacterial species was as follows; Ca<sup>2+</sup> > Zn<sup>2+</sup> > Na<sup>+</sup>. The findings revealed the potential application of *Bacillus* and *Pseudomonas* spp. for the removal of heavy metals from industrial wastewaters under the conditions where salts of Sodium, Zinc and Calcium are present.

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**IDENTIFICATION OF UNKNOWN BACTERIAL PATHOGENS THROUGH VARIOUS BIOCHEMICAL TECHNIQUES**

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Bacterial pathogens may be identified in the clinical laboratory by routine morphological and biochemical methods. Currently the traditionally microbiologic methods were used to examine the presence of bacterial species in the infected samples. These methods including culture, selection, isolation, and morphologic and biochemical characterization provide high sensitivity and specificity. For the current study the human infected samples (urine, blood, pus and serum), spoiled fish sample, earthworm and infected chicken liver were collected and streaked on the selected different mediums. Several biochemical methods were used for the identification and screening of gram (+) and gram (-) bacterial pathogens *i.e.* gram staining, Ziehl Nelson staining, citrate, catalase, coagulase, indole, methyl red, oxidase, urease, motility, carbohydrate test, and Voges proskeur test, respectively. Ziehl Nelson staining was used for the identification of acid fast bacterial pathogens. *Pseudomonas aeruginosa* and *Shigella flexneri* showed oxidase positive, *Streptococcus*, *Enterococcus faecalis*, and *Enterococcus faecium* were catalase negative, *Staphylococcus aureus* was coagulase positive. It was also observed that *Klebsiella pneumonia*, *Serratia marcescens*, *Pseudomonas syringae*, *Serratia odorifera*, *Salmonella typhirium* were indole positive whereas

*Enterobacter amnigenus*, and *Shigella flexneri* were indole negative bacteria. Accurate and definitive microorganism identification is essential for correct disease diagnosis, treatment of infection and trace-back of disease outbreaks associated with microbial infections. Bacterial identification is used in a wide variety of applications including microbial forensics, criminal investigations, bio-terrorism threats and environmental studies.

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### MORPHOMETRIC ATTRIBUTES OF EPIDIDYMAL SPERMATOZOA OF OBSTRUCTIVE AZOOSPERMIC PATIENTS.

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The present study was conducted in 7 obstructive azoospermia males visiting Fatima Memorial Hospital during Jan–July 2011. After taking prior written consent from these patients semen sample were taken to determine the effects of long term obstruction on the epididymal spermatozoa using the Kruger's strict criteria for sperm morphology evaluation. Morphometric measurements of the sperms from the epididymal fluid were done as recommended by WHO laboratory manual (2009/2010). The specimen slides were Giemsa stained and 100 spermatozoa per slide were manually read in an arbitrary fashion under 100X magnification. Percentage Ideal Forms (PIF's) showed 4% strict morphology score for 1 patient only. The remaining six patients had <3 PIF's indicating severe abnormalities in their epididymal spermatozoa. The mean for head lengths was  $4.99\mu\text{m} \pm 4.04$ , for head widths the values were  $3.65\mu\text{m} \pm 0.90$ , the mean for length/width ratio was  $1.34 \pm 0.32$ , for mid-piece lengths the values were  $5.50\mu\text{m} \pm 2.91$ , mid-piece-width was  $0.97\mu\text{m} \pm 0.16$  while the tail lengths had a mean value of  $28.55\mu\text{m} \pm 10.99$ . Confidence interval (CI) was also calculated for difference for the entire data. The values found were 4.69:5.29, 3.65:3.50, 1.31:1.36, 0.97:0.96, 5.50:5.28, and 28.55:27.73 for head lengths, head widths, length/width ratio, mid-piece width, mid-piece length and tail lengths respectively. The results were found to be statistically significant ( $P = 0.000$ ) for all the parameters of Sperm Morphology Evaluation Using Strict Criteria (MEUSC). It is concluded that long term obstruction had caused highly significant abnormalities in the epididymal spermatozoa, particularly tail and mid-piece lengths.

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### IMPACT OF CHLORPYRIFOS ON SOME ENZYMES OF FISH (*APHANIUS DIPAR*)

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Pollution of marine environment by variety of toxic substances has been a major environmental concern. The major pollutants entering into the coastal environment of Pakistan are oil, sewage, garbage, pesticides, toxic chemicals, heavy metal, radioactive waste, thermal pollution and nutrients. Pesticides and the pollutants enter the food chain and tend to accumulate in organisms at higher trophic level. Monitoring of acetylcholinesterase (AChE) inhibition has been widely used in freshwater and marine ecosystems as an indicator of pollutants for e.g. pesticides exposure and effects. The aim of this study was to assess the toxicity of chlorpyrifos

(organophosphorus pesticide) on *Aphanius dispar*. In the present study acute effect of chlorpyrifos pesticide on enzyme activity of juveniles of fish (*Aphanius dispar*) were carried out. The enzymes, acetyl cholinesterase (AChE), alanine aminotransferase (ALT), aspartate aminotransferase (AST), were used. Enzyme analysis kit was used for quantitative determination of different enzymes. The results depict both elevation and inhibition of enzymes in the treated fish. Levels of alanine aminotransferase (ALT) activity were increased (118.33%) significantly ( $p < 0.05$ ) in chlorpyrifos pesticide treated fish. The acetyl cholinesterase (AChE) activity levels decreased (90.7%) significantly ( $p < 0.05$ ) and aspartate aminotransferase (AST) activity was also decreased (90.56%) significantly ( $p < 0.05$ ) in chlorpyrifos pesticide treated fish. Analysis of biochemical parameters in the fish may be useful in environmental biomonitoring. The present work is expected to help in understanding biochemical basis of pesticide effect on fish and hence planning strategies for release of chemicals in the aquatic medium.

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**ANALYSIS OF SENSITIVITY OF POULTRY ASSOCIATED *PSEUDOMONAS AERUGINOSA* AGAINST MEDICINAL PLANTS AND STANDARD ANTIBIOTICS**

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*P. aeruginosa* is the most common avian pathogens and it produces a variety of toxins and enzymes that may contribute to pathogenicity. *P. aeruginosa* was isolated from the chicken liver and identified by biochemical methods. In present study the antibacterial activity of chloroform and isoamylalcohol extracts of medicinal plant including *Cinnamomum zylanicum* (Cinnamon; Dalchini), *Cuminum cyminum* (Cumin; Zeera), *Syzygium aromaticum* (Clove; Loang), *Curcuma long Linn* (Turmeric powder), *Trachyspirum ammi* (Carom seeds; Ajwain) were investigated against chicken liver associated *P. aeruginosa*. Various antibiotics and the n-Hexane, chloroform, ethanolic, methanol and ethyl acetate extracts of *Momordica charantia* (both seeds and green parts Bitter gard) were also analyzed against *P. aeruginosa* through agar disc diffusion method. It was observed that *P. aeruginosa* was susceptible against Norfloxacin, Chloramphenicol, Streptomycin, Gentamycin, Tobramycin, and Ciprofloxin. Whereas moderately susceptible in case of Oxytetracycline, Neomycin, Lincomycin, and Sulfomethoxazol. It was also analyzed that Ampicillin, Tetracycline, Pencillin G and Trimethobrim had no effect. Among the plants tested *C. zylanicum*, *C. cyminum*, *T. ammi*, *S. aromaticum* and green part of *M. charantia* were most active. The highest antibacterial activity was exhibited by isoamylalcohol extract of *C. zylanicum*, *C. cyminum*, *T. ammi*, *S. aromaticum*, and etanolic and methanol extract of green part of *M. charantia* against *P. aeruginosa*. Interestingly the chloroform and isoamylalcol of *Curcuma long Linn* and all extracts of seed had no effect on the growth of *P. aeruginosa*. This study indicated that these medicinal plants and organometallic compounds could be the potential source for antimicrobial agents. Hence, these medicinal plants can be further subjected to isolation of the therapeutic antimicrobials and further pharmacological evaluation.

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**OCCURRENCE RETAINED FOETAL MEMBRANES, ABORTION, DYSTOKIA  
GENITAL PROLAPSED IN BUFFALO IN AND AROUND FAISALABAD**

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Data of 10564 Nili Ravi buffaloes brought to the clinic of Animal Reproduction Department, University of Agriculture, and Faisalabad for the treatment of retained foetal membranes, abortion, dystokia and Genital Prolapsed were analysed to evaluate the relative occurrence and temporal distribution. Retained foetal membranes, abortion, dystokia, genital prolapsed was found to be most common (50.37%). The prepartum vaginal prolapsed was higher (66.34%) than postpartum genital prolapsed (33.64%). Genital prolapsed was more prevalent during 3<sup>rd</sup> lactation (33.99%) followed by 4<sup>th</sup> lactation (23.90%), 5<sup>th</sup> & above (17.56%), 2<sup>nd</sup> (16.10%) and 1<sup>st</sup> lactation (8.78%) respectively. The prevalence of genital prolapsed was 45.8, 22.94, 19.51, and 11.71 percent during summer, autumn, spring and winter respectively. Analysis of variance revealed significant ( $P < 0.05$ ) relationship between the type, number of lactation and season in the occurrence. Retention of foetal membranes was recorded 16.95% in buffaloes. A higher incidence of retained foetal membranes was seen during 4<sup>th</sup> lactation (26.09%) in summer season (42.03%). Abortion was recorded in 15.48% buffaloes being most prevalent in 3<sup>rd</sup> lactation (26.98%) and during winter (42.27%). Dystokia was observed in 10.08 percent of obstetrical problems of which 63.41 percent was due to torsion of uterus and 36.59 percent as faulty disposition of the foetus. The disease was more prevalent in 1<sup>st</sup> calvers (26.83%) and during summer (34.15%).

**HISTOPATHOLOGICAL STUDY OF LIVER AND KIDNEY IN *CYPRINUS CARPIO*  
AFTER THE EXPOSURE OF DIFFERENT DOSES OF POTASSIUM DICHROMATE**

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The liver plays vital role in metabolism and act, as a storage center of many substances while kidney function is to maintain homeostasis. Any damage to liver and kidney tissue will induce abnormalities in this process and ultimately can result in mortality of fish. The objective of present study was to identify the degree and damage to the histological changes of *Cyprinus carpio* liver and kidneys exposed to Potassium dichromate ( $K_2Cr_2O_7$ ). Fish samples were exposed to 2 doses viz 5 and 10 mg.L<sup>-1</sup> designated as P<sub>1</sub>, P<sub>2</sub> while P<sub>0</sub> was control. 16 days exposure of Potassium dichromate ( $K_2Cr_2O_7$ ) induced changes in liver tissue of fish were coagulative necrosis, focal area

of necrosis, dilation and congestion in blood sinusoid, aggregations of inflammatory cells between the hepatocytes, and degeneration of parenchyma cell while haemosiderin and aggregations of inflammatory cells, dilation in the capillary tubes of renal tubules and haemorrhage were observed in kidney of *Cyprinus carpio* when exposed to sublethal concentration of Potassium dichromate ( $K_2Cr_2O_7$ ) with respect to control showing normal histology. Present study results showed that Potassium dichromate ( $K_2Cr_2O_7$ ) is highly teratogenic metal and its deteriorating affects increase with increase in its concentration.

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### INSECTICIDAL AND ANTIBACTERIAL INVESTIGATIONS OF ORGANOMETALLIC COMPOUNDS AGAINST PATHOGENS

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The purpose of this study was to investigate the bioactivities, such as insecticidal and antibacterial activities of some organometallic compounds because the global food supply has been a great concern for several decades. Thus, studies to identify and evaluate new materials should have been performed to develop safe and effective chemicals which protect food from various diseases and insect damages. In present research the antibacterial and insecticidal activity of extracts of organometallic compounds such as Triphenyl, Tri-*n*-butyl (Solid), Tri-*n*-butyl (Liquid), Dimethyl, Diphenyl and HL ligand were investigated. Bacterial pathogens such as *Pseudomonas aeruginosa* spp. (Chicken liver and earthworm), *Shigella flexneri* (spoiled fish samples), *Streptococcus* (Human infected sample), *Enterococcus faecalis* spp. (water), *Enterococcus faecium* spp. (water), *Staphylococcus aureus* spp. (Human infected sample), *Klebsiella pneumonia* (Human infected sample), *Serratia marcescens* (Human infected sample), *Pseudomonas syringae* (Human infected sample), *Serratia odorifera* (spoiled fish samples), *Salmonella typhirium* (spoiled fish samples), and *Enterobacter amnigenus* (spoiled fish samples), respectively were isolated and identified. It was acquired that the growth of *S. flexneri* was inhibited by all tested extracts of organometallic compounds (average zone of inhibition 18-30 mm) while Triphenyl and HL ligand had no effect on *S. typhirium*. The extracts of Dimethyl and Diphenyl showed higher zone of inhibition (24 and 20mm) against *S. flexneri* than *S. odorifera* (22 and 16 mm) whereas moderate zone of inhibition was measured against *E. amnigenus*. It was observed that Dimethyl and Diphenyl significantly inhibited the water associated pathogens *E. faecalis* and *E. faecium* (average zone of inhibition 16-28 mm). On the other hand Tri-*n*-butyl (Liquid) had no effect on the *S. aureus* while Dimethyl showed significant antibacterial activity against *S. aureus* (50 mm) and *P. aeruginosa* spp. (30-32 mm). Insecticidal activity was also measured by using all organometallic compounds and it was scrutinized that all compounds showed higher insecticidal activity against *Bactocera zonata* (fruit fly) larvae and food storage insects. It was acquired that most of these compounds were found active against pathogenic bacteria as well as have insecticidal activity.

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**EVALUATION OF ANTIBACTERIAL ACTIVITY OF ANTIBIOTICS AND MEDICINAL HERBS AGAINST SOME HUMAN BACTERIAL PATHOGENS**

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Infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health. Their impact is particularly large in developing countries due to relative unavailability of medicines and the emergence of widespread drug resistance. Current research focuses on plants since they can be sourced more easily and be selected based on their ethno-medicinal uses. Medicinal plants are used by 80% of the world population as the only available medicines especially in developing countries. An experiment was carried out to study the antimicrobial activity of chloroform and isoamylalcohol extracts of medicinal plant including *Cinnamomum zylanicum* (Cinnamon; Dalchini), *Cuminum cyminum* (Cumin; Zeera), *Syzygium aromaticum* (Clove; Loang), *Curcuma long Linn* (Turmeric powder), *Trachyspermum ammi* (Carom seeds; Ajwain) by agar disc diffusion method. The tested bacterial strains were *Staphylococcus aureus*, *Klebsiella pneumonia*, *Serratia marcescens*, *Streptococcus*, and *Pseudomonas syringae*. Zone of inhibition produced by different extracts against the selected strains was measured and compared with various antibiotics. The present study demonstrated that Chloramphenicol, Streptomycin, Gentamycin, and Ciprofloxacin have higher antibacterial activity (average 30-35 mm zone of inhibition than Nelidixic acid and Neomycin (average 20-25 mm zone of inhibition) Whereas Oxytetracycline, Ampicillin, Penicillin G and Amoxylin showed low antibacterial activity (average 2-8 mm zone of inhibition) against some tested pathogens. It was acquired that the isoamylalcohol extract of *Curcuma long Linn* indicated better results against *S. marcescens* (27 mm) and *P. syringae* (27 mm) whereas the chloroform extract of *S. aromaticum* and isoamylalcohol extract of *C. cyminum* showed significant antibacterial activity against *S. marcescens* and *Streptococcus*, respectively. The results obtained in the present study suggest that the different extracts of medicinal plants revealed a significant scope to develop a novel broad spectrum of antibacterial herbal formulations against human pathogens.

**FREQUENCY OF ANEMIA DURING PREGNANCY IN FAISALABAD DISTRICT**

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Iron deficiency is the most prevalent nutritional problem in many parts of the world and the most common cause of anemia in Faisalabad, especially among pregnant female. The current study aimed to estimate the frequency of anemia during pregnancy in Faisalabad women and the associations between anemia and iron-status, dietary history and socio-economic status of the patients who attended Usama Clinic, Faisalabad and Gynecology Unit of Allied Hospital, Faisalabad during April-June, 2011. Descriptive study consisted of 100 pregnant women in the age group (17-40) years and questionnaire was completed at the time of blood collection. CBC test was performed for determining the haemoglobin level by the hematology analyzer SYSMEX KX-21

and the collected data was analyzed using SPSS program. The overall prevalence rate of anemia was (75%). The frequency of anemia was higher in third trimester of pregnancy (89.3%), illiterate females (36%) and younger age group (<30 years). Differences in the prevalence rates were of non-significant level. Out of 75 anemic patients 65 (86.7%) were having poor dietary habits, thirty seven (49.3%) were not taking any iron supplement and sixteen (21.3%) were facing the menorrhagia problem. In conclusion, although the adopted strategy concerning primary health care seems to be not well planned as no noticeable effect on the control of the prevalence of anemia and there is a great need for further health education promotional programs as well as supplementation programs, especially for pregnant women.

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### A POSSIBLE CROSS TALK BETWEEN *K RAS* AND ADHERENT JUNCTION MEDIATED SIGNALING???

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Incidence and prevalence of Colorectal cancer (CRC) varies from country to country, particularly, depending on different genetic and environmental factors present in that area. CRC has relatively low incidence in Asia and Africa, but high in western countries, including Northern Europe, New Zealand and Australia. Different aspects of CRC have been studied in the other countries of the world, but in Pakistan, relatively little or almost no information is available about different aspects of this disease at molecular level. Experimentally induced mutations in *Kirsten ras sarcoma viral oncogene homologue (K ras)* stimulates the migration of human CRC cells (Caco-2) cells by decreasing the level of E-cadherin/beta-catenin/p120 protein (adherent junction) complex formation so to study the molecular mechanism of CRC, mutational spectrum and presence of interaction or cross-talk between different proteins like K ras and adherent molecules mediated signaling cascades is very necessary. In present study molecular analysis of *K ras* with reference to its association with adherent molecule ( $\alpha$ -catenin,  $\beta$ -catenin and E-cadherin) was addressed in two colorectal cancer cell lines *i.e.* Caco-2 with wild type *K ras* (control) and DLD1 with heterozygous mutation at codon 13 of *K ras*. Real time PCR and western blotting were used as major techniques for comparison of expression at message and transcriptional level. As compared to Caco-2, *K ras* showed slightly higher expression while  $\alpha$ -catenin showed a slight decrease,  $\beta$ -catenin and E-cadherin showed significantly lower expression in DLD1 cell lines. Our results present an idea of presence of a possible cross talk between *K ras* and adherent junction mediated signaling due to presence of mutation (G to D) in codon 13.

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### PRODUCTION, PURIFICATION AND CHARACTERIZATION OF CELLULASES FROM *ASPERGILLUS HEMICOLA* IN SOLID STATE FERMENTATION

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Fungus has the ability to produce large amount of industrially important extracellular enzymes. Microorganisms are commonly used in biotechnological and environmental process

through exploitation of their natural catalytic activities. The current study was designed for the production of cellulases from *Aspergillus hemicola* using corn cobs as a substrate. All three cellulases e.g. endoglucanase, exoglucanase and beta-glucosidase were produced at optimum conditions. These were then subjected to purification and characterization for pH, temperature and kinetics parameters. Endoglucanase show maximum activity (5.16 IU/mL/min) after 96 hours at 50 °C, 5.0 pH, 3mL of inoculum size and 50% moisture level. Maximum activity of exoglucanase (5.24 IU/mL/min) was calculated after 96 hrs at 50 °C, 5.5 pH, 4mL of inoculum size and 60% moisture level. In case of beta-glucosidase, maximum activity was observed after 96hrs at 50 °C, 5.0pH, 3 mL of inoculum size and 70% moisture level. Addition of 0.3% of glucose and 0.4% of peptone in cultural media increase the production of endoglucanase and betaglucosidase. In case of beta-glucosidase addition of 0.3% fructose and 0.2 % of peptone increase the production. Other best cultural conditions are 0.3% tween-80 and 0.2% yeast extract for endoglucanase, 0.2% tween-80 and 0.4% cane molasses for exoglucanase and 0.3% tween-20 and 0.3% of cane molasses for beta-glucosidase. The cellulases produced at optimum conditions were subjected to purification by ammonium sulphate precipitation, gel filtration chromatography. Maximum purification was observed at 50 %, 60% and 60 % of ammonium sulphate for endoglucanase, exoglucanase and  $\beta$ -glucosidase respectively. All three cellulases showed maximum activity at 50 °C temperature and 5.0 pH. The  $K_m$  and  $V_{max}$  was found to be (4.86  $\mu$ M/min and 9.74 mM, 3.34  $\mu$ M/min and 10.29 mM, 4.04  $\mu$ M/min and 8.54 mM) for endoglucanase, exoglucanase and  $\beta$ -glucosidase, respectively.

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#### BIOPROCESSING OF CORN STOVER FOR THE PRODUCTION OF BETA-GLUCOSIDASE BY *ASPERGILLUS FUMIGATUS*

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Cellulases are the most important group of industrial enzymes. These are produce and secrete externally by certain cellulolytic microorganisms. The current study was designed for the optimal production of Beta-glucosidase by *Aspergillus fumigates*. The enzyme produce at optimized conditions was further purified and characterized for pH, temperature and kinetic parameters. Partially purified beta-glucosidase can be used in different industries. Corn stover was used as a lignocellulolytic substrate of *A. fumigates* for beta-glucosidase production. Maximum activity (5.60 IU/mL/min) of beta-glucosidase was observed after 96 hours at 50 °C temperature, 70 % moisture level, 5 mL inoculum size and 5.0 pH. Maximum beta-glucosidase production was observed when 0.3% of fructose as carbon source and 0.4% of peptone as a nitrogen source were added in cultural media. Further, 0.3% of tween-20 as a surfactant and 0.2% of yeast extract as mediator enhances the production of beta-glucosidase by *A. fumigatus*. Maximum purification of was observed by addition of 60% ammonium sulfate. Beta-glucosidase was purified by gel filtration chromatography using sephadex G-100. Purified enzyme was then subjected to characterization analysis. The  $K_m$  and  $V_{max}$  was found to be 4.50  $\mu$ M/min and 11.23 mM respectively. It has maximum activity at 50 °C temperature and 5.5 pH using salicin as a substrate. Metal ions like  $Ca^{2+}$ ,  $Mg^{2+}$  and  $Zn^{2+}$  has positive effect on beta-glucosidase activity.

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**EVALUATION OF *LISTERIA MONOCYTOGENES* THROUGH PCR IN DIFFERENT DAIRY PRODUCTS OF RAWALPINDI-ISLAMABAD**SANA IQBAL<sup>1</sup>, TARIQ MASUD<sup>2</sup> AND MAZHAR QAYYUM<sup>1</sup><sup>1</sup> Department of Zoology/Biology, PMAS-Arid Agriculture University, Rawalpindi<sup>2</sup> Department of Food Technology, PMAS-Arid Agriculture University, Rawalpindi

The present study was conducted to find out the prevalence of *L. monocytogenes* in different dairy products purchased from retail markets of Rawalpindi-Islamabad. The identification was made on the basis of morphological, cultural and biochemical characteristic of the *L. monocytogenes*. The confirmation of *L. monocytogenes* was carried out by using PCR based primers forward 5'- GCA GTT GCA AGC GCT TGG AGT GAA-3' and reverse 5'- GCA ACG TAT CCT CCA GAG TGA TCG- specific to 3' hemolysin gene (hly A). The bands were observed at 730pb region. The results of the present study revealed that the prevalence of *L. monocytogenes* is highest in the homemade soft cheese (10%) followed by raw milk (6.66%), while the lowest was observed in ice-cream (3.33%). The locally prepared ice-creams showed the presence of *L. monocytogenes* as compared to branded ice-creams. The prevalence of virulent *L. monocytogenes* may cause outbreaks of listeriosis in twin cities of Rawalpindi-Islamabad. It is suggested that the proper inspection of dairy food items must be ensured during their processing in manufacturing units. Furthermore quality assurance tests of homemade foods should be taken into consideration. In rural areas where raw milk has been used to make lasi, dahi and butter, without examining the quality of the raw milk, this warrant proper inspection in these food items.

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**DIETARY HABITS OF THE HOUSE RAT (*RATTUS RATTUS*) IN URBAN RAWALPINDI, PAKISTAN**SYEDA RUBAB NAQVI, MUHAMMAD MUSHTAQ, MUHAMMAD SAJJID NADEEM,  
AMJAD RASHID KAYANI AND MIRZA AZHAR BEG

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The dietary habits of the house rat, *Rattus rattus*, were studied in the urban areas of Rawalpindi city. Rat specimens were live trapped from two types localities of the city *i.e.*, stores and houses, and were brought to the laboratory, of the zoology department, PMAS, AAU Rwp. Reference slides of the relevant materials (grains and cereals) and the stomach contents slides were prepared following the modified methods of William (1962) and Ward (1970). Results reflected that the wheat was recorded as the most frequent food item in most of the samples. In addition, chickpea, millet, barley, moong, masoor, maize, sorghum and peanut were also recovered in the stomach samples. In addition to the cereals and grains, sand/soil particles, animal matter and some unidentified materials were also recorded. There was a non-significant difference in the diet of the males and females, as well as the winter and the summer seasons. The diversity index of the stomach contents showed more diversity in the diet of the rats of houses (3.56) as compared to the rats collected from the stores (2.87) of grains and cereals. The results of the study suggested that house rat is a granivore species in the environments of Rawalpindi urban, which consumes heavy amounts of the cereals and grains, which need to be managed.

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**INFLUENCE OF DIFFERENT PLANT BASED FEED INGREDIENTS ON THE GROWTH PERFORMANCE OF *LABEO ROHITA***

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A 60 days research experiment was conducted to study the effects of different plant based ingredients (soybean meal, canola meal, sunflower meal and rice polish) on the growth performance of *Labeo rohita* fingerlings. Aquariums of size 3 x 2 x 1.5 feet were used to rear fish in Fisheries Laboratory at the Department of Zoology, PMAS-Arid Agriculture University Rawalpindi, Pakistan. The water temperature was maintained at 28°C with the help of water heaters and aerators were used to maintain dissolved oxygen in water. Physico-chemical characteristics *i.e.* temperature, pH, dissolved oxygen, bicarbonates and total dissolved solids were studied on weekly basis. The fish were fed by four different feeds in groups; each feed prepared having one major plant based ingredient (soybean meal, canola meal, sunflower meal and rice polish) with the combination of fish meal, maize gluten, fish oil and vitamin pre mix. The weight gain was recorded on weekly basis to feed the fish on the basis of 3% body weight. The growth performance of *Labeo rohita* was compared and statistically analyzed to determine the influence of different diets. The group of fish fed with canola feed gained the maximum weight while the minimum weight gain was recorded in groups fed with sunflower followed by soybean and rice polish. The average total body length was observed higher with canola meal while the lower was recorded with sunflower meal followed by rice polish meal and soybean meal. The survival rate and growth performance of *Labeo rohita* fingerlings revealed that plant based feed ingredients of fish feed resulted in favorable growth response. Due to the low cost of the plant based feed ingredients it gives encouraging results in reducing the financial cost on fish farmer, and it is highly beneficial for the fish farming sector.

**MOLECULAR BASIS OF METABOLIC RESISTANCE IN THE DENGUE FEVER VECTOR *Aedes Aegypti* TO THE NEONICOTINOID IMIDACLOPRID**

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Mosquitoes are vectors of several major human diseases and their control is mainly based on the use of chemical insecticides. Resistance of mosquitoes to organochlorines, organophosphates, carbamates and pyrethroids led to a regain of interest for the use of neonicotinoid insecticides in vector control. In this context, the present study investigated the

molecular basis of neonicotinoid resistance in the mosquito *Aedes aegypti*. A strain susceptible to insecticides was selected at the larval stage with imidacloprid. After 8 generations of selection, larvae of the selected strain (Imida-R) showed a 5.4-fold increased resistance to imidacloprid while adult resistance level remained low. Microarray chip having more than 14000 transcripts was used for gene screening. Transcriptome profiling identified respectively 344 and 108 genes differentially transcribed in larvae and adults of the Imida-R strain compared to the parental strain. Comparative analysis of their biological functions revealed cuticle proteins, hexamerins as well as other proteins involved in cell metabolism and a high proportion of detoxification enzymes. Among detoxification enzymes, cytochrome P450 monooxygenases (CYPs) and glucosyl/glucuronosyl transferases (UDPGTs) were over-represented. Bioassays with enzyme inhibitors and biochemical assays confirmed the contribution of P450 enzymes with an increased capacity to metabolize imidacloprid in Imida-R strain. Comparison of substrate recognition sites and imidacloprid docking models of six CYP6s over-transcribed in the Imida-R strain together with *Bemiscia tabaci* CYP6CM1vQ and *Drosophila melanogaster* CYP6G1, both able to metabolize imidacloprid, suggested that CYP6BB2, CYP6N12 and CYP6Z8 are good candidates for imidacloprid metabolism in *Ae. aegypti*.

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#### **REDUCTION OF HEXAVALENT CHROMIUM BY USING IMMOBILIZED BACTERIA ISOLATED FROM CHROMATE CONTAMINATED ENVIRONMENT**

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Chromium resistant bacteria have the potential to reduce hexavalent chromium so these can be used for the removal of chromium metal from environment. Chromium is a toxic heavy metal and found in industrial effluents (tannery, electroplating, and paint industry). Immobilized bacterial strains have a large potential to reduce this metal as compared to mobilized strains. Chromium resistant bacteria were isolated from the soil of a tannery located in Sialkot, Pakistan. These strains were grown and screened for chromate resistance ability. The selected bacterial strains were characterized morphologically and biochemically. The optimum growth temperature and optimum growth pH was determined for all the isolates. Later on, effect of different immobilizing matrix such as agarose, sodium alginate and gelatin was evaluated on the chromate reduction ability of these bacterial strains.

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#### **PREVALENCE OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY IN BALUCHISTAN**

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Glucose-6-phosphate Dehydrogenase (G6PD) deficiency is the commonest disease, producing enzyme disorder of human being. In most patients with G6PD deficiency, there is no anemia in the steady state. G6PD deficiency results in neonatal jaundice and in some cases are

associated with chronic nonspherocytic hemolytic anemia. 400 million people are affected by this enzyme deficiency in healthy Pakistani population of different regions. The reported incidence varies from 2-3%. However no scientific study has been conducted in Balochistan, therefore exact frequency of this disease is not known. This study attempts to find out G6PD deficiency in neonates in Balochistan. This study was conducted in Bolan Medical Hospital Quetta. Out of 70 neonates included in the study, 62 (88.57%) had normal G6PD enzyme level and 8 (11.43%) had deficient level of enzyme. There were 40 males and 30 females included in the study. Result showed that 5 males and 3 females had low G6PD enzyme on screening test. Out of 70 babies 25 (35.70%) were pashton, 10 (14.30%) were afghan refugees, 9 (12.85%) were settler mostly Punjabi and 1 (1.40%) was Hazara. It was found that 6 out of 25 Brohi/Baloch (24%) and 2 out of 25 (8%) Pathans had Glucose-6-phosphate deficiency. On probing the parents 71% gave history of use of high risk drugs while 54% had reported jaundice in other siblings or close relatives. 11% had positive history of genetic defects in the family.

**AN EXPERIMENTAL STUDY TO ELUCIDATE THE EFFECT OF EMAMECTIN  
SYNERGIZED WITH PIPERONYL BUTOXIDE ON SEVERAL ENZYMES IN  
DELTAMETHRIN RESISTANT AND SUSCEPTIBLE STRAINS OF *TRIBOLIUM  
CASTANEUM***

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Development of resistance against number of insecticides in red flour beetle *Tribolium castaneum* becomes a risk for stored grain products. Dose-mortality bioassays were conducted on 6<sup>th</sup> instar larvae collected from different cities of Pakistan, whereas biochemical assays were conducted to determine role of different enzymes responsible for resistance development. RESULTS: all the collected field strains were found susceptible to emamectin whereas deltamethrin resistant strain was found 4.61 folds resistant. Emamectin became even more toxic when mixed with Piperonyl butoxide (PBO) in 1:2 ratio by reducing LC<sub>50</sub> value from 5.12 to 1.90ppm against R-MDA strain. Biochemical assays revealed that catalase, amylase, phosphatases (alkaline and acidic) and acetylcholinesterase activities were positively correlated with resistance to deltamethrin and emamectin. CONCLUSION: the understanding of synergism with PBO and enzymatic involvement in emamectin and deltamethrin cross resistance provides an opportunity to design new resistance management strategies restoring the efficacy of emamectin-based programs.

**TESTING EFFICACY OF VARIOUS OILS ON *CULEX QUINQUEFASCIATUS*  
MOSQUITOES**

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Oils in water stop mosquito's respiration and larvae die due to suffocation. Use of kerosene oils in water for mosquito's larval control has been abandoned due to their environmental hazards.

Various oils of safe nature from plants; Cinnamon- *Cinnamomum zeylanicum*, Eucalyptus- *Eucalyptus globules*, neem- *Azadirachta indica* Clove- *Eugenia caryophyllata*, and Jamama- *Eruca sativa* were tested for their mortality on 4<sup>th</sup> instar larvae of *Culex quinquefasciatus* mosquitoes. In a laboratory bioassay at 48hrs post treatment, the LD 50 and LD 95 values were calculated using SAS version 9. LD 50 for these oils as recorded were; Jamamma oil 2.45ppm, neem oil, 2.53ppm, cinnamon oil 1.23ppm, clove oil 1.42ppm and eucalyptus oil, 123ppm respectively. LD 95 values were 4.05, 3.51, 2.05 and 2.33ppm respectively. Both cinnamon and eucalyptus oils were more toxic to 4<sup>th</sup> instar larvae of mosquitoes than neem or jamama oils. Based on these results, we recommend the use of environment friendly oils as mosquito larvacides in Pakistan as a future strategy in the control of dengue and malaria vectors.

**AEROMONAS PUNCTATA PNS-1: A PROMISING CANDIDATE TO CHANGE THE ROOT MORPHOGENESIS OF ARABIDOPSIS THALIANA IN MS AND SAND SYSTEM**

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Model system of sand comprising of *Arabidopsis* plant inoculated with PNS-1 strain was used to evaluate the bacterial effect in modulation of root structure of the plant root at tertiary level as compared to MS media which changes the root morphogenesis only at secondary level when inoculated with PNS-1 strain. Inoculation with *Aeromonas punctata* PNS-1 strain subjected to increase the primary root length and number of lateral roots both in MS and Sand system significantly ( $P < 0.05$ ). But these strains modulate the root structure in sand environment in complex manner, which will be important for further incitation of the interaction of microbe with plants, which may depict the close image to natural environment. In order to find out whether this change in root morphology is due to auxin dependent, transgenic line was used to reveal the change in homeostasis of endogenous auxin. Inoculated with transgenic lines of auxin (DR5: GUS), PNS-1 enhanced the endogenous auxin in primary root apices and lateral root. For confirmation PNS-1 was evaluated for auxin production in vitro which showed increase in auxin production with amendment of L-tryptophan. ACC deaminase activity of PNS-1 was checked to confirm whether the primary root elongation is influenced by ethylene concentration which might be lowered by ACC deaminase activity and in turn elongate primary root length. In the present study *Aeromonas punctata* PNS-1 is potential candidate for triggering the change in root morphogenesis of *Arabidopsis thaliana* with the involvement of auxin and ACC deaminase production.

**EFFECT OF VITAMIN E PRE-TREATMENT ON CARBON TETRACHLORIDE (CCL<sub>4</sub>)-INDUCED TOXICITY IN CHICKS (*GALLUS DOMESTICUS*)**

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Present study was designed to investigate the toxic effects of carbon tetrachloride (CCL<sub>4</sub>) (0.5ml/kg b.w. intraperitoneally) on the blood and liver of chicks (*Gallus domesticus*) and the

effects of vitamin E pretreatment (2mg/kg b.w. intraperitoneally) which is known antioxidant on (CCl<sub>4</sub>) –induced toxicity in these organs of the chicks. Study included the estimation of the enzymes activities like ALAT (alanine aminotransferases) and ASAT (aspartate aminotransferases) and some of the biochemical components like glucose, urea, total lipid, cholesterol and protein in both the blood and liver. In liver besides these components, DNA & RNA contents were also investigated. CCl<sub>4</sub> treatment resulted in significant decrease in hepatic ALAT activity and significant increase in ASAT activity. Among biochemical components, glucose, lipid, cholesterol and protein contents in blood and glucose, urea, total lipid, soluble protein, total protein & DNA contents were significantly changed in liver. Hepatic glucose and DNA contents were decreased whereas rest of the components increased. Vitamin E pretreatment has prevented CCl<sub>4</sub>-induced increase in total lipid contents but failed to abolish the increase in hepatic urea content. Vitamin E treatment alone caused significant decrease in plasma ASAT activity and significant increase in blood glucose and DNA contents in liver indicating the role of Vitamin E in glucose metabolism. In addition, prevention of CCl<sub>4</sub>-induced changes in some biochemical components indicate that CCl<sub>4</sub> caused both oxidative and non-oxidative damage.

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#### PHYTOSTIMULATION BY RHIZOBACTERIA CONTAINING ACC-DEAMINASE ACTIVITY

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Major mechanisms utilized by plant growth-promoting rhizobacteria (PGPR) to facilitate plant growth and development is the lowering of ethylene levels by deamination of 1-aminocyclopropane-1-carboxylic acid (ACC) which is the immediate precursor of ethylene in plants. ACC-deaminase enzyme hydrolyses ACC to alpha-ketobutyrate and ammonia. In the present work, five bacterial strains of *Providencia*, *Bacillus* and *Alcaligenes* genera were screened for 1-aminocyclopropane-1-carboxylate (ACC) deaminase, auxin production and phosphate solubilization. All of these strains were considered to be PGPR based on the ability to promote the growth of *Vigna radiata* (L.). The treatment of plant seeds with these bacteria reduced the amount of ACC in plants, thereby lowering the concentration of ethylene. *Bacillus pumilus* Sol-1 (430 nmol h<sup>-1</sup>), *Alcaligenes* sp. Mal-4 (390 nmol h<sup>-1</sup>) and *Providencia vermicola* Ama-2 (377 nmol h<sup>-1</sup>) exhibited the highest ACC-deaminase activity. Auxin production by rhizobacteria showed significant positive correlation (up to  $r = 0.965$ ;  $P = 0.01$ ) with increasing L-tryptophan concentrations. Bacterial ACC-deaminase activity significantly enhanced root length (up to 50%) and number of roots (up to 47%), over control. On the other hand, L-tryptophan dependent auxin production showed significant negative and positive correlation for root length (up to  $r = -0.992$ ;  $P = 0.01$ ) and number of roots (up to  $r = 0.979$ ;  $P = 0.01$ ), respectively. In pot trials, *Alcaligenes* sp. Mal-4 recorded maximum increase for shoot length (57%), shoot fresh weight (85%), shoot dry weight (96%), number of pods (64%) and seeds weight (19%). The ACC-deaminase trait could be employed as an efficient tool for screening of effective PGPR, which could be successfully used as biofertilizers to increase the growth of inoculated plants. The results showed that *Alcaligenes* sp. Mal-4 could be used as biofertilizer to enhance the vigor and yield of leguminous plants.

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### EFFECT OF TANNERY EFFLUENT WASTEWATER ON BLOOD VESSEL PROLIFERATION

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This project was designed for the appraisal of changes caused by tannery effluent wastewater on physiological angiogenesis using Chorioallantoic Membrane (CAM) model. On 5<sup>th</sup> day of incubation, 200 $\mu$ l of three dilutions of tannery effluent wastewater (TEWD1, TEWD2 and TEWD3), potassium dichromate (PDC), chromium chloride (CHC) and phosphate buffer saline (PBS) were inoculated in chicken eggs. After 24hrs, 10% skim milk was injected into the CAMs of all groups to enhance contrast. Pictures of CAM were taken with a novel system based on high resolution Lebeca cam. For quantification of changes in development of blood vessels, Scan Probing Image Processing software was utilized. TEWD1 and TEWD2 and PDC proved antiangiogenic when compared to control. Moreover, microphotographs of CAMs (TEWD1 and PDC) have revealed decreased number of capillary plexuses along the ectoderm and damaged mesodermal collagen mesh work.

### PHARMACOKINETICS OF KETOPROFEN IN HEALTHY PAKISTANI GOATS

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The plan of present research was to study the pharmacokinetic parameters of Ketoprofen (KTP), a non-steroidal anti-inflammatory drug (NSAID) in healthy Pakistani Goats. For this purpose, eight healthy goats were administered, a single intravenous bolus of ketoprofen at the dose of 3.0 mg/kg body weight through jugular vein. Blood samples (3-5ml) were drawn, pre-medication at zero-hr and then at 0.08, 0.17, 0.25, 0.5, 0.75, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 24.0, 48.0, 60.0, 72.0, 84.0, 96.0 hrs post medication in heparinized vacutainers. The blood samples were centrifuged at 4000rpm for 10 minutes for separation of plasma. The plasma was collected in serum collection tubes (eppendorf tubes) and stored at -80 $^{\circ}$ C till analyzed. The concentration of ketoprofen in plasma was determined by a previously developed and validated HPLC (high performance/pressure liquid Chromatography) method. The concentration versus time profile of ketoprofen of each goat was prepared semilogarithmically. The plasma concentration versus time data of each goat was taken and analyzed by computer soft ware. The pharmacokinetic parameters of ketoprofen in goats were calculated by using computer based pharmacokinetic software APO, Version 3.02, as Mean  $\pm$ SEM AUC (Area Under the concentration time Curve)  $\pm$   $\mu$ g.h.ml<sup>-1</sup>, CI (Clearance)  $\pm$  L.hr

<sup>1</sup>.Kg<sup>-1</sup>, t<sub>1/2</sub> (Half Life) ± hr<sup>-1</sup>, VD (Volume of Distribution) ± L<sup>-1</sup>, VD<sub>ss</sub> (Volume of distribution at Steady State) ± L.Kg<sup>-1</sup>, and K<sub>el</sub> (Elimination Rate Constant) ± L.hr<sup>-1</sup> respectively.

**AUXIN PRODUCTION AND ACC-DEAMINASE ACTIVITY OF *PSEUDOMONAS* AND THEIR PHYTOSTIMULATORY EFFECT ON *VIGNA MUNGO* (L.)**

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Production of auxin by *Pseudomonas* spp. is one of the main mechanisms to promote plant growth and yield. In the present study, *Pseudomonas* strains exhibiting auxin production and 1-aminocyclopropane-1- carboxylate (ACC) deaminase activity were evaluated for their growth promoting effects on *Vigna mungo* (L.). All the selected strains were shown to produce variable amounts of the plant growth hormone indole-3-acetic acid (IAA) in the presence of different carbon sources. Colorimetric analysis revealed that *Pseudomonas alcaliphila* AvR-2, *Pseudomonas* sp. AvH-4 and *Pseudomonas aeruginosa* As-17, respectively, produced 40.30, 32.90 and 36.50 µg auxin ml<sup>-1</sup> in the presence of 6% of glucose, sucrose and fructose. Similarly, *Pseudomonas* sp. AvH-4 expressed highest ACC-deaminase activity (355 nmol h<sup>-1</sup>) as compared to *P. alcaliphila* AvR-2 (115 nmol h<sup>-1</sup>) and *P. aeruginosa* As-17 (197 nmol h<sup>-1</sup>). Antibiotic sensitivity pattern of these rhizobacteria also showed resistance against oxytetracyclin, erythromycin and penicillin. Inoculation of *V. mungo* with these plant growth promoting *Pseudomonas* strains significantly enhanced plant growth in pot trials. In laboratory experiments (under axenic conditions), *P. aeruginosa* As-17 was the most effective at enhancing shoot length (70.90%), seedling fresh weight (185.70%) and root length (84.20%). Pot trials conducted under natural environmental conditions showed up to 45.60, 54.10 and 72.50% increases in shoot length, root length and number of pods, respectively, over control.

**BIOFILM FORMATION AND BINDING SPECIFICITIES OF CFA/I, CFA/II AND CS2 ADHESIONS OF ENTEROTOXIGENIC *ESCHERICHIA COLI* AND CFAE-R181 MUTANT**

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Enterotoxigenic *Escherichia coli* (ETEC) strains are leading causes of childhood diarrhea in developing countries. Adhesion is the first step in the pathogenesis of ETEC *E. coli* infections and ETEC pili designated colonization factor antigens (CFAs) are believed to be important in the biofilm formation, colonization and host cell adhesions. As a first step, we have determined the biofilm capability of enterotoxigenic *E. coli* expressing various types of pili (CFA/I, CFA/II and CS2) and tip mutated piliated CFA/I strains. Further, enzyme-linked immunosorbent assay

(ELISA) assay were developed to compare the binding specificity of CFA/I, CFA/II (CS1 - CS3) and CS2 of ETEC *E. coli*, using extracted fimbriae and fimbriated bacteria. CFA/II strain as well as extracted pili exhibited significantly higher binding both in biofilm and ELISA assays compared to non piliated and mutant/wild recombinant strains. This indicates that co-expression of two or more CSs in the same strain is more efficient in increasing adherence compared to those having one only. Significant decrease in binding specificity of CS2 strain with deleted *cotD* and *CfaE*-R181 tip mutant strain indicated the important contribution of minor tip proteins in adherence assays. In addition no effect was observed on agglutination of bovine erythrocytes in R181-CotD mutant strains of CS2 showed that minor tip protein may not be important as adhesions in these strains. Isolated CFA/I, CFA/II and CS2 pili as well as bacteria expressing particular antigens on their surface bound to several intestinal cell membrane structures and play a significant role in host cell colonization. In summary, our data suggest that pili, their minor subunits are important for biofilm formation and adherence mechanisms. Overall, the functional reactivity of strains co expressing various antigens, particularly minor subunit antigen observed in this study suggest that fewer antibodies may be required to elicit immunity to ETEC expressing a wider array of related pili.

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#### **EVALUATION OF POTASSIUM SUPPLEMENTATION IMPACT ON WEIGHT GAIN AND TISSUE CONTENTS OF BROILERS**

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The objective of this work was to investigate the impact of potassium supplementation on the weight gain and tissue potassium contents in the broiler chickens. For this purpose one hundred and eight (one day old) broiler birds were randomly divided into four experimental and two control groups. Each of the experimental group was given with a different level of potassium supplementation (in the form of potassium solution in water ranging from concentration of 100ppm to 600ppm) whereas one control group was given with tap water and another control group was given with a water that contained no potassium at all. Weight of all birds was recorded before the start of experiment. On weekly intervals, five chickens from each group were slaughtered, weight of whole birds and individual tissues was recorded and potassium level in stomach, liver, kidney, heart, leg and chest skeletal muscles were measured. Moreover, potassium amount was quantified in serum and feces too. Atomic absorption spectrophotometer was used to determine the amount of potassium in the samples after their digestion in aqua regia. Among all, a maximum weight gain was observed for the group that was given with 400ppm potassium supplemented water. It was observed that the potassium level in the tissues of chicken has increased steadily for first three weeks approximately and then a decline in this level was seen till sixth week. Detected potassium contents in feces followed no regular pattern. Strikingly, potassium supplementation showed a good impact on weight gain of broiler chickens. So it is recommended that moderate level of potassium should be offered to chicken for enhancing their weight by poultry industry.

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**FLAGELLAR BASAL BODY PROTEIN FLII: CLONING AND EXPRESSION IN  
SALMONELLA TYPHIMURIM**

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Biofilms formation is a major hazardous problem from both clinical and environmental perspective. Flagellum-mediated motility is important for biofilm formation by several gram-negative bacteria. >50 genes are involved in flagellar biosynthesis and function in *Salmonella typhimurium*. The flagella basal body is a representative of Type III protein secretion systems; used by several gram-negative bacterial pathogens to colonize foreign tissues and substrates. The mechanism of flagellar assembly was analyzed in *S. typhimurium*, using bioinformatics analysis to identify conserved structural elements. In this study, FliI a flagellar protein that is needed for flagellar assembly and may be involved in a specialized protein export pathway was cloned and over expressed. Using vital dyes, visualization of single and motile was established based on optical microscopy techniques which will extend initial evidence that flagellum-mediated rotation is critical for biofilm formation. The flagellar basal body is a particularly convenient drug target, since the architecture of most its components has been determined near atomic resolution and it is an ancient evolutionarily conserved macromolecular assembly. The knowledge gained will also have implications for elucidation of the mechanistic design principles underlying protein secretion complexes.

**ANTIOXIDANTS PROFILING AND RESISTANCE MECHANISM OF  
CUPRIAVIDUSMETALLIDURANS CH34 AND PSEUDOMONAS PUTIDA MT2 UNDER  
CADMIUM STRESS**

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In this research study, *Cupriavidusmetallidurans* CH34 and *Pseudomonas putida* mt2 were used to study their antioxidative and resistance mechanisms under cadmium (Cd) stress. Tube dilution method revealed minimal inhibitory concentration of *C. metallidurans* CH34 for Cd to be 30mM and *P. putida* mt2 was 1.25mM. Tube dilution analysis yield that among various heavy metals *P. putida* mt2 resisted only Zn<sup>2+</sup>, while *C. metallidurans* CH34 resisted Ni<sup>2+</sup> > Zn<sup>2+</sup>> Cu<sup>2+</sup>>Hg<sup>2+</sup>> Cr<sup>2+</sup>> Pb<sup>2+</sup>. Cadmium stress induced more reduced glutathione (GSH) and non-protein thiols content (NPSH) in *C. metallidurans* CH34 than in *P. putida* mt2. The antioxidative enzymes profiling showed that strain mt2 used superoxide dismutase and ascorbate peroxidase while strain CH34 used only catalase to combat cadmium stress. To study Cd resistance mechanism in both isolates, bacterial cultures were exposed to two metabolic inhibitors; DNP and DCCD. *Cupriavidusmetallidurans* CH34 showed intracellular cadmium accumulation via ATPase efflux system as studied previously but *Pseudomonas putida* mt2 showed significant biosorption, adsorption and intracellular cadmium accumulation in the presence of Cd, DNP and DCCD. These results for the first time highlighted some unknown cadmium efflux mechanism in *Pseudomonas putida* mt2 which is yet to be discovered.

**CHARACTERIZATION AND OPTIMIZATION OF LIPASE FROM BACTERIA  
INHABITING OIL CONTAMINATED ENVIRONMENT**

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Lipases are an important group of biotechnologically relevant enzymes and they find immense applications in food, dairy, detergent and pharmaceutical industries. Lipases are hydrolases, which are able to act under aqueous conditions on the carboxyl ester bonds present in triacylglycerols to liberate fatty acids and glycerol. Three bacterial isolates were obtained from oil contaminated effluents of different industries on Sheikhpura Road and showed high production of lipase by the formation of zones of hydrolysis on PYA olive oil agar medium. On the basis of biochemical and molecular characterization the isolates have been identified as *Pseudomonas aeruginosa*, *Serratiasp.* and *Bacillus megaterium*. *P. aeruginosa*, *Serratiasp.* and *B. megaterium* showed lipase activity of 800, 600 and 1800 EU/ml, respectively. Optimum growth temperature for *P. aeruginosa* and *B. megaterium* was 37°C, while for *Serratiasp.* it was 28°C. All bacterial isolates showed maximum growth at pH 7. Olive oil and Tween80 were found to be inducers of lipase while concentration of oil greater than 2% retarded the growth of the isolates. These bacterial strains were also able to produce alkane hydroxylase and biosurfactants, which adds to their lipolytic activity and biodegradation potential. These bacterial strains have substantial potential for industrial lipase production.

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**DETERMINATION OF GENETIC DISTANCES AND PHYLOGENETIC  
RELATIONSHIP AMONG THE MEMBERS OF FAMILY PHASIANIDAE BASED ON  
THE MITOCHONDRIAL CYTOCHROME b GENE**

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The Phasianidae is a family of birds which consists of the pheasants and partridges, including the junglefowl (including chicken), Old World Quail, francolins, monals and peafowl. The family is a large one, and is occasionally broken up into two subfamilies, the Phasianinae, and the Perdicinae. Mitochondrial cytochrome b (cytb) is among the most extensively sequenced genes to date across the vertebrates. We employed nearly 111 cytb gene sequences of family Phasianidae from GenBank to calculate and compare levels of genetic distance between sister species, congeneric species, and confamilial genera within and across the major subfamilies and genus of family Phasianidae. Based upon nucleotide sequence comparisons of Phasianidae mitochondrial cytochrome b genes, we established Molecular Phylogenetic analysis by Maximum Likelihood method and the evolutionary history was inferred using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) and Neighbor-Joining methods. The results of these analyses are parallel and reinforce some of the principal trends in genetic distances estimates previously reported.

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**ANALYSIS OF SERUM CHOLESTEROL IN THE GOATS OF GILGIT-BALTISTAN**

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In goats and other ruminants cholesterol is naturally produced in liver and intestinal walls. It is also acquired from supplement food. Cholesterol is source of energy and is precursor of steroids hormones, bile acids and also required for normal cell function. It circulates in the blood with the help of lipoproteins. The level of serum cholesterol is generally lower in goat breed as compared to other cattle's. The present study was proposed and conducted on goats (both male and female) having the age of two years based on teeth composition in three districts of Gilgit-Baltistan viz, Ghezir, Baltistan and Hunza-Nagar. Samples of blood randomly taken from 480 goats and serum were obtained by using centrifuge machine. Quantity of cholesterol in mg/dl of serum was determined by analyzing the serum in Micro-lab 300. The result reveals that the average serum cholesterol in male goats was 59.18 mg/dl, 75.78 mg/dl, and 72.45 mg/dl in Ghezir, Baltistan and Hunza-Nagar respectively. Average serum cholesterol in female was 65.54 mg/dl, 76.26 mg/dl and 77.64 mg/dl in Ghezir, Baltistan and Hunza-Nagar respectively. Analysis variance showed that goats of different regions have significant difference at ( $P < 0.05$ ) level.

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**ISOLATION AND CHARACTERIZATION OF ARSENIC RESISTANT BACTERIA AND THEIR ROLE IN BIOREMEDIATION OF HEAVY METALS**

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Industrial effluents are polluted with large number of poisonous heavy metals out of which arsenic is the most lethal, possessing toxic health effects and contaminates the environment making the drinking water non-portable. Three arsenic resistant isolates of IIB-As 1, IIB-As 2 and IIB-As 3 were isolated from industrial effluents of Kala shah Kaku, Raiwand and Kasur. Bacterial isolates IIB-As 1 and IIB-As 3 were identified by 16SrRNA gene (ribotyping). They showed 98% homology with *Pseudomonas aeruginosa*. For the growth of these arsenic resistant bacterial isolates optimum temperature is 37°C for IIB-As 1 and 35°C for IIB-As 2 and IIB-As 3. IIB-As 1 and IIB-As 2 show optimum growth at pH 7 while the optimum pH for IIB-As 3 is 7.5. These arsenic resistant bacterial isolates have ability to reduce metal, their ability is determined with the help of atomic absorbance spectrophotometer. The data obtained from spectrophotometer recommend that these isolates are capable of removing arsenic from the environment and it can be used in arsenic removing projects for arsenic bioremediation.

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**ISOLATION AND CHARACTERIZATION OF ARSENIC-RESISTANT YEAST FOR UPTAKING ARSENIC**

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The indication to the significant amount of increasing rates of environmental reservoir pollution, heavy metals are considered the most obnoxious of all. Of these we carried out a study

on bioremediation trends and patterns of eukaryotic systems and isolated yeast species as arsenic resistant, eukaryotic systems from industrial effluents of Lahore Canal Drain and effluents from Kasur Tanneries, Raiwind and Kala Shah Kaku. These were tested for the arsenic resistance, morphological changes under this metal stress and hyphal transformations, and their optimal growth parameters under metal stress were responsively analyzed as well. Also these were checked for co-metal or cross-metal resistance with combined effect of arsenic along with fellow heavy metals on their growth. The isolates were evaluated by 18S rRNA verification and identified by homology search tools. The yeast species isolated under metal stress, characterized biochemically and hence able to detoxify arsenic revealed to be *Saccharomyces cerevisiae*, *Candida tropicalis*, *Yarrowia lipolytica* and *Kluyveromyces acetii* by the ibotyping and morphological assessment results. This study can therefore help in bioremediation as the isolates bio-accumulate arsenic and also give an insight how notably the eukaryotes respond to arsenic stress.

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#### CHARACTERIZATION OF ANTIMICROBIAL METABOLITES PRODUCED BY *PSEUDOMONAS AURANTIACA*

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A rhizobacterium *Pseudomonas aurantiaca* was characterized for the production of antimicrobial metabolites. *In vitro* antifungal activity of *P. aurantiaca* was evaluated against fungal pathogens of chickpea (*Fusarium oxysporum*) and rice (*Helminthosporium oryzae*) by dual culture assay. The inhibition zone between the bacteria and the fungus ranged from 18-23 mm. Inhibition of fungal mycelium around the bacterial colony was used as an indication of the extent of antagonism. The suppression of the growth of economically important phytopathogens by antimicrobial compounds indicates biocontrol potential of *P. aurantiaca*. In order to investigate the growth promoting effect of *P. aurantiaca*, bacterial inoculant was applied on rice seedlings at the transplanting time in Net-house. Genotypic variations were observed in growth and yield response to bacterial inoculum. Among three rice varieties, a 45% increase due to beneficial effect of *P. aurantiaca* was observed in grain yield of Basmati-385 compared to control.

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#### IN-VITRO AMPLIFICATION AND PARTIAL SEQUENCING OF INTERFERON REGULATORY FACTOR ONE (IRF-1) OF *CIRRHINUS MRIGALA*

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Interferons are well acclaimed for their wide-ranging implications in immunological responses and are transcriptionally regulated by a set of genes called as Interferon Regulatory Factors (IRFs). It is noteworthy that IRFs represent tissue specific and differential expression. The present study was conducted for *in-vitro* amplification of IRF-1 of various tissues of *Cirrhinus*

*mrigala*. The fish was anesthetized in bezocaine solution. The tissues from its spleen, liver, gills and head kidney were taken and preserved in trissure to soften the tissue and prevent RNA degradation. Then RNA was extracted from these tissues and cDNA was prepared by reverse transcription. The cDNA was amplified by polymerase chain reaction. The PCR products were sorted by 1% gel electrophoresis and bands were observed in gel imaging system. Gel banding pattern highlighted the fact that IRF-1 displayed selective expression in various tissues. These bands were extracted from the gel and purified PCR product was sent for sequencing. Differential display points out undeniable role of IRF-1 in regulation of interferon gene expression in various tissues. However, underlying mechanisms which repress or stimulate a context dependent expression of these genes need further investigations.

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#### **IMPACT OF CHLORPYRIFOS ON SOME FISH (*APHANIUS DIPAR*) TISSUE ENZYMES**

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Marine environment is prone to pollution through agricultural, industrial and domestic pollutants which find its way into the sea through rivers and other tributaries and drainage system. The major pollutants entering into the coastal environment of Pakistan are oil, sewage, garbage, pesticides, toxic chemicals, heavy metal, radioactive waste, thermal pollution and nutrients. Pesticides and other pollutants are known to bioaccumulate in the food chain at higher trophic levels. Generally acetylcholinesterase (AChE) inhibition has been widely used as an indicator of pollutants, e.g. pesticides exposure and effects, in the aquatic ecosystem. The aim of this study was to assess the toxicity of chlorpyrifos (organophosphorus pesticide) on *Aphanius dispar* (killi fish). In this study acute effects of chlorpyrifos pesticide on tissue enzyme activity (acetyl cholinesterase (AChE), alanine aminotransferase (ALT), aspartate aminotransferase (AST)) were assessed in fish juveniles using enzyme analysis kit. The results depict significant elevation in ALT activity by 118.33% ( $p < 0.05$ ) as compared to control and inhibition in AChE and AST enzymes in the treated fish by 90.7% ( $p < 0.05$ ) and 90.6% ( $p < 0.05$ ), respectively. Analysis of biochemical parameters in the fish is useful in the environmental biomonitoring. The results of this study will help understand biochemical basis of pesticide effect in fish.

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#### **ISOLATION AND CHARACTERIZATION OF COPPER RESISTANT BACTERIA FROM INDUSTRIAL EFFLUENTS**

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Industrial waste water is polluted with a number of heavy metals possessing hazardous health affects and makes water unsuitable for drinking by contaminating the environment. Three copper resistant isolates of *Planomicrobium* sp., *Klebsiella pneumoniae*, and *Enterobacter* are isolated and screened from effluent of steel industry and Rohi Nala. The optimum temperature for growth of all copper resistant bacterial isolates is 40°C and optimum pH for IIB.Cu1 and IIB.Cu2 is 7.5 while for IIB.Cu it is 8.0. The strains were identified by the molecular analysis of the genomic DNA of the isolates and by sequencing 16S rRNA gene. Plasmid was detected in bacterial isolates.

Curing was performed to determine the copper resistant gene. Bacterial strains were grown on metal stress after curing indicated the gene for copper is located on chromosome as no plasmid was detected in cured strains. By taking absorbance using atomic absorption spectrophotometer their metal processing ability was determined having a range of 50-60%. Through data it is implied that by removing 60% of copper IIB.Cu1 is most efficient thus it can be utilized for bioremediation of copper from waste waters.

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### BLOOD LEAD LEVELS DURING PREGNANCY IN FAISALABAD

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Lead poisoning is one of the oldest and the most widely studied occupational and environmental hazards. The purpose of the present study was to monitor lead concentration in blood of the pregnant women of Faisalabad. One hundred women blood samples were taken and performed wet digestion. Lead level in blood was determined by flame atomic absorption spectrophotometry. Results indicated that the mean concentration were higher in those were not taken proper calcium dairy products and supplements (Mean  $\pm$  SE)  $2.67 \pm 0.27$  and  $1.46 \pm 0.23$  as compared to women those were taken proper dairy products and administered calcium supplements  $0.72 \pm 0.08$  and  $0.73 \pm 0.09$  respectively. Blood lead concentration was significant association with women of older age groups, those who administered or not calcium rich food and supplement. About 56% women showed  $<1$  mg/dl and 44%  $>1$ mg/dl lead level respectively. They were having mean lower level of lead from its threshold level  $5 \mu\text{g/dL}$  as described by the CDC and WHO. It concluded that improving the educational status, dietary supplementation, nutritional standards and decrease in pollution could help improve the health problems of pregnant women in the rural as well as in urban areas.

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### EARLY-ONSET PRIMARY DYSTONIA IN CONSANGUINEOUS PAKISTANI FAMILIES

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The dystonia are a group of movement disorders in humans defined by sustained muscle contractions or twisted postures. Both early-onset and adult-onset forms are known, of which the latter are more common and the former are usually more severe. Dystonia can be classified according to a variety of factors. These include anatomic distribution, age of onset and etiology. The genetically inherited cases are termed as primary dystonia. We recruited 6 isolated and 4 familial cases of early-onset, primary dystonia from Punjab. There was no response to Levodopa in any patient which thus excluded dopa-responsive dystonia in the affected individuals of all families. The most common genes for primary dystonia, *DYT1* and *THAPI*, were sequenced for all patients but no mutations were detected. All other known loci of dystonia were excluded for familial cases of dystonia. We used genome-wide linkage analysis to map a new locus for a family with recessively inherited focal dystonia. Work is underway to molecularly characterize the phenotype in the remaining families.

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**MICROBIOLOGICAL AND PHYSICO-CHEMICAL ANALYSIS OF WATER SAMPLES  
IN CHEMOBARI LAKE NALTAR, GILGIT BALTISTAN, PAKISTAN**

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The present research was conducted to study aquatic microorganism, fish and physico-chemical parameters in order assess the water quality of high altitude lake (Chemobari, Naltar) in Pakistan. A total of 30 water samples were collected and tested for bacterial contamination and electrical conductivity in the laboratory where as Physico-chemical parameters like pH, temperature, dissolved oxygen (DO) were tested on the spot using various probes. Along with it 05 fish specimens were also collected and analyzed for bacterial contamination. Analysis of the results for all samples revealed values for pH (6.5 to 9.6), temperature (7.1°C to 9.2°C), dissolved oxygen (5.13 mg/l to 18.50 mg/l) and electrical conductivity (112 µS/cm to 138 µS/cm). The microbiological community of lake was found to be composed completely of bacteria with *Escherchia Coli*, *Pseudomonas*, *proteous* and *salmonella* where as *shigella* was found occasionally whereas the bacterial species on fish abdomen and skin were composed of *Escherchia Coli*, *Pseudomonas*, *proteous* and *salmonella*. As a whole the water quality of Dirlay Lake is not good, because it is threatened by anthropogenic disturbances. Further sampling and analysis should be done in this lake to determine any additional microbial community and the function of species with in microbial community.

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**UTILIZATION OF ECONOMICALLY CHEAP RAW MATERIALS BY YEASTS FOR  
BIOFUEL PRODUCTION**

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Three xylanase producing yeast isolates designated as 2-KLP1, 26-KLP3 and 29-S1B were isolated from industrial wastewater. These yeast isolates were observed for their ability to grow in higher concentrations of xylose, sodium pyruvate, acetaldehyde, and carboxymethyl cellulose. They were further checked for their ability to grow in 1% of agro waste materials *i.e.*, rice husk, wheat bran, sawdust and sugarcane bagasse as the only carbon source in the medium. The yeast isolates showed fairly good growth on all these substrates. The most suitable temperature for 2-KLP1 and 29-S1B was found to be 30°C while it was 37°C for 26-KLP3. Maximum growth for 2-KLP1 and 26-KLP3 was observed at pH 7 while 29-S1B showed maximum growth at pH 8. The yeast isolates proved to produce enzyme both intra- as well as extra-cellularly but the dominant form found to be extracellular. The effect of different substrates on the production of xylanase demonstrated that there was only marginal difference in xylanase production when grown in the presence of different substrates. Yeast isolates showed maximum enzyme production after 5 days of incubation. In order to determine the enzyme stability, the crude enzyme was allowed to incubate at a range of temperatures and pH. The optimum temperature and pH for the activity of xylanase

obtained from the yeast isolates were found to be 50°C and 5, respectively. The effect of metal ions on xylanase activity was also determined. The present study indicates that isolated yeasts, when growing anaerobically, were capable of simultaneous saccharification and fermentation of complex polysaccharides present in the medium. The yeast isolates may be used in biofuel production from cheap industrial waste material containing hemicellulose.

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**ANALYSIS OF ANTIBACTERIAL ACTIVITY OF GINGER AND TURMERIC  
EXTRACTS AGAINST THE STRAINS ISOLATED FROM DENTAL UNIT WATER  
LINES**

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The present study showed the antibacterial potential of ginger and turmeric extracts belonging to the family Zingiberaceae. Preliminary phytochemical screening had shown the presence of various bioactive compounds such as phenolics, alkaloids, tannins, anthraquinones, glycosides and terpenoids. Plant extracts were obtained using methanol, ethanol, ethyl acetate, hexane and water. These extracts were tested in vitro against selected bacterial strains isolated from dental unit water lines by agar well diffusion method. The results had indicated that methanolic extract was more effective as compared to other extracts and maximum inhibition was observed against the strain *Bacillus cereus*. Minimum inhibitory concentration values for ginger extract ranged from 6.25-12.5 mg/ml while for turmeric extract it was 12.5-25 mg/ml. GC-MS analysis of methanolic extracts indicated the presence of a variety of compounds including sesquiphellandrene, tumerone, zingiberene, orixane, farnesene, myrcene and gingerol. Furthermore, the effect of organic contaminants on the antibacterial activity of plant extracts was also investigated. Organic contaminants such as skimmed milk powder and baker's yeast resulted in a significant loss of antibacterial potential of the extracts as indicated by an increase in MBC (minimum bactericidal concentrations). The results showed that both the extracts possess antibacterial activity and could be used as traditional folk remedy for the treatment of bacterial infections.

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**STUDIES ON THE ACTIVITIES OF ANTIOXIDANT ENZYMES IN FISH VIZ. LABEO  
ROHITA AND CIRRHINA MRIGALA**

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During present study *Labeo rohita* and *Cirrhina mrigala* were used to study the activities of antioxidant enzymes viz. superoxide dismutase and catalase at different water temperature and pH. Antioxidant enzymes are the stress monitoring biomarkers. Stressful condition of fish is not only due to a single factor, it is due to a synergistic and cumulative effects of many factors such as pH, temperature, crowding and dissolved oxygen. During this study it was observed that *Cirrhina mrigala* show more activity of superoxide dismutase (72.90±4.77 U/ml) and catalase (661.90±246.50 U/ml) followed by *Labeo rohita* that shows least activity of superoxide dismutase and catalase (65.49±3.37 U/ml and 427.30±128.35 U/ml, respectively). Highest mean activity of

superoxide dismutase and catalase was observed in gills followed by liver and kidney. Differences in species, organs for enzymes activity were statistically significant. The interaction between species and organs was highly significant at  $p < 0.01$ . The physico-chemical parameters viz. water temperature, pH, electrical conductivity, carbon dioxide, total ammonia, hardness, sodium and potassium contents was also analyzed and maintained during present research work. Fluctuations in temperature condition and pH were observed and maintained throughout the test trial.

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**STUDIES ON THE DIGESTIVE ENZYME, AMYLASE IN FISH VIZ. CHANNA  
MARULIUS AND CTENOPHARYNGODON IDELLA WITH DIFFERENT  
NUTRITIONAL HABITS**

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An enzyme is a biological catalyst which can be used to speed up favorable chemical reactions or to control the chemical reactions. During present research work the activity of digestive enzymes viz.  $\alpha$ -amylase and  $\beta$ -amylase in carnivorous and herbivorous fish species were studied to see the differences between nutritional habits. During this study it was observed that *Ctenopharyngodon idella* showed more activity of  $\alpha$ -amylase ( $48.31 \pm 1.18$  U/ml) and  $\beta$ -amylase ( $0.43 \pm 0.58$  U/ml) than *Channa marulius* that showed least activity of  $\alpha$ -amylase ( $0.44 \pm 0.02$  U/ml) and  $\beta$ -amylase ( $0.02 \pm 0.01$  U/ml) in test media. Highest mean activity of  $\alpha$ -amylase and  $\beta$ -amylase was observed in digestive tract and least in liver. Stress of cadmium was also given to the both fish species for 30 days and its effects on activity of  $\alpha$ -amylase and  $\beta$ -amylase were compared with control conditions. The results showed a strong correlation between the cadmium and activity of digestive enzymes ( $\alpha$ -amylase and  $\beta$ -amylase). The physico-chemical variables viz. temperature, pH, alkalinity, hardness, calcium, magnesium, carbon dioxide and total ammonia were also fluctuated significantly in control and cadmium exposed test media.

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**MORINGA OLEIFERA LAMK., A HIGHLY ANTIMICROBIAL AND MILK AND  
GROWTH PROMOTING IN RUMINANTS**

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The present study was undertaken to assess antimicrobial activity and milk and growth promoting ability of *Moringa oleifera* Lamk. (Sohanjana) of family Moringaceae. The crude extract of powdered material of different plant parts like flowers and leaves were obtained in non polar and polar solvents (petroleum ether, chloroform, methanol, distilled water etc.) and tested for antimicrobial activity. All the extracts belonging to the said plant parts produced well defined zones of inhibition against pathogenic microbes i.e. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Aspergillus niger*, *Aspergillus oryzae* and *Trichoderma viridae*. The results indicated that Sohanjana was potent antifungal and antibacterial. The flower extract of *M.oleifera* showed comparatively high antimicrobial activity against the microbes tested. Moreover, *Moringa* was found best fodder for the cattle as it not only enhanced

the milk yield of buffalos *i.e.* two liters but also improved the health of buffalos. It was also found best fodder for small goats as it resulted in increasing meat production.

**PREVALANCE OF ANEMIA AMONG PREGNANT FEMALES AND ITS CORELATION WITH FETAL MORTALITY IN DISTRICT MANDI-BAHAUD-DIN PAKISTAN**

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During present study the prevalence of anemia among five hundred pregnant females and its correlation with fetal mortality was studied in district Mandi-Bahaud-Din. Overall 65.4% females were anemic. On basis of Hb level 6.4% were severely anemic, 19.2% were moderately and 39.8% were mild anemic. The PCV of 86.6% while MCV of 46.4% and MCH of 41.8% was less than normal values. Significant correlation was observed in prevalence of anemia and daily intake of tea. A similar significant correlation was also found between anemia, vegetarian diet and poor diet. Age group of pregnant females was also observed significantly correlated with prevalence of anemia and maximum anemic females were in the age group of 35 >40. Over all 17.7% fetal mortality was recorded with significantly highest among severely anemic group that gradually decreased with increase in Hb among moderately and mild anemic groups. The TLC in 13.6% pregnant females showed higher than normal. Significant difference in neutrophils, monocytes, lymphocytes and eosinophils were observed among pregnant females.

**GENETIC DIVERSITY OF *PLASMODIUM VIVAX* IN KHYBER PAKHTUNKHWA, PAKISTAN**

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*Plasmodium vivax*, the second most prevalent malaria parasite has resistance to drugs and monitoring the situation would be helped by molecular methods that can be used to characterize parasites in field studies and drug efficacy trials. Blood from 207 patients of district Kohat, Karak, Bannu and Dera Ismael Khan Khyber Pakhtunkhwa province Pakistan, were diagnosed for malaria (*P. vivax*) infection and were genotyped based on their merozoite surface protein (*mSP*) genes (especially *pvmSP-3α* and *pvmSP-3β* genes) by PCR-RFLP. Analysis revealed that *P. vivax* populations in the study areas are genetically diversified. In *P. vivax*, genotyping of *PvmSP-3α* and *PvmSP-3β* genes showed a high level of allelic polymorphism. For *PvmSP-3α* four different allele groups A (78.98%) being most prevalent one, B (8.52%), C (5.68%) and D (2.84%) with 3.98% mixed-strain infections. Type A showed high level of epidemics in Bannu (83.02%), B and D (10.53%, 5.26%) respectively in D.I.Khan and C (6.98%) in Kohat. Further restriction polymorphism by *AluI* for *PvmSP-3α* yielded seven genotypes for type A (A1=7.95%, A2=17.61%, A3= 24.43%, A4=7.39%, A5=10.80%, A6=6.25% and A7=4.55%), two for type B (B1=4.55% and

B2=3.98%) and two for type C (C1 and C2= 2.84% each). Similarly, amplification of the *P. vivax msp-3β* locus produced two allele groups, A (70.45%) and B (21.02%) with 8.52% mixed-strain infections followed by highest epidemics for type A (72.09%) in Kohat and C (23.68%) in D.I.Khan, while restriction analysis by *Pst*I for *Pvmsp-3β* yielded five alleles for type A (A1=10.80%, A2=14.20%, A3=19.32%, A4=15.34% and A5=10.80%) and three distinct alleles for type B (B1=9.66%, B2=7.39% and B3=3.98%) respectively, with a combined mixed genotype infections of 14.5%. These results indicate that the genotyping analysis by PCR-RFLP can be useful in the evaluation of drug efficacy particularly *in vivo* conditions and clinical manifestations conducted in endemic areas for epidemiological studies of *P. vivax* infections.

**MUTATIONS IN THE E2-PePHD REGION OF HEPATITIS C VIRUS GENOTYPE-3a AND CORRELATION WITH RESPONSE TO INTERFERON AND RIBAVIRIN COMBINATION THERAPY IN PAKISTANI PATIENTS**

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Hepatitis C is a major health problem affecting more than 200 million individuals in the world. Current treatment regimen consisting of interferon alpha and ribavirin does not always succeed in eliminating the virus completely from patient's body. One of the mechanisms by which virus evades the antiviral effect of interferon alpha involves protein kinase (PKR) eukaryotic initiation factor 2 alpha (eIF2a) phosphorylation homology domain (PePHD). This domain in genotype 1 strains is reportedly homologous to PKR and its target eIF2a. By binding to PKR, PePHD inhibits its activity and therefore cause virus to evade antiviral activity of interferon (IFN). Many studies have correlated substitutions in this domain to the treatment response and lead to inconclusive results. Some studies suggested that substitutions favor response while others emphasized that no correlation exists. In the present study we therefore compared sequences of PePHD domain of thirty one variants of six hepatitis C virus patients of genotype 3. Three of our HCV 3a infected patients showed rapid virological response to interferon alpha and ribavirin combination therapy whereas the remaining three had breakthrough to the same combination therapy. It is found that PePHD domain is not entirely conserved and has substitutions in some isolates irrespective of the treatment response. However substitution of glutamine (Q) with Leucine (L) in one of the breakthrough responders made it more identical to HCV genotype 1a. These substitutions in the breakthrough responders also tended to increase average hydrophilic activity thus making binding of PePHD to PKR and inhibition of PKR more favorable.

**PREVALENCE OF ABO BLOOD GROUPS, RH FACTOR AND BLOOD DISEASES REPORTED AT DHQ HOSPITAL GILGIT, GILGIT BALTISTAN, PAKISTAN**

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The study aimed to investigate the frequency of ABO Blood Groups, Rh factor in male and female and also to determine the prevalence of Blood diseases such as Malaria, Anemia, Leukemia,

Hemophilia, and Thalassemia patients reported at DHQ hospital Gilgit. Blood group determination was carried out a period of 11 months from May 2009 to March 2010 and encompassed 348 total individuals, in which 122 (35%) were males and 226 (65%) females belonging to 12 different ethnic tribes. The dominated ethnic tribes were Yashkun (55.4%) followed by Sheen (32.2%). Blood were screened on a daily basis in patients reporting at OPD, DHQ hospital Gilgit to investigate their ABO blood group, Rh factor and prevalence of different blood diseases. Our result reveal that the most common blood group was A<sup>+</sup> (34%) followed by O<sup>+</sup> (27%), B<sup>+</sup> (22%) and then AB<sup>+</sup> (21%) . The least frequent blood groups were A<sup>-</sup> (1.7%), O<sup>-</sup>(0.86%) and AB<sup>-</sup> (0.28%). Rhesus positive blood group comprised (97%) and Rhesus negative were (3%). These results reflect the Blood group picture of the patients reporting at DHQ Hospital Gilgit, the prevalence of ABO blood groups and Rh factor most commonly or rarely in the area of Gilgit. During blood screening 280 patients were positive for different diseases, out of which the most common was anemic 265 patients (male 66, female 199), 3 cases of plastic anemia, 10 cases of malarial parasitic infection, and 2 cases of leukemia; however no any case of hemophiliac and thalassemia were recorded during our study period. The studied population exhibited a predominance of group A<sup>+</sup> followed by O<sup>+</sup> in the order of B<sup>+</sup> > AB<sup>+</sup> > A<sup>-</sup> > O<sup>-</sup> > and AB<sup>-</sup> as well as Rh positive antigen for male (96%) and female (98%) subjects within the population, with Rh negative men and women being 4% and 2% respectively. Among blood diseases the most common blood disease was anemia and their prevalence is common in female (75%) than male (25%) reported during our study period.

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#### EFFECTS OF POST HARVEST HANDLING TECHNIQUES ON PRODUCTION OF AFLATOXIN IN BROWN RICE

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Aflatoxins are noxious metabolites produced by certain fungi in foods and feeds. They are probably the best known and most intensively researched mycotoxins in the world. Aflatoxins have been allied with various diseases, such as aflatoxicosis, in livestock, domestic animals and humans throughout the world. It is a problem, particularly in developing countries. Aflatoxins are produced primarily by some strains of *Aspergillus flavus* and by most, if not all, strains of *A. parasiticus*, plus related species, *A. nomius* and *A. niger*. It is discovered that there are four major aflatoxins: B1, B2, G1, G2. Aflatoxin development in many stored cereal grains has hampered the availability of good quality grains. It may cause severe health hazards to human, animals as well as can create great economical problems. In this study hundred samples of paddy rice were tested for aflatoxin contamination. Fifty samples were self dried in one step while fifty samples were let to be dried conventionally by the huskers *i.e.* first semidrying up to 16 -18 % moisture contents and then final drying up to 12-14 % moisture level after certain storage period depending upon paddy purchase period of each husking unit. The aflatoxins was detected in paddy rice by using VICAM Series-4EX Fluorometer<sup>TM</sup> which is a based on monoclonal antibody- based affinity chromatography. Almost all the self dried samples have aflatoxin level below the European maximum permissible level while the rice samples dried conventionally at husking units were contaminated with aflatoxin more than the European maximum permissible level for brown rice. So such conventional methods of drying should be avoided to create any serious threat to human & animals.

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**ANTIMICROBIAL EFFECT OF BEE HONEY AS COMPARED TO ANTIBIOTICS ON ORGANISMS ISOLATED FROM INFECTED BURNS**

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A study was conducted on blood samples from burn wounds aiming to evaluate the antimicrobial effect of bee honey on organisms isolated from infected burns in comparison to the antibiotics used in treatment of burn infection, and to evaluate the effects produced when bee honey is added to antibiotic discs. The culture media used for microbes were CLED, Blood, SS and MacConkey agar and Nutrient agar. Four major bacterial strains including *E-coli*, *Klebsiella* spp, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were isolated and Antibiotic sensitivity pattern was measured against these microorganisms by using following 3 antibiotics *i.e.*, Amikacin (AK), Zinacef (CXM), Linzolid (LZD). Results shows that *Klebsiella* spp was more sensitive to honey mixed with antibiotic and least for honey alone as compared to other isolates. Where as *Pseudomonas aeruginosa* (61%), *Staphylococcus aureus* (56%) and *E.coli* (51%) were more sensitive to honey only as compared to honey mixed with antibiotics.

**BIOCHEMICAL VARIATIONS IN THE HONEY OF *APIS DORSATA* COLLECTED FROM DIFFERENT FLORAL SOURCES**

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A total of 24 samples was collected from three different localities (n= 3, Nankan Sahib, n= 10, Faisalabad and n=11, Kalar Kahar) and were analyzed for moisture content, pH, electrical conductivity, free acidity, lactone content, total acidity, proline content and HMF content for quality evaluation of *Apis dorsata* honey. The average moisture content were 18.86%, 22.97% and 19.13% from Nankana Sahib, Faisalabad and Kalar Kahar, respectively. pH and free acidity values fell within the prescribed range. Lactone content was 33.44meq/kg from Nankana sahib, 16.53meq/kg from Faisalabad and 12.63meq/kg from Kalar Kahar. Total acidity calculated for honey samples of Nankana sahib was 60.22meq/kg, 55.16meq/kg for Faisalabad and 50.60meq/kg for Kalar Kahar region. The Proline content from all the samples was higher then minimum International Honey Standards limit. The HMF Content was within the maximum International limit.

**STRUCTURAL ANALYSIS OF *CUS REGULON* OF *KLEBSIELLA PNEUMONIAE* INVOLVED IN COPPER RESISTANCE**

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*Klebsiella pneumoniae* is one of the major microflora found in stressed non-clinical environments. It has strong potential for bioremediation of some heavy metals such as mercury and cadmium. The aim of the present study was to explore its potential to resist copper, at molecular level. A copper resistant *Klebsiella pneumoniae* was isolated from aquatic environment receiving

industrial wastewater in Kot Lakhpat area. Six genes namely *cusA*, *cusB*, *cusC*, *cusF*, *cusR* and *cusS* were amplified from its genomic DNA and cloned, followed by sequencing. Analysis of sequences obtained showed that these genes are present in the form of two operons; *cusC*, *cusF*, *cusB* and *cusA* forming one operon and *cusR* and *cusS* forming the other operon. Both these operons are regulated divergently by a same promoter and constitute *cus* regulon, a characteristic genetic determinant of family Enterobacteriaceae. Based on deduced sequences of the corresponding proteins, tertiary structures of these proteins were determined through homology modeling method using SWISS model server. The structural and functional domains of each protein and important residues involved in specific functions were also identified. Membrane topology of each protein was predicted through hydropathy profile of each of the six proteins through online available software SOSUI.

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### ROLE OF PEPTIDE APTAMERS AFFECTING THE P53 PATHWAY PROTEIN ANTERIOR GRADIENT-2 IN BREAST CANCER CELLS

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Breast cancer is the most common form of cancer diagnosed in women worldwide. One of the most commonly mutated and silenced gene in human cancers is P53. Oncogenic inhibition of the p53 tumor suppressor is a key feature of human cancer. Pro-metastatic protein AGR2 is a novel type of p53 inhibitor, found upregulated in a number of different types of cancers including breast cancer and has been proved to attenuate p53 activity by suppressing its phosphorylation after DNA damage. In this context, it is very crucial to determine whether AGR2 is a potential drug target for reactivating the p53 pathway in cancer cells. The use of concepts in the peptide therapeutics field has led researchers to develop bioactive peptide aptamers that bind to AGR2. In the present study, effects of these bioactive peptide aptamers on breast cancer cell lines (MCF-7 cells) were explored. The cells were treated with varying concentrations of peptide aptamers for different time intervals. Untreated cells were also included in the study for comparison. The cells were then harvested, lysed and protein quantification was done by Bradford assay. Expression of AGR2 alongwith other p53 pathway proteins was analyzed by western blotting.

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### SOIL CONTAMINATION DUE TO RAPID SUGAR INDUSTRY IN SOUTHERN PUNJAB: A CASE STUDY

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District Rahim Yar Khan has the industries like Ghee mills, Food industries, Fauji Fertilizers, Fatima Fertilizers; and five sugar mills. However, there has been little published research in this area. This study was conducted for assessment of pollution caused by effluents of sugar mills. The research was done throughout the year on fields in district Rahim Yar Khan. The parameters studied were electrical conductivity (EC), and soil pH. All the parameters varied in both space and time. The assessment of soil electrical conductivity (EC) and pH may be useful in

agriculture production, for they relate to factors that affect productivity of a habitat and consequently its fauna and flora.

**AMPLIFICATION AND CLONING OF *CUEO* AND *CUER* GENES RESPONSIBLE FOR COPPER RESISTANCE IN *KLEBSIELLA PNEUMONIAE***

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Copper is essential for life but highly reactive and thus a potential source of cell damage when present in excess. So the cells need a tight control of copper homeostasis. Bacteria have been found to have various efficient mechanisms for tolerating metal toxicity. *Klebsiella pneumoniae* is a bacterial system that possesses different genes involved in copper homeostasis. *CueO* is a multi-copper oxidase involved in the detoxification of copper. The expression of *CueO* is regulated by *CueR* gene. These two genes along with another gene *CopA* constitute Cue system. DNA was isolated from *K. pneumoniae* followed by RNase treatment. Primers were designed for both the genes *CueO* (1599 bp) and *CueR* (411 bp) which were amplified by polymerase chain reaction. The amplification of genes was confirmed by agarose gel electrophoresis. Both the amplicons were gel purified and ligated to PTZ57R/T vector by employing dA.dT technique. *E.coli* DH5- $\alpha$  competent cells were prepared by using CaCl<sub>2</sub> method and transformed with the ligated DNA products of both genes. Positive transformants were identified by blue/white screening method in the presence of ampicilline. Ligated products were isolated by mini-prep. Presence of the insert was confirmed through restriction digestion analysis. Double digestion for *CueR* was carried out with *Xba* I and *Hind* III or with *Eco* RI and *Pst* I. Single digestion was performed for *CueO* with *Xba* I or *Eco* RI. Restricted products were run on agarose gel electrophoresis and their sizes were confirmed in comparison to DNA ladder mix by fermentas.

**A TRIAL OF ASCORBIC ACID AND TOCOPHEROLADD-ONTHERAPY IN EPILEPTIC CHILDREN ON VALPROATE MONOTHERAPY: EFFECTS ON GLUTATHIONE PEROXIDASE, CATALASE, SUPEROXIDE SCAVENGING POTENTIAL OF PLASMAAND TOTAL ANTIOXIDANT ACTIVITY**

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Evidence regarding role of reactive oxygen species (ROS) in Idiopathic epilepsy has accumulated during recent times. The study was aimed at monitoring and comparing the effect of

Ascorbic acid (Vitamin C) and Tocopherol (Vitamin E) as add on therapy in Valporate treated and untreated epileptic children. The study group was divided into three groups, healthy individuals (n=18), Valproate treated (n=18) and untreated epileptics(n=18). Six individuals from each group were either given Vitamin C (500mg), Vitamin E(200mg) or both. Body weights, erythrocytic Glutathione Peroxidase, Catalase, superoxide scavenging potential of plasma, total antioxidant activity using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay and ferric reducing ability of plasma (FRAP) assay were assessed before and after fifteen days of vitamin administration. Both the treated and untreated epileptic patients of either sex were found to have a faulty antioxidant enzyme system. This suggests that oxidative stress is implicated in the pathogenesis of epilepsy and Valporate therapy compromises the antioxidant system of the body. The combined add on therapy of Vitamin E and C was found to be most effective in relieving oxidative stress in both treated and untreated group. These findings suggest the usage of antioxidants along with Valporate therapy in epileptic children.

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#### AMPLIFICATION AND CLONING OF *COPA*, A COPPER TRANSLOCATING P-TYPE ATPASE IN *K. PNEUMONIAE*

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*Klebsiella* is a gram-negative, rod shaped and non-motile bacterium of family Enterobacteriaceae. *Klebsiella pneumoniae* is found in humans, animals, sewage, soil, industrial effluent and vegetation. *K. pneumoniae* is able to survive in significantly higher levels of environmental copper and is termed as copper resistant. *CopA* gene regulate export of copper in *K. pneumoniae* that is a Cu(1) translocating p-type ATPase. It is central component in copper homeostasis. *CopA* (2.555kb) gene was amplified by using COPA-F and COPA-R primers. The amplicon (2.930 kb) was run on 0.8% agarose gel and later on excised through gene clean procedure. It was ligated in PTZ57 R/T vector through T/A cloning and was transformed in competent cells of *E.coli* DH5 $\alpha$ . Blue and white colonies appeared on Ampicillin, XGAL, IPTG agar plates. Plasmid was isolated by using white colonies. Presence of insert in transformants was confirmed through double restriction analysis of the isolated plasmid by using *Hind* III and *Eco* RI in the presence of 2x Y tango buffer.

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#### ISOLATION, IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF CILIATES

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Like all other ciliates, the genus *Paramecium* is considered to be of fundamental importance in aquatic ecosystems. These are being used in water quality assessment and the determinations of saprobic levels. Based on the indicator values of representative species of *Paramecium*, the water quality of a particular water body may be determined. Due to these and other cytological and molecular applications, an unambiguous identification of these unicellular

eukaryotes is very essential. Current knowledge of the variation and diversification of the species of this genus is limited. Worldwide continuous discovery of different species are proof of the fact that the list of *Paramecium* species is still incomplete. For this purpose the small subunit rRNA (SSrRNA) gene of eight different strains (FT1, FT3, FT4, FT5, FT6, FT7, FT8 and FT9) was sequenced. Phylogenetic comparison of the sequences of these strains with 23 closely related *Paramecium* species from GenBank Database was performed. Three clades representing three different *Paramecium* species were supported by phylogenetic tree. One clade showed maximum similarities (97.8-98.4%) of FT1, FT3, FT5 and FT6 with *P. multimicronucleatum* and second with maximum affinities of FT4 (99.6%) with *P. jenningsi*. However, third one does not support the close association of FT7, FT8, and FT9 with any other species. This group fall at quite distance from other species showing its separate position in the tree, indicating that these species might be the new ones.

#### **SILVER RECOVERY FROM DISCARDED X-RAY AND PHOTOGRAPHIC FILMS BY ACIDIC PROTEASE**

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Silver (Ag), one of the precious and noble metals is used in the photographic and x-ray industry extensively. The films possess gelatin silver halide emulsion layer. The present study describes the recovery of silver by acidic protease produced by *Bacillus sp. X-70*. The X-ray and photographic films were collected from Kodak photo shop and were cut into 1 x 1 cm square chips and immersed in enzyme solution for silver recovery. Different parameters were optimized for silver recovery. The maximum silver was recovered at pH 5.0, temperature 75°C after 60.0 minutes with 10.0 ml enzyme solution. 7.5583 mg/L of silver was successfully recovered at 180.0 rpm from a single x-ray film chip that is cost and time effective.

#### **IN-VITRO AND IN-VIVO ANTIBIOTIC TRIALS IN NATURALLY STRANGLES AFFECTED MULES IN PAKISTAN**

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Strangles is an infectious malady of equidae characterized by upper respiratory tract infection, dyspnea, anorexia, regional suppurative lymphadenitis and causes high morbidity and low mortality. Considering the significance and utilization of equines in our country and the substantial losses rendered by Strangles, the present study was designed to evaluate the efficacy of various antibiotics under laboratory and field conditions. In-vitro testing of Antibiotics: In-vitro antibiotic sensitivity of *S. equi* to various antibiotics (Procaine penicillin, ceftriaxone Na, cephadrine, erythromycin, ampicillin, tetracycline, chloramphenicol, sulfamethoxazole, trimethoprim + sulfadiazine and gentamycin.) was determined by Kirby-Bauer Antibiotic Sensitivity Test. In-vivo Antibiotic Trials: Based on the above in-vitro sensitivity test four top ranking antibiotics were selected. Out of 250 mules forty mules positive for strangles were randomly divided into four

groups A, B, C and D each comprising of 10 mules. The efficacy of antibiotics was checked on the basis of disappearance of clinical signs. The results of in-vitro antibiotic sensitivity test revealed, that *S. equi* was sensitive to Procaine penicillin followed by ceftiofur Na, cephadrine, erythromycin, ampicillin, tetracycline, chloramphenicol, sulfamethoxazole, trimethoprim + sulfanomides and gentamycin in mules whereas the results of in-vivo antibiotic trials revealed that mules suffering from strangles without abscess formation were sensitive to Procaine penicillin followed by ceftiofur Na, cephadrine and erythromycin whereas those mules who developed abscess were ineffective. From the results of present study it is concluded that Procaine penicillin is most effective in-vivo antibiotic followed by ceftiofur Na and cephadrine.

**CATTLE BABESIASIS: DETECTION AND PREVALENCE IN SOUTHERN BELT OF KHYBER PAKHTUNKHWA PAKISTAN**

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Babesiosis is a tick-transmitted disease of veterinary and medical importance causes significant morbidity and mortality in cattles. This study is proposed to analyze/detect the active infection of babesiosis in cattles by PCR and microscopic screening. 200 blood samples of clinically suspected cattles (100 calves and 100 cows) were collected from veterinary hospitals of district Karak and Kohat Khyber Pakhtunkhwa Pakistan. Thick and thin smear slides were examined under microscope. DNA was extracted from serum and was amplified by PCR using species specific primers. Amplified product was analyzed after electrophoresis under UV transilluminator. over all prevalence of Babesiosis with microscopy was 17.5% (16% calves and 19% cows), while by PCR was 25.5% (24% calves and 27% cows). *B. bovis* was detected in 14.5% (10% calves 17% cows) and *B. bigamina* in 11% (14% calves and 10% cows) cattles. The study reveals that Babesiosis is prevalent in the study area and proper preventive measure should be taken.

**OPTIMUM PROTEIN LEVEL OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*) CULTURED IN LOW SALINITY WATER**

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The present study was conducted to evaluate optimum dietary protein level of juvenile Nile tilapia (*Oreochromis niloticus*) reared in tanks (35 liters water capacity) for 42 days. The initial body weight of fish juvenile was  $1.0 \pm 0.01$  gram. Three types of diets containing 25, 30 and 35 % were composed of fish meal, mustered oil cake, rice bran, wheat bran, rice protein and wheat flour etc. the juvenile were hand fed with formulated crumble feed. Fish were fed three times per day at the rate of 3% body weight. The water quality parameters were temperature 28 C, dissolved oxygen 5 mg/lit, salinity 15 ppt, pH and ammonia 0.01 mg/lit. The results showed that growth of fish with

30 and 35 % protein levels were significantly higher than diet containing 25 % protein. Feed conversion ratio (FCR) remained same for all diets. The survival remained 100 % throughout the experimental period. Thus the results suggested that the optimum requirement of Nile tilapia (*Oreochromis niloticus*) lies between 30 to 35 % protein. Since dietary protein is important and costly component of feed, then less amount of protein in diet should be considered for good growth. In this study, 30% protein level is enough for the optimum growth of Nile tilapia (*Oreochromis niloticus*) juvenile under the conditions of the present study.

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### AMPLIFICATION OF GC-RICH DNA FOR HIGH THROUGHPUT FAMILY BASED GENETIC STUDIES

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Researchers face a significant problem in PCR amplification and sequencing of large DNA fragments with GC contents exceeding 80%. Many genes have to be sequenced for candidate or positional cloning efforts in order to identify genes involved in pathogenesis of disorders. Molecular characterization of inherited disorders in large pedigrees also involves mutational screening of DNA from several individuals in the family to establish co-segregation of a mutation with the genotypes. Additionally, hundreds of controls must be screened if a putative disease-causing mutation is identified in order to eliminate polymorphisms or determine allele frequencies. This can be problematic if the mutation lies in a GC-rich sequence. We have established an economical protocol for simultaneous amplification of specific products from DNA samples with 65-85% GC contents and of sizes up to 850 base pairs. It involves the use of a PCR buffer formulated for amplification of DNA with other co-solvents and yields products which can be sequenced using standard techniques.

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### ISOLATION AND CHARACTERIZATION OF CYPERMETHRIN DEGRADING BACTERIA

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Pesticides are an additional support to agricultural practices in Pakistan. The unused pesticides are the source of pollution, which is a serious threat for our environment. Detoxification of these xenobiotic through biological source is receiving attention now a days. The main object of the presenting work is to isolate and identify Cypermethrin degrading bacteria from agricultural soil having many years of using pesticides. The bacterial strain Bn3c(E3) was purified from a Cypermethrin exposed soil by enrichment technique. The bacterial strain was named as *Bacillus subtilis* by morphological, physiological and biochemical tests. The further confirmation was achieved by 16S rDNA characterization. The degradation ability of *Bacillus subtilis* determined by shake flask technique which revealed that strain Sw-1b degrades Cypermethrin up to 92.12 % within three days. The degradation potential of bacterium was studied by using Thin Layer chromatography (TLC), High performance Liquid Chromatography (HPLC) and Bioassay techniques.

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**FIELD EVALUATION OF LETHAL OVITRAPS FOR THE CONTROL OF DENGUE VECTORS IN LAHORE PAKISTAN**

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Field evaluation of lethal ovitraps (LOs) containing various concentrations of *Bacillus thuringiensis serovar israelensis* (*Bti*), buprofezin (insect growth regulator) and integration of *Bti*+buprofezin (1:1) in water and 10% hay infusion against dengue vectors was carried out during August-October 2009, at two municipalities (Samanabad and Mughalpura) of Lahore, Punjab-Pakistan. Each municipality was divided into two blocks; control versus treatment block, with 18 randomly selected houses. Each block received 36 ovitraps/LOs with and without any treatment. In total 15 weekly collections, 10,152 *Aedes aegypti* eggs were recovered 5,351 and 4,801 from treatment and control block respectively, indicating that different treatments did not affect oviposition. However, hay infusion recovered more eggs (6,548) as compared to tap water (3,604). Ovitrap positive index (OPI) was higher in hay infusion as compared with tap water. Moreover, there was no significant difference in egg density index (EDI) in tap water compared with hay infusion. Effect of lethal ovitraps treated with different concentrations of *Bti* indicated that 100 and 10 ppm of this biocide completely inhibited pupal formation, while against 1 ppm 41 and 60% pupae were formed in Samanabad and Mughalpura respectively. LOs treated with buprofezin indicated that different concentrations of buprofezin were more effective in inhibiting the pupae-adults emergence as compared to *Bti* where lethal effect was more on larval stage. Integration of *Bti*+buprofezin in hay infusion was highly effective in reducing pupal formation and inhibiting adult emergence. There was complete inhibition of adult emergence at all concentrations (100-1 ppm) in integrated lethal ovitraps, indicating the most effective tool for controlling *Aedes* populations under natural conditions.

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**CHARACTERIZATION OF HEPATITIS B VIRUS (HBV) GENOTYPES IN CHRONIC HBV PATIENTS IN KHYBER PAKHTUNKHWA, PAKISTAN**

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Hepatitis B virus (HBV) infection is the most common blood-borne liver infection globally. Approximately 2 billion people in the world are infected with HBV including 350 million people with chronic HBV infection. HBV can be classified into 10 genotypes (A-J) but in Pakistan, no such large scale data is available previously on the HBV genotypes. Therefore, the present comprehensive study was conducted to confirm different Hepatitis B Virus markers in chronic HBV patients, HBV DNA identification by PCR, Genotyped the Confirmed HBV DNA PCR patients by multiplex PCR, Correlation of different HBV markers with HBV DNA positivity, Correlation of HBV markers with different genotypes and HBV genotypes relation with blood count in the chronic HBV patients in Khyber Pakhtunkhwa, Pakistan. A total of 713 individuals with chronic HBV infection were studied. The liver function tests (LFTs) especially ALT and AST were performed using Microlab 300 (Merck USA) using ALT and AST kit (Diasys Diagnostic

System Germany). HBV DNA was isolated with GF-1 nucleic acid isolation Kit (Vivantus, USA). PCR reactions were carried out in a thermal cycler (Nyxtech USA) with 5UTaq DNA polymerase (Fermentas USA). For the evaluation of genotypes, HBV DNA-positive samples were selected, confirmed by nested PCR, and were performed by genotype specific PCR using type specific primers. The amplified DNA was subjected to electrophoresis. Of the total samples 240 (33.66%) were classified into genotype A, 210 (29.5%) were genotype D, 15 (2.1%) genotype C while 10 (1.40%) had genotype F, which means that genotype A is predominant in the Khyber Pakhtunkhwa followed by genotype D. In 75 (10.52%) samples, mix genotypes A+D were detected. One hundred and sixty three (22.86%) samples remained untypable. The distribution of genotype A (70%) was mostly found in Mardan division while genotype D (60%) was found in D.I.Khan division. In Hazara division all the four genotypes, A (10%), C (5%), D (40%) and F (10%) were found. Similarly the sex wise distribution of genotypes A, C, D and mix genotype A+D were found greater in males than in females patients. Untypable genotypes were recorded greater in males (61.35%) than in the females (38.65%). The distribution of genotypes in the age group (16-30years), A (39.58%), D (40.48%) and mix genotype A+D (40%) were found greater than other age groups. Lower distribution of the genotype A (4.17%) and genotype D (7.14%) were recorded in the age group (>60 years). Untypable genotypes were greater (47.24%) in the age group (16-30 years) and lower (10.43%) in the children with age group of 1-15 years. No other genotype, mix genotype and untypable genotype was recorded in the old age patients with age group of >60 years.

#### **TONGUE ROLLING AND TONGUE FOLDING TRAIT IN A PAKISTANI COMMUNITY**

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A study was conducted amongst unrelated volunteers students of Islamabad who were all Muslims aged between 18 years and above. Each of the subjects was asked to roll the two lateral edges of the tongue without the aid of teeth. Those who could roll the tongue were designated as tongue rollers and those who could fold the tongue were designated folders. Data was analyzed by applying chi-Square test -

#### **IGF-1 WAS SIGNIFICANTLY LOW AMONG NEWLY DIAGNOSED TYPE 2 DIABETIC PAKISTANI SUBJECTS**

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Diabetes mellitus refers to a range of conditions that are all characterized by elevation of the blood glucose level due to insulin deficiency or impaired insulin action. The aim of this study was to determine the level of insulin like growth factor-1 among type 2 diabetic mellitus (T2DM) patients. The IGF-1 is an important and preferred biomarker of diabetes. This study was done to evaluate the levels of IGF-1 (pg/ml) among T2DM patients with potential risk factors of diabetes including glycemic levels, BMI, WHR and lipid profile. Total 88 subjects were included in this study in which 29 subjects were male and 59 subjects were females with age range of 36-70 years. These subjects have higher BMI and were obese. The subjects were stratified into 3 categories on

the basis of glycemic status. Serum IGF-1 level (pg/ml). and Insulin level were assessed by ELISA technique. In present study, all the diabetic patients have significantly lower levels of IGF-1 ( $p < 0.05$ ). It was concluded from present study that there is a positive correlation between IGF-1 and insulin sensitivity ( $r = 0.58, p < 0.05$ ) while an inverse correlation between IGF-1 levels and Glycemic status ( $r = 0.746, p < 0.05$ ) was observed.

**TO STUDY THE BIOREMEDIATION POTENTIAL OF *HALOMONAS* SP. AND *ARTHROBACTER* SP.**

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Environmental stresses such as toxic metals represent serious hazards for the survival of living organisms. Chromium is one of the most common heavy metal found in the effluents from industries such as tanneries etc. and exerts serious damage to the environment by affecting soil and water. Cr(III) is an essential micronutrient for human diet and is relatively less toxic and less soluble whereas Cr(VI) is toxic, mutagenic and carcinogenic. Different heavy metal tolerance mechanisms have been discovered in various bacterial strains. In the present study, inoculation of bacterial strains in the plants has been used as a bioremediation tool which can influence the solubility and bioavailability of metals to the plant, thus modifying the efficiency of accumulation process. Cr (VI)-resistant bacterial strains were isolated from the local environment and were used to inoculate seeds of *Vigna radiata* and *Triticum aestivum* and effect of bacterial inoculations on growth improvement of plants grown under chromium stress was observed by recording various plant growth parameters. The bacterial strains used were identified through 16S rDNA sequence analysis and were found to belong to *Halomonas* and *Arthrobacter* sp. Both the *Halomonas* and *Arthrobacter* spp. enhanced percentage germination as well as seedling growth of *Vigna radiata* and *Triticum aestivum* and caused reduction in chromium content of seedlings under chromium stresses as compared to the non-inoculated treatment. Generally bacterial strains tend to decrease the adverse effects of chromium in both *Vigna radiata* and *Triticum aestivum* and can be successfully utilized for growth improvement of plants under chromium stress.

**COMPARATIVE ASSESSMENT OF BACTERIA AND PARASITES IN PIT LATRINE SAMPLES, AGRICULTURE FIELD AND VEGETABLES IN OSHKANDAS VALLEY GILGIT- BALTISTAN**

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This study was aimed to find out the comparative assessment of bacteria and parasites in pit latrines, agriculture field and vegetables. This research helped to know the potential harmful impacts posed by the human manure on human health used as an organic fertilizer to yield higher and better crops. This study was launched from October, 2011. So far eight house owners were

taken into confidence from different sectors for sampling who had established pit latrine. Fifty samples have been processed from pit latrines. The samples were brought to lab in sterilized plastic containers and processed for both bacteriological and parasitological analysis. The microbiology was done by serial dilutions culturing on MacConkey and S S agars and organisms were identified by biochemical tests. The parasitology of the samples was done by flotation techniques. The microbiological results reveal that in all the household samples *Escherichia coli* was from  $2.8 \times 10^7$  to  $3.8 \times 10^7$ . *Shigella* species were found in 17(48.57%) samples and *Salmonella* sp in all the samples (100%). The parasitic analysis shows that *Ascaris lumbricoides* were found in all the household samples and *Tricurious tricurria* in 10 (28.57%) samples.

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**PHYSICAL AND MICROBIOLOGICAL ANALYSIS OF DRINKING WATER OF  
OSHIKHANDASS VILLAGE OF GILGIT-BALTISTAN, PAKISTAN**

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Water quality is often directly connected to the morbidity and mortality losses in developing countries and often in rural settings inadequate quality of drinking water supply leads to water borne diseases namely gastroenteritis, diarrhoea, typhoid, dysentery. A study is launched to determine the physical and microbiological analysis of drinking water in Oshikhandass village. So far we have analyzed total 104 water samples microbiologically by Standard Plate Count Technique. Out 104 samples 12 samples are from out of inhibited area, 6 samples each from inlet of water reservoirs (WR-I and II), 6 samples from outlet of each WR. Ten water samples from the distribution of WR-I, 12 from WR-II, 14 from storage containers, 21 samples from water pits and 11 from water channel. The water contamination level out of inhibited area was from 0.00 to  $37 \times 10^5$  CFU/ml, followed by inlet of WR-I  $3 \times 10^5$  to  $119 \times 10^5$  CFU/ml, WR-II 0.00 to  $80 \times 10^5$  CFU/ml, outlet of WR-I  $6 \times 10^5$  to  $113 \times 10^5$  CFU/ml and WR-II 0.00 to  $83 \times 10^5$  CFU/ml respectively. In the distribution system of WR-I the faecal coliform contamination level is  $25 \times 10^5$  to  $141 \times 10^5$  CFU/ml and in WR-II this level is from 0.00 to  $133 \times 10^5$  CFU/ml, while in storage containers  $15 \times 10^5$  to  $90 \times 10^5$  CFU/ml, Water pits from 0.00 to  $213 \times 10^5$  CFU/ml and water channel from 0.00 to  $48 \times 10^5$  CFU/ml. In fact this high percentage is putting the community health at risk. For physical parameters samples were studied for turbidity, temperature, pH and  $Cl_2$  level. Physical analyses results were within the drinking water guideline and variations during this time period were insignificant whereas about 18.26% samples had turbidity values greater than guideline of 25 NTU. The temperature fluctuates with the ambient temperature. The sensitivity of isolated bacteria was carried out against seven antibiotics by Kirby-Bauer disc diffusion method in which Cefixime shows 77.77%, Ciprofloxacin 91.6%, Lincomycin 48.61%, Ampicillin 8.33%, Amoxicillin 16.66%, Cephadrine 66.66%, and Tetracycline 29.16% against the isolated *E.coli*. While the *Salmonella* spp. shows 85.00% sensitivity against Cefixime followed by Ciprofloxacin 95.00%, Lincomycin 50.00%, Ampicillin 15.00%, Amoxicillin 25.00%, Cephadrine 75.00% and Tetracycline 20.00%. Study indicated high incidences of thermotolerant *E.coli* and *Salmonella* spp. in drinking water due to faecal contamination or open defecation in village and therefore makes it unsafe for drinking purposes.

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**A COMPARATIVE STUDY ON PHYSIO-CHEMICAL AND BIOLOGICAL ANALYSIS OF TAP WATER SUPPLIED BY WASEP AND GOVERNMENT IN TEHSIL PUNIAL**

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A comparative study was conducted to evaluate the physical, chemical and biological quality of drinking water supplied by WASEP and PWD at Grunjur and Gahkuch Paen respectively, villages of District Ghizer from July to December 2011. Delagua water testing kit was used to determine the fecal contamination. Chemical contaminants were tested by UV Spectrophotometer and Wegtech Metalyser and the physical parameters were tested by Multi-probe unit, PH comparator and Strips, turbidity tubes and hardness strips. During this study a total 180 samples were collected from the study areas and samples were analyzed at WASEP Water Quality Lab. The observed results from both villages were compared with each other and also with WHO standards and PSI standards. This study reveals that there is no contamination was observed in water supply of WASEP on comparison with supply of water of PWD. Water samples of Gahkuch were found to be highly contaminated with thermo tolerant coli forms during the months of July (66-105cfu/100ml water), August (38-65cfu/100ml water), October (04-12 cfu/100ml water), November (02-10cfu/100ml water) and December no fecal coliforms were found. Water samples of Grunjur were found to be suitable for human consumption. In September (1-3cfu/100ml water) and no coli form was observed in other sampling months. In Gahkuch Paen the chemicals contaminants like heavy metals (Pb, Cu, Hg, Cd, As), Nitrate, Nitrite and Fluoride were not detected, Salinity (0.1%), Chromium (0.005mg/l), Iron (0.05) and Physical contaminants include Conductivity (200 $\mu$ S/cm), TDS (106.5mg/l), PH (7.6), Hardness (<80mg/l) and Turbidity (<5 NTU). In Grunjur the chemicals contaminants like heavy metals (Pb, Cu, Hg, Cd, As), Nitrate and Nitrite were also not detected, Fluoride (0.21mg/l), Salinity (0.1%), Salinity, Chromium (0.02 $\mu$ g/ml), Iron (0.05) and Physical contaminants include Conductivity (178.9 $\mu$ S/cm), TDS (95.1mg/l), PH (7.0), Hardness (<80mg/l) and Turbidity (<5 NTU).

**PREVALENCE OF ANAEMIA IN PREGNANT WOMEN AND GENERAL POPULATION OF HYDERABAD DISTRICT AND ITS ADJOINING AREAS. (SINDH – PAKISTAN)**

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The study on six hundred (600) pregnant women attending at Liatat University of Medical and Health Sciences was carried out to evaluate the percentage of anaemia cases present in the District Hyderabad and its surrounding adjoining areas among pregnant women. Haemoglobin (Hb) levels less than 10.0g /dl was observe in 59% of the cases. And 41% were with maximum of 11.5 g/dl. The 90% cases reported belonging to middle or poor class were classified as anaemic and were at the risk of abortion. 11 cases of still birth were also reported as they had less than 5g /dl. During second phase of study, 1460 healthy individuals, including 380 male, 660 females and 420 children's, aged from 12 to 50 years, belonging to different socio-economic and educational groups were studied. The results showed that percentage of haemoglobin among non-pregnant woman was below 10 g /dl, whereas males had 13.8 g/dl and children had average of 9.5 g/dl. The main cause of anaemia's seems socioeconomic status of general population and improper knowledge of maintenance of pregnancy.

**ISOLATION AND CHARACTERIZATION OF ARSENIC RESISTANT CILIATES FROM THE INDUSTRIAL WASTES**

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The frequent presence of protozoans in the wastewater elicits their importance in processing of wastewater and suggests their exploitation in bioremediation. To isolate arsenic resistant ciliates, industrial wastewater samples were collected from Muridke. Samples were inoculated in Bold Basal medium. *Tetrahymena* was isolated from the culture using drop method. The ciliates acclimatized under laboratory conditions showed an optimum growth at 27±2°C and pH 7. The ciliates showed more sensitivity to pH than temperature. The *Tetrahymena* tolerated arsenic upto 40ug/ml. However its growth was slowed down in arsenic stressed conditions. The *Tetrahymena* was characterized through microscopy and molecular analysis. The 18S rDNA gene sequencing of the isolate showed its 98% homology with *Tetrahymena thermophila*. SDS-PAGE was carried out to check the expression of metallothionein under As stressed conditions. Appearance of low molecular weight (14kDa) novel band of protein under stressed conditions explains the inducible nature of metallothionein gene.

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**OXIDATIVE STRESS AND ANTIOXIDANTS THERAPY IN FIBROMYALGIA PATIENTS**

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Fibromyalgia syndrome (FMS) is characterized by musculoskeletal pain of unknown etiology and is often accompanied by many psychological symptoms. This study was designed to assess oxidant/antioxidant status in fibromyalgia patients, with the aim of evaluating the importance of antioxidants (vitamin E, vitamin C and *Nigella sativa* seeds) therapy in management of this disease. Fifty female patients having mean age of 37.87±1.68 suffering from FM were enrolled in the study. Role of oxidative stress was determined by measuring antioxidant enzymes including Superoxide dismutase (SOD), Glutathione peroxidase (GPx), antioxidant capacity by ABTS (2,2'- azinobis-(3-ethylbenzo-thiazoline-6-sulphonic diammonium salt) and FRAP (ferric reducing antioxidant capacity) assay in plasma and catalase in erythrocytes. The FM patients were also supplemented with antioxidants (Vitamin C, Vitamin E and *Nigella sativa* seeds) for two months to assess the impact on activity of FM with the help of visual analogue scale (VAS) (0 being no pain and 100 being severe pain). It was found that FM patients have low activity of SOD, GPx and low antioxidant capacity (AOC) than healthy controls. It was further observed that after two months supplementation with antioxidants (vitamin C, vitamin E and *Nigella sativa* seeds) the level of SOD, GPx and AOC increased and their mean VAS 90.30±1.52 at baseline decreased to mean VAS of 77.80±1.65 after supplementation with antioxidants. The antioxidant supplementation for two months resulted in significant improvement in FM patients and it may help in minimizing the effects of the oxidative stress.

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**LINKAGE STUDY OF PRIMARY MICROCEPHALY IN PAKISTANI KINDRED**

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Microcephaly is heterogeneous, autosomal recessive trait with reduced head circumference of at least 4 SD below age and sex means due to reduction in neuron production. The brain of microcephalic patient is architecturally normal but severe to mild mental retardation. It is rare disease affecting 2-2.5% of total population specifically in Asia and Arab where the incidence of cousin marriages is relatively high. From seven known currently mapped loci *ASPM* is found to be the frequent causative agent. In the current investigations exclusion mapping of a microcephalic family was done. DNA from all blood samples was extracted using standard procedure and after gene specific PCR amplifications, 8% non-denaturing PAGE was done. Linkage was observed at MCPH5 locus where *ASPM* is a candidate gene on chromosome 1q31. The results of DNA sequencing showed G to A transition and Leucine (CTG) to Leucine (CTA) was noted. There are six triplet codons which differ by single nucleotide encoding for Leucine. Hence, no overall change in the effect of protein expression was observed due to the degeneracy of codons. Therefore, the sequencing of the entire *ASPM* gene with intervening sequences was suggested in order to find the actual cause of microcephaly.

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**SCIENTIFIC EVIDENCES THAT PIG MEAT (PORK) IS PROHIBITED FOR HUMAN HEALTH**MUHAMMAD FIAZ QAMAR<sup>1</sup> AND IFRAH RAZA<sup>2</sup><sup>1</sup>*Department of Zoology, GC University, Lahore – Pakistan*<sup>2</sup>*University College of Veterinary & Animal Sciences, The Islamia University of Bahawalpur*  
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Among all animal meats pork is the filthiest diet to consume by human beings. Pig is the cradle of harmful germs. Scientific evidences prove that pig meat is least healthy having different harmful agents like Cholesterol and Fatty Acids, Bacteria and Toxins and a number of parasites. The pig meat is high in fat and cholesterol that causes the cardiovascular diseases, obesity, the incidence of large intestine cancer. Bacteria and Toxins associated with pigs spread many diseases like *salmonellosis*, which leads to the acute gastroenteritis and diarrhea. Many other diseases like, *Tuberculosis, Yersiniosis, Listeriosis, Leptospirosis, Brucellosis, Small Pox, Influenza, Anthrax, Balantidial dysentery, Foot rot, Cholera and Erysipeloid* are attributed to pork consumption. Parasitic Diseases *Ascaris, Ancylostomiasis, Toxoplasmosis, Trichinellosis, Cysticercosis* showing signs of mental disorders, pneumonia, bleeding of the lungs (haemoptysis), which may lead to death or madness. The patient may become blind and deaf. Nitrates used in pork and pork products as additives are converted into nitrosamines which cause hepatic cell tumors. Flesh of the pork is hard to digest and may lead to chronic digestive disturbances. Pimples, boils, cysts are common in pork eaters. The pig excretory system secretes 2 percent of its uric acid that is injurious for the human health. Pork consumption seriously affects human health and adversely injurious one's moral values. A person gets pig like characteristics by eating pork, Indecency, obscenity and vanished honour of women.

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**A STUDY ON THE EFFECTS OF ALKALINE pH ON THE EPIDERMAL MUCUS OF  
*LABEO ROHITA* (RAHU)**

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*Labeo rohita* (Rahu) is an indigenous freshwater carp and an economically important species found abundantly throughout the freshwater ponds, lakes and the riverine tracts of Pakistan. The commercial production of Rahu is through intensive and semi-intensive aquaculture farming. The soil pH of Pakistan is generally alkaline in nature and may influence the growth of this species. The following study was carried out to investigate the effects of alkaline pH on the epidermal mucus of *Labeo rohita*. Epidermal mucus was obtained from fish divided into two groups; the control group yielded 150±11.78ml mucus whereas the experimental group subjected to alkaline pH produced 480±24.25ml mucus. Both groups of mucus exhibited differences in their odors, viscosity and color. A High performance liquid chromatography (HPLC) analysis revealed a rich array of proteins in the mucus obtained from the control group however a marked reduction of protein concentration was observed in the experimental group. Similarly, lectin activity (4 units) exhibited by the control group was found to be altogether absent in the mucus extracted through alkaline stress. GCMS of the mucus samples detected the presence of acetic acid, cyclohexane, ethylbenzene, heptane, hexane, o-xylene, p-xylene and toluene. However, alkaline pH was found to have no adverse effects on the fatty acid composition of the mucus. Further study is required to determine the impact of alkaline pH on the immunological parameters of *Labeo rohita*.

**PROSPECTIVE SURVEILLANCE OF FEBRILE ILLNESS FOR DENGUE-EPIDEMIC IN  
LAHORE-2011**

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I-A multi-centred study was designed to collect dengue epidemiologic data from government and registered private hospitals/clinics. II-Maintained archive of frozen specimens at The Institute of Molecular Biology and Biotechnology, The University of Lahore, forms the basis for public health measures to define disease epidemiology and future dengue epidemic control program. September, 2011-December, 2011. 101 confirmed blood samples (By ELIZA IgM antibodies test) of dengue patients referred to pathology lab Jinnah Hospital Lahore were subjected to assess dengue epidemiology by evaluating the biochemical, physiological and alterations of circulating antioxidants status. All the patients presented with fever and there was no significant difference in the duration of fever. The duration of hospitalization in DF, DHF and DSS were significant (P=0.016). The study showed non-significant difference among low and high income residents of Lahore city but subjects with age of 20-30 years specially males (81%) were at high risk of dengue infection (DF=91%, DHF=6% and DSS=3%). Significant increase in serum ALT (P=0.025), AST (P=0.015), ALP (P=0.001), TBARS (P=0.015) along with decrease in TP

( $P=0.032$ ), albumin ( $P=0.021$ ), GSH ( $P=0.034$ ), SOD ( $P=0.015$ ) CAT ( $P=0.045$ ) and sialic acid contents ( $P=0.023$ ) were observed. A positive correlation existed between bound sialic acid levels, liver enzymes and circulating antioxidants ( $r=0.656$ ,  $P=0.032$ ). Considering dengue fever as a model for the metabolic response to an acute, self-limiting tropical viral infection, the present findings suggest slight turbulence of the antioxidant system that may be a response to or a consequence of the viral inflammatory process.

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**THE ANTIDIABETIC AND ANTIHYPERLIPIDEMIC EFFECT OF *AEGLE MARMELLOS* AND ANTIOXIDATIVE EFFECT OF SILYMARIN ON ALLOXAN INDUCED DIABETES IN MICE**

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This study was designed to evaluate the antidiabetic, antihyperlipidemic and antioxidative effect of *Aegle marmelos* and silymarin on alloxan induced diabetes in mice. Total 30 mice were used divided into five groups as A, B, C, D and E. Group A was treated with normal chick's diet. Alloxan were given at 150mg/kg body weight through abdominal region of the remaining Groups (B, C, D and E). Group C treated with silymarin (200 mg/kg of body weight), Group D treated with standardized extract of *Aegle marmelos* @ 180.0 mg/kg of body weight and Group E received silymarin and standardized extract of *Aegle marmelos* respectively @ 200 and 180 mg/kg of body weight. The result of the present study showed that *Aegle marmelos* and silymarin reduced the oxidative stress by increasing the reduced levels of GSH, SOD and CAT and decreases the levels of MDA. It was summarized from the present study that *Aegle marmelos* and silymarin have a synergistic effect to minimize the alloxan induced toxicity (diabetes) in mice.

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**EFFECT OF *ALOE VERA* GEL ON LIPID PROFILE IN ALLOXAN INDUCED DIABETIC MICE**

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The ethanolic extract of *Aloe Vera* gel was studied for their hypolipidemic and hypoglycaemic activity. Twenty four mice were taken divided into four groups. 1<sup>st</sup> group act as control and remaining groups were induced diabetes by administrating alloxan @ 150 mg/kg body weight. 2<sup>nd</sup> group served as diabetic control and 3<sup>rd</sup> group treated with glibenclamide (600 $\mu$ g/kg body weight). 4<sup>th</sup> group were received ethanolic extract of *Aloe Vera* gel @ 300 mg/kg body weight for 21 days. The antihyperglycaemic (93.40 mg/dL,  $P=0.037$ ) and hypolipidemic (101.37 mg/dL,  $P=0.04$ ) activity of *Aloe Vera* gel and was compared with group treated with the standard oral hypoglycaemic agent. Treatment with ethanolic extract of *Aloe Vera* gel caused the significant change when compared to untreated animal with respect to blood glucose level and lipid profile. The present study clearly indicate a significant reduction in blood glucose level, plasma level, LDL, cholesterol and increased plasma level of HDL in alloxan induced diabetes in mice.

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**HEPATOPROTECTIVE RESPONSE OF ROOTS, LEAVES AND SEEDS EXTRACTS OF  
*SILYBUM MARIANUM* ON CCl<sub>4</sub> INDUCED LIVER DAMAGE IN RATS**

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The present work was designed to investigate the hepatoprotective response of *Silybum marianum* (Roots, Leaves and Seeds Extracts @ 50 mg/kg Body Wt.) against the carbon tetrachloride induced liver injury in rats. Total 45 albino rats were used divided into five groups (A, B, C, D and E). Group A served as negative control and liver injury was induced by CCl<sub>4</sub> @ 1ml/kg Body Wt. in all the remaining groups. Group B served as positive control and groups C, D and E was supplemented with roots, leaves and seeds extracts along with carbon tetrachloride. The result of the present study showed that roots, leaves and seeds extracts of *Silybum marianum* reduced carbon tetrachloride induced toxicity by increasing reduced levels of GSH, SOD, CAT and TP. It was also observed that these extracts also decrease the levels of MDA, AST, ALP, ALT, Glucose and TG, which was raised as a result of carbon tetrachloride. It was summarized from the present study that seed extract of *Silybum marianum* surpassed to alleviate liver injury induced by CCl<sub>4</sub> followed by leaves and root extracts respectively.

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**THE EFFECTS OF SALINE, hCG AND GnRH ON THE PROGESTERONE AND  
PROTEIN CONCENTRATIONS IN THE OVARIAN FOLLICLES OF GOAT**

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The aims of this study were to determine the effects of saline, hCG and GnRH on the progesterone and protein concentrations of the caprine follicles. For this study reproductive tracts were obtained from the slaughter house and immediately brought to the laboratory (Institute of Pure and Applied Biology, B. Z. University Multan). Ovaries were isolated and follicles were dissected and cultured with the addition of saline, hCG and GnRH for two and half hours at 37°C. Progesterone and proteins were extracted and concentrations determined by ELISA. The concentrations of progesterone were 0.766±0.07, 0.466±0.05 and 0.586±0.05ng/ml in saline, GnRH and hCG group respectively. Significant reduction (P < 0.05) in the concentrations of progesterone was found as a result of GnRH and hCG treatment. Moreover, the decrease in progesterone level was large in GnRH group (P < 0.05) as compared with hCG group. The concentrations of proteins were 0.326 ± 0.01, 0.356±0.007 and 0.398±0.008mg/g in saline, GnRH and hCG groups respectively. Significant increase (P < 0.001) in the concentrations of protein was found as a result of GnRH and hCG treatment. Moreover, the increase in protein level was large (P < 0.05) in hCG group as compared with GnRH group. It is concluded that both hCG and GnRH effect the progesterone and protein concentrations in the follicles. In case of progesterone GnRH suppresses its level more effectively than the hCG, while hCG increases the protein concentrations in the follicle more than the GnRH.

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**ISOLATION AND CHARACTERIZATION OF YEAST STRAINS FROM FLOWERS AND ITS EVALUATION FOR ALCOHOL PRODUCTION**

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Alternative substrates to produce useful chemicals such as biofuel have been attractive now a days. Present study aims to search bioethanol producing yeast strains. A total of 65 yeasts were isolated from a variety of flowers, out of these, 12 isolates were able to assimilate glucose by anaerobic liberation of CO<sub>2</sub>. Only 5 yeast strains were able to ferment glucose and produce ethanol up to 2.05% by using 0.08% Mg<sup>2+</sup> ions in the medium. Based on molecular characteristics the ethanol-producing strains were identified as member(s) of the genera *Metschnikowia* and *Meyerozyma*. The present work deals with the bioconversion of rice into ethanol by using the methods of acid hydrolysis and fermentation by selected 5 yeast strains. In *Metschnikowia cibodasensis* Y34, consistent ethanol production was observed for day 2-4 (1.80%) and day 5-6 (1.65%) and 2.2 % by *Meyerozyma caribica* Y42 at day 4-6 then gradual decrease were started. When two strains were incubated with 1% NaCl and at different pH then *Metschnikowia cibodasensis* Y34 and *Meyerozyma caribica* Y42 grew well and highest ethanol production was observed at pH 3 i.e., 1.75% at day 3, 5 and 2.05% at day 1, 3 respectively. Growth and ethanol production at pH 4 and 5 was near to pH 3 while slight increase was observed in *Metschnikowia cibodasensis* Y34 at pH 4 up to day 3.

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**THE ASSOCIATION OF COMMON GENETIC DIGRESSION IN FTO GENE WITH T2DM IN PAKISTANI POPULATION**MUHAMMAD ISMAIL<sup>1\*</sup>, QAISAR MANSOOR<sup>1</sup>, AMARA JAVAID<sup>1</sup> AND NIGHAT BILAL<sup>2</sup><sup>1</sup>*Institute of Biomedical and Genetic Engineering (IBGE), Islamabad, Pakistan.*<sup>2</sup>*Department of medicine, Pakistan Institute of Medical Sciences, Islamabad, Pakistan.*Email: [m.ismail02@gmail.com](mailto:m.ismail02@gmail.com)

Type 2 diabetes mellitus (T2DM) has become a challenge for the developed and developing societies of the world. Though environmental imbalances trigger the metabolic disturbances; the focus on genetic players has shown discrete associations with the metabolic disorders like diabetes and obesity. Genome-wide association studies for T2DM recognized fat mass and obesity associated (FTO) gene as a locus granting augmented risk for obesity in populations with European ancestry. However the association of FTO gene with T2DM and related metabolic disorders has not been established for Pakistani population. The aim of our study is to find the association of single nucleotide polymorphism (SNP) rs11076023 A/T of FTO gene with T2DM in Pakistani population. Blood samples of 1000 T2DM patients were collected besides controls from primary care hospitals in Islamabad. DNA was extracted using organic method. ARMS PCR to screen rs11076023 A/T SNP in FTO gene; using the SNP specific primers has been done for 203 T2DM patients. The results were statistically analyzed by SPSS 14. Screening for more samples is in progress. In our study population the AT genotype has been found significantly high (55.7%) than TT (32%) and AA (12.3%) genotypes. In conclusion, FTO polymorphism at rs11076023 A/T is associated with

T2DM in Pakistani population which may be of important clinical and public health implications in Pakistani population.

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### CHEMICAL AND FATTY ACID PROFILES OF FRESHWATER FISH INHABITING RIVER INDUS

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Chemical compositions and fatty acid profiles of three commonly consumed and commercially important freshwater fish species *Cyprinus carpio*, *Labeo rohita* and *Oreochromis mossambicus* from two locations of the River Indus in Mianwali District, Pakistan were investigated. Species specific significant differences were observed in moisture, fat, crude protein and ash contents ( $P < 0.01$ ). There was no significant difference between locations ( $P > 0.05$ ) for the nutrient compositions of fish except fat contents which were greater at upstream than the downstream ( $P < 0.01$ ) of River Indus. Amongst the fatty acids that were analysed by Gas chromatography, palmitic acid (C16:0) was found to be the most abundant fatty acid ranging from 32.4 to 45.9%. Essential polyunsaturated fatty acids such as docosahexaenoic (DHA) (C22:6), eicosapentaenoic (EPA) (C20:5) and arachidonic acid (C20:4) were the other important fatty acids found in these fish species. The fatty acid profiles of these fish species did not show any significant difference between upstream and downstream of the River Indus. Generally, *Labeo rohita* was found to be the richest in protein and poly unsaturated fatty acid compositions. It appears that these fish species contained appreciable levels of Omega-6 polyunsaturated fatty acids and are therefore suitable for their use as an unsaturated low-fat source in human diets. As there was no authentic information about the nutritional value of freshwater fish from this study area, this new information could be very valuable to determine the quality of local fish as a significant contributor to the healthy diet of the rural communities of this area and beyond.

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### EPIDEMIOLOGY OF PARALYSIS IN A HOSPITAL POPULATION

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An epidemiological study was carried out in a hospital population of Faisalabad city including Allied Hospital, District Head Quarter Hospital, Sahil Hospital, Mian Muhammad Trust Hospital and Medicare Hospital. A standard questionnaire was used to collect information including different parameters like, Age, sex, birth order, exact diagnosis, genetic relationships, education, socioeconomic status, smoking, physical activities, allied diseases, locality and family history of patients. Out of 300 paralytic patients scored in this study, 52.33% were males and 47.66% were females. Eight types of paralysis were encountered during this study in which hemiplegia was the most common type (70%) among paralytic patients followed by facial paralysis (8.67%) and monoplegia (7.33%). The highest number of paralytic patients (24%) was recorded in

50-59 and 60-69 years age groups. Most paralytic patients (94.66%) were married. The paralysis was more common in patients having nil education (60%). Education of patients had highly significant effect ( $P < 0.001$ ) on affliction of disease. The highest percentage of paralytic patients was observed in retired /jobless category (66.66%). Lowest percentage of paralytic patients was observed in professional and managerial category (3.33%). The socioeconomic status of paralytic patients had highly significant effect on the affliction of disease ( $P < 0.001$ ). Patients with lower socioeconomic status (66.66%) suffered more from paralytic disease than with higher socioeconomic status. Paralysis was more common (56%) in subjects who do not take exercise than those who take exercise (44%). There was a highly significant ( $P < 0.001$ ) difference among smokers, non-smokers and ex-smokers in paralytic patients. The first cousin parental relationship (38.66%) was more common among marriages than non related marriages. Consanguinity has a significant effect on the development of the disease ( $P < 0.05$ ) in paralytic patients. Majority of the patients (64.33%) were living in urban areas and 35.66% were living in rural areas. With regard to allied diseases, 58.33% of paralytic patients were hypertensive and 41.66% were non-hypertensive while 41.33% were diabetic and 58.66% were non-diabetic.

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#### EFFECT OF HEAVY METALS ON FRESHWATER FISH INHABITING THE RIVER CHENAB

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The effect of heavy metals as industrial pollutants (Pb, Zn, Cd, Ni, and Cr) on the total oxidant status (TOS), total antioxidant status (TAS) and histology of liver of *Labeo rohita* domiciled in the River Chenab was investigated. This river is affected by the effluents from leather, plastic, textile, printing, soap and sugar industries that are discharged into the main Paharang drain that falls into the River Chenab. This completely randomised study involved three polluted locations (Thatha Muhammad Shah, Pattan Draaj and Head Trimu) along the stretch of River Chenab in Jhang and a relatively less polluted site of Kot Khera was selected as the control. Water and fish samples of similar size (about 1000g) were collected as replicates from four selected locations. The fish were dissected to collect livers for the estimation of their heavy metals, TOS, TAS and histology. Heavy metals in water and fish livers were determined by Atomic Absorption Spectrophotometer, TOS by using a novel automated measurement method, TAS by the automated colorimetric method and histological studies by using haematoxylin and eosin staining technique. The data were statistically analysed by using ANOVA in Minitab software version 16 to see the effect of sampling sites on the metal profile, TOS and TAS of fish livers. Results showed significant differences ( $P < 0.01$ ) in metal profile of water that was sampled from four selected locations. The concentration of Pb, Cd, Ni and Zn were higher at the three sampling locations than the WHO maximum permissible levels of heavy metals for aquatic life. Conversely, the most metal levels except Ni at the control site were within the safe limits. There were significant differences in heavy metal profile and total oxidants of fish liver sampled from selected locations ( $P < 0.01$ ). The concentration of heavy metals in fish livers at sampling sites exceeded the WHO standards of food fish and control fish except Zinc. Consequently the fish of this study area may cause metal related disorders in these fish and their consumers. Moreover the mean metal concentration in fish livers was several times higher than the corresponding samples of water. Non significant differences

( $P > 0.05$ ) were observed for total antioxidants of fish kidneys being sampled from the selected locations. The antioxidants were high in control as compared to the exposed fishes to heavy metals. Clear histological alternations were observed in the liver of fish collected from the polluted sites compared with control. These include necrosis, thrombus in blood vessels, atrophy, pyknotic nuclei, hemorrhage, coagulative heamorrhage and degeneration in hepatocytes. It can be concluded that proper measures should be taken to avoid the contamination of the River Chenab by Main Paharang Drain which are responsible for the increased levels of metals and total oxidants which may be damaging to the fish health and other aquatic life.

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**DIETARY NAFLD (NON ALCOHOLIC FATTY LIVER DISEASE) INDUCED  
THROMBOCYTOPENIA AND EFFECTS OF *NIGELLA SATIVA* AND *PLANTAGO  
OVATA***

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High fat diets are considered to be the main cause of non alcoholic fatty liver disease (NAFLD). Among a few diagnostic parameters of NAFLD Platelet (PLT) count is given the most preliminary importance. Thrombocytopenia (decrease in PLT count) is reported in NAFLD. From the past few decades herbal products are being recommended by the scientist to reduce the fatty contents of the body. In the present study *Rattus norvegicus* weighing about 200g were divided into two groups based on gender. Each group was further divided into four groups designated as Con, I, II, III with a prefix F for females and M for males. For 16 weeks control groups were fed on normal rat chow, I, II and III were fed on high fat diet X (33% carbohydrates, 33% fats, 34% rat chow). Groups II and III were additionally provided with 5% *Nigella sativa* and 5% *Plantago ovata* / kg of X respectively. The results revealed by analysing the PLT count showed significant differences in all the three experimental groups of both males and females. In FI, FII and FIII significantly lower PLT ( $P < 0.001$ ) were observed as compared to control. In case of intergroup comparison there was a significant decrease of platelets in MIII ( $P < 0.01$ ) in comparison with MII and FII ( $P < 0.05$ ) in comparison with FIII. It can be said conclusively that on the bases of genders, fat plummeting agents have varying effects on the thrombocytopenia induced by the diet induced NAFLD.

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**CYTOTOXIC EFFECT OF CIGARETTE SMOKE CONDENSATE ON MICE AND RAT  
MESENCHYMAL STEM CELLS AND HELA CELLS**

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Cigarette smoking results in deaths of millions of people annually. The constituents of cigarette smoke produce oxidants in the cells that result in inflammation, carcinogenesis and apoptosis. We studied the effect of cigarette smoke condensate (CSC) on HeLa cells and mouse and rat mesenchymal stem cells (mMSC, rMSC). CSC was collected on filter papers, dissolved in DMSO and added 10-100  $\mu\text{g/ml}$  to HeLa cells, mMSCs and rMSCs in 96 well plate. Cytotoxic effect of CSC was measured after 48h by neutral red uptake assay. Images of cells were taken after growing in neutral red medium. There was no significant change in cell morphology after exposing

the cells to CSC for 48h. Beside that the growths of HeLa cells, mMSCs and rMSCs were reduced by 14, 38 and 36%, respectively. The present study demonstrates the growth inhibitory effect of CSC on stem cells compared to mature HeLa cells. The study also proves that CSC does not promote proliferation.

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### EPIDEMIOLOGY OF CATARACT IN A HOSPITAL POPULATION

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An epidemiological study of 500 cataract patients was carried out in hospital population of District Faisalabad, including Rasheeda Ali Trust Eye Hospital, Allied Hospital and District Head Quarter Hospital Faisalabad. A standard questionnaire was used to collect information including different parameters like surname, sex, age at diagnosis, genetic relationships, education, economic status, smoking status, locality, birth order, allied diseases and genetic history of patients. Out of total 500 patients 203 were males and 297 were females. The common types of cataract observed in this study were bilateral cataract 44.4%, left cataract 27%, right cataract 26.8% and congenital cataract 2%. Female cataract patients (59.8%) were significantly more affected with cataract than male cataract patients (40.4%)  $P < 0.01$ . The highest numbers of cataract patients were observed in age group 60-69 years (26.8%) than other age groups. In age groups of 60-69 years (26.8%), 50-59 years (20.4%) and 40-49 years (17.6%) cataract were more prevalent in females than males ( $P < 0.01$ ). Highest percentage of cataract was found in birth orders 1 (32.2%), 2 (20%), and 3 (19.6%). The highest percentage of cataract patients was seen in unskilled people (47.4%). Uneducated patients were significantly more affected with cataract than educated people ( $P < 0.01$ ). Rural population was significantly more affected with cataract than urban ( $P < 0.01$ ). The outcome of first cousin marriages showed high percentage of cataract affliction than in unrelated couples. Regarding allied diseases the maximum number of cataract patients was affected by hypertension (16%) and least number of cataract patients was affected by allergy (4%). The highest percentage of cataract was seen in Rajputs (24.8%) and the lowest in Khan (2%).

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### INHIBITORY EFFECTS OF SOME COMMONLY USED SPICES ON RESISTANT ENTERIC BACTERIAL PATHOGENS

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Antibacterial activity of different spices including black pepper, cloves, red pepper and black cumin was evaluated and compared with commercially available antibiotics penicillin G, rifampicin, polymixin B, piperacillin, tetracycline, amoxicillin and norfloxacin. Aqueous, ethyl acetate and chloroform extracts of the spices were used. Growth inhibition effects of the extracts and their 5x concentrates were evaluated by filter paper disc diffusion method against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Klebsiella sp.* Aqueous extract of black pepper and its 5x concentrate showed diameter of growth inhibition zones (GIZ) around 12 mm and 16.33 mm against *Escherichia coli*, respectively. Largest GIZ appeared (17.33 mm) for

ethyl acetate of black pepper against *Staphylococcus aureus*. The 5x concentrate of black pepper extract in chloroform yielded GIZ of 15.33 mm against *Staphylococcus aureus*. Maximum results (14.83 mm) were obtained with 5x aqueous extract of cloves against *Escherichia coli*, while ethyl acetate extract of cloves yielded very vivid antifungal inhibition zone of 12.67 mm against *Escherichia coli* and its 5x concentrate yielded 16.83 mm against *Salmonella typhi*. Best results expressed for the chloroform extract of cloves against *Staphylococcus aureus* with GIZ of 16.33 mm. 5x concentrated aqueous extract of black cumin gave 16 mm GIZ against *Staphylococcus aureus*. Largest growth inhibition zone appeared (15.17 mm) for 5x ethyl acetate of black cumin against *Salmonella typhi*. Chloroform extract of black cumin and its 5x concentrate showed diameter of growth inhibition zones (GIZ) around 13.83 mm and 14.33 mm against *Escherichia coli*, respectively. Largest growth inhibition zone (12.67 mm) appeared for aqueous extract of black cumin. while its 5x concentrate showed (GIZ) around 15.67 mm against *Staphylococcus aureus*. Ethyl acetate extract of red pepper gave GIZ of 15.17 mm against *Escherichia coli* while chloroform extract yielded a GIZ of 12.17 mm against both *Staphylococcus aureus* and *Salmonella typhi*. Minimum inhibitory concentrations (MIC) of aqueous extracts of the spices were also worked out.

#### EPIDEMIOLOGY OF HEPATITIS C VIRUS IN RISK GROUPS OF KHYBER PAKHTUNKHWA PAKISTAN

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Hepatitis C Virus (HCV) is a major cause of chronic liver disease, which infects approximately 170 million worldwide and in Pakistan alone, it is exceeding 10 million. There is a significant variation in the prevalence of HCV genotypes in different geographic regions. The aim of this study was to determine the prevalence of antibodies to hepatitis C virus (anti-HCV), prevalence of HCV viremia (HCV-RNA) and the prevalence of HCV genotypes in risk groups of Khyber Pakhtunkhwa (KPK), Pakistan. A total of 1514 individuals [824 health care workers (HCWs), 384 hemodialysis HD), 198 injection drug users (IDUs), 68 Thalassemia, 40 hemophiliacs) were subjected to anti-HCV test with a screening method (ICT). In case of positive results, antibodies were confirmed by ELISA, and all these subjects were tested for HCV-RNA by polymerase chain reaction. HCV RNA positive individuals were analysed for HCV genotypes by multiplex PCR. Of the total, 238 (15.72%) were positive for anti-HCV (34 HCWs, 112 HD, 63 IDUs, 17 thalassemia and 12 haemophiliacs). The results of HCV RNA test were positive in 193 (12.75%) subjects. HCV genotypes distribution was: 1a in 22 (11%), 1b in 5 (2.6%), 2a in 30 (16%), 2b in 2 (1%), 3a in 72 (37%), 3b in 28 (15%), genotype 4 in 9 (4.7%) and 25 (4.7%) were untypable while genotype 5 and 6 were not detected in any of the sample. The prevalence of anti-HCV and HCV-RNA in risk groups of KPK was found to be 15.72% and 12.75%, respectively and HCV genotype 3a was the most prevalent.

**INSULIN ADMINISTRATION INCREASED SARCOMERE LENGTH AND NUCLEOLI NUMBER PER MYOBLAST IN FREELY GRAFTED EXTENSOR DIGITORUM LONGUS MUSCLES IN RATS**

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Extensor digitorum longus (EDL) muscle was autotransplanted in saline injected control and insulin administered (1U/0.1 ml/100gb.w./day) rats starting from the day after transplantation. The grafts were recovered at weekly intervals within one month period postgrafting. The grafts were then processed for histological analysis. Haemotoxylin and Eosin stained longitudinal sections (8µm) were observed in a calibrated ocular micrometer fitted microscope with the help of 100 oil immersion objective. Number of nucleoli / myonuclei within a regenerating/regenerated muscle fibre were counted upto 100 units of the micrometer. The data from replicates of a given group were averaged. Sarcomeres became conspicuous at 2-week stage and their lengths were measured in µm. At 2 week stage the hormone supplemented grafts had attained 206% higher values of the length of sarcomeres of the muscle fibres as compared to the respective control values of 0.16µm. At the end of experimental period the hormone supplied regenerated muscle fibres had 82% higher sarcomere lengths as compared to the control value of 0.39 µm. Regarding the enumeration of nucleoli / myoplast it was found that throughout the experimental period the insulin treated grafts had higher counts. The differences were 149%, 174%, 48.8 folds and 41.67 folds higher as compared to the data obtained for the control for one through four week old control muscle regenerates, respectively. These observations confirm the anabolic and growth promoting role of the exogenously supplied insulin in freely grafted regenerating skeletal muscles.

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**CULTIVATION OF CHITINOLYTIC BACTERIA BY USING AGRO-DAIRYINDUSTRIAL WASTES**

FAIZA JABEEN AND JAVED IQBAL QAZI

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Chitin is the second abundant biopolymer in marine as well as terrestrial environments. Chitinolytic enzymes have been utilized in different industries for various applications. Commercial interest for the provision and utilization of chitin and its degradative enzymes has revealed the intense need for inexpensive sources of chitinases. At present available purified chitinases are quite expensive. It is imperative to increase the supply of chitinases, at low cost. In this present work, sugarcane bagasse and whey have been used separately and in combination for the cultivation of chitinolytic bacteria. Growth, chitinolytic activity and protein content of all strains increased when the media ingredients were provided with 2% each of bagasse and whey and their combination. So it is concluded that bagasse and whey can be utilized for chitinolytic enzymes' production. The biotechnological exploitation of these abundantly availability wastes is likely to provide the produce at low-cost and eliminate/reduce the environmental pollution associated with such wastes' disposal.

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**UTILIZATION OF CELL WALL CHITIN OF *SACCHAROMYCES CEREVISIAE* FOR CHITINOLYTIC BACTERIAL CULTIVATION**

FAIZA JABEEN AND JAVED IQBAL QAZI

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Chitin is produced in enormous quantities in the biosphere, chiefly as the major structural component of arthropods and most fungi. Chitin is an essential structural polysaccharide in fungi that is required for cell shape and morphogenesis. Its natural degradation is achieved by bacteria and fungi. The chitinolysis is made possible by chitinases of the microbial origin. Cell walls of *Saccharomyces cerevisiae*, consist largely of the polysaccharide chitin. Depending on growth conditions, dry weight of the cell walls may account for about 10–25% of the total cell mass. For the production of chitinolytic bacteria and their enzymes *Saccharomyces cerevisiae* can be used because of its 22-40 % chitin content. In this work *S. cerevisiae* was isolated and cultivated in nutrient broth as well as in some non-conventional inexpensive media. Biomass of the yeast cells was used as medium for chitinolytic bacterial production. It is concluded that *S. cerevisiae* can be used for production of chitinolytic bacteria and their enzymes to replace expensive market available media for cultivating chitinolytic bacteria.

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**PARTIAL PURIFICATION OF CHITINASES PRODUCED BY *BACILLUS* SP.**

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Three chitinolytic strains of the genus *Bacillus* were isolated on a select agar medium. They were cultivated in the medium according to respective optimum growth conditions and the supernatants of 5-day old cultures were processed by ammonium sulfate precipitation mediated partial purification employing sigma dialysis membranes. Thus characterized enzymes had a higher chitinolytic profiles as compared to the crude enzymes' preparation. As an outcome of this investigation local bacterial diversity for provision of chitinases for select application is preserved.

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**FRUITS AND VEGETABLE PEELS' COMPOSTING POTENTIAL OF CELLULOLYTIC, AMYLOLYTIC AND NITROGEN FIXING BACTERIUM *BACILLUS ENDOPHYTICUS-ZA2***

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Owing to the amount and nature of Municipal Solid Waste generation in cosmopolitan cities, solid waste management is a problem of the day. In developed countries the material is treated biologically before its disposal. While in developing countries it exists as a loathsome problem. Thus an effective economically and environmentally sustainable solid waste management system is inevitable. Composting, one of the technologies of integrated waste management strategies, solves two problems concomitantly. That is the waste disposal is managed with beneficial recycling of organic matter content. In the present study owing to the appealing attributes of the bacterium *Bacillus endophyticus-ZA2*, the isolate was cultivated in M-IV broth and

inoculated in the sterilized chopped potato peels, banana peels and wheat straw mixed in ratio of 2:3:4 under controlled conditions with a continuous supply of humidified filtered sterile air upto 16 days. The compost's samples were taken at every 4<sup>th</sup> day starting from the zero hour and analyzed for various parameters. Rise in pH and ash content values of compost was observed as the composting progressed with initial values of  $6.06 \pm 0.057$  and  $8.36 \pm 2.158$ , respectively. Rise in percent germination index values was also observed with initial value of  $10.13 \pm 1.32$  to a final value of  $34.15 \pm 6.75$  at last sampling point. About 54 % decrease in percent volatile solids and 53.38 % decrease in percent carbon contents were noticed at the end of experiment.

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### MATURE FRUIT AND VEGETABLE WASTES' COMPOST ENHANCES GROWTH AND NITROGEN UPTAKE IN *ZEA MAYS*

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Composted organic material is applied in agricultural field as an amendment to provide nutrients and also to enhance the organic matter contents and to improve the physical, chemical and biological properties of the cultivated soils. Incorporation of composted municipal solid waste MSW to soil exhibits positive effect on growth and yield of wide variety of crops. Plant bioassays are one of the most appropriate tests for assaying maturity of composts as they indicate compost phytotoxicity level and this response is used as an index of compost maturity. In the present study compost prepared from potato peels, banana peels and wheat straw obtained from three Bin experiments i.e. Bin Exp.I (uninoculated), Exp.II (with 1% molasses addition) and Exp.III (inoculated with *Bacillus endophyticus*-ZA2 bacterial strain) of 16 days was further matured for a period of six months with weekly turning activity to provide air. This mature compost was then used in pot experiment using *Zea mays* as experimental plant. Mature compost of Bin Exp. I, Exp. II and Exp. III was mixed in soil in ratios of 25:75, 50:50 and 75:25 respectively. Plants were also grown in 100 % each category of the compost and soil. These plants were cultivated after 30 days and analyzed for shoot length, root length, shoot fresh and dry weight and root fresh and dry weight. Analysis of nitrogen content of plants from pot experiments confirmed that mature composts inoculated with the bacterium *Bacillus endophyticus*-ZA2, showed positive influences. Nitrogen contents of plants cultivated in compost-soil mixtures (Ratio 3 and Ratio 2) of Exp. III had 12.5 % and 7.5 % greater nitrogen contents, respectively as compared to control. All other studied parameters of mature compost of Bin Exp. III showed better results as compared to Exp. I and Exp. II.

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### LAB SCALE COMPOSTING OF FRUITS AND VEGETABLE WASTES EMPLOYING FORCED AERATION

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Solid waste management is one of the biggest environmental challenges facing the world today. Continuous accumulation of solid wastes a consequent of increasing population and urbanization, has become a problematic source of land, water and air pollutions. In developing countries thousands of tons of wastes are disposed off in low-lying areas, on out skirts of towns,

cities and along the rivers' banks without any treatment. The situation is the worst in countries like Pakistan, wherein public concerns and Governmental efforts are far beyond declaring the urban environment hygienic. Tons of solid waste comprising mainly of kitchen and restaurant left over are produced daily in the city Lahore. Their utility in raising fruitful composting will lessen the solid waste disposal problem. In the present study peels of Apple, Banana, Oranges and Potatoes, were composted in glass jars under aerobic condition. Filtered Aeration was provided with the help of electric air pumps. Four jars including one control (containing autoclaved substrate) for each substrate were kept at (37°C) temperature for 21 days. Samples were taken at zero and every seventh day for analysis of pH, EC, ash, moisture contents, seed germination index and bacterial C.F.U. pH and ash content of all the four compost samples increased, while increases in EC of sample B and D and decreases in sample A and C were observed. A significant increase in seed germination assay of sample B and D was observed. Significant reduction of *E-coli* was observed in all samples within 14-days of composting. The simple managements of proper aeration and incubation temperature appear promising in terms of enhancing seed germination index and reducing the wastes into a value added phytotoxin free compost fertilizer, which can escalate the agricultural output.

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#### PLANTS GROWTH PROMOTING COMPOST DERIVED FROM PEELS OF BANANA AND POTATOES

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The world has experienced unprecedented urban growth in recent decades. Municipal solid waste is a permanent problem of urban life, irrespective of the fact that a city is whether industrial or non-industrial one. The municipal solid waste is generated in relatively huge amounts corresponding to the population load and life standards. Thus proper management of municipal solid waste is inevitable because improper treatment and disposal have negative impacts, not only on the environment but also on human health directly. Composting of degradable solid wastes not only solves the problem of municipal solid wastes (MSW) but also results in quality end products that have high fertility values. In Pakistan MSW is generated in larger cities in huge amounts corresponding to the population load. There is acute need to compost this resource for rendering it to a valuable product. This would achieve the solid waste management goal along with incentives. In the present study composting was performed in three plastic bin composters (length 53 cm, width 28 cm). Each container had a hole 8 cm above the bottom to provide compressed air. A stainless steel mesh was fixed 10cm above the bottom. A vertical manually driven all steel made agitator was fixed at the center of the steel mesh for daily mixing the compost mix. Compost mix true in each bin consisted of 3.375 kg (dry weight) of potato peels, banana peels and wheat straw in a ratio of 2:3:4. Compost bin No. 1 was provided only water while compost bin No. 2 and No. 3 were provided with 1 % molasses and 10 % inoculum of bacterial isolate *Bacillus endophyticus*-ZA2, respectively along with the moisture. Compost material of each bin was mixed daily with the help of agitator. The samples were taken every fourth day upto 16 days and processed for the determination of Ash contents, Bulk Density, Volatile Acids, seed germination assay, microbial assay (cellulolytic, amylolytic, nitrogen fixing and coliform bacteria) and Respirometric analysis. pH, temperature and moisture contents were monitored daily. Percent germination index values were highest for Compost from bin No. 3 i.e., 57.9 %, while Compost bin No. 1 and 2 had low % GI values i.e. 6.10 and 23.44%, respectively. Regarding microbial analysis of the experiment decline in coliform was observed in Exp.I, Exp.II and Exp.III from initial C.F.U./g values of 152.6

$\times 10^6 \pm 34$ ,  $103.6 \times 10^6 \pm 8.22$ ,  $282.3 \times 10^6 \pm 11.9$  to final values of  $41.6 \times 10^6 \pm 5.49$ ,  $33.33 \times 10^6 \pm 1.76$  and  $33 \times 10^3 \pm 1.156$ , respectively. These results explain the compost quality enhancing ability of the ZA2 bacterium but through augmenting activities of the natural microbial community.

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### **EFFECT OF IRON EMISSION PARTICULATE INHALATION ON VISCERAL ORGANS OF MICE**

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Workers' health safety measures in general are not taken into account in industrial sector in Pakistan. The situation is the worst especially at small scale operational units. One such example is hundreds of whetting units at Wazirabad. At each unit several workers are exposed to continuous inhalation of iron particulates for several hours and mostly the workers do not use masks. For monitoring health damaging effects of such a work place, twenty adult male mice were kept in the centre of several closely situated whetting units for forty days. The animals were provided with commercially prepared food and drinking water *ad libitum*. The cage was roomed at evening and kept to the work place on every morning. A comparable set of animals was housed at Department of Zoology, University of the Punjab, Lahore. On the termination of experiment, the animals were slaughtered and weight of their different organs as well as their bodies were recorded and the tissues processed for histological analyses. Prominent differences in the somatic indices of lungs of control and experimental groups appeared with values of  $0.6 \pm 0.04$  and  $1.88 \pm 0.47$  respectively. More than three folds increase in the parameter was mainly attributed to the deposition of iron particles within the lungs. Histological dearrangements of the lung tissue taken out from the experimental animals have already been reported. Liver and testes also show increased somatic indices and the elevations were 11.61% and 13.33% higher than the respective control tissues. Histological analysis of liver sections revealed diminished sizes of cells as well as their nuclei as compared to the hepatocytes of control animals.

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### **BIOTECHNOLOGICAL CONVERSION OF WASTE POTATOES INTO CLEAN FUEL**

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Due to high temperature and absence of scientific post harvesting management, huge amounts of potatoes are wastes each year in this country. In this study some purple non sulfur bacterial (PNSB) isolates were screened for utilizing potato starch as major nutritive substrate for production of hydrogen at 30°C at pH 7.0 under illumination of 100W with inoculum size of 10% (v/v). Of the four PNSB strains designated as SS-6, SS-7, SS-8 and SS-9 the SS-7 yielded maximum H<sub>2</sub> upto 580 ml of H<sub>2</sub> L<sup>-1</sup> and SS-6, SS-8 and SS-9 produce 210, 470 and 370 ml of H<sub>2</sub> L<sup>-1</sup>, respectively with amylase activities upto 1.51, 1.50, 1.42, 1.41 µg/ml, respectively. Results of this investigation showed that the starch rich vegetable waste is one of potential sources of renewable biomass for the provision of hydrogen. A technoeconomical plant for utilizing the waste starch source employing solar illumination and suitable hydrogen yielding microbes of local origin is under consideration in this laboratory.

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**BIOHYDROGEN PRODUCTION POTENTIAL OF LOCAL ISOLATES OF  
RHODOBACTER AND RHODOPSEUDOMONAS SP.**

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Four strains of purple non sulfur bacteria representing *Rhodobacter* and *Rhodospseudomonas* sp. isolated from agribased based habitates showed compareable hydrogen production when cultivated in sugar cane bagasse (SCB). The isolate *Rhodobacter capsulatus*-PK and *Rhodospseudomonas palustris*-SS produced substantial amount of hydrogen after cultivation in the agroindustrial wastes. The isolates designated as SS-6 and SS-9 yielded moderate level of H<sub>2</sub> upto 320 ml/L. Sugarcane bagasse, 2.5% appeared suitable substrate without addition of any other carbon source. Whereas 1.5% of sugarcane bagasse needed presence of sodium succinate as additional carbon source. For the isolate SS-8, 10% inoculum size (v/v), pH 7 and 30 ± 2.0 °C were optimum conditions for the hydrogen yield. Sodium glutamate and yeast extract were employed for optimizing C/N ratio. These results indicate suitability as well as economic feasibility for obtaining biohydrogen from an abundantly available agroindustrial waste in this country along with meager amounts of the nitrogen sources employing the reported bacteria.

**OPTIMIZATION AND CHARACTERIZATION OF PURPLE NON SULFUR BACTERIA  
ISOLATED FROM RICE PADDY FIELD**

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Gram negative facultative anaerobic, rod shaped, motile purple non sulfur bacteria with different colonial morphology were isolated after enrichment in Biebl and pfenning medium of rice paddy field sample. The 3 isolated strains were designated initially as SS-8 and SS-7 and SS-9 were identified as *Rhodobacter capsulatus*-PK and *Rhodospseudomonas palustris*-SS with 99%, respectively, similarity as a result of 16s rDNA analysis. The isolates were optimized for growth conditions for 15 days under incandescent illumination of 100W. Inoculum size 30% (v/v), pH 7 and 30 °C temperature appeared optimum growth conditions. These bacteria utilize glucose, fructose, mannose, sucrose, sodium citrate and sorbitol as a carbon sources. While asparagine and glutamic acid appeared excellent nitrogen source for their growth.

**DISTRIBUTION OF PURPLE NON SULFUR BACTERIA (PNSB) IN SOIL AND WATER  
SAMPLES FROM LAHORE**

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Purple non sulfur bacteria (PNSB) are distributed in diverse environments. Owing to their hydrogen producing potential PNSB are ranked high biotechnologically for provision of the biofuel. In this investigation 10 PNSB were isolated from agricultural soil, industrial effluents, pond and canal water. These samples were screened on Biebl and pfenning medium and incubated

under illumination at  $30 \pm 2.0$  °C in an aerobic jar employing oxoid anaeroGen kits. Purple to pink colonies appeared after 7-10 days of incubation. Following pure culturing, the bacterial isolates did show H<sub>2</sub> producing growth under facultative anaerobic, heterotrophic and illuminated conditions. These bacteria add further to the microbial biodiversity of Pakistan.

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### **BACTERIOCHLOROPHYLL PROFILING OF LOCALLY ISOLATED PURPLE NON-SULFUR BACTERIA**

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Purple non sulfur bacteria (PNSB) are phenotypically diverse group of microorganisms which produce bright red blooms following enrichment in specific culture media. This yellow to purple pigmentation is one of important characteristic of PNSB. These pigments indicate the presence of bacteriochlorophylla and carotenoids. The colors of cell suspension vary from species to species indicating the presence of major carotenoid types and bacteriochlorophylls. Absorption spectra of cell suspensions yield primary information on these pigments. This color trait is useful in initial characterization of these species. Ten locally isolated PNSB were subjected for determining absorption spectra of bacteriochlorophyll a and carotenoids with UV-VIS scanning spectrophotometer after making cell suspension in sucrose. Bacteriochlorophylla was characterized by the appearance of prominent peaks at 380, 590, 805 and 860nm and the carotenoids were characterized by the absorption maxima at 490 (rhodopin) and 530nm representing rhodopsin and lycopene, respectively. These bacteria are appealing model for converting visible range solar radiations into usable biohydrogen at expense of suitable carbon and nitrogen sources.

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### **PURPLE NON SULFUR BACTERIA AS A SOURCE OF SINGLE CELL PROTEIN**

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Temperature controlled photobioreactor was studied for a month to determine biomass production of different strains of purple non sulfur bacteria. Cell mass of purple non sulfur bacteria (PNSB) may prove a good alternative of manure, fish feed and agricultural supplement because of its richness in proteins and vitamins. In the present study single cell protein (SCP) production was studied for 30 days under optimized growth conditions. PNSB designated as SS-1 produced total protein up to  $1.62 \pm 2.17$  g/l and  $1.67 \pm 3.10$  g/l in MI and MII after 24 and 28 days of cultivation, respectively. While SS-4 yielded  $1.80 \pm 3.20$  g/l and  $1.79 \pm 4.66$  g/l of protein in MI and MII, respectively under illumination of 100W, pH  $6.8 \pm 2.0$  and  $30 \pm 2.0$  °C. This study proved that photosynthetic bacterial biomass could be recovered as useful products after the cessation of original fermentation. In this context these bacteria have a potential use as a protein source in animal feed. Therefore, purple non sulfur bacteria have an advantage over other bacterial groups as fish feed as being non-pathogenic to the animals. Such co-benefits add to the economics of biohydrogen process.

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**CELLULOLYTIC PURPLE NON SULFUR BACTERIA; CANDIDATES OF  
TRANSFORMING PLANT BIOMASS TO BIOHYDROGEN**

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Cellulose is most abundantly produced carbohydrate in the biosphere. Large numbers of cellulose rich renewable non-cost resources are available on earth. Effluent from various food industries, agricultural and municipal wastes are appealing substrates for provision of economic bioenergy. Many aerobic, facultative anaerobic and anaerobic bacterial species are reported for their cellulolytic activity. Hydrogen producing purple non sulfur bacteria is highly versatile and can metabolize structurally diverse compounds that are constituents of degrading plants and animals wastes. In present study purple non sulfur bacteria isolated from rice paddy field, paper mill effluents which are rich with cellulose showed promising cellulolytic activity. The isolates from Rice paddy field designated as SS-7, SS-8 and SS-9 expressed cellulase activity upto 0.42, 0.74, 0.30 enzyme units after 9 days which was raised upto 3.41, 3.50 and 2.43 units respectively, after 15 days. While the isolates SS-2 and SS-3 from paper mill effluents manifested 1.0 and 1.4 enzyme units after 9 days of incubation. This ability of purple non sulfur bacteria can be exploited for beneficial usage of cellulose rich wastes which may be then converted into simple sugars and H<sub>2</sub>.

**IMPACT OF ANTHROPOGENIC ACTIVITIES HEAVY METALS AND MINERALS  
UPTAKE IN MUSCLE OF CATLA CATLA FROM RIVER RAVI, PAKISTAN**

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Freshwater fish Thaila, *Catla catla* were collected from four selected sampling stations viz., Siphon (A), Shahdera (B), Sunder (C) and head Bolloki (D) of the river Ravi during low and high flow seasons. Freeze fish muscle were transported to UK and after digestion, analyzed using Varian Vista-MPX CCD Inductively coupled plasma optical emission spectroscopy (ICP-OES). Majority of the trace and macro elements in fish muscles increased with the increasing pollution loads from the upstream to the downstream sites of this river. All macro elements mean concentrations were higher during low flow than high flow and pattern among the sites was site C > site B > site D > site A excepting the Mg. The order of mean concentration of these element was Ca > P > Na > Mg > K. The order of trace metal bioaccumulation in muscle was zinc > iron > manganese > chromium > copper > lead > nickel > cadmium. The fish muscles that were sampled from site C accumulated higher Cd (467 %), Cr (438 %), Cu (77 %), Pb (1626 %), Mn (374 %), Ni (386 %), Zn (122 %) and Fe (78 %) as compared with the respective values for fish collected from the upstream site (A) during low flow season. The tremendous increases in the levels of all the investigated minerals in fish muscles from the polluted sites raise concerns about the long term health of the river Ravi ecosystem and consequently the fish and its consumers' health. The results contradict the opinion of the local population that the riverine fish are natural, more health promoting and precious than the pond fish. Therefore, we strongly argue for the utilization of an effect-based monitoring approach to alleviate the detrimental effects of anthropogenic activities on fish and the fish consumers' health.

**CULTIVATION OF YEASTS IN PEELS OF APPLE, MANGO AND WATER MELON AND SUGARCANE BAGASSE.**

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Being an agricultural country Pakistan produces huge amounts of agricultural wastes. Owing to high temperature in most part of the year, the agro/food industrial wastes get partially decomposed in dumps or contaminate natural water systems. Whereas such wastes possess biological resource material which might be upgraded with the help of suitable strategies involving useful microorganisms. In the present study, four yeast strains were attempted for cultivation in water extracts of water melon, mango and apple and sugarcane bagasse. The yeasts grew up to 0.8 O.D. at 600 nm without any supplementation within 24 hrs of incubation. These observations are suggestive to utilize these wastes for provision of yeast for food and bacteriological media (yeast extract) preparation in an economic way. High biological value dietary protein deficiency in poor masses can be removed through provision of low-cost and crude yeasts preparation.

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**ISOLATION OF LACTIC ACID AND COLIFORM BACTERIA FROM MARKETED LABEO ROHITA MEAT**

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*Labeo rohita* is a highly preferred edible fish. Preparation of meat and its handling involves situations that may contaminate the produce and/or permit the growth of already present bacterial flora. Due to poor sewerage in this country, coliform contamination of waters is a usual phenomenon. The fish meat sampled from different markets expressed tremendous growth of coliform bacteria on EMB agar. Most of the colonies were of *E. coli* with green metallic sheen and some dark purple nucleated without sheen. While others were of pink color showing the presence of *Enterobacter* and *Klebsiella* species. The fish meat also contained lactic acid bacteria (LAB) on MRS agar showing moderate number of colonies. Some LAB colonies were off-white in color while others were had yellow nuclei. These observations indicated presence of beneficial and harmful bacteria together and dictate for quality control measures and educating the concerned workers. Such efforts are required for improving public health, especially of occupational personals in this area.

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**SEASONAL VARIATIONS IN PROXIMATE AND FATTY ACIDS' COMPOSITION OF MUSCLES OF CIRRHINUS MRIGALA, LABEO ROHITA AND CATLA CATLA FROM RIVER RAVI, PAKISTAN**HAFIZ ABDULLAH SHAKIR<sup>1</sup>, ABDUL SHAKOOR CHAUDHRY<sup>2</sup> AND JAVED IQBAL QAZI<sup>1</sup>*Microbial Biotechnology Lab., Department of Zoology, University of the Punjab, Lahore.*<sup>2</sup>*School of Agriculture, Food and Rural Development, Newcastle University, UK*

Muscle proximate and fatty acid (FA) composition of three largely consumed freshwater fish species *Cirrhinus (C) mrigala*, *Labeo (L) rohita* and *Catla (C) catla* netted during low and

high flow season from river Ravi catch area (Head Balloki), Pakistan were investigated. Crude protein and fat contents ranged from 19.30 to 20.97 % and 1.62 to 1.89 % showing significant ( $P < 0.05$ ) seasonal dependency. Among 38 identified FA, 49.01 to 60.97 % were saturated FA (SFA), 31.39 to 44.83 % monounsaturated FA (MUFA) and 3.39 to 10.21 % polyunsaturated FA (PUFA). Palmitic acid was identified as major SFA with a mean values of 35.78 % for *C. catla*, 34.52 % for *C. mrigala* and 33.49 % for *L. rohita*. Oleic acid with mean values of 13.48 to 25.89 % was the major MUFA in all three species. Mean total PUFA were higher (9.68 %) in *L. rohita* than *C. mrigala* (7.63 %) and *C. catla* (4.77 %). However, total PUFA reduced upto 1.81, 1.28 and 1.06 folds while Omega ( $\omega$ )-3 decreased upto 2.07, 1.25 and 1.13 times in *C. catla*, *C. mrigala* and *L. rohita* respective at low flow season as compared to the high flow.  $\omega$ -6 measured upto 4.65 % and 6.05 % for *C. mrigala*, 5.16 % and 5.77 % *L. rohita*, 1.97 % and 3.86 % for *C. catla* during low and high flow seasons, respectively. These results demonstrate that rates of river flow, through affecting the process of diluting of industrial / urban pollutants, can change fatty acids profiles and proximate composition of fish meat. In this regard significantly reduced PUFA contents of meat of the three carps sampled during low flow (and this high pollution) period of the river clearly demonstrate diminished nutritive value of fish from polluted waters.

**HEPATIC METAL ACCUMULATION IN CATLA CATLA FROM ANTHROPOGENICALLY AFFECTED SEGMENT OF THE IN RIVER RAVI, PAKISTAN**

HAFIZ ABDULLAH SHAKIR AND JAVED IQBAL QAZI

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Bioaccumulation of cadmium, chromium, copper, iron, lead, zinc, manganese, nickel and mercury has been studied in liver of freshwater fish, *Catla catla* netted from three polluted downstream sites (Shahdera=B, Sunder=C and Balloki headworks=D) during high and low flow seasons and compared with less polluted upstream site (Siphon=A). The order of bioaccumulation was  $Zn > Fe > Mn > Cu > Pb > Cr > Hg > Ni > Cd$ . The liver of fish sampled from polluted site C showed highest accumulation of Cd (457 and 900 %), Cr (195 and 280 %), Cu (68 and 115 %), Fe (68 and 84 %), Pb (1400 and 1250 %), Zn (112 and 96 %), Mn (327 and 342 %), Ni (577 and 336 %) and Hg (2578 and 1922 %) during low and high flows, respectively when compared with site A. The entire sampling sites showed higher metals' concentrations in fish liver during low flow than high flow season.

**NITRITE REDUCTASE GENES IN CHROMIUM REDUCING DENITRIFYING BACTERIAL ISOLATES FROM TANNERIES EFFLUENTS' EXPOSED SOIL**

SUMAIRA ASLAM AND JAVED IQBAL QAZI

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Chromium exposed soil from an area adjoining tanneries was investigated for denitrifying bacteria. Isolates were assessed for their chromium reduction potential. Select chromium reducing bacterial isolates designated as ASD1 and ASD9 reduced 100mg/L of chromium (VI) in 24 hours. They were evaluated further for their ability to reduce nitrate and nitrite or accumulation of nitrite with the help of ion exchange chromatography Dionex ICS 3000 employing AS18 column and AG

18 pre-column. It was found that ASD1 reduced only nitrate and resulted in accumulation of nitrite participating in the initial step of denitrification while ASD9 completely reduced nitrate and nitrite. When ASD1 and ASD9 were assessed for genetic heterogeneity of nitrite reductase genes (*nirK* and *nirS*), *nirK* was absent in both isolates while *nirS* was present only in ASD9. The isolate's potential for complete denitrification in the presence of chromium renders it the pollutant water purifying agent.

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#### **MOLECULAR CHARACTERIZATION OF DIVERSE PHYSIOLOGICAL BACTERIAL GROUPS FOR CHROMATE REDUCTASE GENES**

SUMAIRA ASLAM AND JAVED IQBAL QAZI

*Microbial Biotechnology Lab., Department of Zoology, University of the Punjab, Lahore.*

Seven isolates representing denitrifying, nitrogen fixing and cellulose degrading bacteria were evaluated for their chromate reduction activity. Isolates showed multiple metals resistance against Pb (1250 mg/L), Hg (80 mg/L) and Cu (1000 mg/L). When these isolates were characterized for chromate reductase gene through partial sequencing, it was amplified only in one of the nitrogen fixing isolates. The chromate reductase gene did not appear in denitrifying and cellulose degrading bacteria. This study reports that chromate reductase (*ChrR*) is involved in chromium (VI) reduction in nitrogen fixing isolate. While mechanisms other than *chrR* seems to be helpful for chromium (VI) reduction in denitrifying and cellulose degrading bacteria. The chromate reductase possessing nitrogen fixer may prove a promising candidate for soil fertility improvement programmes addressing rehabilitation of metals contaminated sites.

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#### **CULTIVATION OF ACIDOPHILIC AND ALKALOPHILIC BACTERIA IN AGRO AND FOOD WASTES**

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Bioenergy is a growing field in contest of rapidly depleting fossil fuels. Plant biomass, especially agro industrial/food wastes are very attractive substrates for provision of fermentable sugars. While for large scale operational set up maintenance of aseptic conditions is one of the cost-incurring parameter. Going back to the ages, aseptic handling for select fermentations is being re-considered for reducing price of the product. In this regard exploitation of extremophiles is important. In this study commonly found agro/food wastes; the peels of water melon, apple and mango and sugarcane bagasse, have been used for making extracts on which acidophilic and alkaliphilic bacterial growth is manifested. The growth of the bacteria in such substrates is indication of their cellulolytic potential in essentially low and high pH environments in which pathogenic microbes are discouraged. This model of utilization of the agroindustrial waste paves the path for obtaining bioethanol from the waste in an aseptic way, rendering the process economics permitting feasibility for the activity

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**ANTIOXIDANT ACTIVITY (*IN VITRO*) OF METHANOLIC LEAVES EXTRACT AND FRACTIONS OF THE HILL TOON, *CEDRELA SERRATA* (ROYLE)**

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This study was conducted in Dhodial Pheasantry, Mansehra from November 2010 to February 2011. The purpose of study was to identify the common respiratory diseases in Dhodial Pheasantry. In Mansehra, it is very cold weather and humidity increase, therefore, several respiratory diseases of pheasants were exploited. Some times, these infection leads to serious disease. All respiratory disease can be identified by its external symptoms. During study time 18 special cases which are (2.4%) of total pheasants (697) of Dhodial Pheasantry were studied in which one pair (0.27%) of Siamese fire back, *Lopura diardi* (Bonaparte, 1856) were infected by chronic respiratory disease (CRD), also four (0.55%) male lady amherest's pheasants, *Crysolophus amherstia* (Linnaeus, 1758) one black shoulder peacock, one peahen, which are 0.27 % and two (0.41%) ring nicked pheasant, *Phasianus colchicus* (Linne, 1758) were infected by chronic respiratory disease and slight fungal infection, Three cheer pheasants, *Caterus walichi* (Hardwicke, 1827) which are (0.41%) were infected by conjunctivitis. Three postmortems were conducted which showed other diseases which are not concern with respiratory diseases. The number of total pheasants died with respiratory disease in 2008, 2009 and 2010 are 30, 12 and 26 respectively. The present study it shows that common respiratory diseases in Dhodial Pheasantry are chronic respiratory disease and conjunctivitis, infected birds should be kept in separate cages there for, it may prevent the spreading of disease.

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**CLINICAL AND EVOLUTIONARY SIGNIFICANCE OF TRANSLOCATION BREAKPOINTS BY FUDR INDUCIBLE CHROMOSOMAL FRAGILE SITES IN SHEEP (*OVIS ARIES*)**

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Fragile sites are only sporadically studied and their exact significance in animal species is not known yet. However, these are believed to be the hot spots on mammalian chromosomes which appear as a regular feature and some of them bear clinical and evolutionary significance both in humans and animals. This study was carried out to investigate the coincidence of spontaneous and FUDR induced fragile site data in sheep chromosomes by comparing it with reported break sites of clinical and evolutionary significance such as reciprocal and Robertsonian translocations in sheep genome using standard cytogenetic and G-banding techniques. Correlation of break sites of centric fusion translocations and reciprocal translocation with fragile site data in the present study and elsewhere suggests high coincidence of break sites between the two phenomena. A partial coincidence in t2, t4 and t5 suggests that the two phenomena may be correlated. The absence of the evidence in case of chromosomes 5, 6 and 7 may be attributed to breed differences, as different types of centric fusions are common in different breeds. Four reciprocal translocation break sites coincided with reported fragile site data in the present study. This coincidence supports the

hypothesis that the two phenomena may be correlated.

### STUDY OF PREVALENCE OF DENGUE FEVER INFECTION IN SOME URBAN AND RURAL AREAS OF SINDH

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Dengue fever is one of the most rapidly spreading mosquito-borne disease in the world. This disease is transmitted to human being principally by the bite of infected female two species of *Aedes*; mostly by *Aedes aegypti* and rarely by *Aedes albopictus* mosquitoes. According to Sindh Dengue Surveillance report, the number of patients suffered from Dengue fever during 2011 was three hundred and three. Presently a detailed survey of different areas mainly of lower and upper Sindh was conducted about dengue patients from September to November 2011 and it was observed that in local hospitals patients were examined preliminary and almost all Dengue fever positive patients from different areas of lower and upper Sindh except Karachi were brought to Dengue Ward of Liaquat University Hospital Hyderabad for further confirmation and treatment. The total number of Dengue positive patients recorded was ninety nine, out of which eighty five were only from Hyderabad and its adjoining areas, three from Badin, three from Sanghar, two from Thatta, two from Benazirabad and two from Sukkar, Dadu one from each. Out of these total ninety nine patients, seventy three were male and twenty six were female.

### SCREENING OF ANTIBACTERIAL ACTIVITY OF MEDICINAL PLANTS AND ANTIBIOTICS AGAINST FISH PRODUCTS AT RETAIL OUTLETS

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Many studies have been conducted on the antibacterial activity of medicinal plants against human pathogens. However, little has been done on fish pathogens. Bacterial pathogens *Enterobacter amnigenus*, *Serratia odorifera*, *Salmonella typhirium* and *Shigella flexneri* were isolated from freeze or storage Rita Rita fish, identified and confirmed by conventional microbiological procedures. Differentiations and characterizations of isolates were based on biochemical reactions and gram-staining technique. In present study the n-Hexane, chloroform, ethanolic, methanol and ethyl acetate extracts of *Momordica charantia* (both seeds and green parts Bitter gard) were also investigated. On the other hand the antibacterial activity of chloroform and isoamylalcohol extracts of medicinal plant including *Cinnamomum zylanicum* (Cinnamon; Dalchini), *Cuminum cyminum* (Cumin; Zeera), *Syzygium aromaticum* (Clove; Loang), *Curcuma long Linn* (turmeric powder), *Trachyspermum ammi* (Carom seeds; Ajwain) were investigated against fish pathogens isolated from Rita Rita by agar disc diffusion method. The results of present study showed the presence of wide spectrum of antibacterial activities against all tested pathogens.

It was analysed that Pencilline G, Ampicillin, and Amoxline had no effect on *S. flexneri* and *E. amnigenus*. However, the highest zone of inhibition of 61 mm was observed by Ciproflaxin against *S. typhiriu*, whereas 55 mm by Gentamycin and 51 mm by Streptomycin against *S. flexneri*. The seed extracts of *M. charantia* showed better results as compared to the green parts. The tested plant extracts may contain antimicrobial constituents, and further phytochemical and pharmacological studies are necessary to isolate the active constituents and evaluate the antimicrobial activity against a wide range of such microbial populations.

**ASSESSMENT OF EXTRACTS OF *MOMORDICA CHARANTIA* AND ANTIBIOTICS AGAINST WATER ASSOCIATED *ENTEROCOCCUS FAECALIS* AND *ENTEROCOCCUS FAECIUM***

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Enterococci are readily recovered from vegetation and surface water, probably because of contamination by animal excrement or untreated sewage and leading to cause nosocomial bacteremia, surgical wound infection, and urinary tract infection. *Enterococcus faecalis* and *Enterococcus faecium* were isolated and identified through biochemical reactions. We acquired that the significant zone of inhibition was measured by using the extracts of medicinal plants as well as antibiotics through disc diffusion method against all the strains of both *E. faecalis* and *E. faecium*. The antibacterial activity of selected antibiotics indicated that *E. faecalis* and *E. faecium* are sensitive to Gentamycin, Ciproflaxin and Tetracycline, whereas resistant against Penicillin G, Trimethobrim and Sulfomethoxyzol, respectively. The results of *M. charantia* showed significant zone of inhibition in case of n-Hexane, ethanol and methanol extracts of green parts against (A) and (C) strain of *E. faecium*. On the other hand the ethyl acetate and methanol extracts of seed parts of *M. charantia* indicated considerable results against (B) and (A) strain of *E. faecium*. This study showed that fruit part of *M. charantia* could be potential sources of new antimicrobial agents.

**NEUTROPHILIA AND TRANSAMINASE ACTIVITY DURING THIOACETAMIDE INDUCED ACUTE PHASE REACTION IN RATS**

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Thioacetamide (TAA), liver fibrotic agent, has been used extensively in the development of animal models of chronic liver injury. The present study was aimed with the objective to assess

hematological profile in acute condition after single injection of TAA in rat. TAA (300 mg/kg body weight) was injected intraperitoneally in Wistar rats (Group I) (n=3) N.T.C. (non-treated control: no injection) (Group II) (n=3). The rats were kept under standard conditions with 12 h light/dark cycles and at an ambient temperature of  $22 \pm 1^\circ\text{C}$ , with food and water available *ad libitum*. All the animals were anesthetized and scarified at two different time points of 12 h and 24 h after TAA injection. Blood was drawn through direct cardiac puncture for hematological analysis. The administration of TAA lead to acute hepatic toxicity observed histopathologically. Different blood indices as well as activates of hepatic enzymes, aspartate and alanine aminotransferases were also studied. The leucocytes especially the absolute neutrophil count, showed a significant increase ( $P=0.0442$ ) with maximum count at 12h as compared to the N.T.C. Furthermore, a statistically significant increase in ALT ( $P=0.0015$ ) and AST ( $P=0.0045$ ) was observed. Taken together these results we can conclude that single TAA injection may induce a prominent change in hematological profile which can lead neutrophilia.

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**ANALYSIS OF HEAVY METALS IN LIVER AND MUSCLES OF *RASTRELLIGER KANAGURTA* FROM THE COAST OF KARACHI, PAKISTAN**

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The levels of some heavy metals (Zn, Mn, Cu, and Fe) were determined in liver and muscles of 72 specimen of *Rastrelliger kanagurta*, collected during (April 2010-March 2011) from the fish harbour of Karachi on monthly basis. The concentrations of different metals were found to be higher in liver than in muscles of fish. This study indicates that accumulation of heavy metals is rapid in liver. The maximum highest mean concentrations of metals were as Fe (45.668 u/g), Zn (23.36 u/g), Cu (5.006 u/g), Mn (3.273 u/g) and minimum lowest mean concentrations of metals were as Fe (5.893 u/g), Zn (4.527 u/g), Cu (1.935 u/g), Mn (0.17 u/g). Liver of the fish examined highest concentration of all the measured metals, while muscles retained the lowest. The concentrations of all heavy metals were varied in the calendar months probably due to seasonal changes.

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**INTERLEUKIN-6 IS SIGNIFICANTLY ASSOCIATED WITH INSULIN RESISTANCE AMONG TYPE 2 DIABETIC PAKISTANI SUBJECTS**

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Cytokines are small cell signaling proteins that play an important role in inflammation. IL-6 is a pleiotropic cytokine with involvement in diverse physiological and pathological functions as innate immunity, acute phase response and hematopoiesis. The present study was conducted to assess the serum concentration of IL-6 and determine its relationship with insulin resistance among newly diagnosed type 2 diabetic subjects. Patients with age group 37-75 years, (both sexes) were included in the study. Out of which 54 were males and 46 were females. On the basis of BMI three groups were made as normal weight, over weight and obese. Different demographic parameters as

age, BMI, WHR, blood pressure, personal history and socioeconomic status were recorded. The biochemical parameters including fasting blood glucose, random blood glucose, HbA1c, lipid profile were assessed by chemistry analyzer while serum IL-6 and insulin level was assessed by ELISA. Insulin resistance was measured by HOMA-IR. The level of IL-6 was significantly correlated with BMI, fasting blood glucose, LDL-cholesterol and triglycerides, fasting insulin and insulin resistance ( $p < 0.05$ ) while non significant association of IL-6 was observed with WHR, HbA1c and B.P (both systolic and diastolic), cholesterol and HDL-cholesterol ( $p > 0.05$ ). IL-6 showed significant association with insulin resistance ( $p < 0.05$ ). This underscores the significance of inflammatory marker IL-6 in newly diagnosed diabetic subjects.

#### HEMATOLOGICAL AND SEROLOGICAL CHANGES DURING *NERIUM OLEANDER* (N.O), INDUCED ACUTE-PHASE RESPONSE

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The objective of this work was to stimulate the acute phase response (APR) during induction of *Nerium oleander*, (N.O), a potent, anticarcinogen, leave extract to assess hematological profile and Hepatic enzymes activities L-alanine aminotransferase (ALT) and L-aspartate aminotransferase (AST). Induction of acute inflammatory response by N.O. leaves extract was carried out intramuscularly on Wistar rats of about 180-200 gms of body weight. The rats were randomly assigned to four groups (n=4); A (injected with 5ml/kg), B (injected with 10 ml/kg), Sham-operated control 1 (SOC-1) (injected with pyrogen-free normal saline, 5ml/kg) and Sham-operated control 2 (SOC-2) (injected with pyrogen-free normal saline, 10 ml/kg). The rats were kept under standard conditions with 12 h light/dark cycles with *ad libitum* access to fresh water and food pallets. All the animals were anesthetized and sacrificed after 6h of injection and blood samples were drawn through direct cardiac puncture and processed for red cell indices and Hepatic enzymes activities. The intramuscular injection of N.O. induced an acute condition with a significant increase in WBC and with granulocytosis ( $P < 0.0001$ ) observed in group B ( $P = 0.0045$ ). The ALT activity was significantly increased at both doses whereas there was a notable change in AST ( $P > 0.05$ ). Excessive use of ethnomedicines, like N.O. may induce the acute conditions that can significantly affect the hematology and serology.

#### RELATIONSHIP OF IL-10 WITH OBESITY AND INSULIN SENSITIVITY IN OBESE DIABETIC PAKISTANI WOMEN

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Obesity is accompanied by a state of low-grade systemic inflammation that may contribute to insulin resistance. Obesity is a condition of inflammation in which adipose tissue produce

several chemokines and cytokines. Obesity is epidemic problem globally and results in insulin resistance, type 2 diabetes, hypertension and cardiovascular disease. The study was based on the normal weight, overweight and obese diabetic women. The present study was conducted to assess the IL-10 level in the serum of diabetic women of different BMI groups. The women with age ranging between 35-70 years were included in the study with mean BMI  $27.9 \pm 0.49$  kg/m<sup>2</sup>. Other parameters like fasting blood glucose, systolic and diastolic blood pressure, HbA1c and lipid profile were also included in the study. The IL-10 concentration and fasting serum insulin was measured by ELISA and its relation was found with study parameters. Insulin sensitivity and insulin resistance (HOMA-IR) were calculated. It was found that IL-10 concentration in the serum was negatively correlated with BMI kg/m<sup>2</sup> ( $r = -0.526$ ,  $p < 0.01$ ) and WHR ( $r = -0.266$ ,  $p < 0.05$ ). A positive correlation was observed between IL-10 and insulin sensitivity ( $r = 0.494$ ,  $p < 0.01$ ). Insulin sensitivity significantly decreased with increase in weight ( $r = -0.237$ ,  $p < 0.05$ ), waist ( $r = -0.355$ ,  $p < 0.01$ ) and WHR ( $r = 0.266$ ,  $p < 0.05$ ).

**EFFECT OF DIETARY L-ASCORBYL-2-POLYPHOSPHATE ON ANTIOXIDANT ENZYME STATUS IN BRAIN AND MUSCLES UNDER HYPOXIC AND HYPEROXIC STRESS IN JUVENILE SILVER CARP (*HYPOPHthalmichthys MOLITRIX*)**

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The effect of graded levels of dietary L- ascorbyl -2-polyphosphate on activities of antioxidant enzymes at different saturation levels of oxygen in juvenile silver carp (*Hypophthalmichthys molitrix*) was studied. The two-way analysis of variance of data showed that the activities of antioxidant enzymes, catalase (CAT), superoxide dismutase (SOD) and glutathione reductase (GR) were significantly affected by dietary APP ( $P < 0.001$ ) and dissolved oxygen levels ( $P < 0.001$ ) in brain and muscle tissues. These activities were increased with low ascorbic acid concentration in hypoxic condition whereas decreased in hyperoxic stress with high levels of dietary APP. The two interactions between dissolved oxygen and dietary APP reflect the interdependence of these parameters on one another.

**ASSOCIATION BETWEEN POLYMORPHISM OF MYOGENIC FACTOR 6 WITH MEAT QUALITY TRAITS IN CHINESE INDIGENOUS CATTLE**

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Beef is the third most common in the world of meat consumption, accounting for about 25% of the world's meat production, pork and poultry, to 38% and 30%, respectively. China is the world's largest beef producer in the world after the United States and Brazil and is one of the primary meats utilized in the cuisine of the South Asia (China, Pakistan and Afghanistan), Middle East, Australia, Argentina, Brazil, Europe and United States. It is also very important in Africa and

most of the Asian countries. The Chinese beef cattle industry had endured many changes throughout the years but encountered some of the most dramatic challenges in history over the past several decades. With the rapid development of the economy and the gradual improvements in the people's living standards, demand for good-quality beef products is increasing and therefore, improvements is essential by breeding the specialized beef herds. Myogenic factor 6(MyF-6) is the member of Myogenic determination gene family and encodes for straight muscle-specific transcription factors with conserved basic helix-loop helix domain. It is considered as a key gene for meat quality traits in cattle. Therefore, the objective of present research was to investigate single nucleotide polymorphisms (SNP) in Myogenic factor 6 and to investigate the effect of this mutation on meat quality traits and to estimate the Allelic frequencies from five Chinese indigenous cattle breeds, namely Jia-xian red (JX), Luxi (LX), Nan-yang (NY), Qinchuan (QC), Xia-Nan (XN). Primarily, the mutation was detected by PCR and sequencing, thereafter, confirmed by single stranded confirmation polymorphism (SSCP) method. Least square analysis reveals a T → G synonymous mutation at position 141bp was detected in exon2 of MyoD 1 gene in cattle and showed two types of genotypes named JJ and JK.  $\chi^2$  test showed that, genotypic distributions in all the population involved were in Hardy-Weinberg equilibrium ( $P>0.05$ ) except Xianan. Association analysis of this mutation showed a significant relationship with Loin eye area (LEA) and Meat tenderness ( $P<0.05$ ). Moreover, our results also revealed that JJ genotype had the higher mean value than JK genotype for all the traits involved and it might be the favorable genotype. Allelic frequencies J/K in five breeds were 0.870/0.129, 0.914/0.096, 0.860/0.250, 0.814/0.195, and 0.704/0.195, respectively. Our results suggest that the T141G mutation of the Myogenic factor 6 (MyF)6 genes does influence meat quality traits in Chinese indigenous cattle breeds. We concluded that this SNP can be used as an efficacious genetic marker for meat quality traits in native Chinese cattle breeds. Moreover, this research could be equally applicable in Pakistani cattle breeds.

#### ACE G2350A POLYMORPHISM AFFECTS NOISE INDUCED PREHYPERTENSION AND HYPERTENSION IN PAKISTANI POPULATION

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Response of allele carriers of ACE G2350A in the form of prehypertension and hypertension on noise exposure was assessed in 476 male volunteers of Pakistan exposed to sound level  $\leq 80$  dB (no noise group) or sound level  $\geq 81$  dB (noise group). ACE G2350A polymorphism was not found to be associated with prehypertension and hypertension in noise group. However in no noise group, it was associated with hypertension ( $p=0.003$ ). Homozygous 2350A allele affected the risk of hypertension in no noise group (OR, 5.786; 95% CI, 1.917-17.46) and prehypertension in noise group (OR, 2.116; 95% CI, 1.088-4.315). In homozygous 2350G, chances of prehypertension (RR, 2.869; 95% CI, 1.264-6.508) and hypertension (RR, 4.197; 95% CI, 1.738-10.134) were significantly high due to noise. Risk values also elevated for the prehypertension (RR, 3.371; 95% CI, 1.362-8.341) and hypertension (RR, 2.723; 95% CI, 1.135-6.528) in GA allele carriers on noise exposure. In homozygous 2350A allele carriers, risks of prehypertension was increased due to noise exposure (RR, 8.636; 95% CI, 1.010 -73.793). In same allele carriers, no risk increased for the hypertension due to noise (RR, 0.779; 95% CI, 0.235-2.573). On removing the effects of polymorphism, increased risk of prehypertension (RR, 3.645; 95% CI, 2.051-6.478) was

noted on noise exposure. For hypertension, value of relative risk was also high (RR, 2.626; 95% CI, 1.543-4.471). These findings suggest that noise increases the chances of prehypertension and hypertension depending on the ACE G2350A polymorphism in Pakistani population.

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**EFFECTS OF DIFFERENT LEVELS OF DIETARY ASCORBIC ACID ON GROWTH, LIVER VITAMIN C AND RESPONSE TO HYPOXIC AND HYPEROXIC STRESS IN JUVENILE SILVER CARP (*HYPOPHthalmichthys molitrix*)**

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This study examined the effect of three dietary levels of L- ascorbyl - 2 -polyphosphate, APP<sub>50</sub>, APP<sub>100</sub> and APP<sub>200</sub> per kg diet feed on the growth and liver concentration of ascorbic acid (AA) in juvenile silver carp (*Hypophthalmichthys molitrix*) reared in water with three different saturation levels of dissolved oxygen; Hypoxia (50% oxygen saturation), Normoxia (100%) and hyperoxia (150%), after 30 and 70 days of feeding. Three ways analysis of variance showed that that growth rate of fish were significantly affected by time (P<0.001), dissolved oxygen (P<0.001) and dietary APP levels (P<0.005). The three way interaction between these parameters suggests their influence on one another. After 30 days, growth performance(FCE, SGR and weight gain ) of fish fed APP<sub>200</sub> and reared in hyperoxia was significantly better as compared to fish fed APP<sub>50</sub> and reared in hypoxic condition. After 70 days, in normoxic and hyperoxic condition, no significant difference in growth of fish fed with APP<sub>200</sub> and APP<sub>100</sub> was observed. In hypoxia, fish fed with APP<sub>50</sub> and APP<sub>100</sub> showed comparable but significantly reduced growth. However improvement in growth was observed when fed APP<sub>200</sub> supplemented diet. Liver ascorbic acid concentration were significantly effected by dietary APP levels (P<0.001) and dissolved oxygen (P=0.001). There was a significant interaction between the two parameters for liver AA. In liver, AA concentration significantly decreased with decreasing dietary AA but in hypoxia decreased was statistically more pronounced. These findings suggest that APP supplementation have beneficial effect on growth in hyperoxic and normoxic rearing environment and in hypoxic condition APP requirement increased due to increased rate of liver AA degradation.

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**ANTIOXIDANT, INSECTICIDAL AND ANTIBACTERIAL INVESTIGATIONS OF ORGANOMETALLIC COMPOUNDS**

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The purpose of this study was to investigate the bioactivities, such as insecticidal and antibacterial activities of some organometallic compounds because the global food supply has been a great concern for several decades. Thus, studies to identify and evaluate new materials should

have been performed to develop safe and effective chemicals which protect food from various diseases and insect damages. In present research the antibacterial and insecticidal activity of extracts of organometallic compounds such as Triphenyl, Tri-*n*-butyl (Solid), Tri-*n*-butyl (Liquid), Dimethyl, Diphenyl and HL ligand were investigated. Bacterial pathogens such as *Pseudomonas aeruginosa* spp. (Chicken liver and earthworm), *Shigella flexneri* (spoiled fish samples), *Streptococcus* (Human infected sample), *Enterococcus faecalis* spp.(water), *Enterococcus faecium* spp. (water), *Staphylococcus aureus* spp. (Human infected sample), *Klebsiella pneumonia* (Human infected sample), *Serratia marcescens* (Human infected sample), *Pseudomonas syringae* (Human infected sample), *Serratia odorifera* (spoiled fish samples), *Salmonella typhirium* (spoiled fish samples), and *Enterobacter amnigenus* (spoiled fish samples), respectively were isolated and identified. It was acquired that the growth of *S. flexneri* was inhibited by all tested extracts of organometallic compounds (average zone of inhibition 18-30 mm) while Triphenyl and HL ligand had no effect on *S. typhirium*. The extracts of Dimethyl and Diphenyl showed higher zone of inhibition (24 and 20mm) against *S. flexneri* than *S. odorifera* (22 and 16 mm) whereas moderate zone of inhibition was measured against *E. amnigenus*. It was observed that Dimethyl and Diphenyl significantly inhibited the water associated pathogens *E. faecalis* and *E. faecium* (average zone of inhibition 16-28 mm). On the other hand Tri-*n*-butyl (Liquid) had no effect on the *S. aureus* while Dimethyl showed significant antibacterial activity against *S. aureus* (50 mm) and *P. aeruginosa* spp. (30-32 mm). Insecticidal activity was also measured by using all organometallic compounds and it was scrutinized that all compounds showed higher insecticidal activity against *Bactocera zonata* (fruit fly) larvae and food storage insects. It was acquired that most of these compounds were found active against pathogenic bacteria as well as have insecticidal activity.

#### EFFECT OF GONADOTROPHINS, OESTRADIOL AND INSULIN ON CUMULUS EXPANSION OF NILI RAVI BUFFALO OOCYTES

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The aim of this study was to investigate the cumulus expansion (cytoplasmic maturation) in Nili Ravi buffalo oocytes during in vitro maturation in the presence of gonadotrophins, oestradiol and insulin. The cumulus oocytes complexes (COCs) were collected from 2-8mm follicles from local abattoir ovaries. Buffalo COCs were cultured in a control defined medium TCM-199 supplemented with 2 µg/ml oestradiol (E<sub>2</sub>), 0.05 IU/ml recombinant human follicle stimulating hormone (rhFSH), 2 IU/ml human chorionic gonadotrophin (hCG), and 0.12 IU/ml insulin (I) alone or together with their combinations. Cumulus expansion of COCs were recorded after the interval of 8, 16 and 24 h of incubation. Supplementation of medium with single hormones showed significantly increase in mean diameter of COCs with E<sub>2</sub>, rhFSH and hCG except insulin after 24h (P<0.001; P<0.001 and P<0.05 respectively). With rhFSH even at 8h, significant increased (P<0.001) in cumulus expansion was observed. By the addition of double hormones combinations, highly significantly increase in mean diameters of COCs was observed but the maximum increase was observed with E<sub>2</sub>+rhFSH (P<0.001). The cumulus expansion of COCs was highly significantly increased by supplementing the TCM-199 with combinations of E<sub>2</sub>+rhFSH+hCG (P<0.001) and E<sub>2</sub>+rhFSH+hCG+insulin (P<0.001) after 16 and 24h. In conclusion, addition of E<sub>2</sub>+rhFSH in culture media has highly significantly effect on cumulus expansion of Nili Ravi buffalo cultured *in vitro*.

**EFFECT OF DIETARY L-ASCORBYL-2-POLYPHOSPHATE ON ANTIOXIDANT ENZYME STATUS IN BRAIN AND MUSCLES UNDER HYPOXIC AND HYPEROXIC STRESS IN JUVENILE SILVER CARP (*HYPOPHTHALMICHTHYS MOLITRIX*)**

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The effect of graded levels of dietary L-ascorbyl-2-polyphosphate on activities of antioxidant enzymes at different saturation levels of oxygen in juvenile silver carp (*Hypophthalmichthys molitrix*) was studied. The two-way analysis of variance of data showed that the activities of antioxidant enzymes, catalase (CAT), superoxide dismutase (SOD) and glutathione reductase (GR) were significantly affected by dietary APP ( $P < 0.001$ ) and dissolved oxygen levels ( $P < 0.001$ ) in brain and muscle tissues. These activities were increased with low ascorbic acid concentration in hypoxic condition whereas decreased in hyperoxic stress with high levels of dietary APP. The two interactions between dissolved oxygen and dietary APP reflect the interdependence of these parameters on one another.

**HIGH LEVELS OF FEMALE SEX HORMONES ENHANCES CLEARANCE OF HEPATITIS C VIRUS RNA DURING INTERFERON PLUS RIBAVIRIN COMBINATION THERAPY**

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Different viral and host factors are involved in the successes of anti-viral treatment. Recently the role of estrogen has been documented in the development of fibrosis. However no research is available on the role of female sex hormones including estrogen in treatment outcome. This research reveals correlation of interferon treatment response with female sex hormones in hepatitis C patients. Total 70 hepatitis C virus infected female patients who had planned to receive interferon plus ribavirin therapy were enrolled: Their blood samples were collected and sent by consultants to Gellome Centre for Molecular-based Diagnostics & Research (GCMDR) for HCV RNA viral load and other laboratory investigations. The levels of estrogen and progesterone, hormone were estimated by ELISA method in the baseline samples at Zeena tMedical Lab. These hormone levels were correlated with patient treatment response such as rapid virological response (RVR), early virological response (EVR), end of treatment response (ETR) and sustained virological response (SVR) It was observed that the patients with higher estrogen level have maximum positive treatment response at each stage, which reveals that estrogen and progesterone may play a positive role in the clearance of HCV in female patients during treatment. Based on the results of the current study, it is concluded that female sex hormones such as estrogen and progesterone have positive role in the treatment of hepatitis C viral infection.

**EFFECTS OF DIETARY SELENIUM ON CYPERMETHRIN INDUCED OXIDATIVE STRESS IN JUVENILE MAHSEER (*TOR PUTITORA*)**

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This aim of this study was to investigate whether selenium supplemented diet have any protective effect on cypermethrin (CYP) induced oxidative stress in mahseer (*Tor putitora*) which could be imposed due to irrational use of this pesticide. Fish were fed selenium supplemented diets (1mg Se/kg and 2mg Se/kg diet) for fifteen days before exposed to one fifth (12µg/L) of the lethal concentration (60µg/L) of CYP. Enzymatic antioxidant activities (catalase, superoxide dismutase, glutathione reductase and lipid peroxidation) and total protein contents were determined in liver, brain and muscle tissues. Cypermethrin exposure caused significant (P<0.001) decreased in total protein contents & enhanced the activities of antioxidant enzymes in the tissues. While a significant (P<0.05) increased in protein contents and decreased in CAT, POD, GR activities were observed in liver, brain & muscle tissues of fish fed with selenium supplemented diet prior to CYP treatment. The result suggest that CYP can induced oxidative stress and feeding of selenium supplemented diet prior to CYP exposure reduced the sensitivity of CYP induced oxidative stress.

**COMPARATIVE PRODUCTION OF LYSINE BY *ESHCHARCHIA COLI* AND *KLEBSIELLA* ISOLATES**

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Lysine is an essential amino acid not biologically synthesized in the body. It has pharmaceutical applications as well as great potential for improving the protein quality of cereal based human in diets especially in third world countries. The present study was conducted to determine comparative production of lysine by *Eshcharchia coli* and *Kalabsiella* isolates. *Eshcharchia coli* and *Kalabsiella* isolates were obtained from PIMS (Pakistan institute of medical sciences) Islamabad and ten isolates were tested for their lysine producing ability. All of these isolates were found to produce considerable amount of lysine in L6 fermentation media containing different Carbon (C) sources. Five carbon sources namely glucose, sucrose, fructose and lactose were used. Glucose was found as most suitable source for lysine production, followed by sucrose, lactose, fructose and maltose. Maltose was found to show the minimum production of lysine. Maximum lysine was produced by *Kalabsiella* isolates K1 6.45g/l after 96hrs of incubation when glucose were used as C source followed by 6.0 g/l by *Kalabsiella* isolates K3 after 72hrs of incubation when sucrose was used as Carbon source and 37°C temperature. Optimum incubation periods were between 48-96hrs for better lysine production. Maximum lysine production was shown at 96hrs by *Kalabsiella* and *E. coli* followed by 48hrs. So comparatively form *Kalabsiella* and *E.coli*, *Kalabsiella* showed better lysine production.

**STUDY OF PREVALENCE OF DENGUE FEVER INFECTION IN SOME URBAN AND RURAL AREAS OF SINDH**

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Dengue fever is one of the most rapidly spreading mosquito-borne diseases in the world. This disease is transmitted to human beings principally by the bite of infected female mosquitoes, mostly by *Aedes aegypti* and rarely by *Aedes albopictus*. According to Sindh Dengue Surveillance report, the number of patients suffered from Dengue fever during 2011 was three hundred and three. Presently a detailed survey of different areas mainly of lower and upper Sindh was conducted about dengue patients from September to November 2011 and it was observed that in local hospitals patients were examined preliminarily and almost all Dengue fever positive patients from different areas of lower and upper Sindh except Karachi were brought to Dengue Ward of Liaquat University Hospital Hyderabad for further confirmation and treatment. The total number of Dengue positive patients recorded was ninety nine, out of which eighty five were only from Hyderabad and its adjoining areas, three from Badin, three from Sanghar, two from Thatta, two from Benazirabad and two from Sukkar, Dadu one from each. Out of these total ninety nine patients, seventy three were male and twenty six were female.

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**SCREENING AND OPTIMIZATION OF EXTRACELLULAR ALKALINE THERMOSTABLE PROTEASE PRODUCTION FROM *BACILLUS* SPP. AND CHARACTERIZATION OF PROTEOLYTIC ACTIVITY**TANZEELA RIAZ,<sup>2</sup> NAAZ ABBAS,<sup>1</sup> SHAH JAHAN BAIG<sup>2</sup> AND SYED SHAHID ALI<sup>1</sup><sup>1</sup>*Biochemistry and Toxicology Lab., Department of Zoology, University of the Punjab,*<sup>2</sup>*Food & Biotechnology Research Center, PCSIR Labs., Complex, Lahore*

In recent times, the products of biological origin, especially enzymes are attracting the attention of researchers all over the world. They are playing a vital role in several biological and commercial processes. Among all the enzymes, proteolytic enzymes occupy an important niche; they are degradative enzymes which catalyze the cleavage of peptide bonds in other proteins. Alkaline proteases are the main enzymes among proteases and constitute 60 to 65% of the global industrial enzyme market. Extracellular alkaline thermostable proteolytic bacteria BCTL-PS-127 was isolated from soil and identified as *Bacillus* spp. on the basis of biochemical and physical properties. Maximum enzyme production was achieved when casein, glucose, tryptone are used along with some salts in cultivation medium. Protease production was highest at pH 9 after 72 hours of incubation at 39°C with 1% (w/v) casein concentration. Enzyme was found to be active at 30 - 100°C and maximal activity was exhibited at 50°C. Substrate specificity tests indicated that protease is substrate specific and casein at 1% concentration was found to be the best substrate among the four substrates tested (casein, peptone, skim milk and BSA). The enzyme substrate reaction time was evaluated to be 10 minutes. The protease was tested for its thermostability by giving heat and cold shocks and it was found to be exhibiting considerable thermostability at 30-100°C for 30, 60 and 120 minutes of incubation and did not denature. The protease enzyme worked efficiently over a wide range of pH 8-13 for 60 minutes incubation and showed significant pH stability at the above range of pH. This alkaline and thermostable protease was tested as detergent

additive and it was found to have the ability to be used as detergent additive. It may play an important role in detergent and leather industry.

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**EVALUATION OF ACUTE TOXICITY OF PESTICIDE, KARATE AND ITS  
SUBLETHAL EFFECTS ON PROTEIN AND ACETYLCHOLINESTERASE ACTIVITY  
IN *CYPRINUS CARPIO***

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Karate is a locally used pesticide has lambda cyhalothrin as an active ingredient. To determine the acute toxicity, *Cyprinus carpio* were exposed to 0.00, 0.08, 0.16, 0.2, 0.24, 0.32 and 0.4 $\mu$ l/L of karate for 96 hours in a static bioassay. The 96h LC<sub>50</sub> was determined using Arithmetic method and found to be 0.160 $\mu$ l/L. There was significant decrease in total protein content and acetylcholinesterase activity in brain, liver and muscle tissues of fish exposed to sublethal concentrations (one fifth; 0.032 $\mu$ l/L; one tenth 0.016 $\mu$ l/L of LC 50) of pesticide. Based on concentration and exposure durations, 0, 1, 24, 48 and 72 hrs, there was step-wise decreased in AChE activity and maximum reduction was observed in brain follow by muscles and liver tissues.

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**COMPARATIVE STUDY OF SPERMATIC VEIN HISTOLOGY IN PATIENTS VISITING  
FMH WITH AND WITHOUT VARICOCELE**

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The aim of this study was to investigate and compare histological characteristics of left spermatic veins in patients with and without varicocele, which is a major cause of male infertility. Between January - July 2011, period of six months seven patients of Fatima Memorial Hospital Lahore who were having fertility problems volunteered to conduct the study. After taking prior written consent from these patients sample tissues of spermatic veins were obtained performing micro-surgical procedures under local anesthesia. Histological sections of veins were made and slide stained with haematoxylin and eosin. The stained slides were observed microscopically under calibrated ocular micrometer and measurements taken for diameter of Intima and thickness of Tunica Media and Tunica Adventitia of cross sections of veins. The study demonstrated that Intima, Tunica Media and Tunica Adventitia seem to be increased significantly in these varicocele patients when compared with normal subjects values. According to T-test, the thickness difference of Intima and media is highly significant (p :S 0.000) and Adventitia is also significant (p :S 0.005) as compare to the control group.

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**SUBLETHAL EFFECTS OF LOCALLY USED PESTICIDE, KARATE ON MDA CONCENTRATION AND ANTIOXIDANT ENZYMES IN FRY OF FRESH WATER CYPRINID *CYPRINUS CARPIO***

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Karate, locally used pesticide, had sublethal effects on the antioxidant enzymes and MDA concentration in the liver, brain and muscle tissues of the fry of *Cyprinus carpio*. Fish were exposed to two sublethal concentrations, 0.016 and 0.032 $\mu$ l of LC50 (96 hrs.), for 25 days in a semistatic system. The water of aquaria was changed after every 72 hrs and different concentrations of pesticide were restored. No mortality was observed. The MDA concentration and antioxidant enzymes (CAT, SOD, POD and GR) activities were increased in the tissues in a dose dependent manner. The results clearly indicate the defensive mechanism of cells against oxidative stress.

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**SUB-LETHAL EFFECTS OF CYPERMETHRIN ON TOTAL PROTEIN AND ACETYLECHOLINE ESTRASE ACTIVITY IN DIFFERENT TISSUES OF *TOR PUTITORA* FED WITH SELENIUM SUPPLEMENTED DIET**

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Fish were fed for fifteen with graded levels of selenium supplemented diet prior exposure to sublethal concentration of cypermethrin (CYP). The total protein and acetylcholine esterase activity (AChE) in brain, muscle and liver tissues were estimated after 15 days of treatment. There was significant decrease in total protein and AChE inhibition in all tissues but the impact was more pronounced in fish fed with basal diet as compared to 1 and 2 mg Se kg<sup>-1</sup> supplemented diet. The results clearly indicate the protective role of selenium in reducing the oxidative damage probably caused by CYP exposure.

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**LEVELS OF SERUM CORTISOL IN CANCER PATIENTS**

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In an investigation of cortisol level's variation in cancer patients the blood samples of 35 patients diagnosed for different types of cancer were obtained from Institute of Nuclear Medicine and Oncology, Lahore (INMOL). The different categories of cancer patients included acute lymphoblastic leukemia, breast cancer, non-Hodgkin's lymphoma, stomach cancer and lung cancer.

Blood samples of nine healthy subjects of comparable age group were also collected for control comparisons. The blood samples of each patient and healthy subjects were obtained in morning and evening. The sera of cancer patients and healthy subjects were separated and stored at -20°C. Later the serum was used to determine the cortisol level by Enzyme Linked Immunosorbent Assay (ELISA) technique. Our results demonstrated that mean serum cortisol levels in the morning and evening samples were significantly elevated in all categories of cancer patients than in control group.

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#### **ACID PRETREATMENT OF LIGNOCELLULOSIC BIOMASS OF CORNCOB FOR THE EFFICIENT PRODUCTION OF BIOFUEL**

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Lignocellulosic biomass of corn cob has been found as best option for producing biofuel as it is waste material that is produced in thousands of tonnes every year around the world. Dilute acid pretreatment of corn cob increases the feasibility of such feed stock for the production of biofuel. As acid pretreatment increases the digestion of corn cob and produces fermentable sugars by breaking molecules of lignin, hemicellulose and also cellulose in the biomass. This method further increases the efficiency of hydrolytic processing of remaining cellulose content in the biomass, which increases the overall yield of biofuel. In the present study, dilute acid pretreatment of corn cob was done by using different concentrations of sulfuric acid ranging from 0.5% to 5% and reaction time 30 to 180 mins to find the best reaction condition, resulting in high breakdown of lignin seal. The substrate concentration and temperature of the reaction vessel was constant i.e. 15gms and 121°C. The best condition where maximum delignification occurs was found to be 180 mins and 4% of acid concentration.

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#### **BIOFUEL PRODUCTION BY OPTIMIZED ALKALI PRETREATMENT OF LIGNOCELLULOSIC BIOMASS OF CORN COB**

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By depolymerization of lignocellulosic biomass of corn cob with the help of alkaline pretreatment such as dilute sodium hydroxide, the production of biofuel can be increased as overall yield of biofuel (bioethanol) depends particularly on the pretreatment process. In the present study, dilute sodium hydroxide pretreatment of corn cob was done by applying different concentrations of NaOH solution and reaction time. To find out the effectiveness of the process, weight, lignin content, cellulose, total sugars, reducing sugars was calculated after treatment at each condition. Overall result indicated that sodium hydroxide has strong effect on delignification of lignocellulosic biomass which is the main score in each pretreatment type. Higher delignification increases overall yield of fermentable sugars for bioethanol production. The best optimum condition was found to be 0.5M of sodium hydroxide concentration and 180 mins.

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**STUDY ON ANTIBIOTIC SENSITIVITY IN MILK OF BUFFALOES AND COWS  
SUFFERING WITH MASTITIS IN DISTRICT LAHORE**

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The present study was designed to determine the prevalence of mastitis and affect of antibiotics on mastitis causing bacterial agents in buffaloes and cows. Total of 216 milksamples including 108 from buffaloes and 108 from cows were collected randomly and tested for prevalence of mastitis with the help of Surf Field Mastitis Test and symptoms of the animals. The prevalence of mastitis in buffaloes and cows was 18.50% and 23.14% respectively. Mastitis was more prevalent at the age of 9-10 years both in buffaloes and cows where it was reported 35% and 32% respectively. Physical appearance of milk in buffaloes indicated that among affected samples 65% contained watery fluid more than any other such as blood, pus and mucus whereas mucus and watery fluid both were 36% in affected samples of cows where blood content and pus were reported 16% and 12% respectively. Prevalence of sub-clinical mastitis was 55% and 56% in buffaloes and cows while acute mastitis was prevalent as 5% and 4% in buffaloes and cows respectively. Right fore teat was frequently affected than other part of udder both in buffalo and cows. To control mastitis by four commonly used antibiotics a comparative study was done where Norfloxacin was found to be most effective for mastitis control than Ciprofloxacin and Gentamycin, but bacterial agents were resistant against Penicillin.

**ASSESSMENT OF THE RELATIONSHIP BETWEEN THE BASAL METABOLIC RATE  
AND VARIOUS DEVELOPMENTAL STAGES IN BOYS OF RAWALPINDI AND  
ISLAMABAD**

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The vital functions of the body required for survival of an individual including growth and reproduction are underlined by energy metabolism. The metabolic rate is the measure of the rate of energy turnover. The rate at which energy is produced under resting condition is termed as the basal metabolic rate (BMR), which is the largest component of daily energy demand and it represents the minimum cost of living. The present study was designed to assess the BMR among boys from infancy through puberty and adolescence in our local population of Rawalpindi and Islamabad. The height and weight of the boys were measured and other relevant information was obtained through a questionnaire. The BMR was calculated using the equation described by Harris and Benedict. The present study shows that there was a progressive increase in the mean height, weight, body mass index (BMI) and BMR of boys with advancing age. The increase in height, weight, BMI and BMR was remarkable at mid and late puberty, whereas the height, weight, BMI and BMR steadily increased during prepuberty, early puberty and through adolescence to adulthood. In conclusion, the current study demonstrates that there is an increase in the BMR as the

energy requirements of boys are augmented for accelerated growth at the time of puberty.

### HEPATITIS C TREATMENT: CURRENT AND FUTURE PERSPECTIVES

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Hepatitis C virus (HCV) is a member of *Flaviviridae* family and one of the major causes of liver disease. There are about 175 million HCV infected patients worldwide that constitute 3% of world's population. The main route of HCV transmission is parental however 90% intravenous drug users are at highest risk. Standard interferon and ribavirin remained a gold standard of chronic HCV treatment having 38-43% sustained virological response rates. Currently the standard therapy for HCV is pegylated interferon (PEG INF) with ribavirin. This therapy achieves 50% sustained virological response (SVR) for genotype 1 and 80% for genotype 2 & 3. As pegylated interferon is expensive, standard interferon is still the main therapy for HCV treatment in under developed countries. On the other hand, studies showed that pegylated IFN and RBV therapy has severe side effects like hematological complications. Herbal medicines (laccase, proanthocyanidin, Rhodiola kirilowii) are also being in use as a natural and alternative way for treatment of HCV but there is not a single significant report documented yet. Best SVR indicators are genotype 3 and 2, < 0.2 million IU/mL pretreatment viral load, rapid virological response (RVR) rate and age <40 years. New therapeutic approaches are under study like interferon related systems, modified forms of ribavirin, internal ribosome entry site (HCV IRES) inhibitors, NS3 and NS5a inhibitors, novel immunomodulators and specifically targeted anti-viral therapy for C compounds. More remedial therapies include caspase inhibitors, anti-fibrotic agents, antibody treatment and vaccines.

### AN ASSESSMENT OF THE BODY SURFACE AREA FOR DETERMINATION OF DRUG DOSAGE FOR TREATMENT OF VARIOUS DISEASES IN GIRLS IN DIFFERENT STAGES OF THEIR PUBERTAL DEVELOPMENT

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The body surface area (BSA) is the area covered by one's skin, the largest organ of the body. The BSA is required for determination of dosage of medicines for treatment of various diseases. It also has its application in situations such as chemotherapy/radiotherapy for cancer and treatment of burn cases. The present study is designed to determine the reference ranges of BSA in girls from prepuberty through puberty and adolescence in our local population. The height and weight of the girls were measured and other relevant information was collected through questionnaire especially designed for the study. The BSA was calculated using formula described by Schlich *et al.* The current investigation shows that the mean body weight, height, body mass index (BMI) and BSA of girls progressively increased from 3 to 20 years of age. A steady increase in the height, weight, BMI and BSA is observed in girls at prepuberty, early puberty and through adolescence to adulthood. Nevertheless, a significant increase in the height, weight, BMI and BSA

was noticed at mid and late puberty. In conclusion, the present study provides rationale for measurement of BSA for determination of dosage of medicines for treatment of various diseases.

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**DETERMINATION OF THE BASAL METABOLIC RATE FOR ASSESSMENT OF ENERGY REQUIREMENT FROM PREPUBERTY THROUGH PUBERTY TO ADOLESCENCE AMONG GIRLS IN TWIN CITIES OF RAWALPINDI AND ISLAMABAD**

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Energy metabolism underlies all processes contributing to reproduction and survival. Consequently, metabolic rate is the measure of the rate of energy turnover. The basal metabolic rate (BMR) provides the information about energy requirements to maintain the body weight. The basal metabolic rate may undergo changes in accordance with the energy demand and growth and development at the time of puberty. The height and weight of the girls was measured and other relevant information was collected through questionnaire especially designed for the study. The BSA was calculated using equation described by Harris and Benedict. The results were tabulated and ANOVA was applied for interpretation of results. The mean body weight and height of girls gradually increased from 3 to 20 years of age. The mean BMI progressively increased from early to adulthood in all the girls. The increase in the mean BMR was gradual during prepuberty, early puberty and late puberty/adolescence through adulthood, whereas BMR dramatically increased during mid and late puberty. Most of the girls included in this study belonged to middle and upper middle class and were taking three regular meals daily. In conclusion, the present study demonstrates that BMR increases as the energy requirements of girls increases for growth and development at the time of puberty.

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**DEVELOPMENT OF PERIPHERAL DIABETIC NEUROPATHY IN TYPE 2 DIABETES MELLITUS PATIENTS: A GENETIC SCREENING**

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Diabetic peripheral neuropathy is a serious complication of type 2 diabetes mellitus (T2DM) which lowers the quality of life in diabetes mellitus patients. The presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes have been regarded as diabetic neuropathy. Very few studies have found the association between the development and progression of diabetic peripheral neuropathy in type 2 diabetes mellitus and *alu* repeat insertion/deletion (IID) polymorphism in angiotensin converting enzyme (ACE) gene. The ACE is an important molecule in the rennin angiotensin system (RAS). The objective of the present study is to find the association of ACE gene I/D polymorphism with diabetic peripheral neuropathy in T2DM. . In this study ACE gene I/D polymorphism was screened

through PCR using ACE gene I/D specific primers. The subjects include 276 type 2 diabetes mellitus patients with peripheral neuropathy, 496 type 2 diabetes mellitus patients without peripheral neuropathy and 331 control subjects. ACE gene II genotype was significantly higher ( $p$  value <0.05) in type 2 diabetes mellitus without diabetic peripheral neuropathy. The higher incidence of ACE gene II genotype reveals a caring effect on the development of diabetic peripheral neuropathy in T2DM patients. This finding concludes a possible role of RAS in the regulation of nerve structure and function in T2DM.

#### MOLECULAR CHARACTERIZATION OF TWO ISOFORMS OF COPPER METALLOTHIONEIN GENE(S) FROM A LOCAL ISOLATE OF TETRAHYMENA

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Metallothioneins are cysteine rich low molecular weight proteins having the property to chelate metal ions. Thus supporting the organisms to survive in heavy metal contaminated environment. In the present study both isoforms of tetrahymena Copper metallothionein are characterized from genomic DNA of locally isolated tetrahymena. Cloning and DNA sequencing reveals that both amplified sequences are of 327 and 208 base pairs. First isoform showed a close homology to CuMT-1 while second isoform is relatively more identical to CuMT-2 genes of *Tetrahymena Tropicalis* and *Tetrahymena thermophila* respectively. Both isoforms have three to four metallothionein specific CXXXXCXXXXXC tandem repeats. Homology based predicted three dimensional structure show the metal binding motifs and explains their role in bioremediation of heavy metals from contaminated water.

#### PATTERN OF PEROXIDASE (PRX) ISOZYME IN THE MUSCLES OF DIFFERENT SPECIES OF PORTUNID CRABS, FOUND IN COASTAL WATERS OF PAKISTAN

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Portunid crabs were collected from different localities throughout its distribution range in the Sindh and Balochistan coast, Pakistan. Morphological and morphometric data were collected for each specimen. Two species of family portunidae, *Portunus spp.* and *Scylla spp.* were determined by the use of native PAGE, vertical slab gel electrophoresis. The band patterns of peroxidase (PRX) isozyme were obtained in the muscles of crabs. Muscle samples of each crab were homogenized, centrifuged and run on a 10% vertical polyacrylamide gel in discontinuous buffer system. Peroxidase isozyme comprising 5 loci were investigated from individual's specimen. Peroxidase pattern in two species of portunids *Portunus spp.* and *Scylla spp.* were differentiated from each other. The relative mobility (Rf) of peroxidase isozymes bands were found to be in *Portunus spp.* (Rf = 0.095, 0.365, 0.746) and in *Scylla spp.* (Rf = 0.525 and 0.576) respectively.

**DETERMINATION OF HEAVY METALS IN THREE SPECIES (*MACROPHTHALMUS BOSCI*, *PARASESARMA PLICATUM*, AND *UCA URVILLEI*) OF CRABS FOUND IN MANGROVE AREAS OF KARACHI PAKISTAN**

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The three species of brachyuran crabs (*Macrophthalmus bosci*, *Parasesarma plicatum*, and *Uca urvillei*) were selected to determine the bioaccumulation of heavy metals. Their sedentary lifestyle, feeding and burrowing activities expose these species to water, dietary and sediment-derived contaminants. The levels of Cobalt (Co), Copper (Cu), Chromium (Cr), Cadmium (Cd), Nickel (Ni), Iron (Fe), Lead (Pb), Zinc (Zn), has been evaluated. The selected crab species were collected from the Sandspit, Korangi creek and Port Qasim mangrove areas of Karachi and were analysed for the above metals using Atomic Absorption spectrophotometer. The data indicated variable levels of the metals in each crab species. The analyses of variance show significant differences ( $P < 0.05$ ) for the accumulated concentration of metals among the species and among the sites. Higher concentrations of some metals (Co, Cu, Zn) were observed in *P. plicatum* and *U. urvillei* than *M. bosci*. The ability to accumulate the relatively high level of Co, Cu and Zn suggest that the *P. plicatum* and *U. urvillei* can be the potential biomonitors of Co, Cu and Zn pollution in the mangrove ecosystem.

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**A COMPERATIVE STUDY ON PHYSIO-CHEMICAL AND BIOLOGICAL ANALYSIS OF TAP WATER SUPLIED BY WASEP AND GOVERNMENT IN TEHSIL PUNIAL**

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A study was conducted in Dalnaty and Gulmuti, villages of district Ghizer. During this study a comparative analysis of drinking water was carried out in order to determine physico-chemical and biological contamination. The study was carried out from July 2011 to December 2011. The observed results from both villages were compared with each other and also with WHO standards and PSI standards. Thermo tolerant coliforms were counted in 100ml of water sample by Delagua water testing kit to determine fecal contamination. Turbidity, TDS, Conductivity and Hardness were determined to evaluate physical quality and chemical quality were assessed using different tests were made for Fluoride, Salinity, Fe, Cr, Nitrate, Nitrite and heavy metals (Cd, Pb, Hg, Cu, As). Water samples of Dalnaty were found to be highly contaminated with thermotolerant coliforms during the months of July (96-180 fecal coliforms/100ml water), August (36-196 fecal coliforms/100ml water), and October (87-200 fecal coliforms/100 ml water). No coliforms were observed in November and December. Water samples of Gulmuti were found to be suitable for human consumption because no coli form was observed in drinking water during the study period. Tests for chemical parameters showed that Fluoride was UMR, Salinity 0.0, Fe 0.01mg/l, Cr 0.003µg/ml <LOD for heavy metals, nitrate and nitrite were found to be 0.0ppm in Dalnaty. In water samples of Gulmuti nitrate, nitrite, salinity and heavy metals showed the same results as that of water samples of Dalnaty but Cr was 0.02µg/l, Fe 0.01mg/l and Fluoride 0.22mg/l. Test for physical parameters showed that water samples of both villages follow WHO guidelines.

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**ASSESSMENT OF VULNERABILITY OF YOUNG URBAN POPULATION TO SYMPTOMS OF METABOLIC SYNDROME AS CONSEQUENCE OF CHANGING LIFE STYLE**

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The population ranging between 17 and 50 years of tertiary education institutions of urban Karachi have been studied for their vulnerability to symptoms of metabolic syndrome as consequence of changing life style. This segment of the society is most exposed to nutritive and activity habits causing metabolic syndrome at the later age. The body mass index (BMI), waist hip ratio (W/H ratio), systolic blood pressure (SBP) and diastolic blood pressure (DBP) and fasting blood sugar levels were measured from well over 150 volunteers of both the genders. The mean age of the subjects was  $27.67 \pm 0.52$  years, and the average value of each pertinent characteristic for metabolic syndrome was BMI:  $32.2 \pm 0.41$ ; SBP:  $116.8 \pm 1.0$  mmHg; DBP:  $81.4 \pm 0.7$  mmHg and fasting blood sugar level:  $98.71 \pm 1.0$  mg/dl. The data predicts significant correlation between BMI and systolic, diastolic blood pressures and fasting blood sugar level in all obese subjects on the Asiatic criteria. The gender wise data analysis in female showed mean age:  $27.18 \pm 0.742$  years; BMI:  $31.94 \pm 0.577$ ; W/H ratio:  $0.82 \pm 0.006$ ; SBP:  $113.66 \pm 1.48$  mmHg; DBP:  $78.18 \pm 1.04$  mmHg and FBP  $98.94 \pm 1.39$  mg/dl. The results exhibit significant correlation between BMI and SBP ( $P > 0.033$ ), DBP with BMI ( $P > 0.012$ ) and SBP ( $P > 0.00$ ), FBG with BMI ( $P > 0.005$ ), SBP and DBP ( $P > 0.000$ ). Male subjects illustrate the mean age of  $28.22 \pm 0.691$ , BMI:  $32.3 \pm 0.599$ , W/H ratio:  $0.92 \pm 0.01$ , SBP:  $119.96 \pm 1.28$ , DBP:  $84.618 \pm 0.932$  and FBG  $98.31 \pm 1.59$ . The significant correlation exists between DBP with SBP ( $P < 0.00$ ) and FBG only with BMI ( $P > 0.061$ ). In all control individuals the average age studied was  $22.54 \pm 0.752$ , BMI:  $21.17 \pm 0.488$ , SBP:  $109.26 \pm 1.25$ , DBP:  $79.41 \pm 1.7$  and FBG  $95.71 \pm 1.25$ . The significant correlation displayed only between SBP and FBG ( $P > 0.041$ ) and DBP and SBP ( $P > 0.00$ ). All the studies parameters are in females and males are in normal range. Significant correlation exists only in DPB and SBP ( $P > 0.00$ ) in females and in males there is no correlation between any parameter.

**PHYSICO-CHEMICAL AND BACTERIOLOGICAL ANALYSIS OF DRINKING WATER QUALITY OF NOMAL GILGIT, GILGIT-BALTISTAN**

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This study was undertaken in Nomal Village located in 25km from Gilgit district with its geographical coordinates  $36^{\circ} 4' 23''$  North,  $74^{\circ} 15' 16''$  East. Nomal Valley has an approximate 850 households consisting of 6000 people. The aim of this study was to analyze the state of drinking water quality in Nomal area. Water samples were collected in April month from seven sites in sterilize bottles. The water samples were immediately subjected to both chemical and biological analysis. The results of this analysis were compared with water quality standards of WHO. The following water quality parameters were determined namely pH, temperature, turbidity, odor, taste, total hardness, alkalinity and E.coli. The data present indicated that pH of all samples is 7, turbidity varied from 5-10 NTU and temperature is 9-25°C. The research results showed that physical and chemical parameters of drinking water falls within recommended limits of WHO. Biological water

quality analysis indicated that channel water was highly contaminated showing presence of high fecal coliform. The maximum colonies of coliform present in channel-2 (TNTC) too numerous to count and in tap water 93 E.coli colonies were analyzed. It indicates that biologically water is not suitable for human consumption. The chemical and physical quality of drinking water does not have any health hazard problems this time so for.

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**STUDIES ON TESTICULAR ORGANIZATION AND MATURATION IN RELATION TO CIRCULATING CHOLESTEROL IN CYPRINID FISH *LABEO ROHITA***

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Studies were made on the spawning male *Labeo rohita*. The fish collected from the hatchery, mean body weight ( $1453.82 \pm 129.93$ ) gm. The testes during the spawning season were examined. The results reveal that, the testes at the commencement of spawning season were milky white and the weight was increased mean testes weight ( $45.76 \pm 12.73$ ) gm. Histological examination reveals that, initially before the release of milt most of the cysts in seminiferous lobules were filled with spermatozoa. The spermatozoa get capacitated by gaining tail and milt in the end of May and start their journey from lobules to the opening of the body. The spawning continued till early August. During this phase the leydig's cells were not found any where in the interstitial space. However the appearance of leydig's cells was noticed during the process of milting. Serum levels of cholesterol were at peak when the spermatogenesis takes place. It starts decreasing as the spermatozoa goes out of the body. According to morphological, histological and circulating levels of cholesterol data it can be said that the actual timings of spawning in the male *Labeo rohita* are from late May to early August.

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**EFFECT OF PROFENOFOS, AN ENDOCRINE DISRUPTING CHEMICALS ON SERUM LEVEL OF TESTOSTERON IN HUMAN AND LEYDIG'S CELLS IN RABBITS**

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An attempt was made to know the effects of profenofos, as endocrine disrupters on the serum level of the testosterone in human and leydig's cells in rabbits. In this context, profenofos, an organophosphate was applied through olfaction as well orally in experimental animal. Significant change in serum level of Androgen (Testosterone) was noted  $5.24 \pm 0.68$  ng/ml and  $4.25 \pm 1.12$  ng/ml before and after the application of the referred pesticide in the crop field to the farmers/ spray workers with age group 22-48 years. Insignificant change in the levels of testosterone  $3.33 \pm 1.25$  ng/ml before and  $3.27 \pm 1.4$  ng/ml after application of profenofos in few of farmers/spray workers aged 25-50 years was also noted. Histological observations reveal that the leydig's cells of the rabbits exposed to profenofos were totally vanished or regressed. Even the interstitial space became compressed. Findings of the studies on the serum level of testosterone in human and the leydig's cells of rabbit indicates the severity of the effect of profenofos.

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**STUDIES ON PRE-OVULATORY STAGES IN THE OVARY OF TILAPIA,  
*OREOCHROMIS MOSAMBICUS***

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An attempt was made to study the pre-ovulatory stages in the ovary of *Oreochromis mosambicus*. The fish, mean body weight  $44.2 \pm 8.0$  were collected from the adjoining waters of Jamshoro during the early August to late September. The ovaries of each fish were dissected out and processed for histological examination. The study of the ovarian sections reveal that the ovary of the fish in early August contained gravid oocytes in majority. However, few developing oocytes were also visible. After 15 days the ovaries of fish were in advanced condition. The oocyte membrane of majority follicles disintegrated and they were ready for ovulation. Beside this the ovarian wall became thin and also ruptured from one side indicating the start of spawning phase. It has also been noted that the oocytes were present in different stages of development and are released as the final maturation takes place. It can be said that *Oreochromis mosambicus* breeds many times in a year in clutches. Hence called as the multi spawner fish.

**TOXIC EFFECT OF CHROMIUM ON LIVER AND GILLS OF CYPRINID FISH,  
*CYPRINUS CARPIO***

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Studies to know the impact of chromium on the gills of *Cyprinus carpio* was made. In this context, *Cyprinus Carpio* body lengths (4-5cm) were brought live in the laboratory. After acclimatization the fish were exposed to four different concentrations 25mg/l, 30mg/l, 35mg/l and 40mg/l. The results were matched with the control. The findings of the present studies indicate that the effect of chromium is dose dependent. The disintegration takes place in every part of the gill. At lower concentration of the compound the gill rakers around gill arch lost their integrity at initial stage and at higher concentration they disappeared completely. The cells of mucosal epithelium, basement membrane and submucosa at the lower dose disintegrated and at higher concentrations showed hyperplasia. Adipose tissue is also disappeared gradually. Primary and secondary gill lamellae also disintegrated initially at lower concentration followed by hyperplasia.

**BEHAVIOURAL AND HISTOLOGICAL CHANGES IN THE FISH EXPOSED TO  
ACUTE TOXICITY OF LEAD AND CADMIUM**

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Toxic effects of lead and cadmium were made in the liver of fish, *Cyprinus carpio*, and mean body length  $5.50 \pm 0.15$  inch. The fish was exposed to lead (10 ml/day) and cadmium (10ml/day) for 30 days. During the experimental period the fish exposed to the groups. Cadmium and mixture of cadmium were less active and skin became gradually rough with the appearance of

abscesses around the cheek. However, the fish exposed to the lead alone and with the cadmium added alternatively caused less effect as compared to cadmium alone and in mixture with lead. As far as the histological results are concerned by liver of the fish exposed to cadmium alone and in mixture with lead caused drastic effect like, hepatocytes became reduced in size / hypertrophied, picnosis was noted in the cells, vacuolation was predominant, sinusoids became wider and hepatoma was also seen at few location. So far as lead alone and lead used as an alternative, the damaged of liver was noticed but not so drastic as was in a group exposed to cadmium alone and in synergism with lead. Hepatic cords were intact and became compressed, kupffer cells disintegrated. The overall findings reveal that the cadmium is more dangerous as compared to lead.

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**THERMO STABILITY ANALYSIS OF ENDOGLUCANASE . ENZYME OF  
ASPERGILLUS FUMIGATGS PRODUCED THROUGH TWO DIFFERENT  
FERMENTATION TECHNIQUES B**

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The present work was performed to find out the Thermo stability of Crude Endoglucanase. Enzyme Produced by *Aspergillus fumigatus* at various temperatures (4°C to 80°C) Usage and Storage. Cellulase was produced through submerged fermentation' and solid state fermentation by using biomass (wheat straw) from *Aspergillus Fumigattus* The specie was previously isolated from Lab of FBRC (Pakistan Council of Scientific and Industrial Research) Labs complex Ferozepur Road Lahore. This wa subcultured on PDA and on Vogel medium plates where we use filter paper as carbon source by inoculation. We got pure colonies by incubating the in-cultated plate in incubator for 7 days at 30°C. Then by using wheat straw chopped and wheat straw ground as a substrate we did submerged fermentation and solid state fermentation respectively.

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**ANALYSIS OF BACTERIAL CONTAMINANTS OF RED MEAD SOLD IN GILGIT CITY  
AND THEIR SENSITIVITY PATTERN AGAINST DIFFERENT ANTIBIOTICS**

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Bacteria associated with meat contamination can be risk factor for meat consumers in terms of food born infections thus contaminated meat is acknowledged source of food poisoning and hazardous for human health. The microbiological study was conducted from 2<sup>nd</sup> May 2011 to 10 October 2011 to determine the bacterial contamination level of red meat sold in different sites of Gilgit City. Out of 115 samples 23 red meat samples were examined from each selected site i.e. Hospital Road, Punial Road, Sabzi Mandi, Cinema Bazaar, and Air Port Chowk Bazaar. The bacteriological quality of red meat was analyzed by WHO Manual for Laboratory Investigations of Acute Enteric Infections (WHO Manual CDD/83.3) with the help of pour plate technique. The highest bacterial contamination was found in red meat samples of Sabzi mandi which was  $34.606 \times 10^5$  CFU/g, followed by Punial Road samples  $32.32 \times 10^5$  CFU/g, Air port Chowk Bazaar samples  $32.29 \times 10^5$  CFU/g, Cinema Bazar samples  $30.71 \times 10^5$  CFU/g and Hospital Road samples  $30.31 \times 10^5$  CFU/g respectively. The Bacterial species isolated from red meat were *Staphylococcus*

*aureus*, *Escherichia coli*, *Listeria monocytogens*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Proteus spp*, *Serratia spp*, *Klebsiella spp*, *Citrobacter spp* *Salmonella spp* and *Shigella spp* which were dominated by *Staphylococcus aureus* with (86 %) infestation. The sensitivity of bacteria isolated against seven antibiotics was carried out by Kribey Bauer disc diffusion method in which Ciprofloxacin, Cifixime, and Ceftrixone were most effective over bacterial species as compared to others.

**BACTERIOLOGICAL ANALYSIS OF RAINBOW TROUT ASSOCIATED WITH RISK ASSESMENT OF WATER CONTAMINATION IN JUGLOT HATCHERY GILGIT BALTISTAN PAKISTAN**

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A study was conducted to investigate the bacteriological quality of water and Rainbow trouts of Juglot Hatchery Gilgit Baltistan, Pakistan along with physico-chemical parameters in September and October 2011. A total of 18 fish were collected from 06 ponds of Juglot hatchery and were analyzed for bacterial contamination on its skin and abdomen respectively. Major bacterial communities found on its skin and abdomen was composed of *Escherichia Coli*, *Pseudomonas*, *Salmonella*, *shigella* and *Proteous Marabilis*, *vibrio cholera* respectively. 14 water samples were collected from the same ponds and 2 from inlet source were analyzed for bacterial contamination. The bacterial community of the water was composed of 05 major species of bacteria, *Escherichia Coli*, *Pseudomonas*, *Salmonella*, *shigella* and *Proteous vulgaris* respectively. Also physic-chemical parameters like pH, Temperature, and Dissolved oxygen were assessed on the spot by using different digital probes. Analysis of the results for all samples revealed values for pH (4.40 to 7.62), temperature (14.3°C to 18.6°C) and dissolved oxygen (10.3 mg/l to 18.0mg/l) respectively.

**SIGNIFICANCE OF LDL-CHOLESTEROL HDL-CHOLESTEROL, FT3, FT4 AND TSH IN HYPOTHYROIDISM**

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The aim of this study was to observe the effects of hypothyroidism on lipoprotein with special reference to LDL-C and HDL-C. We also evaluated the correlation of TSH with LDL-C and HDL-C. The overall 120 female patients of age 20-50 years were investigated for FT3, FT4, TSH, HDL-C and LDL-C. Among these 100 patients were of hypothyroidism and 20 euthyroid for control. The LDL-Cholesterol in hypothyroid patients were significantly higher than euthyroid subjects. The significant positive correlation was present between TSH level and LDL-C. The HDL-C increased insignificantly. The increased LDL-C in hypothyroid patients may contribute to atherosclerotic coronary artery disease.

**BACTERIOLOGICAL ANALYSIS OF LOCAL FRESH FRUITS AND VEGETABLES OF GILGIT CITY AND ROLE OF ANTIMICROBIAL AGENTS ON BACTERIAL GROWTH**

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Fresh vegetables & fruits are exposed to potential microbial contamination. The study was conducted from 2<sup>nd</sup> May 2011 to 10 October 2011 to determine the bacterial contamination level on various fresh fruits and vegetables collected from Gilgit city. 80 samples (48 vegetables and 32 fruits) were analyzed and cultured according to WHO Manual for Laboratory Investigations of Acute Enteric Infections (WHO Manual CDD/83.3) with the help of pour plate technique. The quality of the medium and techniques were confirmed by culturing the standard reference strain *E. coli* ATCC 25922. Major bacterial species isolated from vegetables were salmonella, *E. coli*, *Shigella spp*, *staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella*, *Vibrio cholera* whereas *E. coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella spp* were major bacterial species on fruits. The high contamination and high number of pathogens on the surface of vegetables exposed to the local manure was  $8.24 \times 10^5$  CFU/g as compared to the vegetables which were not exposed to the local manure was  $5.52 \times 10^5$  CFU/g. The high contamination and high number of pathogens on the surface of fruits collected by indirect method was  $7.2 \times 10^5$  CFU/g as compared to the fruits collected by direct method was  $4.14 \times 10^5$  CFU/g. 05 antimicrobial agents with 2% concentration were used to see their effect on isolated bacterial species growth. Among these antimicrobial agents which were used to reduce bacterial growth, Acetic acid was most effective with (92.6%) while citric acid was least effective with (33.46%). Further seasonal sampling and analysis is needed in order to determine different microbial agents and their relationship with the human health.

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**MOLECULAR VIROLOGY OF HEPATITIS C VIRUS POLYPROTEIN CLEAVAGE PRODUCTS**

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Hepatitis C is a major liver disease caused by a bloodborne hepatitis C virus (HCV) which has become a serious threat to the human population. According to WHO there are 170 million people infected world wide, with Egypt (22%), Pakistan (4.8%) and China (3.2%). Since its isolation in 1989 six major genotypes, 72 subtypes and many quasispecies of HCV which are identified from the different regions of the world. The major HCV subtype 3a has been identified in 80% of the infected individuals in Pakistan. Therefore, the current study is aimed to develop effective, economically feasible, and more potent antiviral drug against the surface antigens. For successful accomplishment of this objective, 100 patients were randomly selected from Pakistani population. The cDNA was synthesized after RT-PCR amplification of total RNA which was extracted by using Trizole method. Gene specific primers were designed from the envelop protein (E1, E2) gene sequence of HCV local strain *Pk/173*. PCR amplified genes were cloned in cloning vector *pTZ57RJT* by using T/A cloning method. The *E. coli* DH5a was transformed with this cloning vector and Blue/White screening was done on IPTG, X-gal and ampicillin plate. For the expression and characterization, the gene will be sub-cloned into expression vector pET22a/b. The

expression of genes will be induced with 0.5mM IPTG and the sample will be withdrawn after regular intervals. The samples will be run on SDS-P AGE for their expression analysis. The expressed gene product will be purified to homogeneity and their immunogenicity will be checked by using animal model (Rabbit/Mice).

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#### **EFFECT OF NICOTINE ON HEMATOLGY IN ADULT MALE MICE (*MUS MUSCULUS*)**

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Nicotine is one of the main and toxic alkaloid present in tobacco which is largely absorbed in the body through cigarette smoking. A considerable amount of nicotine is found to be present in the blood of tobacco smokers and snuffers and is largely responsible for various physiological changes. The present study was designed to study the effects of nicotine on various hematological parameters of adult male mice (*Mus musculus*). For this purpose the study mice (n=60) were divided into two groups i.e., control and experimental group. Subcutaneous injections of 1 mg/ kg body weight of nicotine were given to the experimental group (n= 40) and saline solution was given to the control group (n= 20). Hematological parameters such as WBCs , count, RBCs count, Hb concentration, HCT, MCV, MCH, MCHC and PLT count were assessed by using hematological analyzer in the laboratory of LCWD. Statistical analysis was done by using SPSS (Version 19). The WBCs count, PL T count, HCT and MCV were found significantly higher (p<0.05) in experimental group while the significant decrease (p<0.05) was observed in RBCs count, Hgb concentration, MCH and MCHC in the experimental group when compared to the control group. It is concluded that intake of nicotine caused markedly deleterious effects on various hematological parameters.

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#### **EVIDENCE OF A DISTINCT AWAKENING CORTISOL RESPONSE IN YOUNG ADULTS DURING EXAMINATION**

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The measurement of cortisol in saliva provides a reliable tool for investigation of hypothalamus-pituitary-adrenal axis activity in a stress free manner. The salivary cortisol exhibits a clear diurnal variation and circadian rhythmicity with a time course closely paralleled to that of plasma cortisol. Previous studies have demonstrated a well established cortisol awakening response (CAR) in adolescents, young adults and elderly subjects. Various psychological and social stressors are reported to have adverse effects on CAR. Academic examination is also considered as the stressful challenge to many students. The present study was designed to examine the effect of academic stress on CAR in young adults. The saliva samples were collected from young adults at wakeup, 15 min after wakeup, 30 min after wakeup, 1000, 1600 and 2000 hrs two months prior to examination and during final examination for award of M.Sc. degree. Two months prior to examination, the salivary cortisol concentrations, which were low at wakeup, significantly

increased 30 min post awakening and declined thereafter reaching lowest levels at 2000 hr. During the examination, the concentrations of salivary cortisol remained higher at wakeup and did not show a characteristic high CAR. The elevation of salivary cortisol secretion after awakening was significantly lower and CAR remained blunted. Nevertheless, the concentrations of salivary cortisol did not fall as sharply as two months prior to examination. Furthermore, the concentrations of salivary cortisol remained significantly higher at 2000 hr. In conclusion, the present study shows that during examination the salivary concentrations of cortisol are higher at wakeup and exhibit a distinct and blunted CAR and that the levels of salivary cortisol remained elevated at the end of the sampling at 2000 hr.

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**EFFECT OF NEUROMEDIN S (NMS) ON GHRELIN SUPPRESSED TESTOSTERONE SECRETION IN ADULT MALE RHESUS MONKEY**

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In the present study the stimulatory role of NMS was investigated on ghrelin suppressed testosterone secretion in adult male rhesus monkeys. Four adult male rhesus monkeys were used in this study. Fifty nmol of NMS and 2~g/Kg ghrelin were injected through a teflon cannula implanted in saphenous vein. Blood samples were collected individually for NMS and ghrelin 60 min before and 120 min after NMS and ghrelin administration at 15 min intervals. To study the effect of NMS on ghrelin suppressed plasma testosterone secretion samples were collected 45 min before the administration of ghrelin, then NMS was administered after 60 min of ghrelin injection and samples were collected for 120 min after NMS injection. The plasma testosterone concentrations were determined by using specific Enzyme Immunoassay (EIA). Ghrelin significantly ( $P<0.05$ ) decreased plasma testosterone secretion after 45 min and levels remained low till 60 min. NMS blocked this decline caused by ghrelin and further stimulated ( $P<0.001$ ) plasma testosterone secretion from 30 to 60 min after its administration. In conclusion the present study suggests that NMS has not only the ability to restore the inhibitory effect of ghrelin on testosterone secretion but to further augment the testosterone secretion in adult male rhesus monkey. This response might be regulated through HPG axis however further study is recommended to understand the exact mechanism of action of these two peptides in regulation of reproductive behaviour in primates.

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**ANTIOVARIAN ANTIBODIES CONCENTRATIONS: A CONTRIBUTORY FACTOR CAUSING INFERTILITY**

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The Present study was designed to determine the Antiovarian antibodies (AOA) concentrations along with their relation with other hormones like LH, FSH, PRL and E2 among infertile and fertile human females to assess their role in infertility. The studied population consists of (n=17) fertile (without any history of infertility) and (n=65) infertile (Premature ovarian failure

(POF), polycystic ovary syndrome (PCOS), idiopathic, endometriosis) female subjects. Blood samples were collected from each subject and plasma was separated by centrifugation and stored at -20°C. Plasma concentration of anti-ovarian antibodies (AOA), Luteinizing hormone (LH), Follicle stimulating hormone (FSH), Prolactin (PRL) and estrogen (E2) were estimated by EIA. All the hormonal concentrations were compared by using one way ANOVA. Infertile patients with POF had significant ( $P < 0.001$ ) whereas patients with PCOS, idiopathic and endometriosis group had non significant ( $P > 0.05$ ) increase in plasma AOA. FSH, LH and PRL concentrations were significantly higher ( $P < 0.05$ ) in infertile women as compared to fertile women while there was a significant decreased level ( $P < 0.05$ ) of E2 in POF, PCOS infertile female subjects. AOA concentrations showed significant ( $P < 0.0001$ ) positive correlation with FSH and significant negative correlation ( $P < 0.0001$ ) with E2 in patients with POF. From the results it may be concluded that together with levels of reproductive hormones, levels and measures of AOA may contribute additional marker for human female infertility. So testing for the presence or absence of anti-ovarian antibody in women prior to initiation into the IVF-ET program should be recommended as this would help to counsel the patients regarding the reproductive outcome with IVF.

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#### **EGG QUALITY COMPARISON BETWEEN TWO GENERATIONS OF JAPANESE QUAIL BEING SELECTED FOR HIGHER BODY WEIGHT**

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The present experiment was conducted at Avian Research and Training (ART) centre UVAS, in order to observe the response to selection for higher body weight regarding different egg quality parameters. 72 eggs each, from the birds of both generations of four different close-bred stocks at the age of 8 weeks were subjected to egg quality analysis. Regarding egg quality, egg weight, egg shell thickness, egg shape index, albumen and yolk index were studied. The statistical analysis of data using ANOVA with CRD factorial arrangements for interpretation using GLM (General Linear Model) Procedures (SAS9.1) and the comparison of means using Duncan's Multiple Range (DMR) Test, revealed significant differences in yolk index and shell thickness between the two generations while non-significant differences were observed regarding, egg weight, egg shape index and albumen index.

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#### **AN IMMUNOHISTOCHEMICAL ANALYSIS FOR DIFFERENTIAL EXPRESSION OF GPR39 IN PATIENTS WITH REPRODUCTIVE CARCINOMAS**

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GPR39 is a vertebrate G protein-coupled receptor with a 1660 base pair transcript, resulting in a peptide of 456 amino acids and a calculated weight of 52 kDa. The receptor is expressed in a range of tissues including the gastrointestinal tract, kidney, liver, pancreas, reproductive tissues and various brain regions. The aspect of GPR39 signal transduction cascade paints a multifaceted picture of its role in various diseases including diabetes and cancer. Strong tumorigenic ability of

GPR39 has been indicated by recent functional studies. The aim of the present study is to investigate and assess GPR39 expression in benign and malignant human reproductive carcinomas. Biopsies (n=10) of different reproductive carcinomas of breast, prostate and testicular tissues were obtained from male and female patients. These carcinomas included pericanalicular fibroadenoma of breast, testicular teratoma and Intraductal carcinoma of prostate. Tissues were then fixed, embedded in paraffin, sectioned and processed for standard immunohistochemical analysis using an antibody specific to human GPR39. Tissues of human cerebrum were taken as a positive control and negative controls with omitted primary antibody were taken to assure specific binding. Widespread GPR39 immunolocalization was observed in the tumorigenic cells of the malignant tissues (testicular teratoma and intraductal carcinoma of prostate), whereas, level of GPR39 expression was found to be greatly decreased in the benign tissue sections (pericanalicular fibroadenoma of breast). Present results provide preliminary facts and add to the emerging evidence that the magnitude of GPR39 receptor expression in reproductive carcinomas is highly significant and suggests a basis for the possible action of GPR39 in cancer development and progression. The factors involved in the upregulation of GPR39 in the tumorigenic cells are not known, and this issue warrants further studies.

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#### **ROLE OF GLUTATHIONE AND HYDROGEN PEROXIDE ON CRYOPRESERVED SPERM MOTILITY OF NILI RAVI BUFFALO**

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Cryopreservation reduces sperm motility, sperm intracellular thiol levels, which consequently may reduce fertilizing ability. The addition of glutathione (GSH) an antioxidant, prior to cryopreservation for prevention of the sperm from the damages is gaining interest for researchers. On other hand, hydrogen peroxide ( $H_2O_2$ ) is known as reactive oxidant specie (ROS) that also induces adverse effect on sperm motility. The aim of this study was to investigate the effect of cryopreservation and effect of  $H_2O_2$  on sperm motility as well as preventive role of GSH. GSH was added to cryopreservation media prior to sperm cryopreservation of Nili Ravi buffalo bull. To conduct this study semen was collected from five bulls and diluted individually with EYTG extender and 10 groups were prepared from each semen sample. One group studied as fresh without any additives, the 2nd group cryopreserved without any additives and other groups were cryopreserved in the presence of additives (1 mM GSH, .5 mM GSH, 100  $\mu$ M  $H_2O_2$ , 200  $\mu$ M  $H_2O_2$ ) and combination of these additives (1 mM GSH + 100  $\mu$ M  $H_2O_2$ , 1 mM GSH + 200  $\mu$ M  $H_2O_2$ , .5 mM GSH + 100  $\mu$ M  $H_2O_2$  and .5 mM GSH + 200  $\mu$ M  $H_2O_2$ ). After thawing sperm parameters were observed such as total motility, motility gradation *i.e.* 4/4, 3/4, 2/4 1/4 by wet preparation method. Cryopreservation reduced significantly mean percentage sperm total motility from  $80.4 \pm 0.84$  to  $71.28 \pm 2.65\%$ . Additive 100  $\mu$ M  $H_2O_2$  also maintained total sperm motility percentage at  $71.51 \pm 2.18\%$  whereas both concentrations of GSH and combination of GSH +  $H_2O_2$  with different concentrations rather reduced mean percentage of total sperm motility ranging between  $56.41 \pm 4.4$  -  $35.85 \pm 1.50$ . No significant difference was found among groups in each grade. Sperm cryopreservation of Nili Ravi buffalo bull did decrease its total motility but on other hand addition of antioxidant (GSH) with a concentration of 1 mM or .5 mM prior to sperm cryopreservation did not protect sperm total motility. Rather 5 mM GSH concentration decreased sperm progressive motility compared to that of cryopreserved semen without additive.

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**GENETIC GAIN IN 4-WEEK BODY WEIGHT THROUGH MASS SELECTION IN 4  
CLOSE-BRED STOCKS OF JAPANESE QUAIL**

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Avian research and Training (ART) Centre, University of Veterinary and Animal Sciences Lahore, is extensively involved in the breeding and genetic improvement of Japanese Quail. Mass selection procedure is being applied to enhance 4 week body weight. 3 flocks of 15000 birds comprising 5000 each were received from the hatchery of ART, Centre and were subjected to selection for higher body weight. Individual birds were weighed and 400 females and 140 males of the highest body weight category were selected to be the parents of next generation from each batch of 5000 birds. So, a population of 1200 females and 420 males was established. Attaining the age of 14 weeks of age, the youngest flock, the egg were collected, selected and incubated to get the F1. The progeny of F1 responded positively to the selection and the analysis of Data using ANOVA with CRD factorial arrangements for interpretation using GLM (General Linear Model) Procedures (SAS9.1) and the comparison of means using Duncan's Multiple Range (DMR) Test, revealed significant differences in baseline population and F1 at the age of week1,2,3,and 4 respectively. Significant improvement was observed in body weight of all the four weeks due to selection for higher body weight.

**SEQUENCING AND PHYLOGENETIC ANALYSIS ON THE BASIS OF FUSION PROTEIN  
GENE OF PESTE DES PETITS RUMINANTS (PPR) VIRUS CIRCULATING IN PUNJAB**

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Peste des petits ruminants (PPR) is a contagious viral transboundary disease of small domestic and wild ruminants. It affects a big population of sheep and goat especially the trade and nomadic travelling areas in Pakistan each year. It is a major candidate disease to be controlled to establish commercial level farming of sheep and goat. In this study, from five outbreaks of PPR, 32 (~26%) out of 122 samples collected were found to be positive through RT-PCR using both Morbilivirus genus specific NI/N2 (generating 238bp amplification) and PPR virus specific primers pairs F1/F2 (generating 372bp amplification). Nested PCR using F1A/F2A and sequencing were used to confirm the PPR virus. Two outbreaks were attended in the year 2009. In DG Khan (February 2009) 90% morbidity and 50% mortality was observed while in Lahore (June 2009) 80% morbidity and 55% mortality was observed. While in 2010, sampling was done from three outbreaks. In Lahore (March 2010) experimental farm, out of 200 goats, 90% were morbid with 20% mortality. In Sargodha (February 2010) two outbreaks were studied one with 60% morbidity and no mortality while the other presenting 98% morbidity and 30% mortality. PCR amplifications from these outbreaks samples were sequenced to find the lineage of virus circulating in Punjab. The Fusion protein amino acid sequences from these viruses were found to be well conserved and Lineage IV was confirmed after sequencing and phylogenetic analysis. These viral gene sequences

were homologous to other neighboring Asian countries including China, Bangladesh, India, and Iran and even have been reported in Tajikistan. PPR must be controlled through the development of effective vaccine form local virus instead of exotic ones to enable the livestock industry to meet the present food challenges faced by Pakistan.

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#### **HYPOGLYCEMIC EFFECT OF AEGLE MANNELOS LEAF (BAEL) EXTRACT ON ALLOXAN INDUCED DIABETIC MICE**

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*Aegle marmelos* is highly reputed ayurvedic medicinal tree commonly known as the bael tree, because all parts of the tree are used in traditional system of medicine. Thirty two mice were included in the present study divided into 4 groups of eight mice in each two groups of mice were normal and the other two groups were diabetic. Diabetes was induced by intraperitoneally injection of alloxan at a dose of 100 mg/kg body weight. Mice having blood glucose levels in a range of 104-170 mg/dl were considered diabetic. Two groups of mice normal, control and diabetic groups were given orally normal feed. While the other normal and diabetic groups were given normal feed and *Aegle marmelos* leaf extract 300mg/kg body weight as an effective dose against alloxan induced hyperglycemia. The experiment lasted for 45 days and blood samples (0.5 ml to 1.0 ml) were collected from coccygeal vein of rats on 1st day, 21 st day and 42nd day of the experiment in heparinized tubes: Blood glucose levels were measured using spectrophotometer by enzymatic kit at a wavelength of 540nm. It was observed that the *Aegle marmelos* leaf extract is most effective in reducing and maintaining the glucose level in normal and hyperglycemic mice. However *Aegle marmelos* leaf extract did not have significant effect on reducing glucose level in hyperglycemic mice. The data was statistically analyzed by and multiple comparison test among different group which were treated with *Aegle marmelos* leaf extract to study its effect on hyperglycemic mice. Glucometer was also used to measure blood glucose levels for comparison with spectrophotometer reading. Mice body weight were measured by digital upper balance device during experiment. The outcomes of the present study revealed that the administration of *Aegle marmelos* leaf extract has hypoglycemic effects in diabetic mice, therefore it was concluded that *Aegle marmelos* leaf extract is helpful to lower glucose levels in treatment of hyperglycemia.

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#### **EFFECT OF SOY PROTEIN ON SERUM GLUCOSE AND CHOLESTEROL LEVELS IN DIABETIC RATS**

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Soybean is considered as "complete protein" source in that it provides all essential amino acids for human nutrition. Forty eight rats included in this study were divided into six groups of eight rats each. Two groups of rats were normal and the other four groups were diabetics. Diabetes was induced by subcutaneous injection of Alloxan at a dose of 100-mg/kg body weight. Rats having blood glucose levels in a range of 104-170 mg/dl were diabetics. Two groups of rats were

made hypercholesterlemic' by oral administration powder cholesterol 1 % (1g/kg diet). Normal control; diabetic control and diabetic hypercholesterolemia group were given normal feed only. While the other was normal control. Diabetic control and diabetic hypercholesteroleinin groups were given normal feed and soy powder at a dose of "1.0g/d" as 'an effective dose' against alloxan-induced hyperglycemia. The experiment lasted for sixty days and blood samples (0.5 ml to 1.0 ml) were collected from coccygeal vein of rats on day, one, thirty and day, sixty of the experiment in heparinized tubes. Blood glucose level was measured using spectrophotometer by enzymatic kit at a wave length of 540nm. Glucometer was used to measure blood glucose levels or comparison with spectrophotometer readings. Experimental data was subjected to statistical applications according to the Steel & Torrie (1982). T-test was applied to compare mean as a paired sample test (before/after) and was considered statistically significant at  $P < 0.05$ . The results of this study revealed that the administration of soy protein powder showed hypoglycemic and hypocholesterolemic effects in diabetic rats. It was concluded that soy protein powder is helpful to lower cholesterol and-glucose levels in treatment of hyperglycemia and hypercholesterolemia.

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#### **BIOCHEMICAL EVALUATION OF THYROID DISORDERS IN URBAN AND RURAL POPULATION OF SINDH**

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An attempt was made in order to collect the information regarding the thyroid disorders like, Euthyroidism, Hyperthyroidism, and Hypothyroidism. The patients were interviewed at NIMRA (Nuclear institute of Medicine and Radiotherapy) Cancer Hospital, Jamshoro, a center of Pakistan Atomic Energy Commission (PAEC). Blood samples of the patients sought and processed for hormonal assay. Scanning for seeking thyroid size and shape abnormality was done. Ultra sonic studies - were made to get information about internal view, location, and nature of gland. Total 103 thyroid patients were examined between August 6th to September 23rd 2011, of these 57 patients diagnosed Euthyroidism, 25 Hyperthyroid, 21 Hypothyroids. The diagnosis was made as per parameters referred above. The studies reveal that maximum cases of Euthyroids were found in rural areas. Hypothyroid cases were also predominant in the rural areas. However, the cases of Hyperthyroidism were also noted but the prevalence was almost equal in Urban and Rural areas of Sindh.

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#### **OPTIMIZATION OF PROCESS PARAMETERS FOR THE PRODUCTION OF SINGLE CELL PROTEIN AS *CANDIDA UTILIS* BY SOLID STATE FERMENTATION**

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*Candida utilis* was used in the present study for the production of single cell protein (SCP) while wheat bran was used as substrate for the production of single cell protein incorporate with inorganic salts. The major objective of the present study was to optimize the process parameters of single cell protein. The optimized conditions found suitable for maximum production of SCP using wheat bran as a substrate were inoculum size 20% having maximum soluble protein  $2.92 \pm 0.17$

mg/100 ml and crude protein 58.33±1.21 g/100g, media pH 6.5 with maximum soluble protein 3.13±0.20 mg/100ml and crude protein 87.01±0.49g/100g, fermentation period of 96 hours with maximum soluble protein 3.22±0.255mg/100 ml and crude protein 60.91±4.41g/100g, molasses concentration 2.0% with maximum soluble protein 3.49±19mg/100 ml and crude protein 56.76±2.48g/100g and incubation temperature 30°C with maximum soluble protein 4.00±0.233 mg/100ml and crude protein 58.78±0.278g/100 g. Molasses and biotin enhance the production of SCP. The maximum production of SCP in the presence of cheaper substrate at room temperature make the strain as SCP useful source of protein.

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**EVALUATION OF BIOCHEMICAL ALTERATIONS IN SERUM UREA, SERUM CREATININE AND SERUM ELECTROLYTE (Na<sup>+</sup> AND K<sup>+</sup>) LEVELS IN DIABETIC PATIENTS WITH AND WITHOUT RENAL FAILURE**

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The present work was designed to investigate the evaluation of biochemical alterations in serum urea serum creatinine and serum electrolyte (Na<sup>+</sup>, K<sup>+</sup>) levels in diabetic patients with and without renal failure. Total 75 individuals were selected for the present study which were divided into three groups with 25 patients in each group. Group A was control group with normal individuals, Group B was of diabetic patients without renal failure and Group C was consisted of diabetic patients with renal failure. The blood samples were processed and analyzed for the estimation of serum electrolyte (Na<sup>+</sup>, K<sup>+</sup>), serum urea and serum creatinine levels. The data was statistically analyzed by applying ANOVA (Analysis of variance) and LSD (least significant difference). The nonsignificant difference was observed in potassium (K<sup>+</sup>) and sodium (Na<sup>+</sup>) levels between all the groups as P>0.05. A significantly elevated levels of serum urea and serum creatinine were obtained in diabetic patients with renal failure as compare to control group (p<0.05) however the change was in significant in diabetic patients without renal failure. Analysis of alteration of serum potassium (K<sup>+</sup>) and sodium (Na<sup>+</sup>) levels showed insignificant variations in both the case groups as compare to control group as P > 0.05. It was concluded from the results of present study that signs of diabetes are associated with significant alterations in serum urea and serum creatinine levels with renal failure however manifestation of diabetes mellitus has negligible effect on serum electrolyte levels in both the conditions with and without renal failure.

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**EVALUATION OF ALTERATION IN SERUM ALKALINE PHOSPHATASE, SERUM CALCIUM AND VITAMIN D LEVELS IN RHEUMATOID ARTHRITIS AND GOUT**

KALSOOM, WAQAR AZEEM, M.H.QAZI, MUSHTAQ A. SALEEM, RABAIL ALAM, MUHAMMAD AMIN AND AMIR SAEED

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The present study was conducted to investigate the metabolic alterations related to bone health in rheumatoid arthritis and gout patients. Total 150 subjects were selected for this purpose.

The individuals were divided into three groups of 50 individual in each. First group was a control group while second and third groups were comprised of rheumatoid arthritis and gout patients respectively. The drawn blood was completely processed and analyzed for serum vitamin D, alkaline phosphatase and calcium levels by using chemical reagent kits based on CMI, kinetic and endpoint methods. The results were interpreted on the basis of presence or absence of significant differences among the groups. The outcomes of the present study showed insignificant ( $p > 0.05$ ) alteration in vitamin D levels in rheumatoid arthritis and gout patients as compared to control group while due to metabolic changes significant alterations ( $p < 0.05$ ) in serum alkaline phosphatase and calcium levels were observed in rheumatoid arthritis and gout patients. No significant change in vitamin D levels was observed in both the disorders correlated with the fact that the 25 (OH) D is an inactive form of vitamin D and its active form (1, 25 (OH)<sub>2</sub> D) has more stronger impact on the pathophysiology of disease as compared to 25 (OH) D. Further investigation is needed to evaluate the role of 1, 25 (OH)<sub>2</sub> D and PTH in the manifestation of such disorders like rheumatoid arthritis and gout with the focus on metabolic changes in bones which are related with the disease pathophysiology.

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**DETERMINATION OF BIOCHEMICAL COMPOSITION OF BARLEY AND ESTIMATION OF EFFECT OF HIGH FIBER DIET ON HYPERCHOLESTEROLEMIC RATS**

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The present work was designed to investigate the biochemical composition of barley and to estimate the effect of high fiber diet on hypercholesterolemia. Proximate analysis was performed to determine the biochemical composition of barley in which moisture, ash, fat, fiber, fiber profile (cellulose and lignin) and protein contents were estimated. High fiber diet of barley was developed which was administered to albino rats (*Rattus Norvegicus*) to determine the cholesterol lowering effect of high fiber barley diet. High fiber diet of barley was mixed with normal chick starter diet of rats and was administered to hypercholesterolemic rats for the period of one month. Significant reduction in serum cholesterol level was observed in the rats which were administered with high fiber diet of barley. It was inferred from the outcomes that the high soluble fiber intake can be beneficial in lowering cholesterol level and in the prevention of cardio vascular diseases by reducing cholesterol levels in hypercholesterolemic conditions.

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**EFFECT OF NIGELLA SATIVA ( KALONJI) SEED OIL ON ALUMINIUM INDUCED HEPATOTOXICITY AND NEPHROTOXICITY IN RATS (*RATTUS NORVEGICUS*)**

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Aluminium exposure can result in aluminium accumulation in the liver and other organs. The metal can be toxic to the tissues at high concentration. The present study was therefore aimed

at to investigate salubrious effects of *Nigella sativa* to ameliorate aluminium toxicity in aluminium treated rats. 60 albino rats (*Rattus norvegicus*) were selected and were grouped into two groups; AIC<sub>h</sub> fed group and combine AIC<sub>h</sub> fed and *Nigella sativa* seed oil fed group. The aluminium toxicity was generated in rats by the administration of AIC<sub>h</sub> daily at the dose of 200mg/Kg body weight for the period of 30 days. After induction of aluminium hepatotoxicity and nephrotoxicity 1 ml seed oil of *Nigella sativa* was administered to each rat daily for the period of 60 days to evaluate the toxicity alleviating effect of seed oil of *Nigella sativa*. Serum AST (aspartate aminotransferase), ALP (Alkaline Phosphatase), ALT (Alanine transaminase) and serum urea, creatinine levels were estimated as indices of hepatotoxicity and nephrotoxicity respectively. Significant reduction in elevated levels of serum AST, ALP, AL T, urea and creatinine was observed in that group which was administered by *Nigella sativa* seed oil indicating reduction in aluminium induced hepatotoxicity and nephrotoxicity. In the present investigation seed oil of *Nigella sativa* was proved useful in mitigating the toxic effects of aluminium by improving the damage caused under aluminium induced hepatotoxicity and nephrotoxicity.

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#### ANTIOXIDANT ACTIVITY IN FIBROMYALGIA PATIENTS

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Fibromyalgia (FM) is characterized by chronic widespread pain in all four quadrants of body and the axial skeleton. To determine the role of oxidative stress in FM patients and to access the effect of vitamin E, vitamin C and *Nigella sativa* seeds in these patients also to look for reversal of oxidative stress after supplementation with antioxidant. Fifty female patients having mean age of 37.87±1.68 suffering from FM were enrolled in the study. Role of oxidative stress was determined by measuring antioxidant enzymes including Superoxide dismutase (SOD), Glutathione peroxidase (GPx), antioxidant capacity by ABTS (2,2'-azino-bis-(3-ethylbenzo-thiazoline-6-sulphonic diammonium salt) and FRAP (ferric reducing antioxidant capacity assay in plasma and catalase in erythrocytes. The FM patients were also supplemented with antioxidants (Vitamin C, Vitamin E and *Nigella sativa* seeds) for two months to assess the impact on activity of FM with the help of visual analogue scale (VAS) (0 being no pain and 100 being severe pain). It was found that FM patients have low activity of SOD, GPx and low antioxidant capacity (AOc) than healthy controls. It was further observed that after two months supplementation with antioxidants (vitamin C, vitamin E and *Nigella sativa* seeds) the level of SOD, GPx and AOC increased and their mean VAS 90.30±1.52 at baseline decreased to mean VAS of 77.80±1.65 after supplementation FM is associated with low antioxidant capacity and supplementation with antioxidants may help in minimizing the effects of the oxidative stress.

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#### STUDY ON BACTERIAL ISOLATES FOR AMINO ACID OVER PRODUCTION USING MUTANT BACTERIAL ISOLATES

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In the present study the project undertaken was aimed at the exploitation of locally isolated bacteria for the improvement in amino acid production using different mutant strains. For this

purpose 20 strains of amino acid producing bacteria were isolated from the soil samples which were collected from area of sugar factories near Lahore and pattoki. Of these 9 produced significant amount of glutamic acid, lysine, methionine, cysteine, valine, tyrosine, tryptophan and glycine. Majority of the microorganism have natural ability to convert complex organic compounds into amino acids. These isolates were grown on different fermentation media based on glucose, urea, molasses, and vitamins. Isolated strains were subjected to mutation to improve their ability to produce the desired amino acid and increase the yield. For this purpose the different mutagens used according to their mode of action, e.g. ethyl methane sulfonate, ethidium bromide, sodium azide and uv irradiation.

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#### **MORPHOLOGICAL AND MOLECULAR IDENTIFICATION BY DNA BARCODING OF GRASSHOPPER (ACRIDIDAE: ORTHOPTERA) FROM POONCH DIVISION**

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The study was conducted to remove the conflicts between species of acridid grasshopper both on the bases of morphology and at molecular level by DNA barcoding from Poonch division of Azad Jammu and Kashmir. For DNA barcoding tissue sampling was performed and processed for DNA extraction, PCR amplification of barcode region of COI, gel electrophoresis and then sequencing. Nucleotide data from the barcode region of COI supported the grasshopper identifications and phylogenetic relationships performed on the basis of morphological characters. In this study 26 species of family Acrididae were identified and subjected to DNA barcoding made comparisons with the nucleotide data among species and performed phylogenetic analysis. Amplification and sequencing of barcode region of the majority of specimens was performed at the Biodiversity Institute of Ontario. Out of 26 species barcoding results of only eighteen species were obtained. According to the morphological characters these are 17 species identified but barcoding of these species resulted in 18 species. Among them two of the specimens were identified as *Gastrimargus africanus africanus* but according to barcoding one specimen was different from *G. africanus africanus*. Similarly according to barcoding results two identified species *Patanga succincts* and *Patanga japonica* shown minute variation and lies on the same branch of tree as represented the same species.

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#### **EVALUATION OF BIOCHEMICAL ALTERATIONS IN SERUM ELECTROLYTE (Na<sup>+</sup> AND K<sup>+</sup>), TOTAL PROTEIN AND LIPID PROFILE IN HYPERTHYROIDISM AND HYPOTHYROIDISM**

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The present study was conducted to investigate the evaluation of biochemical alterations in serum electrolyte (Na<sup>+</sup>, K<sup>+</sup>), total protein (TP) and lipid profile levels in hypothyroidism and hyperthyroidism. Total 90 individuals were selected which were divided into three groups as control group, hypothyroid group and hyperthyroid group. Each group was comprised of 30 individuals. Blood samples were analyzed for the estimation of serum electrolyte (Na<sup>+</sup>, K<sup>+</sup>), serum

total protein and lipid profile levels. The data was statistically analyzed by applying ANOVA (Analysis of variance) and LSD (least significant difference). Insignificant difference in serum sodium (Na<sup>+</sup>) levels was observed in subjects with hyperthyroid as compared to control as  $p > 0.05$ . Significantly elevated amount of serum sodium (Na<sup>+</sup>) was estimated in subjects with hypothyroid as compared to control group and hyperthyroid group as  $p < 0.05$ . While insignificant difference was observed in potassium (K<sup>+</sup>) level among all the groups in comparison with each other and with control as well as  $p > 0.05$ . For serum total protein level significant difference was observed in hypo and hyperthyroid groups as compared to control group. While in comparison to hypo vs hyperthyroid groups significant difference was recorded. Significant alteration ( $p < 0.05$ ) in all the parameters of serum lipid profile was observed in both types of disorders except serum HDL-C and TG. It was found that both the disorders significantly alters all the basic metabolic entities (serum Na<sup>+</sup>, TP, TC, LDL-C) effectively while having less effect on serum K<sup>+</sup>, HDL-C and TG levels in hyperthyroidism. It was inferred from the outcomes of present study that the manifestations of hypothyroidism and hyperthyroidism have overall profound effect on the metabolism of basic metabolic entities. Although both type of disorder significantly alter and imbalance the basic metabolic units of the body however hypothyroidism is more effective in altering the metabolism of these units as compared to hyperthyroidism.

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#### DETECTION OF DENGUE VIRUSES IN *Aedes* MOSQUITOES FROM DIFFERENT LOCALITIES OF LAHORE

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Dengue viruses are spread through the bites of female *Aedes* mosquitoes to human mostly in urban areas of tropical/sub-tropical countries. Dengue epidemics is annually occurring in Pakistan since 2006. Recently in 2011 dengue became severe epidemic in province Punjab, where >15000 positive cases and >7300 deaths occurred, especially in the highly populated urban city of Lahore. With neither vaccine nor proper treatment for dengue, prevention of the disease depends upon the surveillance and early diagnosis/detection of dengue virus antigens from mosquito vectors which will serve as early warning system for forecasting impending outbreaks. In current study 28 entomological surveys were carried out in various localities of Lahore from March-September, 2011 for the collection of *Aedes* mosquitoes. Two species *Aedes aegypti* and *Aedes albopictus* were found common during this period. However, *Aedes aegypti* were present throughout these months while *Aedes albopictus* appeared in the months of July-August, 2011. In addition various types of natural and artificial breeding containers were also observed for immature stages of *Aedes* mosquitoes in all localities visited during above mentioned period. The most productive containers were automobiles used tyres for larval production with 94% positivity. Collected mosquitoes were screened for dengue viruses using dengue specific monoclonal antibodies (MAB) as antigen capture Enzyme Linked Immunosorbent Assays (ELISAs). Of the 114 pools of *Aedes aegypti* females (n=570) screened, 31 pools were found positive for dengue viruses indicating 27.19% infection rate (MIR). However, of the 04 pools of *Aedes albopictus* females (n=20) screened; only 1 pool was found positive with 25% infection rate (MIR). This is the first report of DENY detection from adult females of *Ae. aegypti* and *Ae. albopictus* collected from different localities of Lahore, Pakistan

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**BLINDNESS AND VISUAL IMPAIRMENT IN RETINITIS PIGMENTOSA: A  
PAKISTANI HOSPITAL-BASED STUDY**

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Present retrospective, analytical study was performed between December 2010 and May 2011 on all retinitis pigmentosa cases seen from Lahore during ophthalmologic consultation at the Layton Rahmatullah Benevolent trust '(LRBT) hospital from the duration of Aug 2008-Jan 2011. The aim of this research was to determine the significance of visual disability associated with retinitis pigmentosa in Punjab province of Pakistan. The techniques used for the diagnosis of retinitis pigmentosa were an examination of ocular fundus using a biomicroscope (fundoscopy) and far visual acuity. Indeed, a drop in visual acuity and blindness were the main reasons for consultation in this study. 500 cases of retinitis pigmentosa (RP) were identified from different areas of Punjab corresponding to hospital prevalence of 1.1/100. The retinitis pigmentosa (RP) was studied in 142 women and 358 men. The average age was 25.56 years in RP patients. Retinitis pigmentosa was bilateral in all cases and isolated in 68.20% of cases. Visual disability is very common in retinitis pigmentosa.

**DETERMINATION OF HEAVY METALS' CONCENTRATION IN FODDER,  
VEGETABLE AND FRUIT IRRIGATED BY HUDIARA DRAIN IN DISTRICT LAHORE**

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The aim of this study was to investigate the occurrence of heavy metals in fodder, vegetable and fruit irrigated by Hudiara drain. So a survey was conducted along the whole length of Hudiara drain (55 Km) to assess the heavy metals accumulation in producers. Different samples of fodder, vegetable and fruit were collected at three sites (Lallo, Mohlanwall and Khurdpur in Distt. Lahore) from the agricultural fields that are being permanently irrigated by Hudiara drain. Significantly high concentrations of heavy metals like Na, Mg, Al, K, Ca, Ti, Cr, Mn, Fe, Ni and Zn were detected in all samples of fodder, vegetable and fruit. The heavy metal concentration shows a significant variation, when compared to permissible international standards. The concentration of heavy metals (Na, Mg, Al, K, Ca, Ti, Cr, Mn, Fe, Ni and Zn) at Lallo was (2724 ppm, 1292 ppm, 959 ppm, 5601 ppm, 2338 ppm, 0, 0, 255 ppm, 0, 0,) in fodder; (3053 ppm, 2668 ppm, 1152 ppm, 35214 ppm, 13826 ppm, 0, 0, 0, 54 ppm, 16 ppm) in vegetable and (1448 ppm, 1255 ppm, 927 ppm, 33362 ppm, 836 ppm, 0, 0, 0, 103 ppm, 0, 106 ppm) in fruit respectively. The concentration of heavy metals (Na, Mg, Al, K, Ca, Ti, Cr, Mn, Fe, Ni, and Zn) at Mohlanwall was (22610 ppm, 6230 ppm, 3493 ppm, 42911 ppm, 14212 ppm, 0, 0, 0, 1202 ppm, 107 ppm, 0) in fodder; (16940 ppm, 10826 ppm, 4740 ppm, 104642 ppm, 49585 ppm, 0, 0, 168 ppm, 470 ppm, 0, 169 ppm) in vegetable and (4413 ppm, 5050 ppm, 1502 ppm, 63124 ppm, 21287 ppm, 0, 0, 0, 264 ppm, 92 ppm, 120 ppm) in fruit respectively. The concentration of heavy metals (Na, Mg, Al, K, Ca, Ti, Cr, Mn, Fe, Ni and Zn) at Khurdpur was (40255 ppm, 16133 ppm, 67967 ppm, 102366 ppm, 64306 ppm, 483 ppm, 0, 0, 3729 ppm, 355 ppm, 0) in fodder; (25024 ppm, 16158 ppm, 27201 ppm, 112038 ppm, 110262 ppm, 0, 0, 244 ppm, 1614 ppm, 0, 179 ppm) in vegetable

and (32151 ppm, 22996 ppm, 16239 ppm, 37820 ppm, 1210 ppm, 241 ppm, 240 ppm, 3630 ppm, 0, 145 ppm) in fruit respectively. The concentration of heavy metals tends to increase from first to third site gradually. It is concluded that Hudhara drain is highly polluted by the addition of untreated industrial effluents and city sewage and heavy metals tend to accumulate significantly in fodders, vegetables and fruits irrigated by Hudhara drain, these results are signals of threat to the entire ecosystem including human population which can receive these pollutants directly or indirectly through food chain. Still the consequences may be wide spread as the drain dumps its polluted water in Ravi River that irrigates lot of agricultural land in the province of Punjab.

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### GENETICS OF LUNG CANCER

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Lung cancer is the leading cause of cancer-related death in both males and females' world wide. Only 13% of lung cancer patients live more than 5 years. Clinically, lung cancer can be divided into 2 groups: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Approximately 75% of lung tumors are NSCLC, which consists of squamous cell carcinoma (SCC), adenocarcinoma (ADC) and large cell carcinoma (LCC). Lung cancer development involves both environmental and genetic factors. Current treatment options consist of surgical resection, platinum-based doublet chemotherapy (a combination of two drugs that includes carboplatin or cisplatin as the backbone), and radiation therapy alone or in combination. Unfortunately, despite these therapies, the disease is rarely curable and diagnosis is poor, with an overall 5-year survival rate of only 15%.

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### INSULIN RESISTANCE STUDY IN DIABETES MELLITUS TYPE II AND ITS CORRELATION WITH OBESITY

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IR is considered to be the main underlying cause in 70-80% type II diabetics. Development of IR being a slow and gradual process, begins with excess weight gain, years before the occurrence of overt diabetes. About 80% of the type II diabetics are obese. The present study was thus intended to evaluate the association of IR with DM II (its major outcome) and obesity (its main causative factor) in Pakistani diaspora. It is a case control study. For the purpose the subjects were taken from both genders, above the age of 30 years and classified into diabetic and non-diabetic groups, further subdivided on basis of BMI into those having BMI  $\geq 25$  (overweight and obese) and  $< 25$  (normal weight). For certain comparisons among different parameters, further division of overweight/obese group was done as overweight subjects with BMI 25-29.9 and obese subjects with BMI  $\geq 30$ , and the diabetics into 2 groups, as diabetics on oral medications and diabetics on insulin. The individuals were classified as IRS and non-IRS on the basis of clinically easily applicable parameters as described by NCEP (2001) definition for IR. If 3 or more of these factors were positive, the case was considered to be clinically insulin resistant: Waist circumference:  $\sim 80$ cm (in females),  $\sim 90$ cm (in males), Blood Pressure:  $\sim 130/85$  mmHg, or on antihypertensive

treatment; Fasting Blood Sugar: 110 mg/dl, or on antidiabetic treatment. Triglycerides:  $\geq 150$ mg/d, or on antilipemic therapy; HDL: <50mg/dl (in females), <40mg/dl (in males), or on antilipemic therapy. The associations between IRS, DM II, obesity, IR parameters, and various relevant history, physiological and biochemical parameters that influence the occurrence of IR in a person were done step wise, and the credibility of IR parameters (FIL, HOMA, QUICKI) and NCEP criteria for evaluating IRS assessed. In the end the risk indicators for IRS were identified. First the associations of bM II with IRS, obesity, IR parameters and relevant history, physiological and biochemical parameters were found and assessed. Secondly, the associations of IRS with obesity, IR parameters and relevant history, physiological and biochemical parameters were found and assessed. Thirdly, the associations of obesity with IR parameters and relevant history, physiological and biochemical parameters were found and assessed. Fourthly, the associations of various relevant history, physiological and biochemical parameters were found

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### **STRATEGY ON THE BINDING OF Bcl2 FAMILY PROTEINS WITH CURCUMIN AND ITS ROLE IN APOPTOSIS IN HELLA CELLS**

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The members of Bcl2 family include both apoptotic and antiapoptotic proteins. Bcl2 and Bcl-xl are known as anti apoptotic proteins. Several studies are in progress to develop such therapeutic agents which can inhibit the activity of Bcl2 and Bcl-xl. The network compounds used so far include curcumin and silibinin. It has been shown that curcumin inhibits both Bcl2 and Bcl-xl. This results in the activation of proapoptotic Bax and Bak. Some studies are shown that curcumin induces apoptosis by inhibiting the proliferative pathways involving AKT and proapoptotic pathways. The activation of later pathway causes oligomerization of Bax on the mitochondrial membrane. The present studies were undertaken to study in detail the binding affinity of curcumin with Bcl2, Bcl-xl, Bax and Bak. In order to find out the efficacy of curcumin as inhibitor of Bcl2 and Bcl-xl. We have shown that curcumin binds both to Bcl2 and Bcl-xl in the hydrophobic of these proteins bordered by BRI, BID and BH3 domains. However, the affinity of curcumin for Bcl2 (-6.2) is higher than Bcl-xl (-5.9). Further curcumin binds all the three domains of Bcl2 in the hydrophobic pocket. The stability of binding of curcumin is documented for the first time in this study. This is related to the formation of a stable bond of curcumin with aspartate III of Bcl2 protein. No such bond is formed with any protein studied (Bcl-xl, Bax and Bak). The binding site of curcumin in the case of Bax is restricted to BRI and BID whereas in the case of Bak curcumin binds to BHI, BH2 and BH3 of the molecule. The binding studies have to some extent fortified with experimental studies using curcumin treated hela cells. The blotting of proteins such as Bcl2 and p53 from the cytosol treated with various dose of curcumin show a decrease of Bcl2 and an increase of p53. These data are supported by microarray data of cells treated with 100 $\mu$ M of curcumin. From the binding studies of curcumin with proapoptotic and antiapoptotic proteins it has been concluded that curcumin is a strong inhibitor of antiapoptotic Bcl2. Thus showing that it acts through the mitochondrial apoptotic pathway. From rise in p53, shown for the first time in this study indicate that curcumin may also be acting through the death receptors DR4 and DR5. The binding studies of curcumin with Bcl2, Bcl-xl, Bax and Bak are being reported for the first time.

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**SIGNIFICANCE OF LDL-CHOLESTEROL, HDL-CHOLESTEROL, FT3, FT4 AND TSH ON OVERT HYPOTHYROIDISM**

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This research was conducted to estimate the significance of FT3, FT4, TSH, LDL-C and HDL-C levels in hypothyroidism. One hundred hypothyroid patients were enrolled for study. Twenty normal, subjects taken as control for comparison. Hypothyroidism is defined as TSH level higher than normal (0.045-4.05 m U/l), usually more than 05 m IU/L along with free T4 level below the lower limit of reference range. Primary hypothyroidism is due to insufficient function of thyroid tissue or impaired synthesis of thyroid hormones. Secondary hypothyroidism or central hypothyroidism is defined as deficient function of thyroid gland due to inadequate stimulation by TSH. This may be due to production of either insufficient or ineffective. TSH from a number of congenital or acquired pituitary and hypothalamic disorders. The lipoproteins are typically spherical in shape. The size ranges from 10-1200 nm. The lipoproteins are composed of both lipids and proteins called apolipoprotein. HDL-cholesterol and LDL-cholesterol are two types of lipoproteins that carry the water insoluble lipids in the blood. The cholesterol which may come from diet or be synthesized in the body is carried through these lipoproteins. The HDL is smallest and dense lipoprotein particle. It is synthesized by both the liver and intestine. The objectives of the study were to evaluate the significance of HDL-C, LDL-C, FT4, FT3 and TSH in hypothyroidism. Also to evaluate the correlation of TSH to LDL-C & HDL-C in hypothyroid patients. In present study the patients of hypothyroidism diagnosed on the basis of raised TSH level (  $39.198 \pm 14.732$  mIU/L ) and lower FT4 (  $3.841 \pm 3.565$  pmol/L ) and FT3 (  $1.298 \pm 0.632$  pmol/L ). The significantly (  $P < 0.05$  ) higher LDL cholesterol level (  $160.50 \pm 24.748$  mg/dl ) were found in patient of hypothyroidism as compared to euthyroid control subjects (  $104.70 \pm 10.332$  ). The HDL-C level insignificantly increased in hypothyroid patients as compared to normal control subjects. (  $40.08 \pm 3.897$  ). The higher LDL-C is associated with increased risk of atherosclerotic coronary artery disease. Therefore, it may considered risk factor and indicator of atherosclerotic cardiac disease which is the clinical significance of our study.

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**PRODUCTION OF ALKALINE PROTEASES BY *BACILLUS* SPECIES**

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Protease producing strain was isolated from the soil near the municipal waste stream water of Lahore by using mineral salt medium, nutrient agar and skim.tned milk medium. The isolated strain was then characterized morphologically and biochemically as *Bacillus* species. The media was than optimized for the selected strain as a factor of mannitol, peptone, NaNO<sub>3</sub>, ZnSO<sub>4</sub>, MgSO<sub>4</sub> and NiCl<sub>2</sub> against different concentration. The mannitol, NaNO<sub>3</sub> and peptone were taken with initial concentrations of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 g/d. The ZnSO<sub>4</sub>, Mg SO<sub>4</sub> and NiCl<sub>2</sub> were taken with initial concentrations of 1.0, 1.5, 2.0 and 3.0 g/d. The results showed that the increases in concentrations of all the factors have direct effect on the growth of *Bacillus* species and on protease production. All the factors except NaNO<sub>3</sub> showed maximum growth found protease production after 120 hours of incubation at 37°C except for NaNO<sub>3</sub> which showed maximum growth and protease production after 96 hours of incubation at 37°C.

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**CORRELATION OF GESTATION LIPID PROFILE WITH NEONATAL BIRTH WEIGHT IN PAKISTAN**

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Published data is limited to the estimation of lipid profile, in the 2nd and 3rd trimester of pregnancy and its relationship to neonatal birth weight. While the objective of the present study was to determine the levels of lipid profile in each trimester and investigate the correlation of maternal lipid profile with neonatal birth weight. The whole work was conducted in the social security hospital, Multan Road Lahore. Total 100 pregnant women were included in the study, all women were enrolled in the 1st trimester of pregnancy. Total Cholesterol(TC), Triglycerides (TGs), High density lipoprotein(HDL), and low density lipoprotein (LDL) levels estimation was done in each trimester, and each variable was correlated with neonatal birth weight. From this study we were able to conclude that TC, TGs and LDL levels are increased during the successive periods of pregnancy. While HDL remained the same throughout pregnancy. TC, TGs and LDL had strong relationship with neonatal birth weight, while HDL had weak relationship or effect on neonatal birth weight. The whole work reflected that correlation was present between TC, TGs, HDL and LDL with Neonatal birth weight. If they are increased during the pregnancy the neonatal birth weight will be increased.

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**PROGNOSTIC AND DIAGNOSTIC SIGNIFICANCE OF SERUM ASPARTATE, ALANINE . AMINOTRANSFERASE AND ALKALINE PHOSPHATASE IN DIFFERENT CANCERS DURING PRE, MID AND POST RADIOTHERAPY**

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This research was conducted to estimate the significance of serum AST, ALT and ALP as biochemical markers in breast, lung, prostate and cervical cancers during pre, mid and post radiotherapy. One hundred of cancer patients were selected and twenty were taken as control. Only confirmed cancer cases with no other chronic disease such as myocardial infarction or diabetes were included in this study irrespective of age and sex. The serum AST, ALT and ALP levels were determined by using commercially available enzymatic kits through photometer. The present study described that levels of AST, ALT and ALP were significantly higher among cancer patients (breast, prostate, lung and cervix) as compared to control. The significant higher mean AST and ALT level during pre-radiotherapy was  $34.05 \pm 6.8$  and  $34.8 \pm 6.8$  respectively in patients of lung cancer as compared to the control. The significant higher mean ALP level during pre-radiotherapy was  $250.80 \pm 92.8$  in patients of prostate cancer as compared to the control. Statistical evaluation also indicated significantly higher differences ( $p < 0.05$ ) in AST, ALT and ALP levels among groups during mid and post-radiotherapy. When the radiotherapy progressed the levels of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase show significant decrease ( $p < 0.05$ ) and approach normal levels among groups of different cancers indicating their significance as prognostic marker.

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**PREVALENCE OF VARIOUS TYPES OF CANCERS IN INDUSTRIAL POPULATION WITH SELECTIVE HISTOPATHOLOGY AND SERUM BIOCHEMISTRY**

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The workers working in different industries are exposed to various chemicals at their work place. Most of these chemical substances act as carcinogens and contribute to etiology of cancer in factory workers. These workers need special preventive measures at their work place to avoid or minimize the exposure to carcinogens. But such measures are not observed up to recommended standards in developing countries. This results in increased incidence of different types of cancers in industrial workers: Exposure to carcinogenic compounds has been noticed more in textile/cotton industry, mechanical manufacturing units, leather/tanneries works, furniture/wood works and rubber/pipe industries. Exposure is not limited to these trades. Exposure have also been noticed in dairy, pharmaceutical and ceramics industries. Even those people who are not working in factories but in other places like hospitals are equally exposed to carcinogens, especially who are exposed to radiation, such as radiographers, and they also suffer from malignancies. In present study, we found lung cancer as largest cancer in industrial population (21 out of 100). Lymphoma (19 out of 100) and sarcoma (14 out of 100) were next to occur. Ca breast remained the major cancer in women (13 out of 35 women). This was seen nearly in all ages. Ca prostate, and Ca liver/gallbladder were also observed in noticeable numbers. Other cancers such as Ca ovary, Ca colon, Ca testis, Multiple myeloma, leukemia, Ca parotid, Ca larynx etc were also recorded but to a lesser extent. Effects of these cancers were observed on liver and kidney functioning. For this purpose, serum Bilirubin and liver enzymes, AST and AL T, of all patients were done for liver functions. Blood urea and serum creatinine were estimated for assessment of kidney function.

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**SIGNIFICANCE OF SERUM TRIGLYCERIDE CHOLESTEROL, TSH, FT3, AND FT4 IN SUBCLINICAL HYPOTHYROIDISM**

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This research was conducted to estimate the significance of cholesterol and triglyceride levels in subclinical hypothyroidism. Subclinical hypothyroidism is defined as increased thyroid Stimulating hormone (TSH) levels from normal reference range but free thyroxin (FT 4) levels and triiodothyronine (T 3) levels are within normal range (Surks *et al.*, 2004 ). Subclinical hypothyroidism is also defined with serum TSH concentration above the clinically defined limits of the reference range when serum free T 4 (FT 4) concentration is within its reference range. The normal reference range for thyroid stimulating hormone (TSH) is in between 0.45mIU/L to 4.12mIU/L. The reference range varied with age, sex and ethnic groups but as the differences were small, there was no need to adjust the reference values. The reference range of normal serum TSH concentration was 0.45mIU to 4.5mIU/L and FT4 concentration is 10.3 -25.7 P moUL (Surkset *al.*, 2004). In patients having few or no definite clinical signs or symptoms of hypothyroidism with subclinical form, the subclinical hypothyroidism is based on laboratory diagnosis (Spencer *et al.* 1996). Objectives of this study were to assess the significance of serum cholesterol, triglyceride in subclinical hypothyroidism diagnosed by estimation of FT3, FT4 and TSH levels and also to find correlation of serum cholesterol and triglycerides with level of TSH in SCH. One hundred

subclinical hypothyroid patients were enrolled in our study. Twenty normal subjects were also taken as control for comparison. In present study the patients of subclinical hypothyroidism showed significantly higher TSH level ( $10.595 \pm 6.270$  mIU/L) than euthyroid subjects and normal FT4 ( $14.544 \pm 17.214$  pmol/L) and FT3 ( $3.267 \pm 0.593$  pmol/L) levels. Significantly ( $P < 0.05$ ) higher cholesterol levels ( $222.11 \pm 28.968$ ) were found in patients of subclinical hypothyroidism and lower cholesterol level observed in euthyroid control subjects ( $140.30 \pm 15.598$ )

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**MATURATIONAL STUDIES OF OOCYTE AND PLASMA CONCENTRATION OF CHOLESTEROL DURING SPAWNING SEASON IN *LABEO ROHITA***

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*Labeo rohita*, a commercial fish collected from fish hatchery. An attempt was made to understand the pattern of development of the oocyte during the spawning season. Vitellogenesis starts in the month of May and reached to the peak up to early July followed by the final oocyte maturation (FOM). The oocyte membrane begins to disintegrate after final oocyte maturation (FOM) and then oocyte starts migrating towards the periphery of the follicle. Migration of oocyte takes 3 to 7 days and then fish spawn. After spawning the ovary contains the oocytes of primary and secondary level including the residual follicles that become atretic. Circulating level of cholesterol increases abruptly when the ovaries were gravid and the oocytes was migrating. The level remained dropped in the spent fish.

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**EVALUATION OF *GLIRICIDIA SEPUM* FOR ITS TOXICITY AGAINST FIELD RATS**

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Rodent pests are a great threat globally to field crops, stored grains, buildings, insulations, electric wirings, wood etc. Rats eat and contaminate stored grains with their droppings resulting in more than 45 diseases to human and live stock. Most of the rodenticides used for their management may be toxic for human, livestock and other non-targeted animals. There is a need to discover natural plant products for rodent management, feasible for traditional farming system. *Gliricidia sepum* [family: *Fabaceae* (Chadhokar, 1982)] is a leguminous, fast-growing, easily propagated, nitrogen-fixing tree used throughout the tropics for many purposes such as, for fence, shade tree, fodder, fuel wood, green manure, animal feed etc. *Gliricidia* can be used as poisonous bait for rodents by mixing with grains. Poison is derived from its roots, bark and leaves. To evaluate toxicological effects of *Gliricidia sepum* on field rats, laboratory study is being carried out, aimed at, to develop safe, economical and environment friendly bio-rodenticides. *Gliricidia sepum* leaves were collected from Coastal Agricultural Research Centre, SARC, PARC, Karachi. Ethanolic extraction of the leaves was carried out by PCSIR Laboratories. The extract was tested in different doses against the field rats, *Bandicota bengalensis*, trapped from agricultural fields of Thatta. Rats were acclimatised in live animal laboratory. After acclimation, mean weight recorded was  $205.48 \pm 0.92$  g for male, whereas  $199.56 \pm 1.20$  g for female rats. *Gliricidia* bait was formulated by mixing *Gliricidia* extract (5 g A.C., 10 g A.C. and 20 g A.C.) in wheat flour and broken rice. All the

formulations were offered to 10 field rats, *Bandicota bengalensis* (5 male, 5 female) for 5 days. Among these formulations, 10 g A.C. *Gliricidia* gave promising results. After 5 days feeding all rats became sluggish/sick, whereas on sixth day and on eight day two rats died. Autopsy examination revealed internal bleeding, which may confirm the presence of anticoagulant compounds in *Gliricidia*. Similarly, *Gliricidia sepium* leaves (crushed and fermented) bait was prepared by following the same procedure and given in 2%, 4%, 8% and 16% concentration to 10 field rats, *Bandicota bengalensis* (5 male, 5 female) for five days. Mean intake (2%) was  $12.25 \pm 1.02$  g for male whereas  $9.80 \pm 1.90$  g for female rats. Mean intake (4%) was  $10.25 \pm 0.82$  g for male whereas  $11.80 \pm 2.20$  g for female rats. Mean intake (8%) was  $8.60 \pm 0.56$  g for male whereas  $7.24 \pm 2.40$  g for female rats. Mean intake (16%) was  $4.90 \pm 1.63$  g for male whereas  $6.20 \pm 1.56$  g for female rats. Mean intake (control) was  $14.25 \pm 1.00$  g for male whereas  $13.40 \pm 1.50$  g for female rats. No rat was died in these trials, however, it seems, *Gliricidia* served as repellent in these trials or this may indicate the requirement for high doses with some additives in bait to attract the rats. These preliminary investigations may be useful in further studies on the role of *Gliricidia sepium* in rodent management.

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#### SCREENING OF PECTIN DEGRADING BACTERIA FROM LOCAL ENVIRONMENT: ISOLATION AND CHARACTERIZATION

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Plants contain large amount and variety of structural polymers which are required for support and protection. Among these polymers very significant proportions belongs to structural polysaccharides like cellulose, hemicelluloses and pectins etc., which are composed of simple sugars. These structural units are the raw material which can be reused or upgraded into high quality products. Enzymes play a key role in degradation of these complex polymers into reusable simple monomer. Micro-organisms are exploited for production of wide variety of required enzymes, which are currently used in industry, medicine, food technology and in several other ways. Pectinases break down pectic polysaccharides of plant tissues are a group of industrially important enzymes and have potential applications in fruit, paper, textile, coffee and tea fermentation industries. In present study screening, isolation and characterization of the pectin degrading microorganisms from the local environment was carried out to evaluate their activity qualitatively and quantitatively. The thirty four bacterial isolates were collected from rotten fruit and vegetable, water and soil samples of different localities of Lahore, Pakistan. They were screened for their pectinolytic activity on citrus pectin containing medium at pH 7. Thirteen isolates were found to possess pectin degrading activity. Pectinase activity of all thirteen isolates was evaluated qualitatively by measuring area of hydrolysis zones of pectin which were in the range of 1.33-6.96cm<sup>2</sup>. On the basis of maximum enzyme production zone (6.96 and 5.19 cm<sup>2</sup>), two isolates (BCTL-SL-154, BCTL-FL-09) were selected for further characterization. Pectinase production by these two isolates was also carried out quantitatively. The isolate BCTL-SL-154 showed 79.30 ug/ml/min while BCTL-FL-09 had 67.30 ug/ml/min pectinase activity. These isolates were further optimized for growth conditions. Bacterial isolates showed optimum growth at 35°C. The optimum pH for BCTL-SL-154 was 5.5 while BCTL-FL-09 showed optimum growth at pH 7.0. After characterization, bacterial strains (BCTL-SL-154 and BCTL-FL-09) were identified as *Bacillus cereus* and *Bacillus macerans* respectively.

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**A RAPID DNA EXTRACTION METHOD FOR *BACILLUS THURINGIENSIS***FAIZA SALEEM<sup>1,2</sup> AND ABDUL RAUF SHAKOORI<sup>1</sup><sup>1</sup> *School of Biological Sciences, University of the Punjab, Lahore*<sup>2</sup> *Department of Biotechnology and Microbiology, Lahore College for Women University, Lahore.*

The Polymerase Chain Reaction (PCR) is commonly used to detect and identify bacteria from various sample types for a variety of reasons. Prior to performing PCR, DNA must be extracted efficiently from samples. An optimal extraction procedure will efficiently extract DNA from any micro-organism present in the sample whilst at the same time removing any protein, compound or chemical which may subsequently inhibit the PCR. The DNA extraction method must be simple, quick and efficient. Safety, cost and DNA quality must also be considered. DNA quality is critical because the efficiency of PCR amplification can be reduced by inhibitors from the matrix. *Bacillus thuringiensis* produces endospores that resist the lysis by usual methods used for other bacteria. SDS - NaOH is mostly used in lysis buffer along with incubations for lengthy time periods. Here we devise a rapid method for DNA extraction from *Bacillus thuringiensis* using tritonX-100 in lysis buffer. After incubation at 60C for 30 min, the lysate is phenol-chloroform extracted. DNA was precipitated with absolute Ethanol, washed with 70% ethanol and dissolved in TE. The entire method did not take than 1 hour. The DNA extracted by this method was utilized in enzymatic reactions including PCR, restriction analysis and produced good results. This method can also be used for DNA extraction from other species including Gram negative as well as Gram positive isolates.

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**ROLE OF BACTERIAL MANNANASE IN BIODEGRADATION OF WASTE:  
SCREENING, ISOLATION AND CHARACTERIZATION**SYED SHAHID ALI, SAMINA SANAULLAH, FOUZIA TABSSUM AND TANZEELA RIAZ  
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Microbial mannanase have become biotechnologically important since they target the hydrolysis of complex polysaccharides like hemicelluloses, mannanase, galactomannans etc., into simple reducing sugars and play vital role in the biodegradation and bioremediation of fruit and vegetable wastes, low cost plant biomass and toxic organic pollutants. The role of mannanase in the paper and pulp industry is well established and recently its application in the food and feed technology, coffee extraction and detergent industry has been reported. For isolation and initial screening of the bacteria for  $\beta$ -1,4-mannanase activity, the samples were collected from soil, water, decaying fruits and vegetables. Screening for enzyme production potential of bacteria was carried out on mineral salt: agar medium containing Locust bean gum (LBG), (galactomannan) as a carbon source as well as substrate for enzyme. Isolated bacteria were screened qualitatively by iodine test for  $\beta$ -1,4-mannanase activity. The isolates which showed maximum zone of hydrolysis on the agar plates following staining were selected for further studies. Thirteen bacterial isolates out of fifty two showed significant enzyme activity (Hydrolysis zone) whose diameter was in the range of 1.5-2.4 cm. Eight out of thirteen isolates possess enzyme production potential. Two bacterial isolates BCTL-FL20 showed maximum biodegradation with protection of 562 and 596mg reducing sugar/dl of the medium at 48 and 72 hours incubation respectively, while BCTL-SL188 produced 60mg/dl sugar which correspond to  $1191.00 \pm 48.6$  and  $119.00 \pm 6.53$   $\mu\text{g/ml/min}$ , respectively ( $n=3$ ).

Growth conditions of these isolates were further optimized for temperature and pH. Both isolates showed maximum growth at 39°C and pH 9. On the basis of morphological and biochemical characteristics BCTL-FL20 and BCTL-SL188 were identified as *Bacillus badius* and *Bacillus cereus* respectively. In conclusion, this study revealed that a number of bacteria in our natural habitat possess enormous potential to degrade agro-based industrial wastes and residual plant biomass by producing extracellular enzymes. They can also play important role in upgradation of wastes and to reduce environmental pollution problem posed by the agro-industrial and domestic residual plant wastes.

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#### IDENTIFICATION OF *CRY2AA* BINDING PROTEINS IN *HELICOVERPA ZEA* USING PROTEOMICS

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*Bacillus thuringiensis* (*Bt.*) is gram positive bacterium which forms endospores and produces parasporal crystals. These parasporal crystals are solubilized in the midgut of insects at alkaline pH and converted to active toxins by midgut proteases. This activated toxin then binds with the receptors (mainly known receptors are alkaline phosphatase (ALP), aminopeptidase N (APN) and cadherin-like) in the brush border membrane vesicles (BBMV) of the midgut microvilli and forms the pores in the membrane of midgut epithelial cells. When pores are formed then water enters the epithelial cells by osmosis and lyse the cells by osmotic pressure, when epithelial cells are lysed then gut is disrupted, larvae stop feeding and die due to septicemia. We have mainly focused on purification of proteins and finding some new receptors/binding proteins in BBMVs from *H. Zea* with *cry2Aa*. *cry2Aa* protein was purified by FPLC from *Bt.* strain known to produce it. Western blotting, ligand blotting and alkaline Phosphatase (ALP) assay was performed to identify binding-proteins/receptors. Multiple bands (30-240KDa) were observed in ligand blots and western blots while isolated bands were seen in ALP assays (68-240KDa). From LC-MS/MS data we identified new proteins, which are possibly important in the mechanism of action of cry proteins.

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#### BIOFILM FORMATION BY *PHOTOBACTERIUM* UNDER CADMIUM STRESS

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Biofilms can be defined as communities of microorganisms attached to a surface. Biofilm producing bacteria mostly live within a matrix of extracellular polysaccharide (EPS). Exopolysaccharides (EPS) are high molecular weight polymers which are long chain composed of sugar residues and secreted by microorganisms into the surrounding environment. *Photobacterium* strains were isolated from marine fish Indian mackerel. Minimum inhibitory concentration (MIC) of these strains was observed to be 800µg/ml. Bioluminescence of these strains were determined in the presence and absence of cadmium. A thick white amorphous EPS matrix was seen surrounding

bacteria, indicating a biofilm but not all *Photobacterium* were biofilm formers. It was concluded that EPS production was higher in EPS medium in the absence of cadmium stress. After carbohydrate and protein estimation of EPS sample, it was noted that carbohydrate percentage was higher as compared to protein in EPS samples.

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**GENETIC ANALYSIS OF HUMAN GROWTH HORMONE GENE REVEALS SEVERAL MUTATIONS IN THE EXON 5 OF LOCAL POPULATION IN PAKISTAN**

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Human pituitary growth hormone (GH-N) consists of a primary transcript of 1632 bp, having five exons separated by four introns. GH-N encodes for a mature peptide of 217 amino acids having a signal peptide of 26 amino acids and a 22-K polypeptide hormone of 191 amino acids. We have amplified a portion of the fifth exon region of GH-N using a pair of primers; the forward primer was designed against the sequence in fourth intron and reverse primer within the fifth exon. DNA was extracted using Blood Genomic DNA extraction kit, from freshly taken blood of a male individual. This DNA was used as a template and the region of DNA was amplified by PCR. Sequence of the amplified product showed total four variations in the coding region of fifth exon of GH-N (accession no. M13438.1). The two transition mutations were at position 1886 (T replaces C) and at 1916 (G replaces A) and were silent mutation. Two transversion mutations were at positions 1905(C replaces G) and at 1985 (G replaces C). That led to two changes in the amino acid sequence, as compared to GH-N. First was Histidine (H) at position 153 located between the loop of third and fourth helix of human growth hormone. For second mutation Methionine (M) at position 179 (located in the fourth helix of peptide 3-D structure) replaced by Isoleucine (I). These changes could affect the micro environment of polypeptide chain. The mutation at position 179 is a frequent mutation with a substitution score 1 (and has been reported by other workers). For second mutation at position 153 is a rare mutation with a substitution score of -1 according to the BLOSUM62 matrix.

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**NESTED PCR BASED PREVALENCE OF MALARIA IN DIFFERENT AREAS OF MIRPUR AZAD KASHMIR**

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Epidemiological study for malaria diagnosis was conducted in different area of Mirpur Azad Kashmir. DNA was extracted from fresh blood for malaria diagnosis. The extracted DNA was used as a template to amplify approximately 100bp species-specific sequence of the small subunit of the ribosomal RNA (18S SSU rRNA) of plasmodium Sp. A total 400 blood samples were collected from general healthy population during August 2011. Nested PCR showed over all 67% result. The *P. falciparum* was more prevalent (37%) was compared to *P. vivax* (17%) and remained infection

(11%). The age group (20-27) young male and age group (5-16) children were more affected. The results suggest that in malaria endemic areas where transmission of both *P. falciparum* and *P. vivax* occurs, nested PCR detection of malarial parasites can be very useful complement to microscopic examination in order to obtain the real incidence of each species and also for the follow-up of patients after specific treatment.

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### SERUM PROTEIN ANALYSIS OF COMMON FISHES OF PESHAWAR THROUGH GEL ELECTROPHORESIS TECHNIQUE

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In this study, native polyacrylamide gel electrophoresis (Native-Page) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS Page) were applied on serum protein of fish *Cirrhinus mrigala* (mori), *Labeo rohita*, *Ctenopharyngodon idella* and *Channa punctatus* taken from Sherabad hatchery Peshawar. The electrophoregram showed that there were similarities in these fish in native page the serum protein of *Cirrhinus mrigala*, *Labeo rohita*, *Ctenopharyngodon idella*, *Channa punctatus* were 1, 2, 2, 3 respectively but in SDS-page in the serum protein band of *Cirrhinus mrigala*, *Labeo rohita*, *Ctenopharyngodon idella*, *Channa punctatus* were 10, 7, 4, 8 respectively. The protein 1, 3, 5, 6 were present in all.

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### ACCUMULATION OF LEAD IN RENAL, GALL BLADDER AND SUPRA GINGIVAL CALCULI OF THE SUBJECTS OCCUPATIONALLY EXPOSED TO

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238 samples of renal, gallbladder and supra gingival calculi of lead recycling workers were collected over the period of seven years (2003-2009), and studied for the accumulation of lead. The results were compared with those of reference subjects who had no history of occupational exposure to lead. Renal calculi were sub grouped as oxalate, phosphate, urate and cystine calculi, gallbladder calculi as pure cholesterol, bilirubinate and mixed calculi and supra gingival calculi as single principal group composed of hydroxyl apatite phase. The lead content of calculi was investigated for its dependence on type and composition of calculi, blood lead, job status and duration of exposure. The effect of lead in calculi derived from kidney (N=80) was also investigated on respective Creatinine clearance rates. The results show that the lead levels in the calculi of lead workers were significantly higher than those of reference (  $p < 0.01$  ). The mean lead levels of three principal groups of calculi significantly differed from each other (  $p < 0.01$  ) and were found as, renal calculi  $66.18\mu\text{g/g}$  (  $\sigma = 29.44\mu\text{g/g}$ , N=94) gallbladder  $56.89\mu\text{g/g}$ , (  $\sigma = 14.29\mu\text{g/g}$ , N=78) and supra gingival calculi  $21.17\mu\text{g/g}$  (  $\sigma = 7.47\mu\text{g/g}$ , N=66). According to mean values of lead content, the subgroups of renal calculi followed the order as cystin > oxalate > phosphate > urate where as in the case of gallstones the corresponding order was noted as

bilirubinate > cholesterol > mixed calculi. The lead content of renal and gallbladder calculi did not depend on blood lead levels, job status and duration of exposure but in supra gingival calculi the results were influenced by job status and duration of exposure. With regard to the composition of calculi lead positively correlated with phosphate content of renal calculi ( $r=0.655$ ) and that of the supra gingival calculi ( $r=0.866$ ). The creatinine clearance rates were found to be inversely related to lead content of kidney calculi and followed regression equation  $=115-0.552 \times \text{lead in kidney calculi}$ .  $R^2 = -70.7\%$  and Pearson correlation coefficient  $r = -0.843$  ( $p < 0.01$ ). It has been concluded from the studies that occupational exposure to uncontrolled lead related activities increases the body lead burden. As with other body pools, the toxic metal has potential to accumulate in human calculi and pose dangers to host organs where calculi are deposited.

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#### **PRODUCTION OF CMCase FROM LOCALLY ISOLATED *NEUROSPORA SP.***

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CMCase is one of the enzyme of cellulases system and well known as endo-1,4-D-glucanases or endo-glucanases. The CMCase is an extracellular enzyme that degrades the cellulosic units and yield glucose as an end product. The effects of different parameters (pH, carbon source, nitrogen source, incubation time, size of inoculum and diluent type) were studied by growing the *Neurospora sp.* through the Solid State fermentation technique. The wheat bran as a carbon source yield maximum CMCase (1.26U/ml/min). Among diluent, malt extract produced 1.453U/ml/min of the enzyme. The inoculum size formed the greater amount of the CMCase units by 5ml of the volume of inoculum size (2.48 U/ml/min). The nitrogen source parameter leads to maximum enzyme units by urea (2.58 U/ml/min). Furthermore, the incubation time 96 hours produced 2.80U/ml/min of enzyme units. The pH factor result 6.06U/ml/min of the CMCase at 6.5 pH. These results are helpful for upscale studies to increase the yield of CMCase which can be utilized for commercial production of glucose from cellulosic materials.

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#### **INFLUENCE OF THE SEQUENCE ENVIRONMENT OF NEIGHBORING AMINO ACIDS ON AMINO-ACETYLATION: *IN SILICO* STUDIES**

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Posttranslational modifications of proteins such as phosphorylation, glycosylation, acetylation, etc. are crucial for various biological functions. Amongst all other modifications acetylation is the most important due to its role in gene expression (posttranslational modification), sub-cellular localization of proteins (co-translational modification), etc. Sequence analysis of surrounded amino acids of modified residues is important to understand the biological activities of the proteins controlled by various modifications. Computational studies are playing very important role to understand the influence of surrounded amino acids on modified residues. MAPRes (Mining Association Patterns among preferred amino acid residues in the vicinity of amino acids targeted for post-translational modifications) is a useful computational tool to analyze the neighbouring environment of modified residues. In this work, MAPRes was utilized to identify significantly preferred amino acids in surrounding of acetylated and non-acetylated residues. MAPRes mined

some useful and novel association patterns that are critical for agreement and disagreement for acetyl group at different residues. The consistency of these patterns validated by comparing patterns with existing literature and computational prediction models for acetylation. These patterns will be helpful for further investigations to unravel and to analyze the manifold effects of acetylation on proteins function.

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***IN SILICO* STUDIES: THE ROLE OF O-GlcNAc MODIFICATION OF P/MSP2 FOR DEVELOPMENT OF A VIABLE ANTI-MALARIAL VACCINE**

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Multi-stage life cycle with stage specific antigens is basic stumbling block to develop a viable vaccine against malaria. Blood stage antigens have been given more attention due to lethal effects of erythrocytic invasion of merozoites. The role of glycosylation in malarial proteins is crucial for the development of a practicable vaccine that has not been considered yet. This approach is still controversial as malarial parasite genome has no genes for glycosyltransferases but it has ability to utilize host metabolic machinery by combinatorial metabolism that is well documented. The merozoite surface protein 2 (MSP2) expressed on merozoites during erythrocytic invasion; stimulate the production of IgG and IgG3 antibodies which can interfere with the invasion and propagation of merozoites. MSP2 can be glycosylated through human erythrocytic O-linked N-acetylglucosamine transferase (OGT) at potential glycosylation sites. 3D structural modeling and docking studies of OGT, MSP2 and UDP-GlcNAc performed by using various homology and non-homology based computational tools. The screening of docking complexes of OGT with UDP-GlcNAc and O-GlcNAc with MSP2 revealed that MSP2 has potential sites for glycosylation. This glycosylated MSP2 may produce better immune response therefore; it can be a suitable vaccine or a subunit of vaccine against malaria.

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**MAPRes 2.0: A COMPUTATIONAL TOOL FOR MINING ASSOCIATION PATTERNS BASED ON POLARITY AND CHARGE IN THE VICINITY OF POSTTRANSLATIONALLY-MODIFIED PROTEIN SITES**

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Several *in silico* methods such as prediction models for posttranslationally-modified sites and association rule mining are available for analyzing the protein sequence. Investigation of protein primary sequences on the basis of amino acid characteristics such as polarity and charge has not yet been considered. Previously, MAPRes was used to investigate the influence of the sequence context on potentially modifiable sites (PTM sites). In the present study, using MAPRes 2.0, we have extended the program to take into account the polarity and charge of the amino acids when mining the association rules/patterns for the PTM sites. The amino acids with side chains (R) such as non-polar aliphatic (L), aromatic (R), polar uncharged (U), negatively charged (N) and positively charged (P) have been considered for association rules/patterns mining. The results show that the patterns mined by MAPRes 2.0 that incorporate structural information regarding polarity and charge of amino acids usefully document the structure-function relationship of amino acids in a

potentially multifunctional protein. MAPRes 2.0 is freely available at <http://www.imsb.edu.pk/Database.htm>

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### **ROLE OF Scc3 PROTEIN IN THE REPAIR OF MEIOSIS SPECIFIC DNA DOUBLE STRAND BREAKS (DSBs)**

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Meiotic recombination in *Saccharomyces cerevisiae* is initiated by the formation of DNA double strand breaks (DSBs), which are created by Spo11 protein. These DSBs are tightly monitored for timely and error free repair. DNA damage, such as DSBs, causes many proteins to be post-translationally modified. Post-translational modification of proteins is an essential process used in all metabolic pathways to regulate protein function. It was found that Scc3 protein is phosphorylated in response to DSBs and identified its kinase. Meiotic DSBs remains un-repaired, when the Scc3 protein is not fully phosphorylated.

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### **PREVALENCE OF P53 MUTATIONS IN BREAST CANCER PATIENTS IN PAKISTAN**

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The tumor suppressor gene *TP53* encodes a nuclear protein that prevents the cells from dividing before DNA damage is repaired. Mutations in *TP53* gene have effects on its biological activities. The objectives of present study aims at determining the frequency *TP53* mutations in sporadic, genetic lineage and analysis of the data i.e. questionnaire collected from breast cancer patients from Pakistan, during the study. Female breast cancer patients were recruited at Shaukat Khanum Memorial Cancer Hospital & Research Centre and Mayo Hospital, Lahore Pakistan, from January 2005 to December 2008. A total of 150 sporadic breast cancer patients and three families with breast cancer cases were included in the study. From all study participants, a blood sample and a piece of tissue of normal and tumor both were collected. DNA was extracted and exons 5-8 (central region) of *TP53* gene were PCR amplified. Each sample was heteroduplexed with a normal control sample (confirmed by sequencing). To screen *TP53* mutations Temporal Temperature Gradient Gel Electrophoresis (TTGE) was performed. The mutations were confirmed by sequencing. Restriction Fragment Length Polymorphism (RFLP) was used for understanding the status of codon 72, exon 4 of *TP53* gene polymorphism (arg/arg) in Pakistan. The data was analyzed using the R15 programme, provided by International Agency for Research on Cancer. Three deleterious mutations were detected in the sporadic breast cancer patients, viz., codon 238 where TGT is mutated to TAT (cys to tyr), codon 248 where CGG is mutated to CAG ( arg to glu), and codon 278 where CCT is mutated to TCT (pro to ser). These mutations were not detected in normal breast tissue and blood samples of these patients. R15 analysis (rARC, 2011) of *TP53* gene mutations showed that the mutations detected in Pakistani breast cancer patients are reported most prevalent somatic mutations (codon 238 = 79 tumors, codon 248 = 779 tumors and codon 278 = 74 tumors) in breast cancer patients of the world. Three-dimensional structures were predicted by 3D Viewer (software given on IARC website) and found that all these three mutations are in DNA binding region of

*TP53* and could change the structure of protein and, therefore, affect its function. *TP53* mutation has not been observed in normal persons and breast cancer families blood samples. One family was detected with Li-Fraumeni syndrome characters but *TP53* mutations are not found in it. Although the polymorphism arg/arg, codon 72, ex on 4 of *TP53* gene is reported as a functional relevant polymorphism that contributes to breast cancer development yet in the present study, genotype arg/pro and pro/pro, both polymorphisms were found more significant in Pakistani breast cancer patients as compared to arg/arg with corresponding ratio of arg/pro (53.3): pro/pro (34.6): arg/arg (12). Normal controls showed about the same difference in ratio of arg/pro: pro/pro: arg/arg, (50:40: 10). Correlation of *TP53* mutations with clinicopathological parameters (data collected by questionnaire) was observed. Patients were divided into two groups; group 1 (*TP53* non mutated) and group 2 (*TP53* mutated). As both groups have not shown any difference so no prominent correlation between *TP53* mutations and clinicopathological parameters was found. It is concluded that the frequency of *TP53* gene mutations in DNA coding region (5-8 exon) is low in Pakistani breast cancer patients. However, present study is in favor of the fact that the frequency of *TP53* gene mutations is different in different geographical areas. Genotype arg/arg is less prevalent in the female breast cancer patients and normal population of Pakistan. There was no significant correlation between *TP53* mutation and tumor aggressiveness *e.g.* nodal status, size, ER/PR, histopathology *etc.* Epidemiologically, no carcinogen was found important as a causative factor of *TP53* gene mutations in Pakistani breast cancer patients.

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## SECTION – I I

### PESTS AND PEST CONTROL

#### DETERMINATION OF INSECTICIDE RESISTANCE AGAINST PEACH FRUIT FLY, *BACTROCERA ZONATA* (SAUNDERS) (DIPTERA: TEPHRITIDAE)

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The peach fruit fly, *Bactrocera zonata* (Diptera: Tephritidae), is one of the most economically harmful pests of fruit and vegetables in Pakistan. The control of this pest is mainly based on cover spray by organophosphate and pyrethroid insecticides. The present research was conducted to determine the resistance level of 5 insecticides viz., trichlorfon, malathion, bifenthrin, lambda-cyhalothrin and methomyl against fourteen field populations i.e., Khadal, Mardan Pur, Bibi Pur, Binda Sandhla, Muzafar Abad, Sultan Pur Humar, Hamid Pur, Jhoke Gamu, Arazi Ghulam Mustafa, Booty wala, Garalia, Riaz Abad, Taty Pur and Matital located in area of Multan, Punjab. Results revealed that trichlorfon was found moderate to high resistance ratio (24.71-fold<RR<32.90-fold) and malathion with moderate level of resistance (11.96-fold<RR<19.78-fold) against 14 field populations. Bifenthrin was observed as low to moderate resistance level (5.68-fold<RR<12.30-fold), whereas, lambda-cyhalothrin was examined as with low resistance level (5.51-fold<RR<9.78-fold) towards all tested populations. The methomyl was remained susceptible against all tested field populations of *B. zonata*.

#### BIOCHEMICAL MECHANISMS OF RESISTANCE IN TWO TRICHLORFON RESISTANT STRAINS OF *BACTROCERA ZONATA* (SAUNDERS) (DIPTERA: TEPHRITIDAE)

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*Bactrocera zonata* (Saunders) is the most harmful insect pests of horticultural crops in Pakistan and in generally is being controlling by different insecticides. The trichlorfon has long history of use for controlling *B. zonata* and preliminary studies showed that this insect had been developed resistance to trichlorfon. Resistance to trichlorfon in *B. zonata* was examined in present study by comparing the metabolic activities of four types of enzymes viz., esterases, cytochrome P450, glutathione S-transferases and acetylcholinesterase in two trichlorfon resistant strains (Laboratory Resistant=LR and Field Resistant= FR) with susceptible strain. Results showed that the esterases, cytochrome P450 and glutathione S-transferases activities were lower in the susceptible strain, whereas, acetylcholinesterase (AChE) activity in the susceptible strain was found higher as

compared to the resistant strains. The activities of these 4 types of enzymes were not significantly different between LR and FR strains of *B. zonata*.

**COMPARISON FOR TOLERANCE AND SUSCEPTIBILITY RESPONSE BETWEEN DIFFERENT COTTON *GOSSYPIUM HIRSUTUM* (L.) GENOTYPES TO PESTS INCIDENCE**

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Resistant crop varieties can effectively reduce and suppress insect pests abundance, therefore, effects of different cotton *Gossypium hirsutum* (L.), genotypes on the population buildup of pests were investigated in the field experiments. Field studies (15 cotton genotypes) on host preference detected low attractiveness of whitefly by plants of genotypes B-2, B-3 & B-4, jassid B-10, B-5 & B-14, and bollworms B-10 & B-13 (squares) and B-10 & B-14 (bolls). The highest germination was obtained in genotypes B-10, B-1 & B-4, whereas, seeds of genotypes B-7, B-13 & B-3 resulted in poor germination. Genotypes B-8 and B-9 were tolerant to cotton leaf curl virus (CLCV), while, B-11, B-5 & B-8 showed an efficient yield potential. Field observations (25 cotton genotypes) indicated that for whitefly genotypes C-2, C-3 & C-5, jassids C-25, C-23 & C-15, and bollworms C-8, C-22 & C-6 (squares) and C-24, C-23 & C-17 (bolls) had lower number of insect pests attracted than other tested genetic material. Studies conducted on germination observed very high rates in genotypes C-5, C-15 and C-19, and low in C-12, C-1 and C-9. Genotypes C-15, C-23 and C-17 showed tolerance to CLCV and C-9, C-7 & C-12 gave maximum yield. The proper utilization of the available host plant resistance can reduce insect pest populations in cotton crop.

**COMPARATIVE EFFICACY OF SOME NEW INSECTICIDES AGAINST JASSID, *AMRASCA DEVASTANS* (DISTANT) (HEMIPTERA: CICADELLIDAE) ON COTTON CROP UNDER NATURAL FIELD CONDITIONS**

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The comparative efficacy of 5 new insecticides, viz., pyridaben 15EC, nitenpyram 10SL, chlorfenapyr 36EC, thiacloprid 480SC and pyriproxyfen 10.8SC was evaluated against jassid, *Amrasca devastans* (Distant) on cotton crop under natural field conditions during the year 2011 at Anjum Farm 87/9L Sahiwal, Punjab. The results showed that nitenpyram 10SL was most effective against jassid populations after 24hours, 72hours and up to 7 days among the all tested insecticides followed by thiacloprid 480SC and chlorfenapyr 36EC. The pyriproxyfen 10.8SC and pyridaben

15EC were observed no significant effect against jassid populations on cotton crop.

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**ASSESSMENT OF BT AND NON-BT COTTON *GOSSYPIMUM HIRSUTUM* (L.)  
GENOTYPES FOR HARBOURING MAJOR INSECT PESTS IN FIELD**

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Insect monitoring on host plant is a key component in any successful integrated management programme. Insect populations surveillance was conducted on conventional cotton NIAB-2010 and Bt cotton B-22, under natural conditions. Both cotton types affected insect populations, regardless of whether these are found in Bt or non-Bt crop fields. Comparison of Bt and non-Bt cotton genotypes against insect pests observed that Bt crop (Bt-22) had considerably less impacts on whitefly and jassid, however, affected more pink and spotted bollworms populations than conventional cotton (NIAB-2010). Accordingly, there were no considerable effects on natural enemies associated with cotton types and the population of predators in Bt field was not negatively affected in comparison with conventional cotton. So, the effects of host plant on the establishment of insect pests and predators can find a range of factors that may be exploited to make cotton a less suitable plant for utilization by herbivorous pests for future management strategy.

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**HOST PREFERENCE OF FRUIT FLIES *BACTROCERA ZONATA* (SAUNDERS) AND  
*BACTROCERA CUCURBITAE* (COQUILLET) (DIPTERA: TEPHTRITIDAE)**

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Host choice experiments were done for flies *Bactrocera zonata* (Saunders) and *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) by using different fruits and vegetables. Host preference of the *B. zonata* fruit fly was studied on mango, peach and apple fruits in field experiments. The mango was recorded as most preferred host followed by peach and apple, due to the maximum pupae formed, pupae weight and emergence of fruit flies. The bitter gourd, brinjal, muskmelon and pumpkin were tested for the relative host preference of fruit fly *B. cucurbitae*. The bitter gourd was found as most preferred host demonstrating the maximum pupae formation, pupae weight obtained and percent adult emergence of fruit flies. Brinjal was observed as moderately preferred host, while, muskmelon and pumpkin were sorted out as least preferred hosts. These results provide experimental support that flies will respond differently to the host experienced in the field and the hosts that are of advantageous to the pests will be adapted. Results further imply that host preference can shift towards less suitable hosts, and if hosts which provide a proper breeding situation become scarce then alternative hosts are accepted.

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**DETECTION AND TRANSFORMATION STUDIES OF BACTERIOGIN PRODUCING  
BACILLUS AND PSEUDOMONAS SPECIES**

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The main aims of present study were to isolate and screen the bacteriocin producing bacterial strains and to know the nature of that compound by various separation and purification techniques especially TLC and SDS PAGE. The transformation of bacteriocin-encoded into the reference *E. coli* JM107 by CaCl<sub>2</sub> method. For this purpose, a total of five bacterial strains (AZS, A2, P, U and LB) showing high bacteriocin activity against the target organism were selected for further studies. The biochemical analysis and API20E strip indicated that isolate P showed 99% homology with *Pseudomonas aeruginosa* while isolate U showed 97.9% homology with *Pseudomonas putida* and rest of the three strains belong to *Bacillus* genera. The bioactivity of the TLC bands with reference to R<sub>f</sub> value (0.70) were checked and the result indicated that the zone appeared in all case except the control which showed no zone against target organism. The stability of the crude extract of bacteriocin to high temperature 121°C, Proteinase K and UV radiation indicated that these compound could be a better option for utilization as a probiotics. The SDS-PAGE analysis indicated that bacteriocin from both *Pseudomonas* (P and U) and *Bacillus* (A2, AZS and LB) genera were of low molecular weight *i.e.* molecular weight of *Bacillus* (A2, AZS & LB) were approximately in the range of 10 to 25 kDa whereas the molecular weight of both *Pseudomonas* (P & U) strains was approximately 40 kDa. The bioactivities of the SDS-PAGE bands were checked against the target organism. The clear zones around the bands indicated that our selection of bacteriocin bands with reference to page ruler™ prestained protein ladder # SM 0671 were correct. The result of transformation studies revealed that bacteriocin producing plasmid gene of *Pseudomonas* (P and U) and *Bacillus* (A2) was successfully transformed in competent *E. coli* JM107 but was not prominent in case of other two strains of *Bacillus*. It was concluded that bacteriocin character was plasmid encoded and the low molecular weight, diverse stability and transformation studies indicated that these compound could be a better alternative to antibiotics.

**DEVELOPMENT OF RESISTANCE TO TRICHLORFON,  
MALATHION AND BIFENTHRIN IN THE FIELD SELECTED STRAINS OF FRUIT  
FLY, BACTROCERA ZONATA (SAUNDERS) (DIPTERA: TEPHRITIDAE) UNDER  
LABORATORY CONDITIONS**

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*Bactrocera zonata* (Saunders) attacks a large variety of fruits and vegetables, which caused huge economic losses. The use of insecticides has been an important source for controlling this pest. Although, the development of insecticides resistance in *B. zonata* has not kept rhythm with that of other insects, which may be due to their trend of high mobility. Recent studies were carried out to access the potential risk of resistance development in *B. zonata* with selection pressure of 3 insecticides *viz.*, trichlorfon, malathion and bifenthrin against three field selected strains *i.e.*, M<sub>1</sub>, M<sub>2</sub> and SWL in the laboratory. Results manifest that the M<sub>1</sub>, M<sub>2</sub> and SWL strains showed as 2.5-

2.3- and 2.4-fold increase resistance towards trichlorfon, while malathion as 2.5-, 2.8- and 2.4-fold increase in their parental resistance up to six generation, respectively. The selection pressure of bifenthrin against M<sub>1</sub>, M<sub>2</sub> and SWL strains was recorded as 3.8-, 3.0- and 4.2-fold increase from their parental RRs. From the findings, it was concluded that *B. zonata* could develop resistance to bifenthrin more rapidly as compared to malathion and trichlorfon.

**EVALUATION OF DIFFERENT IPM-MODELS FOR THEIR PERFORMANCE IN BITTER-GOURD (*MOMORDICA CHARANTIA*) CROP AGAINST MELON FRUIT FLY (*BACTROCERA CUCURBITAE* COQUILLET) PEST**

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*Bactrocera cucurbitae* severely damages the fruits of cucurbits rendering the fruits unfit for marketing and consumption. Cover spray of insecticides which is the most common method for its control, contaminates the readily used cucurbits with toxic residues of highly persistent insecticides. It is, therefore, required to develop an economically sound, environmentally safe and socially acceptable IPM model for *B. cucurbitae* in cucurbit cropping system. The most effective melon fruit flies management tactics, screened through different experimentations, were integrated into different IPM-Models, which were evaluated at two localities, under field condition. The results of these tests showed that variations in the biotic and abiotic factors, of each locality, did not affect the performance of each IPM-Model. The performance of each IPM-Model was found to be similar at Harappa and Faisalabad. However, an IPM-Model-7 (HSM + P x P 45 cm + Early Sowing (15 February) + Sanitation + Resistant variety + Baited pheromone + GF-120 + Food-baited chemical) proved to be a significantly best model, with a fruit infestation  $\approx$  53.5 and 12.1 times less; yield loss/plant  $\approx$  27.9 and 6 times less; marketable fruits/plant  $\approx$  112.9 and 2.6 times more; marketable yield/plant  $\approx$  7.2 and 2.7 times more; expected marketable yield in tonnes/ha  $\approx$  7.2 and 2.7 times more than that of IPM-Model-8 (control) and the conventional IPM-Model-1, respectively and a cost benefit ratio of 1 : 7. Similarly, an IPM-Model-3 (HSM + P x P 45 cm + Early Sowing (15 February) + Sanitation + Resistant variety + Baited pheromone + GF-120 spray), with a fruit infestation  $\approx$  39.8 and 9 times less; yield loss/plant  $\approx$  25.1 and 5.4 times less; marketable fruits/plant  $\approx$  97.5 and 2.3 times more; marketable yield/plant  $\approx$  6.3 and 2.4 times more; expected marketable yield, in tonnes/ha  $\approx$  6.3 and 2.4 times more than that of IPM-Model-8 and that of a conventional IPM-Model-1, respectively and a cost benefit ratio of 1 : 6.8, also, gave significantly better results and was found, statistically, at par with IPM-Model-4 and IPM-Model-6. IPM-Model-4 (HSM + P x P 45 cm + Early Sowing (15 February) + Sanitation + Resistant variety + Baited pheromone + Botanicals spray) and IPM-Model-6 (HSM + P x P 45 cm + Early Sowing (15 February) + Sanitation + Resistant variety + Baited pheromone + Spray of GF-120 6 + Spray of Botanicals) yielded a fruit infestation  $\approx$  30.2-39.9 and 6.8-8.9 times less; yield loss/plant  $\approx$  19.4-23.5 and 4.2-5 times less; marketable fruits/plant  $\approx$  96.7-79.5 and 2.3 times more; marketable yield/plant  $\approx$  6.3 and 2.4 times more; expected marketable yield, in tonnes/ha  $\approx$  6.2-6.3 and 2.4 times more than that of IPM-Model-8 and that of a conventional IPM-Model-1 and a cost benefit ratio of 1 : 6.8. IPM-Model-5 (HSM + P x P 45 cm + Early Sowing (15 February) + Sanitation + Resistant variety + Baited pheromone + Food-baited chemical) yielding fruit infestation  $\approx$  14.5 and 3.3 times less; yield loss/plant  $\approx$  8.7 and 1.9 times less; marketable fruits/plant  $\approx$  93.3 and 2.2 times more than; marketable yield/plant  $\approx$  6.1 and 2.3 times more; expected marketable yield, in

tonnes/ha  $\approx$  6.1 and 2.3 times more than that of IPM-Model-8 and that of the conventional IPM-Model-1, respectively and a cost benefit ratio of 1 : 6.5 also performed better. IPM-Model-2 (Optimum sowing time + Resistant variety + FSM + Chemical control + No sanitation), with a fruit infestation  $\approx$  5.8 and 1.3 times less; yield loss/plant  $\approx$  5.3 and 1.1 times less; marketable fruits/plant  $\approx$  55.4 and 1.3 times more; marketable yield/plant  $\approx$  3.3 and 1.2 times more; expected marketable yield, in tonnes/ha  $\approx$  3.3 and 1.2 times more than that of IPM-Model-8 and that of the conventional IPM-Model-1, respectively and a cost benefit ratio of 1 : 6.2, also, gave good results and was found to be, statistically, different from the conventional IPM-Model-1 and the control IPM-Model-8. The descending order of the performance of tested IPM-Models is: IPM-Model-7 > IPM-Model-3 > IPM-Model-6 > IPM-Model-4 > IPM-Model-5 > IPM-Model-2 > IPM-Model-1 > IPM-Model-8. In conclusion, IPM-Model-3, IPM-Model-4, IPM-Model-5, IPM-Model-6 and IPM-Model-7 can be exercised in the areas where there are hot spots of the melon fruit flies.

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#### EVALUATION OF SOME BOTANICAL EXTRACTS FOR THEIR DETERRENCE TOWARDS MELON FRUIT FLY UNDER LABORATORY AND FIELD CONDITION

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Chemical control of melon fruit fly with cover spray of insecticides results in contamination of cucurbit fruits which are marketed and consumed just after picking and magnification of residues with severe carcinogenic and mutagenic health hazards. Keeping view these facts, some botanicals which are considered safe were evaluated under laboratory and field conditions. Kernel and leaves of neem, *Azadirachta indica* (Meliaceae) and 'dhrak'/bakain, *Melia azadirachta* (Meliaceae), peel of sweet orange fruit, *Citrus sinensis* (Rutaceae), chilli fruit, *Capsicum annuum* (Solanaceae), ginger rhizomes, *Zingiber officinale* (Zingiberaceae) and garlic cloves, *Allium sativum* (Alliaceae) were evaluated for their effect on the deterrence/attraction of melon fruit fly towards treated fruits under the laboratory and treated crop under field conditions. The olfactometric studies revealed that garlic, ginger and neem extracts deterred the maximum number of released fruit flies, from oviposition, (94.54 % to 92.8 % of released flies) for a longer period (8 days) and resulted in least fruit infestation ( $3.4 \pm 3.3\%$  to  $7.13 \pm 4.5\%$ ). After eight days, 13-15 fruit flies (26-30% of released fruit flies) were attracted to the fruits, placed in the small jars of an olfactometer and 16.67% to 33.3% of the fruits, placed in small jars, were found infested on 10<sup>th</sup> day.  $13.4 \pm 3.12$  (26.8% of released flies),  $8.47 \pm 2.18$  (16.94% of released flies) and  $7.93 \pm 2.47$  (15.86% of released flies) fruit flies were attracted to and  $40.47 \pm 12.9$ ,  $56.67 \pm 12.7$  and  $63.33 \pm 10.3\%$  fruit infestation was observed in those fruits, which were treated with chilli, 'dhrak' and citrus, respectively. Field studies also showed that spray of garlic, ginger and neem extracts resulted in fruit infestation  $\approx$  1.8-4.5 times less, yield loss/plant  $\approx$  2.1-4.3 times less, marketable fruits/plant  $\approx$  4.1-6.4 times more and marketable yield/plant  $\approx$  1.8-2.8 times more than control. However, the spray of other botanical extracts resulted in more than 40% fruit infestation, less than 4 marketable fruits/plant, more than 150g yield loss/plant and less than 350g marketable yield/plant. Conclusively, garlic, ginger and neem extracts, which were found comparatively more effective, can be integrated with other non-chemical fruit fly control tactics in cucurbits cropping system to reduce the number of cover spray with insecticides and ensure the provision of cucurbits free of toxic residues of insecticides.

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**PHYSICO-MORPHOLOGICAL PLANT TRAITS INFLUENCING RESISTANCE  
AGAINST APHIDS ON WHEAT CROP**

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Eleven genotypes of wheat (V-05066, V-5BT-006, V-04178, V-04022, V-05603, V-05082, Saher, InqLab-91, Chakwal-5C-011, V-033010 and V-032862) were sown in the field area of Entomological Research Institute, Faisalabad, during 2009 following RCBD replicated thrice to determine the influence of various physico-morphological plant traits viz., leaf area (cm<sup>2</sup>), chlorophyll contents (%), number of nodes per tiller, inter-nodal distance (cm), plant height (cm), number of grains per tiller, number of spikelets, spike length (cm), number of hair on midrib (per cm) and lamina (per cm<sup>2</sup>) and length of hair(um) on midrib and lamina towards resistance against aphids. Significant difference was found among genotypes and dates of observation regarding the population of aphids. The genotype V-05066 was found comparatively susceptible with maximum aphid's population *i.e.*, 18.69 per tiller, whereas V-04178 was found comparatively resistant with minimum population of aphids *i.e.*, 5.73 per tiller. February 24, 2009 showed maximum population of aphids (46.07/tiller) and this population of aphids decreased on the subsequent dates of observation. Variations were found to be significant among genotypes regarding leaf area, chlorophyll contents, number of nodes per tiller and inter-nodal distance. The genotypes under study did not differ significantly in their plant height, number of grain per tiller, number of spikelets, spike length, number of hair and length of hair. All the physico-morphological plant traits under study showed non-significant correlation with the population of aphids, however, number of spikelets per plant was the most important factors which brought maximum per unit change *i.e.*, 7.3% in population of the pest followed by hair density on lamina (5.9%), leaf area (3.6%), plant height (3.5%), hair density on midrib (3.0%), number of grains per tiller (2.2%) and inter-nodal distance (2.0%). The other physico-morphological plant traits showed negligible contribution (0.1 to 0.9%) in per unit change in the pest fluctuation.

**EFFICACY OF SOME OLD AND NEW PESTICIDES AGAINST APHID POPULATION  
ON BRASSICA SPECIES**

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In the present study three insecticides like Methamidophos @ 2.5litre ,Fenpropetherin @ 350ml/hectare and Imidacloprid @370ml/hectare were tested for their efficacy against aphids on different *Brassica* spp.. The experiment was conducted during October 2010 to April 2011 . Crop season following RCBD replicated four times in the Research Farm Of AGRICULTURAL COLLEGE at BZU MULTAN. The data regarding aphids population before spray and 1 Day (24 hours), 2 Days (48 hours), 3 Days (72 hours), 4 Days (96 hours), 5 Days (120 hours), 6 Days (144 hours), and 7 Days (168 hours) after spray was recorded . Mortality of the pest was calculated for the entire post treatment intervals. No simple variety was totally resistant / susceptible to the aphids

attack. Different varieties showed different responses to aphids attack. The population of aphids was at par statistically in all the treatments. The efficacy of different insecticides was increased with the passage of time. Significant variations are found to exist among treatments regarding percent reduction in pest density at all the pest treatment intervals. Methamidophos proved to be most effective insecticides resulted in maximum reduction in aphid's density at all the post treatment intervals. Furthermore, the insecticidal effect was equal statistically at 7 Days after spray. The variety Brassica Oligacea showed minimum population of aphids, Whereas Brassica campestris possess maximum aphids population.

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**FIELD EVALUATION BT COTTON AGAINST *HELICOVERPA ARMIGERA*  
(LEPIDOPTERA: NOCTUIDAE)**

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Cotton plant has been genetically modified to incorporate recombinant DNA from bacterium, *Bacillus thuringiensis* containing delta endotoxin protein. It is commonly known as Bt cotton, because it produces an insecticidal protein (Cry1Ac) to combat the lepidopterous pests, particularly bollworm species. *Helicoverpa armigera* (Hubner) commonly known as cotton bollworm (CBW) is one of the damaging pests of cotton and many other field crops worldwide. A transgenic Bt cotton line, IRFH-901 expressing Cry1Ac insecticidal protein from *B. thuringiensis* Subsp. *Kurstaki* (Berliner) was evaluated for resistance to *H. armigera* under field conditions for two cotton seasons 2006-07 at Postgraduate Agriculture Research Station (PARS), Faisalabad, Punjab. The experimental fields were laid out in a Randomized Complete Block Design (RCBD) consisting of four treatments each with four replications. The results showed that there was no significant difference in egg densities between Bt and non-Bt cotton. However, larval densities were significantly reduced in Bt cotton as compared to conventional non-Bt cotton. No insecticide application was needed against this pest in Bt cotton fields. However the higher larval densities in non-Bt cotton fields demanded insecticide applications. The results indicate that transgenic Bt cotton can play a significant role in reducing the pesticide application for the control of *H. armigera*. However, increased larval densities at later stages of crop may be a critical factor in the non-adoption of Bt cotton in Pakistan. The studies highlight the importance of sustainable temporal plant expression of Cry1Ac in Bt cotton varieties for effective control of lepidopterous pests especially *H. armigera*.

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**BIOLOGICAL ACTIVITY OF METHANOLIC EXTRACTS OF SOME LOCALLY  
AVAILABLE PLANTS AGAINST STORED GRAIN MITE *RHIZOGLYPHUS TRITICI*  
(ACARI: ACARIDAE)**

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Stored grain mites are the major pests of stored commodities especially wheat is greatly damaged by the stored grain mites in storage structures. Research works on mites remained neglected due to their microscopic size. The present studies were carried out to check the efficacy of

methanolic extracts of locally available plants *i.e* Neem (*Azadirachtaindica*), Kanair(*Phoenix careinsis*), Amaltas (*Casia fistula*) and Dhatura (*DaturaStramonium*) against stored grain mites under randomized complete block design. There were six treatments for each botanical (8%, 4%, 2%, 1% 0.5% and control), having three replications of each. The population inhibition was recorded after seven, fourteen, twenty one and twenty eight days. The dose concentration versus mortality data were subjected to workout LC<sub>50</sub>. The population inhibition trend by different botanicals after seven days was Neem (80%)>Amaltas (75%)>Kanair (66%)>Dhatura (61%). After fourteen days trend was Neem(82%)>Amaltas>(78%)>Kanair (69%)>Neem>Dhatura (66%).After twenty one days it was Amaltas (84%)>Kanair (84%)>Neem (82%)>Dhatura (69%) After twenty eight days Amaltas caused 91% population inhibition, Neem 88%, Kanair caused 85% and Dhatura 74% population inhibition. The LC<sub>50</sub> values of Amaltas after seven days was minimum (1.41% solution) followed by Neem (1.51% solution), Kanair (2.57% solution) and Dhatura (5.89% solution). The LC<sub>50</sub> values of Amaltas after fourteen days was minimum (0.40% solution) followed by Kanair (2.37% solution), Neem (1.38% solution) and Dhatura (4.60% solution). The LC<sub>50</sub> values of Amaltas after twenty one days was minimum (0.42% solution) followed by Kanair (0.59% solution), Neem (0.44% solution) and Dhatura (1.79% solution). The LC<sub>50</sub> values of Amaltas after twenty eight days was minimum (0.11% solution) followed by Dhatura (0.24% solution), Kanair (0.28% solution) and Neem (0.38% solution). As the data shows that population inhibition percentage increased with the passage of time. Amaltas proved more toxic to stored grain mites after twenty eight days in the present experiment by causing 91% population inhibition having LC<sub>50</sub> value of 0.11% solution. The LC<sub>50</sub> values decreased with the passage of time. Kanair caused 85% population inhibition after 28 days and LC<sub>50</sub> value was 0.28% solution. Neem caused 88% population inhibition after 28 days and LC<sub>50</sub> value was 0.38% solution for it. Dhatura caused 74% population inhibition after 28 days and LC<sub>50</sub> value was 0.24% solution for it.

#### **EFFECTIVENESS OF *METARHIZIUM ANISOPLIAE* AGAINST DIFFERENT INSTARS OF *HELICOVERPA ARMIGERA* (LEPIDOPTERA: NOCTUIDAE)**

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*Metarhizium anisopliae* the entomopathogenic fungus has potential to cause many infections in many lepidopteran insects including *Helicoverpa armigera* which is a major insect pest of many agricultural and horticultural crops. The study comprised to check the effectiveness of two isolates of *M. anisopliae* (Ma02 and Ma05) at different concentrations *i.e.*,  $1 \times 10^6$ ,  $1 \times 10^7$  and  $1 \times 10^8$  spores/ ml against different larval instars of *H. armigera* under laboratory conditions. The results showed that on 5<sup>th</sup> day of fungal treatment 100% mortality of 2<sup>nd</sup> instar larvae was recorded incase for Ma02, while for the 3<sup>rd</sup> larval instar maximum percent mortality of 70 and 55 was recorded after 7 days incase for Ma02 and Ma05. Both isolates were found different in their effectiveness against different immature stages of the insect. The introduction of entomopathogenic fungi in the insect pest control program can enhance the efficiency of other control measures if are used in combination.

**SURVEY AND CONTROL OF PEACH FLAT-HEADED BORER, *SPHENOPTERA DADKHANI* (OBEN.) IN PLUM ORCHARD**

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Peach flat-headed borer, *Sphenoptera dadkhani*, Oben. (Buprestidae: Coleoptera) is an economic pest of stone fruits in Pakistan. Survey of Peach flat-headed borer infestation in stone fruit orchards at three different areas *i.e.*, Peshawar, Nowshera and Swat was conducted. Among three different areas, maximum gummosis infestation (93.52%) was recorded in plum orchards at Nowshera followed by Peshawar (82.39%) while in Swat infestation was minimum (47.66%). In peaches, maximum infestation was recorded in Peshawar *i.e.*, 88.26% followed by Nowshera (82.02%) and Swat (66.71%). Different insecticides were tested against Peach flat-headed borer in plum orchard. Results revealed that the minimum gummosis infestation was recorded in Cyren and Thiodan treatments *i.e.*, 3.73, 5.43 gum points/m<sup>2</sup> followed by Tenekil plus (8.45), Triazofos (8.70) and maximum in Control (25.92). Cyren was found more effective as compared to other tested insecticides.

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**EFFECT OF DIFFERENT NITROGEN FERTILIZER DOSES TO MAIZE ON THE BIOLOGY OF *CHILO PARTELLUS* (LEPIDOPTERA:CRAMBIDAE)**

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Lepidopteran stem borer, *Chilo Partellus* (Lepidoptera:Crambidae), is the dominant and most damaging pest of maize. Field trials were conducted to assess the effect of five different nitrogen doses (N<sub>0</sub>-N<sub>4</sub>) on the *C. Partellus* on three different varieties *i.e.*, KWS-55, KK-8711 and KS-85. Plant nitrogen varied significantly between N<sub>0</sub>-N<sub>4</sub> in the field plots. Each plot received one of four level of nitrogen *i.e.*, 0, 60, 90, 120, 150 kg/hectare in split doses at two and three weeks after emergence. The field data revealed that maximum infestation was observed on KWS-55 after six week of emergence at nitrogen level (N<sub>3</sub>). The insect were feed on the leaves from different plots of nitrogen applications. The maximum larval and feces weight for the 5<sup>th</sup> larval instar was of 0.085 and 0.018 gm at N<sub>4</sub> treatment applied to the maize variety KWS-55. In contrast to this minimum larval and feces weight *i.e.*, 0.073 and 0.014 gm was observed on KK-8711 for the nitrogen level N<sub>4</sub>. A positive correlation was observed for the increase in nitrogen level and infestation of *C. partellus* on the varieties of maize.

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**INSECTICIDAL EFFICACY OF DIFFERENT EXTRACTS OF THE VARIOUS WOODS AND THEIR PARTS AGAINST TERMITES**

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The two species of termites *Coptotermes heimi* and *Heterotermes indicola* (Isoptera: Rhinotermitidae) commonly found in houses feeding on cellulosic material were forced to feed

upon three different Extracts of *E. cammeldulensis*, *D. sissoo* and *A. arabica* woods and their parts bark, sapwood and heartwood. The extracts of aforementioned wood samples and their parts were taken in water, ethanol-benzene, and chloroform using Soxhlet apparatus adjusting cycle 6 times per hour for 4-5 hours. Filter papers were dipped and then dried to remove solvents and hundred termite workers were forced to feed upon filter papers in Petri dishes impregnated with extracts. The population of termites was counted after each twenty four hours for six days. It was observed that among the three extracts, water soluble proved to be very effective for growth of termites as no any mortality was experienced feeding upon these extracts in all the woods and their parts. The ethanol-benzene and chloroform extracts of three woods and their parts found to be toxic to varying degrees for both species of termites. It indicates that some organic chemicals exhibiting insecticidal properties are found in the woods. This particular component can be identified and isolated using HPLC and GCMS and put into commercial use. Being natural products they will not have any side effects on other vertebrates. To make it cheaper we can use replaceable parts of termite resistant plants like leaves, bark, seeds etc.

**EVALUATION OF SOME ACARICIDES AGAINST TWO SPOTTED SPIDER MITES, *TETRANYCHUS URTICAE* KOCH (ACARI: TETRANYCHIDAE) UNDER LABORATORY AND FIELD CONDITIONS ON COTTON**

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The study was carried out to determine the efficacy of some commercially available acaricides against Two Spotted Spider mites (TSM), *Tetranychusurticae* Koch in the laboratory and field conditions at Department of Entomology, University of Agriculture, Faisalabad, Pakistan during summer 2010. The chlorfenapyr 36 SC, pyridaben 15 EC and fenpyroximate 5 SC proved to be the best miticides among the tested ones for the control of TSM based on their LC<sub>50</sub> values by leaf dip method in the lab and % mortality in the field. All the tested acaricides caused significant mortality both in the lab as well as in the field up to 72 hours after treatment. The chlorfenapyr (87-100 % mortality in the field and the lab) was the most effective one among all whereas dicofol (32-38 % mortality in the field and lab, respectively) was least effective. However, the fenpyroximate showed the lowest LC<sub>50</sub> value (51.8 mg l<sup>-1</sup> at 48 h) in the lab tests showing high effectiveness against mites. The recommended application rates used in the field and the LT<sub>50</sub> values further suggested judicious use of effective acaricides.

**ENTOMOPATHOGENIC FUNGUS *ISARIA FUMOSOROSEA* AS A BIOLOGICAL CONTROL AGENT AGAINST *RHIZOPERTHA DOMINICA* F. (COLEOPTERA: BOSTRYCHIDAE)**

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Lesser grain borer, *Rhizopertha dominica* (Coleoptera: Bostrychidae) is a very important primary pest of stored wheat in world. To evaluate the pathogenicity of entomopathogenic fungus

*Isaria fumosorosea*, an experiment was designed in the laboratory conditions against the adults of *Rhyzopertha dominica*. Spore suspension was made from 15 days old fungus culture on potato dextrose agar medium. The required concentrations of *I. fumosorosea* i.e.,  $1 \times 10^8$ ,  $2 \times 10^8$ , and  $3 \times 10^8$  spores/ml were prepared by serial dilution in 0.05 Tween 80 solution. The insects reared on the clean and dry grains in laboratory conditions were treated by dip method. The data of adult's mortality was recorded after 24 hours for consecutive seven days. The maximum percent mortality of 77.5% was recorded in concentration of  $3 \times 10^8$  spores/ml; in contrast to this minimum mortality percentage i.e., 52.5% was detected by treating the insects with the concentration of  $1 \times 10^8$  spores/ml. Results demonstrated that *I. fumosorosea* has a potential to be used for the control of lesser grain borer and its addition in the integrated pest management programs for the control of stored grains pests.

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**IMPAIRMENT OF GROWTH, DEVELOPMENT AND REPRODUCTION IN *TRIBOLIUM CASTANEUM* (HERBST) (COLEOPTERA: TENEBRIONIDAE) DUE TO LARVAL EXPOSURE TO LUFENURON-TREATED DIET**

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*Tribolium castaneum* is a cosmopolitan stored product pest causing serious infestations in raw stored grain, cereal processing facilities, warehouses, retail stores and home granaries. Present study was conducted for determining the sub-lethal effects of a chitin synthesis inhibitor (CSI), lufenuron at concentrations of 0.02, 0.04 and 0.08 ppm on various stages of *T. castaneum* under laboratory conditions. Lufenuron caused significant effects on larval mortality, larval weight and larval duration. Succeeding development of pupae and emergence of adults were seriously prohibited. When adults emerging from exposed larvae were allowed to oviposit in untreated wheat flour, both fecundity and egg hatchability were reduced significantly at all the concentrations compared to control treatment because of the transovarial activity of lufenuron. Further, subsequent development of surviving F<sub>1</sub> larvae, pupae and adults were also severely interdicted. Results show the potential usefulness of lufenuron in mills, warehouses and food storage facilities for the promising replacement of prevalently used organophosphate and pyrethroid grain protectants.

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**POTENTIAL USE OF *CALOTROPIS PROCERA* (GENTIANALES: ASCLEPIADACEAE) TO CONTROL *MUSCA DOMESTICA* (L.)**

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*Calotropis procera* (milkweed) is very common in uncultivated soil and in dry arid zones in the in Pakistan. In the present study insecticidal property of crude extract of *C. procera* was evaluated against *Musca domestica*. Mortality rate of flies was significantly high in the treated

group compared to the control. The results of present study suggested that extract of *C. procera* is highly effective against the *Musca domestica*. In order to introduce milkweed as potential technology for control of house flies there is a need to characterize its extract and pinpoint the chemicals which are responsible for the mortality.

**BIOLOGICAL ACTIVITY OF CITRUS ESSENTIAL OILS AGAINST *RHYZOPERTHA DOMINICA* (F.) (COLEOPTERA: BOSTRICHIDAE)**

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*Rhyzopertha dominica* is known as most destructive insect pest of various stored grain commodities. A study was conducted for determining the biological activity of essential oils extracted from peels of *Citrus paradisi* and *Citrus reticulata* against two strains of *R. dominica* adults under laboratory conditions. Twenty adults of *R. dominica* were exposed to the filter papers treated with citrus essential oils at concentrations of 3, 6 and 12% at exposure periods of 3, 6, and 9 days in each treatment. At concentration 12%, *C. paradisi* oil exhibited mean percent mortality (49.00%), followed by *C. reticulata* (37.33%) in Multan strain while (40.66%) and (30.00%) in Vehari strain respectively at exposure periods of 9 days. Adults mortality varied significantly with increase in concentration and due to the effect of citrus species. *R. dominica* population buildup was minimum (5.74%) due to effect of *C. paradisi* after 120 days of treatment while in control treatment population buildup was (21.66%). Results showed that *C. paradisi* oil was most effective than *C. reticulata* oil for the control of *R. domonica*. These results exhibited the potential of citrus oils for the management of *R. dominica* in food storage facilities.

**TOXICITY OF SOME INSECTICIDES AGAINST *CHRYSOPERLA CARNEA* STEPH.- AN IMPORTANT PREDATOR OF COTTON JASSID (*AMRASCA DEVASTANS* DIST.)**

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Insecticides not only kill the pest insects but also badly affect their natural predators. Chrysopid larvae are useful predators of various economically important insect pests like aphid, jassid, white fly etc. In this project toxicity of insecticides was observed against cotton jassid and its predator *Chrysoperla carnea* (Steph.). For this purpose, an experiment was laid out in CRD in the lab with eight treatments viz., Talstar, Endosulfan, Trebon, Confidor, Polo and Monocrotophos and two control treatments, replicated thrice, each with two concentrations (0.025% and 0.05%). Each pesticides was applied @ 1.4 ml on third instar larvae of the predator and known number of jassids with Pottar Spray Tower in three different modes (A1- insecticides applied to the substratum, A2- insecticides applied to the substratum+prey and A3- insecticides applied to the substratum + prey + predator). The observations were recorded after 24 hours of each application.

It was found that Monocrotophos and Talstar were highly toxic giving 96 - 100% and 80 - 90% kill of the predatory larvae, respectively. Confidor and Endosulfan were proved moderately toxic while Polo and Trebon found to be somewhat selective in their use against the predator; however, all the insecticides effectively controlled the cotton jassid.

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#### **KHAPRA BEETLE: A MENACE FOR RICE EXPORT**

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Khapra beetle- *Trogoderma granaium* Everts (Dermestidae: Coleoptera) causes 10- 18% grain losses in the country; however, losses attributable from this notorious pest to the global economy ranges from 02 to 70 percent. Resistant to starvation is main feature of its widespread throughout the world. Its increasing and alarming interceptions from the international trade of rice are quite evident from the example of USA alone. In 2005- 06, khapra interceptions were only 4.5 but this figure shoot up to 100 during 2011. Keeping in view their drastic impact on the economy and to safeguard the international trade of rice, a survey was conducted with the help of well defined questionnaire to identify the areas of its spread that can be targeted to control the khapra spread. This paper covers the various aspects regarding phyto- sanitary regulations and measures alongwith short and long term strategies for every stakeholder from miller to exporter to keep this notorious pest out of the rice trade.

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#### **TOXICITY OF DIFFERENT INSECTICIDES AGAINST LARVAL INSTARS OF LEAF WORM, *SPODOPTERA LITURA* (LEPIDOPTERA: NOCTUIDAE) FIELD POPULATIONS UNDER LABORATORY CONDITIONS**

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Cotton leaf worm, *Spodoptera litura* is an important insect pest of more than one hundred plant species with economic importance. Chemical control is considered an important tool to manage its population under threshold levels. Five insecticides namely lambda-cyhalothrin, profenofos, emamectin benzoate, lufenuron and thiamethoxam were tested against six larval instars of two field populations collected from cauliflower crops in Taxila and Rawalpindi during 2011. Leaf dip bioassays were performed and mortality as end point was considered to estimate the lethal concentrations at 50% kill at 48 and 72 hours exposure in laboratory. Emamectin benzoate followed by profenofos was more toxic as compared to that of lufenuron, cyhalothrin and thiamethoxam, respectively for both Rawalpindi and Taxila strains. However, profenofos was more toxic at 3<sup>rd</sup> larval instar. Population comparison revealed Taxila strain to be more tolerant to insecticides than that of Rawalpindi strain. Although lambda-cyhalothrin and thiamethoxam were less toxic yet contribute in early management of *S. litura* on economic crop like cotton, cauliflower, arum etc.

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**INVESTIGATION OF POSSIBLE MECHANISM OF INSECTICIDE RESISTANCE IN  
HOUSEFLY (*MUSCA DOMESTICA* L.)**

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Insecticide resistance in *Musca domestica* is a common phenomenon throughout the world. Present study was designed to evaluate the possible role of esterases and glutathione S-transferases against pyrethroids resistance in houseflies. Susceptibility tests were performed against various concentrations of lambda cyhalothrin (Field dose, 1/10<sup>th</sup> and 1/20<sup>th</sup> of field dose) for one hour and mortality was recorded till 24 hours after exposure. Mortality rate was highest at field rate concentration. For the biochemical estimation flies were divided into two groups, one group was exposed to the sub-lethal dose of insecticide while second group was taken as control. Esterase level for control and treated was determined by Van Asperen (1962). Method of Zhang et al. (2007) was used for the estimation of glutathione S-transferases. We did not observed any difference in the level of esterases between treated and control groups however; level of glutathione S-transferases was significantly higher in the treated group as compared to the control group. On the basis of our results it can be concluded that GSTs might play role in pyrethroid resistance.

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**MONITORING OF *PROCONTARINIA* SPP. AND VARIETAL SUSCEPTIBILITY/  
TOLERANCE OF DIFFERENT VARIETIES OF MANGO AGAINST MANGO LEAF  
GALL MIDGES IN MULTAN**

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Mongo leaf gall midges are serious threat to mango orchards in the Subcontinent. For monitoring adult and larval population, an experiment was performed at the Faiz Chaman Fruit Farm near Bahauddin Zakariya University Multan, Pakistan. For evaluation of the gall midges trapping efficiency of various colored sticky traps, eight different sticky traps (Blue, Green, Orange, Pink, Red, Violet, White and Yellow) and each treatment was replicated four times. The experiment was laid out in a Complete Randomized Block Design. A significantly higher number of *Procontarinia* Spp. Adults were trapped on orange traps as compared to other colored traps. Varietal susceptibility/tolerance of different mango varieties against mango leaf gall midges were evaluated by studying the population dynamics of larvae and its damage. Leaf gall midges attack was observed on all varieties of mangoes. Mango varieties Kala Chaunsa and Anwar ratol were comparatively tolerant showing 66.5% and 60% less larval population on plastic traps respectively than Sufaid Chaunsa. In the same way gall population on leaves of Chaunsa was 40% higher than Kala Chaunsa. Mango variety "Chaunsa" is most susceptible to mango gall midges.

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**FEEDING EFFICIENCY AND DEVELOPMENTAL PERIOD OF *SPODOPTERA EXIGUA* (LEPIDOPTERA: NOCTUIDAE) ON DIFFERENT HOST PLANTS**

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The beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae), is an important pest of numerous crops, and it causes economic damage. Development of efficient strategies for controlling beet army worm requires knowledge of its biological relationships with various host plants. Present study aimed at knowing of feeding efficiency and developmental period of *S. exigua*. On four host plants viz. Brassica, Cabbage, Cauliflower and Turnip. The damage caused by *S. exigua* was 62g, 59.5g, 58g, and 42g respectively from newly hatched 1<sup>st</sup> instar to pupal stage. Brassica was found to be most damaged crop by *S. exigua*. Average damage per instar was 10.33g in brassica followed by 9.96g Cauliflower, 9.91g Cabbage and Least 7g in case of Turnip. Maximum developmental period (25 Days) was calculated in turnip leaves and least on Brassica (21 Days). Thus the most preferred crop was Brassica and Cabbage where it completes its developmental stages in minimum duration as compare to others.

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**EVALUATION OF SOME MAIZE VARIETIES / LINES FOR THE RESISTANCE AGAINST ANGOUMOIS GRAIN MOTH *SITOTROGA CEREALELLA* (OLIV.) (LEPIDOPTERA: GELECHIIDAE)**

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In the recent year, maize breeders throughout the world have developed a very large number of new varieties of maize which have been bred for yield, length of growing season, plant height and resistance to the insect pests. In Pakistan *Sitotroga cerealella* caused great losses to stored maize very few studies have been done on maize resistance/ susceptibility related to biochemical factors. The present project was carried out to identify those resistant varieties by studying resistance index and response toward biology of *S. cerealella*. The experiment was conducted in the Toxicological Laboratory, Department of Agriculture Entomology, University of Agriculture Faisalabad, under the laboratory condition was maintained at 28±2°C and 65±5% R.H, in completely randomized design with ten treatments and three repeats including a control. Ten maize genotypes were used, out of which four were approved varieties, Neelum (Yellow), Sadaf (white), Agaiti-85 (Yellow) and Pak Afgyous (White), four famous hybrid varieties, 32B33 Sp08 (Yellow), 8711 Sp08 (Yellow), 8288 Sp08 (Yellow) and 33W86 Sp08 (yellow) and two basic lines, EV 5098 (Yellow) and EV 6098 (White). Uniform population of *S. cerealella* was maintained. The varieties were fed to the insect for observing adult emergence, developmental period, weight of adult moth, fecundity, hatching percentage, percent damage, percent weight loss and biochemical analysis of grains such as the total phenolic acid, total flavonoids, compounds, tannic acid concentration and percent inhibition were also accounted. Results showed that no variety was completely immune to the grain moth but the comparative resistance was observed in these varieties. 8711 HYB and 32W86 HYB were found to be the resistant with less fecundity, long developmental period, less damage percentage and less weight loss. The linear correlation of the

biochemical factors of the maize grains with insect developmental stages showed that grain weight loss was significantly correlated with total phenolic, total flavonoids compounds while insignificance with tannic acid and percent inhibition.

**INTEGRATED PEST MANAGEMENT OF MELON FRUITFLY *BACTROCERA CUCURBITAE* (COQUILLET) (DIPTERA: TEPHRITIDAE) ON BITTERGUORD**

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The Fruitfly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) is distributed widely in temperate, tropical and sub-tropical regions of the world. It has been reported to damage 81 host plants and cucurbitaceous vegetables, particularly the bittergourd (*Momordica charantia*), muskmelon (*Cucumis melo*), snap melon (*C. melo* var. *momordica*), and snake gourd (*Trichosanthes anguina*) are highly prone to damage by this pest. Due to the difficulties associated with control of this pest by chemical insecticides, farmers experienced great losses in cucurbits. Therefore, a trial on integrated pest management of fruitfly on bittergourd was conducted at Faisalabad during 2009 and 2010. The trial was arranged in RCBD with 3 replications. There were 8 treatments including untreated check. Collection of infested /rotten fruits was done at weekly interval. Pheromone traps were installed at flowering stage and kept in field till the completion of experiment. Spray of insecticides was carried out on the appearance of pest and repeated at 10 days interval. Data regarding fruitfly infestation were recorded by observing 50 fruits per plot selected at random. Minimum fruitfly infestation *i.e.* 4.89% and 6.22% was recorded in the treatments where collection of infested fruits & burying deep in soil at weekly interval + installation of pheromone traps (Methyl eugenol) were practiced. It was followed by 7.11% and 8.00% in the treatment where collection of infested fruits and burying deep in the soil + bait application (Diptrex 1 part + Methyl eugenol 2 parts + molasses 2 parts + vaseline 2 parts) were done as against 19.33% and 22.47 % infestation in untreated check during both years 2009 and 2010 respectively.

**STUDIES ON THE FREQUENCY OF INSECTICIDES APPLICATION ON THE CITRUS NURSERY AGAINST CITRUS LEAF MINER, *PHYLLOCNITIS CITRELLA* STAIN**

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The present studies were carried out to determine the frequency of insecticides viz. emamectin benzoate 1.9% EC, imidacloprid 20% SL, profenofos 50% EC, lufenuron 50% EC, acetamiprid 20% SP, bifenthrin 10% EC, spinosad 24% SC, cyhalothrin 2.5% EC, flufenoxuron 10% DC and triflumuron 20% EC against citrus leafminer and to find the best insecticide which gives maximum protection to citrus nursery and orchard for maximum time against citrus leafminer, *Phyllocnistis citrella* Stainton (Gracillariidae: Lepidoptera), in Randomized Complete Block Design (RCBD) with three replications at nursery area of Institute of Horticultural Sciences, University of Agriculture Faisalabad. The quantity of spray material was applied at 0.1% of each formulation in two seasons (Spring Flush & Monsoon Flush) Before the application of each treatment percent infestation of selected plants was calculated which was 25-35 % leaf infestation in the plots. Data for the percent reduction in leafminer infestation was calculated 24, 48, 72 and 96

hours after spray. Next application was only applied when new attack appeared on the selected plants. Percent larval mortality was corrected by Abbot's formula. Data were analyzed statistically (MStat). The results showed that all chemicals gave significant control of citrus leafminer with highest efficacy being shown by spinosad 24% SC, emamectin benzoate 1.9% EC and bifenthrin 10% EC in descending order. Insect growth regulators such as lufenuron 50% EC gave minimum results as compared to the other treatments. In each season number of spray ranged from 3-4 and time interval between two sprays was 4-10 days. Results can be used for sorting possibilities of replacement of these insecticides with alternative to reduce insecticides application at nursery stages of the citrus plants.

### FUTURE OF RICE CROP IN THE PUNJAB, PAKISTAN

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In the Kallar tract during a survey through face to face interviews of rice farmers their attitudes and thoughts about the future of rice crop were assessed. The imbalance use of fertilizers, rice straw burning, lack of new rice varieties, coverage of large area by a single variety, irrational use of pesticides, high costs of agricultural inputs, ever low support prices for rice produce, unavailability of credit and crop insurance policies, scarcity of irrigation water, increasing age of rice farmers and above all the desperation of farmer community from rice culture resulting in their migration towards the cities has fainted the future of this important crop. In order to make the future bright of this crop the positive change in attitude and philosophy among decision-makers, scientists and others stakeholder to acknowledge the importance of this crop for mankind so that while formulating policies and extension training programs regarding agriculture, hazards of pesticides, increasing costs of rice farming, declining prices of farm products, increasing average age of farmers, encouraging young people to choose farming as a profession and promoting a sense of pride in farmers were suggested.

### SUSCEPTIBILITY OF *LIAPHIS ERYSIMI* (KALT) TO INSECTICIDES ALONE AND IN COMBINATION UNDER LABORATORY CONDITIONS

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A laboratory experiment was conducted in the Toxicology Laboratory to find out the effectiveness and efficacy of systemic insecticides alone and in combinations against the adult females of mustard aphid, *Lipaphis erysimi* (Kalt), (Aphididae: Homoptera), in the Department of Agric. Entomology, University Of Agriculture, Faisalabad. Serial concentrations of carbosulfan 20 EC, acetamiprid 20 SL, triazofos 40 EC, profenofos 50EC and imidacloprid 20SL were found after preliminary laboratory tests. These insecticides were tested alone and in combination keeping carbosulfan as standard in 1:1 ratio with other above mentioned insecticides. Leaf disc method was

used for residual toxicity test by spraying insecticide concentrations with a Potter Tower. Ten adult females of mustard aphid were used in bioassays; which were released on treated leaves and efficacy of insecticide was assessed by counting the aphids' mortality after 24 hours. A concentration-mortality data was subjected to Probit Analysis to determine LC<sub>50</sub> of insecticide alone and in combination. The results obtained after probit analysis showed carbosulfon 20 EC gave the efficient control of Mustard Aphid when was used alone to control the mustard aphid under laboratory conditions. After carbosulfon 20EC, the order of effectiveness of other insecticides was acetamiprid 20 SL, triazofos 40 EC, imidacloprid 20SL and profenofos 50 EC when were used alone in order to find their efficacy against *Lipaphis erysimi*. As far as combinations, carbosulfon + profenofos insecticidal combinations gave the best control followed by carbosulfon + imidacloprid and carbosulfon + triazofos against mortality of *L. erysimi* (kalt).

#### ANTITERMITIC ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF FIFTEEN MEDICINAL PLANTS (SEED)

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The present study was designed to evaluate the antitermitic activity and phytochemical exploration of fifteen selected medicinal plant seed. The quantitative analysis of flavonoids, saponins, tennins and alkaloids were performed by bioassay method in EtOH (ethanol) extracts and the contents were found be ranged form 0.65-15.18 %, 0.54-8.60 % 0.08-27.71 % and 0.34-12.47 %, respectively, while qualitative analysis of methanolic extracts also revealed the presence of glycoside, steroid, cardiac glycosides and terpenoid. The MeOH extracts of fifteen medicinal plant seed showed excellent antitermitic activity and the Lt<sub>50</sub> of *F. vulgare*, *P. harmala*, *P. coryiifolia*, *R. commuis*, *C. tigung*, *mentha sp*, *O. sativum* and *C. frutesoens* was found lower than 10 mg/mL, while *N. stivum*, *a. sativum*, *P. ovata*, *A. indica*, and *M. azadirach* was found to be higher than 35 mg/mL of 15% solution of extracts. The Lt<sub>50</sub> was also investigated of 3%, 5% and 10% extracts, but found significantly ( $P>0.05$ ) higher than 15% extracts solution. It is concluded that these plant can be used as an antitermitic agent and as source of flavonoids, saponins, tennins and alkaloids.

#### IN VITRO TOXICITY OF SOME NEW INSECTICIDES ON *CHRYSOPERLA CARNEA* (STEPHENS)

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The comparative toxicity of some new insecticides *viz.*, Tracer (spinosad, 240 SC), Match (lufenuron, 5 EC), Belt (flubendiamide, 480 SC), Coragen (chlorantraniliprole, 20 SC), Proclaim (emamectin benzoate, 1.9 EC), Lorsban (chlorpyrifos, 40 EC), Decis (deltamethrin, 2.5 EC) @ 198,

494, 429, 62, 494, 2470 and 618 ml/ ha, respectively was tested on different larval stages of *Chrysoperla carnea* in the toxicology laboratory of Entomological Research Institute, Faisalabad during 22 April to 6 June, 2011 at  $27 \pm 1^\circ\text{C}$  and  $65 \pm 5\%$  RH. The observations on mortality percentage of 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> instar larvae of *C. carnea* were recorded from each treatment at 3, 6, 12, 24 and 48 hours of post application intervals. Deltamethrin 2.5 EC and chlorpyrifos 40 EC were found toxic to all the instars of *C. carnea* at all post treatment intervals with mortality % 40 to 96 and 32 to 92% respectively. The application of flubendiamide 480 SC was proved to be safe showing minimum mortality (8 to 16 %) of predator at 1<sup>st</sup> larval stage of the *C. carnea* at all the post treatment intervals except 48 hours (64%). Spinosad 240 SC ranged from (28 to 44 %), lufenuron 5 EC (32 to 56%), Chlorantraniliprole 20 SC (32 to 56) and Emamectin benzoate 1.9 EC (28 to 80%) were intermediate in their effect in almost all the post treatment intervals for the 1<sup>st</sup> instar larvae of *C. carnea*. The application of spinosad and lufenuron were found equal statistically, less effective and proved safe for 2<sup>nd</sup> (20 to 68 and 40 to 56% respectively) 3<sup>rd</sup> instar larvae (20, 40 to 56 and 8 to 44% respectively) of *C. carnea* at all post treatment intervals.

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**COMBINED EFFECT OF SILYMARIN AND GLYCYRRHIZIN TO EVALUATE THE MECHANISM OF OXIDATIVE DEFENCE IN RATS (*RATTUS NORVEGICUS*) RECEIVING CCl<sub>4</sub> FOR ARTIFICIALLY INDUCED LIVER INJURY**

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A time course study was designed to evaluate the effect of Silymarin (SLN) and glycyrrhizin (GLN) in different dosage regimen entail to lessen the oxidative stress, the ultimate cause of hepatic injury in rat models receiving CCl<sub>4</sub> as hepatotoxin. Total sixty Wister male albino rats were used in this study randomly allocated into four groups as A, B, C, D, E and F. Group A was served as control. Groups B, C, D, E and F received a dose of CCl<sub>4</sub> (50% solution of CCl<sub>4</sub> in liquid paraffin, 2ml/kg b.w., intraperitoneally (i.p.) twice a week to induce hepatic injury. Rats in groups C, D, E and F in addition to CCl<sub>4</sub> Silymarin @ 200 mg/kg, Glycyrrhizin @ 50 mg/kg, Silymarin @ 100 mg/kg and 200 mg/kg with Glycyrrhizin @ 25 mg/kg and 50 mg/kg respectively for a period of six weeks. The results of the present study shows that CCl<sub>4</sub> induce hepatic injury by a significant increase in serum ALT (Alanine amino transferase), AST (Aspartate amino transferase), ALP (Alkaline phosphatase), TBARS (Thiobarbituric acid reactive substances) along with decrease in TP (Total protein), GSH (Reduced glutathione), SOD (Superoxide dismutase) and CAT (Catalase) activities. Treatment with different doses of SLN and GLN significantly reduce ALT, AST and TBARS levels in rats receiving CCl<sub>4</sub>. GSH and hepatic enzymes like SOD and CAT were significantly increased in rats treated with Silymarin and Glycyrrhizin in different combinations. The synergistic effect of Silymarin and Glycyrrhizin reduce the oxidative damage at both the combined doses as compared to CCl<sub>4</sub> treated rats but pronounced effect was observed in rats receiving 200 mg/kg of Silymarin and 50 mg/kg of Glycyrrhizin. It was summarized from the present study that Silymarin and Glycyrrhizin have a synergistic and significant time course effects to alleviate the oxidative stress induced by CCl<sub>4</sub> and conform the antioxidative properties.

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**RED COTTON BUG, *DYSDERCUSINGULATUS* (HEMIPTERA : PYRRHOCORIDAE):  
AN EMERGING THREAT TO COTTON IN PUNJAB (PAKISTAN) AND ITS CONTROL**

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Efficacy of five botanicals viz., Neem (*Azadirachta indica*), Sufaida (*Eucalyptus obliqua*), Datura (*Datura alba*), Moringa (*Moringa oleifera*) and Akk (*Calotropis gigantea*) as well as five insecticides Chlorpyrifos 40 EC, Malathion 57EC, Lambda-cyhalothrin 2.5EC, Nitenpyram 10AS and Imidacloprid 20SL were tested against red cotton bug in laboratory. Insecticides were also applied through flooding viz., Chlorpyrifos 40EC, Fipronil 50EC, Malathion 57EC and Imidacloprid 20SL. Among five botanicals maximum mortality after two days of application under laboratory conditions was recorded in Datura (*Datura alba*) leaf extract i.e. 50%. However, mortality in descending order was Datura (50%) > Moringa & Akk (43.33%) > Neem (40%) > Sufaida (26%). Insecticidal application revealed that Chlorpyrifos 40EC @ 800 ml/100 lit water in lab. and 1.5 lit/ac under field conditions gave highest mortality of 97.33% and 96.67% respectively, whereas Imidacloprid 20 SL @ 250 ml/100 lit water (in lab) and 800 ml/ac (in field) resulted in minimum mortality i.e., 89.33% & 58.35% respectively. Toxicity in descending order of different insecticides in laboratory was recorded as Chlorpyrifos 40EC (97.33%) > Malathion 57EC (96.67%) > Lambda-cyhalothrin 2.5EC (90.67%) > Nitenpyram 10AS and Imidacloprid 20SL (89.33%), whereas by flooding method the order of toxicity was Chlorpyrifos 40EC (96.66%) > Malathion 57EC (85.65%) > Fipronil 50EC (72.93%) > Imidacloprid 20%SL (58.35%) > Water Flooding (23.23%).

**EFFICACY OF ESSENTIAL OILS AGAINST AMERICAN COCKROACH  
(*PERIPLANETA AMERICANA* L.)**

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The toxicity, repellency and fumigant activity of three essential oils were evaluated against the *Periplaneta americana* (L.) under laboratory conditions. The essential oil derived from *Cymbopogon citratus* showed the best toxicity, repellency and fumigant activity over the other candidate essential oil. The essential oil of *C. citratus* exhibited complete repellency (up to 100%) against *P. americana* using different concentration (4, 5, 6 and 7%) after 24 hours. While maximum toxicity from 20-100% was observed between 2-24 hour intervals. LC<sub>50</sub> value for *C. citratus* is 8.013, 7.050, 4.897 and 3.391 at 2, 4, 6 and 24 hours respectively. Fumigant activity ranging from 70-100% was observed using *C. citratus* under laboratory conditions against *P. americana*. Satisfactory toxicity 10-100% was found using *Mentha arvensis* at different concentrations between different time interval (2-24 hours). In case of *M. arvensis* LC<sub>50</sub> value for contact toxicity is 8.122, 8.013, 6.004 and 4.640 after 2, 4, 6 and 24 hours respectively. While complete repellency was observed only at 7% concentration. Fumigation value ranges from 50-80% was seen in case of *M. arvensis* at different concentrations. Least toxicity, repellency and fumigant activity was observed in case of *Eucalyptus citrodora*. Percentage mortality from 0-80%

was achieved between 2-24 hours at different concentrations. LC<sub>50</sub> value is 8.268, 10.292, 7.050 and 4.814 after 2, 4, 6 and 24 hours respectively. 40-60% fumigant activity was observed after 24 hours at different concentrations. *Eucalyptus citrodora* proved to be less toxic than other essential oils. Order of toxicity of three oils can be arranged as: *Cymbopogon citratus* > *Mentha arvensis* > *Eucalyptus citrodora*

#### LABORATORY EVALUATION OF METHOPRENE INTEGRATED WITH *BACILLUS THURINGIENSIS* WDG FOR THE CONTROL OF *Aedes aegypti* LARVAE

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In the present study laboratory evaluation of methoprene integrated with *Bacillus thuringiensis* var *israelensis* Vectobac® WDG (water dispersible granules 3000 ITU / mg) in a mixture of 1:2 was carried out against *Ae. aegypti* (early 4<sup>th</sup> instars) larvae collected from Lahore Pakistan. The main objective was to determine the minimum effective dosage of methoprene and *Bti*WDG alone and in a mixture of these two (1:2) for larval mortality along with pupae reduction and adult emergence inhibition (EI) of *Aedes aegypti* reared in laboratory. Probit regression analysis of *Bti*WDG alone indicated LC<sub>50</sub> 0.071 ppm and 0.040 ppm at 24-48 hours post exposure. Whereas, LC<sub>95</sub> was 0.126-0.028 ppm at 24-68h post exposure respectively. Pupae formation was completely inhibited against 100-0.1 ppm while 88% pupae reduction was observed against 0.01-0.0001 ppm. In addition there was complete adult emergence inhibition against all doses (100-0.0001 ppm). Integration of methoprene with *Bti*WDG showed more toxic effects on all developmental stages i. e. larvae, pupae and adults. Probit regression analysis revealed LC<sub>50</sub> 0.019-0.005 ppm at 24-72h respectively, whereas, LC<sub>95</sub> was 0.047- 0.001 ppm at 24-168h post exposure indicating 2X higher larval mortality rate in integrated group. Complete pupae reduction (100%) was observed against 50-0.05 ppm and 92% against 0.00005 ppm while no adult emerged against all doses of methoprene integrated with *Bti*WDG. These results indicated that mortality rate was very rapid and toxic effect was enhanced by 2-3X in integrated control as compared to *Bti*WDG alone.

#### THE EFFECT OF ORAL ADMINISTRATION OF SILYMARIN AND GLYCYRRHIZIN ON LEAD NITRATE INDUCED TOXICITY IN MALE MICE

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The present work was designed to investigate the effect of oral administration of silymarin and glycyrrhizin on lead nitrate induced toxicity in male mice. Total 50 albino mice were used which were divided into five groups. The first group was served as positive control and all the remaining four groups received lead nitrate @ 1mg/kg b.w for seven days. The second group received on lead nitrate and served as negative control. The other two group received Silymarin (SLN) and Glycyrrhizin (GLN) along with lead nitrate @ (200 mg/kg body weight)/(50 mg/kg body weight) respectively for a period of one month. The last group supplemented with Silymarin

(SLN) and Glycyrrhizin (GLN) along with lead nitrate @ (200 mg/kg body weight)/(50 mg/kg body weight) for a period of one month as a combination therapy. GSH, SOD, MDA, CAT, ALT, AST, ALP, TP, Blood Pb contents, kidney Pb contents, serum creatinine and serum urea were estimated. The result of the present study showed that silymarin and glycyrrhizin reduced lead toxicity by increasing levels of GSH, SOD, CAT, TP and decreasing levels of MDA, AST, ALP, Pb contents in blood and kidney, serum creatinine and serum urea which was raised as a result of lead toxicity. It was summarized from the present study that Glycyrrhizin and Silymarin alleviate the lead toxicity when administered alone but profound effects were recorded when SLN and GLN provided in combination

**LABORATORY EVALUATION OF IMIDACLOPRID AS A TERMITICIDE AGAINST TERMITE, *HETEROTERMES INDICOLA***

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Studies were conducted to evaluate the dose response relationship, horizontal transfer and deterrence of Imidacloprid against *Heterotermes indicola* in the Entomology laboratory at Nuclear Institute for Food and Agriculture (NIFA) Peshawar. The studies were initiated with the lower concentrations of Imidacloprid. Doses of 1-10 ppm showed significantly lower mortalities (20-30%) as compared with the doses exceeding 20 ppm. In the next series of experiments, higher doses i.e. 100-500 ppm was chosen. All the doses higher than 100 ppm resulted in significant worker mortality (>80%) within a period of 12 days. ELT 50 values ranged from 4-8 days whereas ELT 90 values ranged from 8 to 16 days. The values of 95% confidence interval, a dose of 100 ppm may take 12 to 27 days to inflict 90% mortality after the termites get a chance to feed on the treated substrate. At a dose of 500 ppm the time may vary from 6 to 10 days. The horizontal transfer of Imidacloprid showed that a concentration of 100 ppm did not produce a high recipient mortality (<30%) when confined with the donor for 10 days. However for the rest of concentrations, significantly higher mortalities were observed in untreated termites (recipients) confined with treated workers. Deterrence studies showed that termites consumed significantly lesser amounts of blotting paper treated with all the concentrations of Imidacloprid except 100 ppm. Termites fed very little on the treated substrate indicating the strong feeding deterrence of Imidacloprid at the concentrations >200 ppm. Mortality recorded was more than 90% in the experimental arenas having a treated blotting paper with 200 ppm or greater concentrations.

**EFFECTS OF TEMPERATURE AND HOST EGG AGE ON QUALITY PRODUCTION OF *TRICHOGRAMMA CHILONIS* ON LEPIDOPTEROUS (*SITOTROGA CEREALLELA* AND *CORCYRA CEPHALONICA*) EGGS**

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The present study was conducted for efficient and quality production of the stingless wasp, *Trichogramma chilonis* Ishii with respect to rearing temperature and host egg age of the angoumois grain moth, *Sitotroga cerealella* (Olivier) and the rice meal moth, *Corcyra cephalonica* (Stainton)

on its biology. Maximum parasitism was observed 95.7 and 84.3% at 28 °C, while minimum parasitism was 61.3 and 39.6% at 32 °C on *S. cerealella* and *C. cephalonica* eggs, respectively. The most favorable temperature was 28°C on which maximum parasitism and adult emergence were obtained from *S. cerealella* eggs. Maximum parasitism was observed 97.4 and 79.4% in 2 h old, while minimum parasitism was 24.6 and 17.3% in 72 h old eggs, of *S. Cereallela* and *C. cephalonica* eggs, respectively. Parasitism by *T. chilonis* decreased with increasing host eggs age. Maximum adult *T. chilonis* emergence was 98.2% in 2 h old eggs, while minimum emergence was 21.5% on 72 h old eggs of *S. cereallela*. Adult *T. chilonis* longevity on the host eggs of different ages of female wasp was non-significantly different to each other except the 2 and 12 h old eggs which were significantly different from rest of the treatments in both hosts' eggs of different ages. Maximum female longevity was 4.0 don 2 h fresh eggs *C. cephalonica*, while minimum was 3.0 don 24–48 h old *S. cereallela* eggs. The female ratio for different host eggs age was almost non-significant to each other except 2 h old eggs, with maximum number of female (64). The results showed that *T. chilonis* preferred young eggs when offered older eggs, simultaneously.

**EVALUATING FIPRONIL AS A SLOW-ACTING TOXICANT AGAINST  
*HETEROTERMES INDICOLA* (RHINOTERMITIDAE: ISOPTERA)**

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Laboratory experiments were done to evaluate Fipronil as a slow-acting toxicant against termite, *Heterotermes indicola*. When exposed to different concentrations of Fipronil (1, 10, 20, 30 and 50 ppm), a very fast mortality rate was observed for doses more than 1 ppm. A dose of 1 ppm however had a slow rate of kill and reached 100% mortality in 12 days. The dose of 5 ppm shows an intermediate response where an initial fast rate of kill was observed which slowed down later, culminating in total mortality at day 8. ELT 50 (Effective lethal time to kill 50% of the treated termites) were estimated to be 7.5, 1.84, 1.41, 1.2, 1.8 and 1.01 days at the doses of 1, 5, 10, 20, 30 and 50 ppm respectively. Deterrence studies showed that at concentration range of 1-20 ppm, no significant difference was observed between the consumption of treated and untreated substrate while at 30 and 50 ppm, termites preferred to feed on untreated food. Studies on horizontal transfer of Fipronil from the workers exposed to different concentrations (donors) and mixed with untreated workers (recipients) for period of almost one week indicated that all the concentrations greater than 1 ppm were able to inflict more than 50% mortality in the untreated workers. Our studies confirm that Fipronil can be used as a non-deterrent and slow-acting toxicant against *H. indicola*.

**SUSCEPTIBILITY OF STORED VARIETIES OF FRESH DATES OF SINDH TO THE  
INFESTATION BY *ORYZAEPHILUS SURINAMENSIS* (COLEOPTERA)**

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The date-palm, *Phoenix dactylifera* L. has been considered the most important plant product in desert regions of north Africa and south west Asia since ancient time, due to its high nutritional value and storage properties, Lee (1963), Riad (2006). Dates are rich in carbohydrates,

minerals and vitamins etc. There is no cholesterol and no fat in dates, Al-Farsi *et al.* (2008). In Pakistan, Sindh is an ideal place for cultivating the varieties of dates such as Aseel, Karbalian, Copro, Fasli, Dadhi etc, Bhambro (2003). Stored dates are facing sever problem of insect infestation, mainly from Sawtoothed grain beetle *Oryzaephilus surinamensis* (L.) (Coleoptera : Silivaniidae) by reducing the quality and quantity (weight loss) of dates, Aldryhim and Adam (1998). Saw toothed grain beetles were reared on their natural food, three stored varieties of fresh dates viz, Kupro, Karbalian and Fasli, for three months from 01-10-20011 to 31-12-20011 at the entomology laboratory, department of Zoology, University of sindh, Jamshoro under the controlled condition. The effect of infestation on the quality and different nutrients of dates was studied. During this period the development of beetles was variable on different varieties. We kept 10 pairs of beetles in each variety. The highest numbers of sawtoothed grain beetles were developed on Kupro and Karbalian varieties, counts 350, 300 beetles respectively and 66% damaged of weight loss was observed in each variety. In Fasly the number of beetles, counts 200 beetles, and 45% damaged of weight loss was observed. The growth rate of larvae of the beetles was much higher on kupro and karbalian than Fasly. This result indicates that increase in the moisture and sugar content in different varieties were corresponded with the increase in infestation by stored beetles. The infestation rate showed significantly positive correlation with moisture and carbohydrates (sugar).

**LARVICIDAL PROPERTY OF ESSENTIAL OILS AGAINST *Aedes aegypti* AND *Culex quinquefasciatus* LARVAE (DIPTERA: CULICIDAE)**

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Five essential oils from various parts of plant species *Acorus calamus*, *Mentha arvensis*, *ocimum basilicum*, *Saussrea lappa* and *Cymbopogan citrates* were investigated for their larvicidal properties against *Aedes aegypti* and *Culex quinquefasciatus*. Essential oils were obtained by steam distillation method. The mosquitoes were reared in laboratory by maintaining conditions and twenty late 3<sup>rd</sup> instar larvae of *Ae.aegypti* and *Cx.quinquefasciatus* were exposed to different concentrations of essential oils ranging from 1.95-1000.00ppm. The larval mortality was observed after 24 hours under the laboratory conditions. Results showed that all the five plant essential oil produced significant larval mortality against two mosquito species. However the highest larvicidal activity was observed in the essential oil from *O. basilicum* against *Ae.aegypti* and *Cx.quinquefasciatus* with the LC<sub>50</sub> values 75.35 ppm and 92.30 ppm respectively. However the LC<sub>50</sub> values for *A. calamus*, *M. arvensis*, *S. lappa* and *C. citrates* against *Ae. aegypti* are 99.41, 114.33, 128.89 and 136.28ppm respectively and against *Cx.quinquefasciatus* are 107.81, 112.18, 141.43 and 148.54 ppm respectively. So according to the larvicidal activity of essential oils against *Ae.aegypti* and *Cx.quinquefasciatus*, the oils were arranged in the following ascending order of preference *i.e.* *O. basilicum* > *A. calamus* > *M.arvensis* > *S. lappa* > *C.citratus*. In this study, it can be concluded that five essential oils which were distilled from *A. calamus*, *M. arvensis*, *O. basilicum*, *S. lappa* and *C. citrates* has remarkable larvicidal properties, which may be considered as a potent source for the production of natural larvicides which would be environmentally safe and alternative to synthetic insecticides.

**EVALUATION OF NATIONAL UNIFORM WHEAT YIELD TRAILS (NUWYT)  
NORMAL FOR RESISTANCE AGAINST CORN LEAF APHID *ROPHALOSIPHUM  
MAIDAS (L.)*.**

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Seedling Bulk Test was conducted for advanced entries of National Uniform Wheat Yield Trails (NUWYT) Normal (N) of year 2010-11, For Resistance against Corn Leaf Aphid (*Rhopalosiphum maidas*). Out of 20 entries one line PR-102 was found to be resistant while BGWS-4 and L. Check were moderately resistant. Six entries, V-07096, V-76309, V-15, V-07067 and DN-92 were found to be susceptible. In case of antixenosis (non preference) studies of same entries results showed that least preferred entries were V-08173, V-07076, V-76309 and V-05BT006 while highly preferred were NARC-2002 and TW 96007 against Corn leaf aphid, While In case of antibiosis (number of nymphs laid) studies of same entries results showed that entries V-07096, V-07076, V-08171, V-0587014, SD-076309, 076377, CT-04192, TW-96007, HB-10, HB-11, PR-102, QS-111, NR-378, NARC-2002 were least fecund (LF). Entries V-07067, V-15 were moderately fecund. Entries NR-379, DN-92, V-07193, and BGWS-4 were highly fecund. In these studies Resistant and Moderately resistant wheat varieties were recommended entries against Bird Cherry Oat Aphid.

**LABORATORY SCREENING OF WHEAT ENTRIES AGAINST WHEAT APHIDS FOR  
THE SOURCE OF RESISTANCE**

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Seedling Bulk Test was conducted for advanced entries of National Uniform Wheat Yield Trails (NUWYT) Rainfed (RF) of year 2010-11, for Resistance against Corn Leaf Aphid (*Rhopalosiphum maidas*). Out of 12 entries three line Nrl-0517, DN-73 and NR-397 were found to be resistant while NR-381, NR-390 and NARC- 2002 were moderately resistant. One Line was found to be susceptible AZRC-2008-1 remaining were moderately resistant. In case of Antixenosis studies (first component of resistance) on same entries results showed that out of 12 entries moderately preferred entries were Nrl-0517, AZRC-2008-1, NR-381, NR-390 and NR-391. In case of antibiosis (second component of resistance) studies results showed that Least fecund (LF) entry was NR-397, moderately fecund (MF) were NRL0517, DN73, 05FJS3075, AZRC-2008-1, 6C016, NR-381, NR-390, NR-391, NR-392 and NARC-2002. In case of Tolerance (third component of resistance) studies results showed that comparatively tolerant entries were KT-50 and NR-397. while least tolerant variety was 05FJS3075.

**SPIDER (ARANEAE: ARANIEDAE) FAUNA OF MANGO ORCHARDS IN DISTRICT  
JAMSHORO FROM SINDH, PAKISTAN**

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Spiders are carnivorous and one of the most abundant predatory group in the terrestrial ecosystem. They play important role in insect population reduction and in agro-ecosystem. The present investigation was carried out to identify the spider's fauna in mango orchard of Jamshoro and its adjoining areas during 2010-2011. Specimens were collected from tree canopy, soil and vegetation of selected mango grooves and were taken to laboratory for identification. High population of spiders was collected during the months of May-September, collection was continued but reduced population was recorded due to the use of insecticides against mango pests. The results revealed that out of the total of 847 specimens collected, 46 species belong to 11 families and 29 genera. The species identified are recorded for the first time from Sindh, Pakistan.

**MEALY BUG PARASITOID (*AENASIUS BAMBAWALEI* HAYAT) AND ITS  
PARASITISM ON COTTON MEALY BUG (*PHENACOCCLUS SOLENOPSIS* TINSLEY)**

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Present study on interactions of cotton mealy bug, *Phenacoccus solenopsis* (Tinsley) (Hemiptera: Pseudococcidae) and its parasitoid, *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae) were conducted under laboratory conditions in mass rearing laboratories at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad. Test insects were provided with sugar solution with natural diet which consists of potato sprouts, okra and cucurbits, under captivity. Results showed that *A. bambawalei* parasitized its host population up to 36-57%. There was no preference observed by parasitoid to 1<sup>st</sup> and 2<sup>nd</sup> instars of mealy bug whereas, wasp parasitism was related to 3<sup>rd</sup> instar of mealy bug. Moreover it has no parasitism to 4<sup>th</sup> instar (adult wingless female) mealy bug and for adult winged male.

**EFFECTS OF BT COTTON PLANTS ON THE LARVAL DEVELOPMENT OF  
ARMYWORMS (*SPODOPTERA LITURA* (F)) (LEPIDOPTERA: NOCTUIDAE)**

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Bioassays of Bt cotton plants against larvae of armyworms, *Spodoptera litura* (Lepidoptera: Noctuidae) were conducted under laboratory conditions to assess efficacy of Bt cotton and its effects. Larvae of armyworms were reared and fed on leaves and other plant parts from hatching to pupation and eventually their moth emergence. Result showed that Bt cotton

leaves gave high protection up to 100% mortality against 1<sup>st</sup> and 2<sup>nd</sup> instar larvae while failed to protect the crop against 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> instar larvae. However sub-lethal effects by continuous feeding on Bt plants, were observed in later instars with decreased larval development, unsuccessful molt to next instar, deformed pupation, and low moth emergence.

**IMPACT OF VARIETIES AND WEATHER FACTORS IN INFESTATION OF MAIZE STEM BORER, *CHILO PARTELLUS* (SWINHOE) ON MAIZE**

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Plant infestation due to Maize borer (*Chilo partellus Swinhoe*) varied among genotypes at various dates of observation. On cumulative basis the genotype FH-932 appeared to be comparatively susceptible showing maximum plant infestation i.e. 8.59 percent where as the genotype FH-985 with minimum plant infestation i.e. 5.27 percent found to be comparatively resistant. The maximum plant infestation was recorded to be 10.56 percent on April 08, 2011 and this infestation was decreased thereafter on the subsequent dates of observation till a minimum level of 1.7 percent on June 03, 2011. Maximum, minimum and average temperature had negative and significant effect on the plant infestation while relative humidity and rainfall exerted non significant effect. Maximum temperature contributed a maximum role in plant infestation i.e. 57.3 percent followed by minimum temperature with 40 percent role in the fluctuation of plant infestation and found to be the most important. The maximum plant infestation caused by maize stem borer was observed to be 10.57 percent on April 08, 2011 with prevailing weather conditions of 32.4°C (maximum temperature), 15.5°C, (minimum temperature), 23.09°C (averages temperature), and 31 percent RH.

**EFFICACY OF LIGHT EQUIPPED POWERED SUCKER AND INSECT KILLER IN COTTON AGAINST SPOTTED BOLLWORM (*EARIAS* PP.) AND ARMYWORM (*SPODOPTERA LITURA*)**

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Present studies were undertaken to determine the efficacy of Light Equipped Power Insect Killer (LEPIK) machine comparing with its different intervals of application and chemical control. Check was also included to evaluate that either the treatments are effective or not. Research was conducted in two consecutive years i.e. 2010 and 2011 during cotton growing season. Pest population data was recorded from the field using Maryo's Method. Hence, the plots served as Check show highest pest population density of Spotted bollworm (*Earias* spp.) (15.790 & 12.723) and Armyworm (*Spodoptera litura*) (13.240 & 11.310) on the basis of post treatment data. Data was collected after each treatment application either it was LEPIK technology treatment or chemical control. Plots treated with LEPIK machine at 3 days interval performed excellently

among other intervals of the machine while comparing with table means. Results can be summarized as for both of the years respectively, 4.200 and 3.067 mean values for 3 days LEPIK machine application for *Earias* spp. and for *Spodoptera litura* 3.33 & 2.027. Chemical though show knock down effect and gave table means such as; *Earias* spp. mean table values as 2.943 & 1.733 and *Spodoptera litura* table mean values as 1.897 & 0.627.

**STUDY ON THE COMBINED INSECTICIDAL EFFECT OF PYRETHROID,  
AZADIRACHTA INDICA AND BORIC ACID ON THE BACILLUS THURINGIENSIS  
EFFICACY IN TRIBOLIUM CASTANEUM**

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The red flour beetle (*Tribolium castaneum*) is a common pest insect known for attacking and infesting stored flour and grain. They are one of the most common and most destructive insect pests for grain and other food products stored in silos, warehouses, grocery stores, and the home. *Bacillus thuringiensis* (*B.t*) is a Gram positive, soil dwelling bacteria commonly used as a biological alternative to a pesticide. The present project was designed for the study of combined insecticidal effect of *Bacillus thuringiensis* along with boric acid, insecticide cypermethrin and the plant leaves powder i.e *Azadirachta indica* against the *Tribolium castaneum* to check the mortality rate of these insects and LC<sub>50</sub> was calculated. Bacteria were isolated from rich soil, pulse dust, grain dust and its growth was checked on the T3 media by applying the microbial techniques. On the other hand the larvae of *T castaneum* were reared in empty glass jars, the third instar larvae and the adults were collected which were used in insecticidal bioassays. At regular time interval of 24, 48 and 72 hours the results of individual bioassay were checked against larvae as well as for adults and LC<sub>50</sub> values of each concentration was calculated by SPSS (Probit) Programme. The results showed synergism when applied in combined concentration of *B.t*, boric acid, cypermethrin and powder of neem leaves in 1 gm of diet. The combined concentration of each gave higher mortality to larvae and the %age mortality was calculated as 33, 16 and 16% after 24 hrs, 41, 25 and 16% after 48 hrs and 50, 25 and 16% after 72 hrs in three different combined concentrations as compared to individual concentration. Likewise, in case of adults the %age mortality was 33, 16 and 13% after 24 hrs, 41, 20 and 16% after 48 hrs and 41, 25 and 16% after 72 hrs of treatment.

**EFFECTS OF PESTICIDE APPLICATION ON THE PEST CONTROL POTENTIAL OF  
SPIDERS RESIDING THE RICE FIELDS OF PUNJAB, PAKISTAN**

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The aim of this study was to investigate the effects of the application of different pesticides on the pest control potential of spiders residing rice fields of Punjab, Pakistan. For this purpose, sampling was conducted at rice fields located at Kasur, Chandrai Village Lahore and Adaptive Research Farm Sheikhpura. The sampling was done using pitfalls and De-Vac suction sampler. Four fields of one acre area were selected at each of the sites. The fields were treated with the pesticides Lambda Cyhalothrin, Monomehypo, Cartap, Ryzelon and Imidacloprid. The results showed 27.5%, 12% and 34% decrease from control in the density of spiders at the sites treated

with Lambda Cyhalothrin, Imidacloprid and Cartap respectively, located at Chandrai Village, Lahore. At Kasur, Lambda Cyhalothrin, Imidacloprid and Monomehypo treated rice fields had 26%, 35% and 29% less spider density as compared to control field. A 41% decrease in spider's density was observed at the site treated with a combination of Ryzelon and Lambda Cyhalothrin. During the study spider populations failed to reach their peak potential in the treated fields during the crop season. These results demonstrate that pest control potential of spider decrease due to their low density, which release the insect pests' populations from the predation pressure.

#### **EFFECT OF LEAF MORPHOLOGY ON WHITEFLY POPULATION IN DIFFERENT COTTON GENOTYPES IN SARGODHA, PAKISTAN**

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The research work conducted to study the effect of leaf morphological characters, i.e. number of hairs, number of gossypol glands and length of hairs on lamina, midrib and vein of leaf surface in four cotton genotypes, i.e. IR-3701, CM-496, FH-113 and BT-802. The result shows that IR-3701 is highly susceptible to whitefly population, maximum number of whitefly population than other genotypes; it has 17.73 whiteflies per leaf. CIM-496 is less susceptible to whitefly population, having minimum number of whitefly population, which is 9.61 whiteflies per leaf. FH-113 has 13.75 whitefly population per leaf and BT-802 has 12.78 whitefly per leaf. Length of hairs on lamina has no significant correlation with whitefly population, length of hairs on midrib and gossypol glands on lamina has significant correlation with whitefly population and length of hairs on vein, gossypol glands on midrib, vein and number of hairs on lamina, midrib and vein has highly significant correlation with whitefly population.

#### **POTENTIAL REPRODUCTIVE HEALTH EFFECTS AND OXIDATIVE STRESS ASSOCIATED WITH PESTICIDE EXPOSURE**

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During the last several decades there has been widespread use of potential pesticides in Pakistan to improve agricultural products. Pesticides used in agriculture cause deleterious effects contributed to dramatic increase in oxidative stress leading to reproductive dysfunction. The present study therefore aimed to investigate potential harmful effects of selected pesticides causing oxidative stress and reproductive toxicity in mice. model. Three pesticides, Chlorpyrifos, Carbofurn and Cypermethrin were selected for the present study. The experimental work was conducted on eighty albino male and eighty albino female mice (*Mus musculus*) which were grouped into four groups of both male and female mice with twenty rats in each group. The experimental groups were control group Chlorpyrifos. administered group, Carbofurn administered

group and Cypermethrin administered group. The treated mice received solution of Chlorpyrifos, Carbofom and Cypermethrin daily at the concentration of 0.5 mg/Kg body weight/ml for the period of sixty days. The blood samples were collected from each mouse at the intervals of twenty days. The samples were processed and analyzed to assess oxidative stress and cellular damage by performing histopathology of testis and ovary. Antioxidant enzymes; catalase, superoxide dismutase and glutathione peroxidase, malondialdehyde as marker of oxidative stress, sperm count and changes in testicular and ovarian histology were the criteria used to evaluate oxidative stress and reproductive toxicity of the treated male and female mice. Result showed high malondialdehyde levels and low levels of antioxidant enzymes catalase, superoxide dismutase and glutathione peroxidase raised lipid peroxidation and oxidative stress. Reduced sperm count and excessive destruction of testicular follicles with destruction of germ line cells, spermatids, Leydig cells and Sertoli cells was observed in all the treated male mice. Regenerative changes in the ovarian tissue viz: destruction of stromal and germinal layers, stromal necrosis and, fibrin necrosis; inflamed and degenerative follicles was observed as an indicative of the reproductive toxicology and damage in female mice however the damage was more severe in testis of male mice as compared to ovarian damage in female mice. It was inferred from the outcomes of present study that the administration of Chlorpyrifos, Carbofom and Cypermethrin at lethal dose causes oxidative stress by the generation of reactive oxygen species (ROS) resulting in reproductive damage at cellular level.

**EFFECT OF BIOLOGICAL CONTROL ALONG WITH OTHER ENVIRO- FRIENDLY TECHNIQUES TO MANAGE THE POPULATION OF FRUIT FLY, *BACTROCERA ZONATA* (SAUNDERS) IN GUAVA ORCHARD**

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Peach fruit fly, *Bactrocera zonata* is a serious threat to the horticultural crops (mango and guava etc) in Pakistan. It can be managed rather than control. For the management of fruit flies, biological control agent, *Oirhinus giffardii* (pupal parasitoid) releases along with male annihilation technique (MAT) and bait application technique (BAT) were applied in guava orchard at Habib farm Hyderabad during 2009-10 and 2010-11. Results revealed that biological control in conjunction with MAT, BAT or both reduced the fly population and fruit incursion. However the significantly reduction of adult fly trap catches and fruit infestation % were recorded in bio+ MAT + BAT followed by BIO + MAT as compared to control.

**EFFECT OF DIFFERENT PLANTING DATES OF BT COTTON GENOTYPES ON THE INFESTATION OF SUCKING AND BOLLWORM PESTS**

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The present research studies were executed to find out the response of five Bt cotton genotypes sown in different dates to the infestation of sucking and bollworm complexes. All the

five transgenic cotton genotypes (IR-443, IR FH-901, IR-448, IR NIBGE-1 and IR-1513) were sown under natural field conditions on different dates in three successive months April, May and June. Data were regularly recorded on the population of sucking complex and bollworms. Results revealed that significantly higher jassid population appeared on Bt cotton genotypes sown in April (early sowing) as compared to medium and late sowing. Maximum thrips and whiteflies infestation was recorded in late sown genotypes followed by medium sowing whereas population of the same was the lowest in early sown Bt cotton genotypes. Pink and spotted bollworm attack was found below ETL in all early, medium and late sown genotypes, however, highest was recorded in the late sown genotypes. Significantly higher yield (kg/ha) was recorded from genotypes sown early (April) followed by medium sowing (May). Among different Bt cotton genotypes tested, IR-443 produced significant results showing lowest pest infestation and maximum cotton yield followed by IR-FH-901. The studies manifested that early sowing in April of the latter two Bt cotton varieties/genotypes should be considered as an important tool to help in managing certain pest populations in an economically viable and environmentally safe manner.

**DETERMINATION OF SUITABLE PUPAL SIFTING AGE OF PEACH FRUIT FLY,  
*BACTROCERA ZONATA* (SAUNDERS) IN THE MASS REARING COLONY**

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Tephritid fruit flies (Diptera: Tephritidae) are among the most destructive agricultural pests in the world. The genus *Bactrocera* and *Ceratitis* have a world-wide status for its negative impact on fruits. The peach fruit fly, *Bactrocera zonata*, one of the most important fruit fly pests, alone has 80.6 % population and is the most abundant specie of Pakistan. Economical and quality production of *B. zonata* under laboratory conditions requires basic practical and scientific knowledge. The present research studies were conducted to standardize the rearing procedure of *B. zonata* for its quality production by reducing adult deformities. The agitation involved in the sifting of pupae of peach fruit fly, *B. zonata*, from the substrate in which they have pupated results in damaging effect on the flight muscles. Results revealed that sifting of *B. zonata* pupae on day 3rd and 4th after popout caused significant damage to flight muscles showing maximum deformity. It was observed that female fruit flies were more sensitive to sifting as compare to males. Overall studies confirmed that sifting done on day 2nd after popout proved very -suitable, giving minimum deformity and maximum emergence percentage.

**SYNERGISTIC EFFECT OF DICHLORVOS + TETRAMETHRIN, *BOUGAINVILLEA GLABRA*, POTASSIUM CHLORIDE AND *BACILLUS THURINGIENSIS* EFFICACY ON *TRIBOLIUM CASTANEUM***

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*Bacillus thuringiensis* (Bt) crystal proteins are pore-forming toxins used as insecticides around the world also known as a biological pathogen. Crystal toxin proteins from the Gram-positive rod shaped bacterium, *Bacillus thuringiensis* (Bt), are used extensively to control insect pests. These range from caterpillars (Lepidoptera) and beetles (Coleoptera) that infest crops to

black flies and mosquitoes (Diptera) that transmit human diseases. The red flour beetle (*Tribolium castaneum*) (Coleoptera: Tenebrionidae) is a common pest of wheat flour and is found worldwide. It also feeds up on dry fruits, pulses and prepared cereal foods where both larva and adult beetles cause damage. The present research is based on determining the combined effect of Tetramethrin+Dichlorvos (3.1% EC), *Bougainvillea glabra*, Potassium chloride and Shaf I *Bacillus thuringiensis* by assaying their bio-toxicity against *Tribolium castaneum*. *Bacillus thuringiensis* was isolated from samples, culturing on different media by applying microbial techniques and then Crystal protein will be isolated from bacteria. The bio-toxicity analysis by SPSS, determined that larvae are more susceptible and shows significant results as compared to adults. Separate bioassays revealed that Shaf I *Bacillus thuringiensis* was more toxic at all concentrations followed by an insecticide DDVP+ Tetramethrin, than come chemical, potassium chloride and then the least toxic was plant, *Bougainvillea glabra*. In the synergistic effect highest mortality percentage was shown by a Bioinsecticide, *Bacillus thuringiensis*. All elements whenever are combined together they proved to be useful for the control of stored product pests.

**EVALUATION OF MANAGEMENT EFFICIENCY OF A MECHANICAL REPELLANT:  
WIND POWERED HAWK EYE ROTATOR (WPPER) IN AGROCECOSYSTEMS**

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Avian pesticides are commonly used to reduce pre and post-harvest losses throughout the world but they are not eco-friendly. Mechanical repellants present a viable strategy for management of avian pests. In a pilot study at Faisalabad, we used wind powered hawk eye rotator (WPPER) as a sustainable strategy to inhibit parakeet depredations at post harvest stages of wheat and maize. Observations were recorded for two consecutive hours in the study period extending from April to May 2009. This management strategy was employed on control (without rotator) and experiment (with rotator) sites for 20 minutes duration with five days each stipulated for assessing the unguarded guarded situations. WPPER resulted in 47% decrease of visitation rate in maize and 51% in wheat. This pilot study proved that use of mechanical devices may lead towards the development of sustainable management strategies for the control of avian pests in agroecosystems.

**SUSCEPTIBILITY OF STORED VARIETIES OF FRESH DATES OF SINDH TO THE  
INFESTATION BY *ORYZAEPHILUS SURINAMENSIS* (COLEOPTERA)**

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The date-palm, *Phoenix dactylifera* L. has been considered the most important plant product in desert regions of north Africa and south west Asia since ancient time, due to its high nutritional value and storage properties, Lee (1963), Riad (2006). Dates are rich in carbohydrates, minerals and vitamins etc. There is no cholesterol and no fat in dates, Al-Farsi *et al.* (2008). In Pakistan, Sindh is an ideal place for cultivating the varieties of dates such as Aseel, Karbalian, Copro, Fasli, Dadhi etc, Bhambhro (2003). Stored dates are facing sever problem of insect infestation, mainly from Sawtoothed grain beetle *Oryzaephilus surinamensis* (L.) (Coleoptera):

Silivanidae) by reducing the quality and quantity (weight loss) of dates, Aldryhim and Adam (1998). Saw toothed grain beetles were reared on their natural food, three stored varieties of fresh dates viz, Kupro, Karbalian and Fasli, for three months from 01-10-70011 to 31-12-20011 at the entomology laboratory, department of Zoology, University of sindh, Jamshoro under the controlled condition. The effect of infestation on the quality and different nutrients of dates was studied. During this period the development of beetles was variable on different varieties. We kept 10 pairs of beetles in each variety. The highest numbers of sawtoothed grain beetles were developed on Kupro and Karbalian varieties, counts 350, 300 beetles respectively and 66% damaged of weight loss was observed in each variety. In Fasly the number of beetles, counts 200 beetles, and 45% damaged of weight loss was observed. The growth rate of larvae of the beetles was much higher on kupro and karbalian than Fasly. This result indicates that increase in the moisture and sugar content in different varieties were corresponded with the increase in infestation by stored beetles. The infestation rate showed significantly positive correlation with moisture and carbohydrates (sugar).

#### EFFECT OF TEMPERATURE ON SURVIVAL OF ENTOMOPATHOGENIC NEMATODES ON *GALLERIA MELLONELLA*

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Entomopathogenic nematodes are effective biological control agents against many soil-inhibiting insect pests over the years. These are present in abundance in the soil and they can exist in a wide range of habitats. One local and five exotic species of entomopathogenic nematodes were evaluated at different temperature for their effectiveness against *G.mellonella*. Mortality of local as well as exotic species was tested at 20°C, 25°C 30°C and 40°C. Different species caused the death of *Galleria mellonella* after different time intervals. At room temperature all the six species showed maximum mortality of *Galleria mellonella*. Local species showed prominent mortality at temperature ranging from 25°C to 35°C. It killed *Galleria* after an average of 35.33 and 34.66 hrs respectively. At temperature 20°C and 40°C, time interval was increased and the death of *Galleria* was caused after 38.33hrs and 39.66hrs respectively. Larvae of *H. indica* were killed after 93.33, 97.33, 72.33 and 97.33 hrs at the subjected temperature followed by *H. bacteriophora* and *S. krusai*.

#### DIFFERENT SOIL AMENDMENTS FOR THE MANAGEMENT OF STEM BORER CAUSING MYCOTOXINS IN CORN

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Different soil amendments were used for the management of stem borer (*Chilo partellus*) as a cause of mycotoxins in corn at the National Agricultural Research Centre (NARC), Islamabad, Pakistan. Experiments conducted with different composts (leaf manure, sheep farm yard manure, 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 31 different corn hybrids, genotypes and variety Islamabad Gold revealed that by soil amendment (neem) reduced the incidence of insect pest *C. partellus* which ultimately increased grain yield (5936 and 4688 kg ha<sup>-1</sup>).

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#### ROLE OF TERMITES IN PROMOTING THE ENZYMATIC ACTIVITY OF FUNGAL PATHOGENS IN GROUNDNUT

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Enzymatic activity (pectinase, cellulase, protease and lipase) of different fungal pathogens causing different diseases in groundnut was determined before and after application of termites. *In-vitro* studies; pathogens viz., *Aspergillus flavus*, *Aspergillus niger*, *Penicillium*, *Trichoderma* and *Alternaria* showed maximum pectinase activity after 48 hrs and then decreased. *Fusarium graminearum* and *F. oxysporum* showed the same pattern of pectinase activity but in variable quantity (66.6 µml<sup>-1</sup> & 77.7 µml<sup>-1</sup>, respectively) after 48 hrs. Cellulase production was not observed at 24 hrs. after inoculation, however, all fungi except *Alternaria* showed cellulase activity at 48 hrs. Protease enzyme was found only in *F. graminearum*. Lipase activity was maximum at 24 hrs. post-inoculation in all fungi, with the exception of *F. graminearum* and *Alternaria*. Differences in enzymatic activity at different intervals may suggest their specificity in causing groundnut pod rot. Moreover, it was observed that Termites *Odontotermes* spp. promoted *A. flavus*, *A. niger*, *Penicillium*, *Trichoderma* and *F. graminearum* resulted in more enzymatic activity and more disease severity in groundnut.

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#### ASSESSMENT OF CEREAL CROPS AGAINST INSECT PEST AND PATHOGENS

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Resistance in different summer crops i.e., maize, sorghum and millet germplasm was checked during summer, 2011 at the National Agricultural Research Centre (NARC), Islamabad, Pakistan. Experiments conducted in both field and laboratory showed the presence of more foliage disease in sorghum, while, least disease was observed in millet. Sorghum plants showed infection of *Macrophomina phaseolina*, *Gloeocercospora* and *Cercospora*. While, *Fusarium moniliforme*, *Fusarium graminearum*, *Cephalosporium acremonium*, *Macrophomina phaseolina* and *Rhizoctonia solani* were commonly isolated from maize. Infestation of stem borer *Chilo partellus*, termites and mites were commonly observed from the tested three crops. Out of 350 tested lines of maize, two were found resistant (R) while, seven were moderately susceptible (MS) and other 341 were susceptible (S) against the test pest and pathogens. Moreover, higher grain yield was found in resistant lines, which, could be recommended to the farmers of the country to produce high production as well as quality seed.

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**COMPARATIVE EFFICACY OF DIFFERENT NEEM PRODUCTS AGAINST WHEAT APHIDS**

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Aphid population on wheat is increasing in Pakistan since many years. As wheat is used as a staple food, application of synthetic pesticides to control these pests is discouraged at Government level. Present study was conducted to provide the farmer alternative control option against wheat aphids in the form of botanicals. Lab toxicological studies reflected that four out of six neem preparations including one aqueous extract (NSKE) and three neem oils proved most effective with LC<sub>50</sub> values ranging from 0.20 to 1.28%. In field-cage experiments almost similar trend was observed, neem oils resulted in maximum mortality of *Rhopalosiphum padi*, and *Schizaphous graminum* was statistically similar to that of imidacloprid followed by NSKE. In case of *Sitobion avenae*, NSO-Hexane was statistically similar with imidacloprid followed by NSO-Expeller and NSKE, while NSO-Ethanol was at par with imidacloprid. On the basis of cumulative insect days in field trials, NSO-Expeller proved as effective as imidacloprid in controlling the aphids, while except NSKE, other treatments were statistically at a par with imidacloprid. No significant differences were found between the population of mummified aphids between control and NSKE, NLE, NSO-Hexane, NSCE and NSO-Expeller. There existed no significant differences in all the neem treatments in comparison to control and imidacloprid treated plots. Maximum increase in wheat yield (7.28 q/ha) was observed in NSKE treated plots followed by those of imidacloprid (7.23 q/ha). Application of imidacloprid resulted in maximum CBR (1:1:34) followed by that of NSKE 5% (1:1.31). If the additional cost of loss of beneficials and environmental risks posed by the application of insecticides is considered, the CBR of neem extract may be regarded as comparable to that of imidacloprid and may be recommended to be applied against wheat aphids.

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**SELECTION OF CORN GERmplasm AGAINST DIFFERENT PEST AND PATHOGENS**

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Corn germplasm including three different inbred lines (A 619, B 73, MO 17), their crosses (A 619 X B 73; A 619 X MO 17; MO 17 X B 73) and backcrosses (MO 17 X B 73) X A 619 and (MO 17 X B 73) X MO 17 were tested against stalk rot pest and pathogens, *Chilo partellus*, *Fusarium moniliforme* and *Macrophomina phaseolina* at the National Agricultural Research Centre (NARC), Islamabad. The plants were inoculated with the pathogens used in combination at later plant growth stage i.e. after silking. Of all inbred lines, MO 17 was found to be resistant against stalk rot showing disease severity of 38.8%, 30.1% and 21.3% during 1993, 1994 and 1995, respectively. Backcross (MO 17 X B 73) X A 619 was found highly susceptible giving more pest infestation and disease severity of 52.7%, 87.1% and 75.6% during 1993, 1994 and 1995 respectively and consequently decreasing the grain yield significantly. More grain yield was observed in backcross (MO 17 X B 73) X MO 17 followed by the cross A 619 X MO 17.

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**EFFECT OF SOIL SOLARIZATION IN REDUCING INSECT PESTS INVOLVED IN PROMOTING AFLATOXINS IN CORN AND GROUNDNUT**

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Strategies for the management of mycotoxins in corn and groundnut using soil solarization were developed at the National Agricultural Research Centre (NARC), Islamabad, Pakistan. Experiments with soil solarization showed a pronounced reduction in weed population soon after removal of the transparent polyethylene sheets. Solarization controlled weeds (87.7%), increased soil temperature by 12.1°C over non-solarized soil at 10 cm depth, reduced soil-borne insect pests, reduced corn and groundnut diseases (72.9% and 67.5%), conserved moisture, increased the availability of essential nutrients in the soil and hence enhanced the growth of groundnut plants. Soil solarization was found an effective method in controlling corn and groundnut diseases. In treated plots, grain yield was more than double due to pathogen control, enhanced available nutrients in the soil as well as no competition with weeds. Soil solarization applied with transparent polyethylene film was found the most effective in reducing the incidence of *Aspergillus flavus* and consequently reduced aflatoxins in fields and stored corn and groundnut. We can avoid the hazardous/ poisonous effects of different chemicals on plant and animal life and thus, soil solarization technology could be used for the management of mycotoxins in food grains in future.

**COMPARISON OF WEB DESIGN OF ADULT FEMALE AND JUVENILE OF *ARGIOPE TRIFASCIATA* (ARANEAE, ARANEIDAE)**

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Body size measurements may have different significance in relation to web design depending on whether the spider is adult or juvenile. Present study was conducted to compare the different web characteristics (mesh height, capture area, capture thread length, horizontal and vertical diameters, upper and lower spirals and number of radii) of adult females and juveniles of *Argiope trifasciata* collected from Sheikhpura and Lahore from September to November, 2010. A total of 110 webs (adults =88, juveniles =22) were selected. After removing the spiders, the webs were sprayed with fine mist of water and corn starch using Knapsack hand sprayer to make the webs more prominent. Web area and mesh height were estimated by formulae designed by Herberstein and Tso (2000) and capture thread length by Venner *et.al.* (2001). 2 sample t-test was used to find difference in web parameters. To find relationship in body measures and web characteristics, Pearson's correlation was applied. A significant difference was found in mesh height, web area, and capture thread length of the webs constructed by adult and juvenile spiders. Moreover, the web characteristics of adults did not show relationship with their body measures (wet weight, carapace width, 4th leg length and total body length) while juveniles were positively correlated with these web characters while showing significant positive correlation of 4th leg length with capture area and capture thread length. When web parameters of both phases (adults and juveniles) were compared a significant positive difference was found in between mesh height,

capture area and capture thread length and a non-significant difference between number of radii. There was also a significant difference found in stabilimentum length and stabilimentum area of the webs of adults and juveniles. Being their larger web size, the adults caught more and large prey as compared to juveniles which trapped a few number and small sized prey.

### SCREENING OF DIFFERENT MAIZE GERMPLASM AGAINST SOUTHERN CORN LEAF BLIGHT, SHOOT FLY AND LEAF MINER

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Resistance in various maize germplasm against Southern Corn Leaf Blight (SCLB) caused by *Helminthosporium maydis*, shootfly and leaf miner was tested at the National Agricultural Research Centre (NARC), Islamabad, Pakistan. In this regard 71 different maize germplasm were screened in the field during spring season, 2010. Plants were inoculated with spore suspension of the causal organism at 4-5-leaf stage before tasseling. Data were recorded on 0-5 scale at tasseling-silking stage of the crop. Mean values of the disease severity and incidence showed that the maize genotypes differ from one another in their reaction to SCLB significantly. Genotypes Soan-3 and Rakaposhi were least affected and showed resistance against the disease as well as against shoot fly and leaf miner under field conditions at NARC, Islamabad. Neem extract was also tested for the management which was found useful against leaf miner and shoot fly.

### FEEDING POTENTIAL AND LARVAL DEVELOPMENT OF *CHRYSOPERLA CARNEA* (STEPHEN) ON DIFFERENT PREY SPECIES UNDER LABORATORY CONDITION

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A laboratory study was carried out to examine feeding potential and larval development of *Chrysoperla carnea* (Stephen) on Banana aphid, *Petalonia nigronervosa* (Coquerel), Safflower, aphid, *Uroleucon carthami* (Theo) alid Jassid, *Empoasca devastans* (Distant) at temperature 29±2 and relative humidity 65±5 during 2011. The result showed that 1 st instar *C. carnea* larvae consumed *P. nigronervosa* 8.6±0.35 nymphs/day. \whereas the 2nd and 3rd instar consumption rate were 16.3±0.54 nymphs/day and 20.3±0.56 nymphs/day respectively. The 3rd instar was voracious feeder as compare to rest instars. There was significance difference in the feeding porential among different instars at (P<0.05). Similarly the consumption rate of *C. carnae* on *U carthami* was observed. It was shown that 1st instar larva consumed 626±031 nymphs/day, followed by 2nd and 3rd instar 12.5±0.37 nymphs/day and 18.6±0.40 nymphs/day respectively. Similarly 3rd instars consumed more nymphs followed by rest instars. There was significance difference in the co'nsumption rate among different instars at (P<0.05). The data depicted that *C. carnea* devoured *E. devastans* in different stage. The consumption of 1 st instar larvae was 6.06±049 nymphs/day, whereas the 2nd and 3rd instars consumed 11.9±050 nymphs/day and 18.3±049 nymphs/day respectively. There was significance difference in the feeding potential among different instars at

( $P < 0.05$ ). There was significance difference in the feeding potential among different hosts *P. nigronervosa* at ( $P < 0.05$ ), but no significance difference were, found among rest of. hosts at ( $P > 0.05$ ). The result further revealed that the larval development in 1st instar 3 days, 2nd instar 2 days and 3rd instar 2 days was recorded same on *P. nigronervosa*, *U. carthami* (Thea) and Jassid, *E. devastans* prey species. There was no significance difference in the development of *C. cdrnea* reared on different prey species at ( $P > 0.05$ ). It was concluded that feeding potential of *C. carnea* reared on *P. nigronervosawas* relatively higher as compared to those reared on *U. carthami* and *E. devastans*. The predation of *C. carnea* on *U. carthami* and *E. devastans* was almost equal, while *P. nigronervosa* attracted more predation as compared to safflower aphid and jassid.

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#### SOIL SOLARIZATION WITH NEEM REDUCED THE INSECT PESTS MITES, THRIPS, ARMYWORMS AND CUTWORMS IN GROUNDNUT

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To investigate productivity and quality of groundnut as well as to increase resistance of test crop groundnut, the developed technology soil solarization was used alone and in combination with soil amendments (neem, chicken farm yard manure, sheep farm yard manure, farm yard manure, leaf manure and biokhad). Trial was conducted at the National Agricultural Research Centre (NARC), Islamabad during summer, 2009. All plots were pre-irrigated, and then mulched treatments were covered with transparent and colored (red, pink, green, yellowish green, yellow, blue, purple, silver and black) polyethylene films (0.04 mm thick) to trap solar heat in the soil. Major tikka disease of groundnut caused by *Cercospora* spp i.e., *Cercospora personate* and *Cercospora arachidicola* was also significantly reduced (17.5%) in solarized plots amended with neem leaves. The combined treatment (soil solarization with neem leaves) was found more effective in reducing the insect pests i.e., mites, thrips, armyworms and cutworms in groundnut, also found safe and economical in comparison with other treatments and thus, could be used for an integrated disease management system in future.

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#### AFLATOXINS ENHANCED IN THE PRESENCE OF INSECT PEST IN GROUNDNUT

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From 40 peanut seed samples collected in Potohar area of Punjab, Pakistan (District Rawalpindi, Attock, Chakwal, Jehlum), fungal species were isolated. The most dominant genera were *Aspergillus* (3 spp.), *Penicillium* (2 spp.) and *Fusarium* (3 spp.). From the preceding genera *A. flavus*, *A. niger*, *P. chrysogenum* and *F. oxysporum* were the most frequent species. Twenty-two isolates belonging to 11 species were tested for production of mycotoxins, after growth on liquid medium containing two carbon sources (sucrose or cellulose). Thin layer chromatographic analysis revealed that the quality and quantity of mycotoxins was higher on sucrose than cellulose. Mycotoxins identified were aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> & G<sub>2</sub>. Results revealed that aflatoxins were enhanced in the presence of insect pests *Helicoverpa*, mites, thrips armyworms and cutworms. These insects were significantly reduced in the treatments applied with neem leaves and neem extract.

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**MYCOTOXIN PRODUCING FUNGI IN GROUNDNUT UNDER STORAGE CONDITIONS**

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A survey for the incidence of mycotoxin producing fungi in groundnut under storage conditions at different locations of Punjab (District Rawalpindi, Attock, Chakwal, Jehlum) and Khyber Pakhtunkhwa (KPK) (Kohat) showed the presence of seven fungi viz., *Aspergillus flavus*, *Aspergillus niger*, *Fusarium graminearum*, *F. oxysporum*, *Penicillium*, *Trichoderma* and *Alternaria* in groundnut at market/ storage centres. Mapping for mycotoxins in groundnut in Punjab (District Rawalpindi, Attock, Chakwal, Jehlum) was done successfully. It was observed that mycotoxins were significantly reduced by controlling the insect pests under storage conditions.

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**DEVELOPMENT AND GROWTH OF *EXOCHOMUS QUADRIPISTULATUS* (COLEOPTERA: COCINELLIDAE): A PREDATOR OF MUSSEL SCALE *LEPIDOSAPHES ULMI* (HOMOPTERA: DIASPIDIDAE) ON APPLE**

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*Exochomus quadripustulatus* L., a polyphagous predatory beetle of both aphids diaspids, is capable of developing on mussel scale *Lepidosaphes ulmi* L. alone. The development of *E. quadripustulatus* from egg to pupa was completed in 94-128 (107.5±8.35) and 88-112 (97.83±3.8) days in male and female, respectively. Adult female has a unique tendency of egg laying with varying numbers of eggs/day under protected places; technique of egg collection is described. A significant linear relationship on the biological measurement was found between head capsule width and the larval body length; size and weight of both larva and adult. Some of the adults *E. quadripustulatus* when fed on *L. ulmi* lived for 114-305 days while other survived 8-35 days under field insectary conditions. It may be concluded that *E. quadripustulatus* is capable of playing its role in reducing the mussel scale *L. ulmi* populations on apple alone even if the other alternate prey (aphid) is not available in the field.

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**USE OF *RHIZOPHORA MUCRONATA* PAIR AGAINST SOME ATMOSPHERIC FUNGAL ALLERGENS**

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*In vitro*, antifungal bioassay of *Rhizophora mucronata* leaves was performed. The extracts were prepared in OMSO, OW, chloroform, acetone and ethanol in 2000, 4000 and 6000 ppm doses and tested against seven allergenic fungi viz., *Alternaria alternata*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Cladosporium herbarum*, *Penicillium notatum* and *Saccharomyces cerevisiae*. The data was compared with negative control against different aforesaid solvents and most of the results

were found as significant ( $P < 0.05$ ). Inhibition in the growth of *Alternaria alternata* was greater followed by *A. niger* and *A. flavus* except in the case of *S. cerevisiae* where the results were recorded as ineffective. Overall, concentration dependent tendency was noted.

### THRIPS AND THEIR NATURAL ENEMIES ON ROSE

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The field experiment to study the Thrips and their natural enemies on rose were carried out at Rizwan Sidiqi Agricultural farm near Tando Jam during Kharif season 2008. The result indicated that the maximum population of thrip was recorded on April 2nd ( $15.78 \pm 0.79/\text{leaf}$ ) and ( $18.32 \pm 0.86/\text{flower}$ ). Whereas the minimum population was observed on May, 28th ( $0.96 \pm 0.20/\text{leaf}$ ) and ( $2.28 \pm 0.30/\text{flower}$ ). It was found that the population of the pest was negatively correlated with temperature ( $r = -0.3659$ ) and relative humidity ( $r = -0.7578$ ). The data further revealed that the population of predatory spiders was maximum on April, 23rd ( $3.27 \pm 0.36/\text{plant}$ ). The spiders showed positive correlation with thrip population ( $r = 0.3751$ ). The feeding potential of predatory spiders was also tested in laboratory at Temp.  $28 \pm 2^\circ\text{C}$  and Relative humidity  $65 \pm 5\%$ . *Thomisus sp.* consumed greater number of thrips ( $6.25 \pm 1.25/\text{day}$ ) followed by *Eusparassus sp.* ( $4.50 \pm 1.06$ ), *Marpissa carinata* ( $3.00 \pm 0.87$ ) and *Marpissa lenebrosa* ( $2.25 \pm 0.75$ ) respectively. There was significant difference in feeding efficiency amongst different predatory spiders at  $P < 0.05$ .

### ECONOMICS LOSSES DUE TO DENGUE FEVER IN LAHORE

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Up to October 2011 about 139 patients die as a result of dengue fever and 13,399 are total reported cases in the hospitals. Where 11,627 cases were reported from Lahore. Our analysis reveals that more than 30,000 people were affected by this infectious disease in the year 2011. This morbidity is expected to become double in the next year i.e. 2012, after the rain fall. Because it is very difficult to destroy the eggs of mosquitoes. Moreover, in some of the cases this pathogen is also present in the vector since birth, where in case of malaria, the Anopheles female will have to pick up pathogen from affected mammal. Hence, the authors feel their responsibility to document the "economics loss due to dengue fever in Lahore". This paper will facilitate dengue control team and economic planners to obtain more funds for controlling dengue's vector. The direct cost paid by total patients is Rs.30,000,000 approximately in the year 2011. As a result of morbidity the productivity of patient losses for two months at least. In turn, this "Productivity loss" caused decline into the GDP of Pakistan. The total income, ownership of house, ownership of vehicle, etc were also taken in the questionnaires obtained from patients in Lahore. This data is present in the form of table in the paper. Instant survey helped the authors to report the percentage of money

income of patient that is spending on disease's treatment. It is also found, that the morbidity is more frequent in the poor patients. This paper also giving a look, on ;the government expenditure on defluge's treatment and control. Finally, the recommendations of paper tells us that health education will be proven more power full tool for controlling dengue in the coming years.

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**FARMERS PERSPECTIVES OF RODENTS DAMAGE IN DIFFERENT CROP FIELDS  
AT HIGHER AND LOWER ALTITUDE OF UPPER SWAT, PAKISTAN**

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A preliminary survey (from July to October 2011) about farmers knowledge and experience was conducted in different crop fields at higher and lower altitude of Upper Swat, Pakistan to understand damage, population density and prevalence of the rodents in pre and post harvest stages of different crops. A total of 24 farmers with 248 family members were interviewed using a questionnaire. 92.8% and 80% of the farmers were said that rodents are frequently distributed at post harvest stage of crops in low and higher altitude respectively. 71.5 % and 90% of the farmers were stated that rodents are widely distributed in rain-fed fields 71.5% and 50% have view that burrows impact on land was bad and 100% were responded that stone bunds provided ideal habitats for rodents' survival. 78.5% and 100% have view that damage was observed as for rodent evidence. 85.5% and 80% of the farmers were said that rodents are the main pests of crops in low and higher altitude respectively. 50%, 50%, 85.7%, 90%, 85.7%, .80%, 64.3%, 60%, 64.2% and 100% of the farmers were stated that rodents damage was observed in fields, stone-bunds, damage was noted at post harvest stage systems/m<sup>2</sup> , estimated damage at pre harvest stage, 500g/m<sup>2</sup>, rodents were control by baiting in low and higher altitude respectively. 64.2% and 100% stated that maize and potatoes' are the main crops grown at low and higher altitudes respectively. 50% and 100% stated that rice and maize are most susceptible crops for the rodents in low and higher altitudes respectively. It was concluded that high altitude was mostly suffering from rodents' depredation than low altitudes (as higher altitude was mostly rain-fed). Maize was the most susceptible crop for rodents attack at higher and lower both altitudes while potatoes was the only crop that was grown at medium high altitude and Ipaize at lower altitudes of upper Swat. The rodent management was mostly done by baiting.

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**SOME OBSERVATIONS ON BIRD DAMAGE TO STANDING WHEAT CROP AT  
THATTA, SINDH**

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Wheat, *Triticum aestivum* (L.) is grown over about 8.33 million hectares in Pakistan with a production of about 21 million tons and constitutes about 80% of the total cereal consumption of the nation. It is one of the most effected crops facing pest problems, such as, insects, rodents and birds. Damage losses to standing wheat crops by birds is one of the greatest problems facing growers globally. The economic damage sustained is usually unquantified. Damage problem varies,

depending on the variety grown and time of ripening of the crop. House sparrow (*Passer domesticus*) is the most destructive pest and inflicts heavy damage to wheat and other grain crops. Shafi (1990) observed 2-11 % wheat damage, being more prominent at maturity stage of the crop. Pakistan is a developing country, where the preliminary estimates of sparrow damage to subsistence and commercial wheat crop ranges from 2-60 % (Bashir, 1978). Rose-ringed parakeet (*Psittacula krameri*) is a major avian pest of oil seeds, orchard and cereal crops. Pest birds inflict heavy damages to wheat, millet, lentil, sorghum and other grain crops (Ahmad, 1979; Qureshi, 1980; Shafi *et.al*, 1990). Some preliminary studies on the extent and pattern of bird damage to wheat fields at Thatta were carried out. At ripening stage, many pest birds affected the crop badly. The field was watched continuously from a suitable, hidden place so that birds could not be disturbed. The data on pattern, timings and pest species was recorded regularly and analysed statistically. For damage estimation, sampling was carried out by using 1 m x 1 m quadrates. Twelve quadrates, six on each side of the road, were laid 10m apart from each other. The number of intact tillers and number of ears showing sparrow damage was recorded in each of the quadrates. Overall 37% damage to the crop at ripening stage was estimated. House sparrow *Passer domesticus* was the major pest responsible for damage (24%) followed by rose-ringed parakeet, *Psittacula krameri* (11%) and others (pigeon, mynah, doves) (2%). The sparrow attacked the crop in large flocks continuously early in the morning and before dusk, however, small flocks were observed during day time. Damage was confined at periphery mostly; however damage was observed all over the field. Parakeet attacked the crop in small flocks early in the morning and before dusk, and solely through the day. Mostly the ears were cut from tillers. Damage was observed over all the crop heads. Some doves, mynah and pigeons were observed picking the seeds from the ears of the standing crop and the wasted/fallen ears (by parakeets) under the trees.

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**POPULATION DENSITY, SPECIES COMPOSITION AND DAMAGE ESTIMATION OF RODENT PESTS OF AGRICULTURAL FIELDS AND ZOONOTIC IMPORTANCE IN DISTRICT SWAT, PAKISTAN**

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During the research studies in crop fields of district Swat, a total of 159 rats (11.4% trap success) were trapped in 1386 (66x21) trap nights in different agricultural crop fields at higher and lower altitudes of upper Swat, K.P.K, Pakistan from July to October 2011. The experiment was designed at a total of about eight acre area. Population density, burrows analysis, species composition and damage estimation were determined. Population density was assessed as: the number of animals caught multiplies by 100 divided by the value of total number of traps and trap nights damage estimation was assessed as: Total number of stem damage multiply with 100 divided by total number of stems counted, thus pre harvest was determined as : 15.6% in potatoes; 13.2% in Maize and 7.11 % in Maize 4.04 % in Rice in higher and lower altitudes of upper Swat respectively. Post harvest damage was estimated as: 3.16% potatoes, 10.5% Maize and 7.41% Maize, 3.92% rice in higher and lower altitude of upper Swat respectively. During the study period a total of 273 burrows including 145 in higher and 128 in Lower altitude of upper Swat were

counted of the total burrows counted 58 (21.2%) were excavated and analyzed recorded different chambers as: nesting, resting and hoarding chambers. The materials hoarded by the rats was identified as mostly the stems of the cultivated crops during pre harvest stage, while cobs and panicles in case of maize and rice were observed as hoarded material after harvesting.

**LARVICIDAL ACTIVITY OF DIFFERENT ESSENTIAL OILS AGAINST  
AEDESAEGYPTI AND CULEXQUINQUEFASCIATUSLARVAE (DIPTERA: CULICIDAE)**

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Five essential oils from various parts of plant species i.e. *Acoruscalamus*, *Menthaarvensis*, *ocimumbacilicum*, *Saussrealappa* and *Cymbopogan citrates* were investigated for their larvicidal properties against *Aedes aegypti* and *Culexquinquefasciatus*. Essential oils were obtained by steam distillation method. The mosquitoes were reared in laboratory by maintaining conditions. Twenty late 3<sup>rd</sup> instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus* were exposed to different concentrations of essential oils ranging from 1.95-1000.00ppm. The larval mortality was observed after 24 hours under the laboratory conditions. Results showed that all the five plant essential oils produced significant larval mortality against two mosquito species. Highest larvicidal activity was observed in the essential oil from *O. basilicum* against *Ae.aegypti* and *Cx.quinquefasciatus* with the LC<sub>50</sub> values 75.35 ppm and 92.30 ppm respectively. However the LC<sub>50</sub> values for *A. calamus*, *M. arvensis*, *S. lappa* and *C. citrates* against *Ae. Aegypti* are 99.41, 114.33, 128.89 and 136.28ppm respectively and against *Cx.quinquefasciatus* are 107.81, 112.18, 141.43 and 148.54ppm respectively. So according to the larvicidal activity of essential oils against *Ae.aegypti* and *Cx. quinquefasciatus*, the oils were arranged in the following ascending order of preference i.e. *O. bacilicum* > *A. calamus* > *M.arvensis* > *S. lappa* > *C.citratus*. From this study, it can be concluded that five essential oils which were distilled from *A. calamus*, *M. arvensis*, *O. bacilicum*, *S. lappa* and *C. citrates* had remarkable larvicidal properties, which may be considered as a potent source for the production of natural larvicides which would be environmentally safe and alternative to synthetic insecticides.

**TESTING OF GRAIN BASED BAIT AS CARRIER OF STRYCHNINE ALKALOID FOR  
THE MANAGEMENT OF INDIAN CRESTED PORCUPINE *HYSTRIX INDICA***

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Field trials were conducted in Abbottabad - Balakot tract of the northern Pakistan for testing the effectiveness of groundnut – maize grain bait in delivering the lethal amount of strychnine alkaloid in controlling the Indian crested porcupine, *Hystrix indica*, which is a severe forest and agricultural pest in different parts of Pakistan. The results suggested that, in case of continuous poison baiting trial, a 10% reduction in burrow activity was recorded in case of saccharin supplemented (5%) groundnut – maize (1:1) grain bait; while no reduction was recorded in case of no saccharin supplementation. In case of pre-baiting trial, a 25% reduction in burrow activity was achieved in case of grain bait supplemented with saccharin and 10% without saccharin.

The results of the study suggested that strychnine alkaloid is an ineffective rodenticide for the control of Indian crested porcupine at least with the present bait and conditions. Further trials with low concentrations of strychnine alkaloid and increasing the pre-baiting duration are suggested.

**EFFICACY OF SOME INDIGENOUS PLANT OILS AS INSECT GROWTH REGULATORS (IGRS) AGAINST RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM* (HERBST)**

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Increasing concern about health and environment has led to the need for searching safe natural resources as alternative to replace toxic pesticides and fumigants being used to control insect pests (Kostyukovsky, 2005). Plant extracts are natural resource of pesticides/insecticides (Raguraman & Singh, 1997; Gbolade, 2001) and insect repellents (Thorsell, *et. al.*, 1998; Oyedele *et. al.*, 2000). Red flour beetle, *Tribolium castaneum* (Herbst) is the most abundant and injurious pest in warehouses and flour mills. In view of determination of synthetic pesticide free control of grain pests with natural products, research was conducted to evaluate comparative toxicological effect of five indigenous plant oils, *Azadirachta indica* (neem), *Valeriana officinalis* (valerian/mushkbala), *Acorus calamus* (sweetflag), *Curcuma longa* (turmeric) and *Saussurea lappa* (costus/kuth) as Insect Growth Regulators (IGRs) against red flour beetle, *Tribolium castaneum* (Herbst). All plant oils reduced growth of the insect during metamorphic stages. Maximum larval inhibition (88.35%) was observed by valeriana oil (0.1%); whereas, maximum pupal inhibition (77.77%) was observed by costus oil (0.05%). Minimum larval inhibition (4.09%) was observed by neem oil (0.025%); whereas, minimum pupal inhibition (6.83%) was observed by neem oil (0.05%). These findings may help in future research on effective, safe and economical Integrated Pest Management of stored grains pests by natural plant products.

**TESTING OF GROUNDNUT – MAIZE BAIT AS CARRIER OF ZINC PHOSPHIDE FOR THE MANAGEMENT OF INDIAN CRESTED PORCUPINE, *HYSTRIX INDICA* KERR (RODENTIA: HYSTRICIDAE)**

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Indian crested porcupine, *Hystrix indica*, is a serious pest of forest plantations, orchards and agricultural crops in different countries of the world. Field trials, conducted in Abbottabad - Balakot (Pakistan), suggested that consumption of groundnut – maize (1:1) bait poisoned with zinc phosphide, offered after three nights of pre-baiting practice, exhibited a decline and negligible amount of bait was consumed on 3<sup>rd</sup> night of poison baiting. Degree of decline in bait consumption gradually increased with increasing zinc phosphide concentration from 1% to 3%. The

consumption of poison bait with or without saccharin (5%) during the poison baiting nights were not significantly different, however, there was a persistent trend where the baits having 5% saccharin, as taste additive, were consumed in higher quantities as compared with those having no saccharin. This trend was equally reflected in the data on average consumption of control bait during each test night. Higher reduction in burrow activity (55%) was recorded with 2% zinc phosphide and 5% saccharin (without saccharin supplementation, 45% reduction was recorded), as compared with 1% (35% with saccharin and 30% without saccharin) and 3% (25% with saccharin and 15% without saccharin). The study suggested that 2% zinc phosphide can be used by using groundnut – maize (1:1) as bait base and 5% saccharin, at least for the initial control campaign of this mammal pest species. Further refinement of the rodenticide and sweetener concentrations may be more useful.

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**ROLE OF AUDIOVISUALS IN SUCCESS OF RODENT PEST MANAGEMENT  
CAMPAIGN IN SINDH.**

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Rodent pests cause immense losses to standing crops stored food and transmit many fatal diseases to human and livestock. According to recent survey, overall damage crops and stored commodity amounts to 2,540 million rupee annually. Pakistan has been gifted with different agro-ecological zones due to variation in altitude, temperatures and rainfall etc resulting in occurrence of number of rat species. To enhance awareness level among farming community, a comprehensive study is being conducted to compare the impact of audiovisual tools, *i.e.* documentary films, literature and lectures in farmers gatherings. During Fy. 2010-11, 14 farmers awareness campaigns were launched in which 960 farmers and NGO's were educated to apply latest developed PARC Rat bait packages to minimize rat damage to crops and other commodities. Post awareness results of campaign indicated 25-30% increase in awareness level to adopt modern rat control technology.

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## SECTION – III

## ENTOMOLOGY

## PRESENT SITUATION OF ARTHROPOD-BORNE DISEASE IN PAKISTAN

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The residents of the country is estimated with 177.1 million in 2011, Pakistan is the sixth most populated country in the world, behind Brazil and ahead of Bangladesh. At 2.03% it has the highest population growth rate among the SAARC countries, resulting in an annual addition of 3.6 million people. Pakistan is situated in sub tropical region, so it has a rich fauna of disease vectors due to its different epidemiological and climatologically characteristics, arthropods vectors are the transmitters of disease, they carry pathogens from one host to another and play an important role and serve as a causative agent of several vector-borne diseases, By common practice, vectors are usually considered to be invertebrate including mosquitoes, sand flies, houseflies and other dipterous flies. In addition, soft ticks, hard ticks fleas, lice, mites, bed bugs and cockroaches cause malaria, filariasis, leishmaniasis, schistosomiasis, onchocerciasis, dengue and other arboviruses are the major public health problems in Pakistan. Chief vector borne diseases present in the country include malaria, leishmaniasis and dengue. Malaria is one of the most important mosquito- borne diseases; it has been a main public health problem and disease still panic for millions of people due to deprived health conditions throughout the country it is also observed that it occurs throughout the year especially in Sindh Province. Main vectors of Malaria in Pakistan are *A. barbirostris*, *A. bariamensis*, *A. gigas*, *A. gigas var similensis*, *A. habibi*, *A. nigerrimus*, *A. lindesayi*, *A. annularis*, *A. culicifacies*, *A. fluviatilis*, *A. maculates*, *A. turkhudi*, *A. multicolor*, *A. moghulensis*, *A. pallidus*, *A. pulcherrimus*, *A. sergenti*, *A. splendidus*, *A. stephensi*, *A. stephensi spp.*, *A. subpictus*, *A. superpictus*, *A. theobaldi*, *A. culicifacies*, *A. maculates* spp, Out of the anopheles species reported from Pakistan, two species, *Anopheles culicifacies* and *An. stephensi* are consider the primary malaria vectors in the country. The prevalence of leishmaniasis is wide spread in many parts of the country; three types of leishmaniasis are found in different areas. Cutaneous Leishmaniasis (CL) is found in the southwestern region, Visceral Leishmaniasis (VL) in the Northeastern region and Anthroponotic Cutaneous Leishmaniasis (ACL) is seen diffused in central region Main vectors of Leishmania in Pakistan *Phlebotomus Papatasi*, *P. salehi*, *Paraphlebotomus Sergenti*, *P. alexandri*, *P. Nuri*, *Larrousius kandelakii*, *L. burneyi*, *L. keshishiani*, *L. Major*, *Adlerius hindustanicus*, *A. salangensis*, *A. longiductus*, *Euphlebotomus argentipes*, *Anaphlebotomus colabaensis*. Dengue viruses are probably endemic in the country; an outbreak of dengue fever occurred in October 2006 in Pakistan. Several deaths occurred due to misdiagnosis, late treatment and lack of awareness in the local population, it has become a dangerous health issues from last many years in Punjab. Main vectors of Dengue in Pakistan *Aedes albopictus*, *Ae. albolateralis*, *Ae. vittatus*, *Ae. pseudotaeniatus* and *Ae. aegypti* (the principal vector in many parts of the world). Crimean-Congo Hemorrhagic fever (CCHF) is an endemic tick-borne viral disease and out breaks occur mainly in the cold, arid regions of Pakistan. The natural hosts include sheep, cattle, goat, camels etc. Ticks belonging to the Genus *Haemaphysalis* and *Hyalomma* were the dominant species. Scrub Typhus an acute febrile, typhus like disease, transmitted by larval trombiculid mites has a wide distribution in eastern and southern Asia. Out breaks of scrub typhus has been reported from Pakistan. Bedbugs and cockroaches are main pests in the ruler and urban areas; scorpion and spider stings are main

concern of public health. Different preventive and control measures should be apply by the Government authorities. It especially depends on environmental management including awareness of citizen, health education, drug treatment, use of insect repellent, permethrin-treated bed nets, install mosquito screens, elimination of breeding sites, Larval mosquito control and abatement such as cans and containers, biological and chemical control of vectors etc.

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**IDENTIFICATION AND DESCRIPTION OF TWO NEW SPECIES OF SUBGENUS  
*PHYTOSEIUS (PHYTOSEIUS) RIBAGA FROM BALUCHISTAN, PAKISTAN***

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Mites of the family Phytoseiidae are known predators of other harmful mites and small insects. They have very efficiently been used in biological control of different pests in different parts of the world. A survey was conducted from different localities of Pakistan to identify the phytoseiid mites. Two new species were collected and identified from different areas of Baluchistan. Different plant parts were shaken on a white paper. The phytoseiid mites fallen on the paper were collected with the help of a moist camel hair brush and stored in small vials containing 75 % ethanol. The collected specimens were mounted on the glass slides in Hoyer's medium. The sketches of different body parts were prepared with the ocular grid and the measurements were done with the help of ocular micrometer. *Phytoseius (Phytoseius) turpis* was collected from Zhob (Baluchistan 4500 ft) from unidentified host plant and *Phytoseius (Phytoseius) varix* was collected from Kharan (Baluchistan) from Mulberry (*Morus alba*). The types were deposited in the Acarology Research Laboratory, Department of Agri. Entomology, University of Agriculture, Faisalabad, Pakistan

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**COMPARATIVE EFFICACY OF SCABICIDAL DRUGS IN THE TREATMENT OF  
SCABIES**

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Scabies is highly communicable dermatosis caused by a microscopic mite species; *Sarcoptes scabiei* var. *hominis*. There are various therapeutic agents available in market to treat scabies infection. Tendency of emergence/reemergence attributes of scabies prompted to design one year study to evaluate the relative efficacy of various scabicial treatments against classical scabies. A comparative trial was accomplished in 360 adult scabies patients divided into six groups. Host showed a high level of resistance to Lindane (85%) followed by Sulphur plus Proteinum (86.66%), respectively. While, combination of Ivermectin with Permethrin proved to be the fastest regimen resulting in 100% recovery in one week. The scenario of resistance is alarming for medical practitioners, enforcing them to design effective control strategies to reduce scabietic infestation.

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**REVISION OF BUTTERFLIES OF FAMILY PIERIDAE FROM HYDERABAD DIVISION**

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Butterflies belong to order Lepidoptera. They are very delicate and look very beautiful and attractive due to their colorful wings. Butterflies are important pollinators for many varieties of plants because they fly for long distance, but at the same time some of them are very serious pest of many crops and fruits. Most of the butterflies in the family Pieridae are small to size medium. They usually have white or yellow color, wings with black edges. Caterpillars of most Pieridae feed on various varieties of mustards (family Brassicaceae), legume and cabbage. Presently we have surveyed Jamshoro, Tando Jam, Matiari localities of Hyderabad division and more than 800 specimens have been collected. Identification has been done by the colouration of fore wings, mouth parts and external and internal male and female genitalia. Their wings have been expanded on the stretching board and leave for twenty four hours on that then preserved in wooden boxes. During this period we have identified six species of butterflies, three species of genus *Colotis*, *C. etride*, *C. vestalis*, *C. amata*, one species of genus *Catopsila*, *C. pomana*, one species of genus *Anapheis*, *A. aurota* and one species of genus *Eurema*, *E. hecabe* of family Pieridae. It has been a new record of these species from surveyed localities of Hyderabad, Sindh, Pakistan.

**REVISION AND NEW RECORD OF (DIPTERA: SYRPHIDAE) IN HYDERABAD DEVISION**DILDAR ALI SOLANGI<sup>1</sup> NASREEN MEMON<sup>2</sup>, JUMA KHAN<sup>3</sup>, BHOJOO MAL<sup>4</sup>*Department Of Zoology University of Sindh Jamshoro**Solangidildar@gmail.com, Nasreen\_kousarbks@hotmail.com*

Hovers flies are attractive group of insect with their striped abdomens. They look like small bees, sometimes called flower flies or syrphid flies. Adult hoverflies are important pollinators, while the larvae are predacious in nature and 40% of these are aphidophagous. Aphids cause damage to the crops, vegetables and fruits. Hovers flies belong to order Diptera, family: Syrphidae which comprises three subfamilies: Syrphinae, Milesiinae, Microdontinae. Each Subfamily consists of many tribes. Present study was conducted on Hoverflies of tribe syrphini from Hyderabad, Sindh. The specimens were collected during January to May 2011. The specimens of hoverfly were randomly collected with help of insect net and hand picking from different field of Hyderabad and then kept into jars, brought to entomology laboratory, department of zoology. Hoverflies were killed by keeping them in to the fridge for five hours, legs and wings of one side of body were stretched and preserved in the insect boxes and then Naphthalene balls were also used to protect and preserved insect from predators (ants) and fungus. During our work we surveyed different fields of Hyderabad division Matiari, Tando Jam , Tando Muhammad khan . Nine hundred specimens were collected; five species of Hoverflies were identified belonged to tribe syrphini *Episyrphus balteatus*, *Sapherophoria scripta*, *Eupeodes luniger*, *E.corollae*, *Epistrophe nitidicollis*. The identification was done by using binocular on the basis of their general coloration and specially stripes, bars and bands on the abdomen, wings venation and internal male and female genitalia. We compare the species with the type material and relevant literature. These species are newly identified in these above mentioned areas.

**A NEW LOOK AT THE BIODIVERSITY AND BIOGEOGRAPHY OF PRAYING  
MANTIDS FROM SINDH**

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The study was undertaken to prepare a comprehensive and updated record of Praying Mantids (Mantodea) belonging to families, Eremiaphilidae, Empusidae and Mantidae from Sindh Province. Biodiversity and biogeography of 16 species of generalist predators Praying Mantids (PMs) in Sindh is discussed. In addition the role PMs as a bio-control agent is highlighted and concluded that PM, diversity is high in this province.

**REVISION OF GENUS *APODIPHUS* SPINOLA (HEMIPTERA, PENTATOMIDAE,  
HALYINI) WITH DESCRIPTION OF NEW SPECIES**

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Spinola described the genus *Apodiphus* to accommodate *Halys amygdali*. After that different workers described and added different species. At present genus *Apodiphus* described with description of new species from Quetta, Pakistan with special reference to metathoracic scent gland ostiole and male and female genitalia and compared it with its closely allied species *A. integreiceps* Horvath. The description is supported by illustration.

**REVISION AND NEW RECORD OF GENUS *EUPEODES* (SYRPHIDAE, SYRPHINI)  
FROM BALOCHISTAN, PAKISTAN**

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Syrphidae is one of the largest families of order Diptera. Syrphid flies are famous with the name of hoverflies and flower flies. They are day time flying insects. It comprises three sub-families. Sub-family Syrphinae have three tribes bacchani, paragini and syrphini. Syrphini looks like small bees. They are considered beneficial flies, the adults flies are important pollinators, while the larvae are predator in nature and these are aphidophagus too. Presently we have surveyed Pishin, Kuchlak and Dusht localities of Quetta, Balochistan and more than 200 specimens have been collected during 2<sup>nd</sup> May 2011 to 30<sup>th</sup> October 2011. The species were identified on the basis of external morphology specially the coloration such as marking on abdomen, tibiae and wings venations and also male and female internal genitalia. Their wings and legs have been expanded on the stretch board and left in that position for 24 hours, then Preserved in wooden boxes. During this period we identified three species of genus *Eupeodes*, *Eupeodes corollae*, *E. luniger* and *E. nitens*. It has been a new record of these species from surveyed localities of Quetta, Balochistan Pakistan.

**FORENSIC APPLICATIONS OF DIEL OVIPOSITION VARIATION IN GREEN BOTTLE FLIES DURING WINTER IN RESPONSE TO MEAT OF DIFFERENT BODY ORGANS OF VARIOUS ANIMALS**

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The present study illustrates the forensic importance of circadian oviposition fluctuations in green bottle flies during winter. The flies were collected from the vicinity of the Department of Zoology, Government Postgraduate College Gojra over minced liver. Green bottle flies were reared and the experiment was conducted under  $25 \pm 2^\circ\text{C}$ , 70% R H and 14D: 10L dark light cycle. Ten green bottle flies were allowed to oviposit for 24 hours and egg counts were carried out every 2 hours daily. The experiment was repeated three times. The oviposition stimuli used were minced meat of intestine, liver, kidney, heart and muscles of chicken, goat, cow, buffalo and rabbit. Oviposition started at 8.00am and kept on increasing until 4.00pm and then declined. No oviposition was observed in green bottle flies from 6.00pm to 8.00am in case of almost all the organs of nearly all the animals tested. Maximum oviposition was observed on meat of different organs of buffalo followed by that of rabbit, cow, goat and chicken. Statistical analysis using ANOVA showed that the time interval had a significant effect while the test meat had an insignificant effect in terms of stimulation for oviposition in green bottle flies. The studies of this sort is useful to assess the post-mortem interval in the field of forensic science.

**BIOLOGY AND FEEDING BEHAVIOUR OF ASIAN SPECIES RED PALM WEEVIL *RHYNCHOPHORUS FERRUGINEUS* ON DIFFERENT VARIETIES OF DATE PALM**

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Date –palm *Phoenix dactylefera* is one of the oldest fruit tree in the world. Date palm *Phoenix dactylefera* is one of the main crop of Sindh province particularly in upper Sindh. It is main source of nutrients such as carbohydrates, vitamins etc. Red palm weevil *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae), is also known as Asian palm weevil, coconut weevil or Indian palm weevil (Faghih 1996). This pest caused serious damage to tree trunk. It is an economically important tissue-boring pest of date palm in many parts of the world. In India this pest first was recorded on coconut palm (Lefory, 1906, Nirula, 1956) and later on it was reported on date palm tree (Lal, 1917; Buxton, 1918). In Pakistan RPW was reported by Baloach *et al.* (1992) from Khairpur, Sindh. Red palm weevil (RPW) *Rhynchophorus ferrugineus* (Olivier) were reared on three mediums (varieties of date palm) in the laboratory during 1<sup>st</sup> September 2010 to 30<sup>th</sup> March 2011. It was observed that Aseel variety was best medium for egg laying. To observe percentage of hatching, four medium were prepared viz One Petri dish, lined with the moist filter paper with piece of stem of Aseel size 3×2cm. remaining three Petri dishes were lined with dry filter paper, each containing a piece of stem size 3×2 cm of Aseel, Fasly and Karbalian as to observe the percentage of hatching on different mediums. Highest percentage hatching observed on Aseel 85 and lowest on Fasly 72. Larval growth was best observed on Aseel variety (82%-92%)

lowest on Fasly variety (72% to 82%). The result indicates that the biology of red palm weevil from egg hatching to the development of larval stages was high on Aseel variety than all remaining varieties which means it causes serious damage to Aseel, therefore, we assume that Aseel has high sugar content as was mentioned by Salama and Abdel-Razek (2002). The number of larval instars is variable in literature that is 7-9 but we have observed seven larval instars in all Varieties at least for three generations.

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**DIURNAL RHYTHMS IN BLUE BOTTLE FLY OVIPOSITION DURING WINTER OVER FLESH FROM VARIETYPES OF ANIMAL SOURCES: IMPLICATIONS FOR FORENSIC SCIENCE**

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The present study demonstrates the diurnal rhythms in blue bottle fly oviposition during winter with forensic applications. The minced liver was used to as a bait to procure the blue bottle flies from a grassy field situated near the Department of Zoology, Government Postgraduate College Gojra, Toba Tek Singh. The environmental conditions under which the blue bottle flies were reared and oviposition was observed, were  $25\pm 2^{\circ}\text{C}$ , 70% R H and 14D: 10L dark light cycle. The oviposition of 10 blue bottle flies was observed for 24 hours daily and eggs were counted at 2 hours interval with three replications. The minced meat of intestine, liver, kidney, heart and muscles of chicken, goat, cow, buffalo and rabbit were tested as stimuli for oviposition. Fluctuations in oviposition pattern were observed from 2.00am to 10.00am where oviposition was at its peak and then kept on increasing until 6.00pm. Oviposition could not be detected in blue bottle flies from 6.00pm to 2.00am in the next morning in response to approximately all the flesh stimuli from different animal sources. Maximum oviposition was observed on meat of different organs of buffalo followed by that of rabbit, cow, chicken and goat. The data were statistical analyzed using ANOVA which indicated statistically significant effect between time intervals and an insignificantly effect between meat stimuli from different animal sources in terms of stimulation for oviposition in blue bottle flies. The present study is helpful in determining the minimum time since death or murder (post-mortem interval = PMI) in the field of forensic science.

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**FORENSIC IMPORTANCE OF CYCLIC LARVIPOSITION PATTERN IN FLESH FLIES DURING WINTER WITH RESPECT TO DIVERSE ARRAY OF MEAT STIMULI**

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The present study was carried out to understand the forensic importance of cyclic larviposition pattern in flesh flies during winter. The minced liver was used as a bait to collect the flesh flies from the neighborhood of the Department of Zoology, Government Postgraduate College Gojra Pakistan. Flesh flies were reared under  $25\pm 2^{\circ}\text{C}$ , 70% R H and 14D: 10L dark light cycle and observations were taken under the same environmental conditions. Fifteen flesh flies were observed for larviposition for 24 hours and larvae were counted after every 2 hours. The experiments were repeated three times. The minced flesh of intestine, liver, kidney, heart and muscles of chicken,

goat, cow, buffalo and fish served as stimuli for larviposition. Larviposition commenced at 8.00am and kept on increasing until 4.00pm with a few exceptions and then dropped. Larviposition did not occur in flesh flies from 6.00pm in the evening to 8.00am the next morning in response to nearly all the meat stimuli. Buffalo flesh served as the strongest larviposition stimuli followed by fish, chicken, cow and goat. The results were analyzed using ANOVA and found that effect of different meat stimuli did not differ statistically from each other while the time interval had a significant effect in terms of stimulation for larviposition in green bottle flies. This type of study is helpful to determine the post-mortem interval (PMI) in forensic science.

**BIOLOGY OF SPOTTED BOLLWORM *EARIAS VITTELLA* (FAB.) (LEPIDOPTERA: NOCTUIDAE) IN LABORATORY UNDER CONTROL CONDITIONS**

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*Earias vittella* is a major lepidopteron pest of cotton in Pakistan and it cause major infestation. Study of biology play key role in the pest management. Present study of biology of *Earias vittella* carried out in laboratory under control conditions (Temperature 26, 31 and 36± 2°C) on their natural diet cotton shoots, flowers and bolls. Highest mortality recoded in First larval instars and Egg 14 and 10 % age the lowest mortality recoded in fourth larval instars and Pupae 1.2 and 1.6 5age, total cumulative %age of survival recoded 62.85age. Life cycle completes 23-47 days. Male is smaller in size then female, male dies after few days of mating. Present study reveals that use control practices from Egg to second larval instars because these are sensitive and easy to control.

**PRELIMINARY OBSERVATIONS ON THE ORTHOPTEROID INSECTS OF NARA (DESERT), KHAIRPUR, SINDH, PAKISTAN**

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The Orthopteroids Insects of Nara desert are studied for the first time. The material was collected from the Choondiko, Gas Field area, Village Muhammad Akram and Achiro Thar localities of Nara desert from October 2011 to January 2012. Following species were identified: Pyrgomorphidae: *Chrotogonus trachypterus trachypterus* (Blanchard), *Pyrgomorpha Bispinosa deserti* (B.Bienko), *Poecilocerus pictus* (F.) Acrididae: Oedipodinae *Acrotylus humbertianus* (Sauss), *A. longipes subfaciatus* (B.Bienko), *Hilethera aelopodiodes* (Uv.), *Sphingonotus savignyi* (Sauss), *S. rubescens rubescens* (Walk), *Locusta migratoria* (L.), Acridinae *Acrida exaltata* (Walk), *Truxalis eximia eximia*, Gomphocerinae *Ochrilidia geniculata* (I. Bol), *O. gracilisgracilis* (Kr.), Eyprepocnemidinae *Heteracris littoralis* (Walk), Cyrcanthacridinae *Shistocerea gregaria* (Forsk), Dericothythidae: Dercorythinae *Dericorys tibialis* (Pallas), Tetrigidae: *Paratettix* sp. Tettigonidea *Euconocephalous incertalus* (Walk), Dermaptera: *Labidura riparia* (Pallas), Mantodea: *Eremiaphila* sp. *Humbertiella* sp. Blattida: *Periplanta orientalis*, Gryllidae: *Gryllus bimaculatus*, *Acheta domestica* Gryllotalpidae: *Gryllotalpa africana*.

**NOTES ON FOOD HABITS AND FEEDING BEHAVIOUR OF SOME ACRIDOIDEA OF  
KHAIRPUR AND ITS ADJOINING AREAS**

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The habitat occupancy, mouth parts adaptations, crop contents and feculae of 10 species of Acridoidea are analyzed during the course of this study on feeding behaviour. The species studied were *Chrotogonus trachypterus trachypterus* (Blanchard), *Pyrgomorpha bispinosa deserti* (B.Bienko), *Gonista sagitta* Uvarov, *Gelastorhinus semipictus* (Walk), *Ochrilidia gracilis gracilis* (Kr.), *Truxalis examia examia*, *Duroniella laticornis* (Kr.), *Sphingonotus savignyi* Sauss. *Acrotylus longipes subfasciatus* (B.Bienko) and *Hieroglyphus perpolita* (Uvarov).

**SCREENING OF WHEAT CULTIVARS FOR THE REARING OF *SITOTROGA  
CEREALELLA* (OLIVIER) (LEPIDOPTERA: GELECHIIDAE)**

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Comparative studies were carried out for varietal preference of 9 cultivars of wheat viz., Inqulab, Shafaq 2006, AS 2002, Aquab, Pasban 90 (Approved varieties) and 3138, 3094, 04189 and 04188 (new candidate lines) for the rearing of *Sitotroga cerealella*, in mass rearing laboratories at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad. Result showed that developmental period, no. of adult emerged and adult weight was attained comparatively better in reared insects after feeding on grains of Inqulab-90. From the chemical analysis of tested grains, it was evinced that crude protein contents were recorded as the highest, 15.7 in 03094, while lowest, 13.5 in 3138, whereas, intermediate 15.3 in Inqulab. Total fats were ranged from 1.3 to 1.6 among the varieties whereas, highest were observed in Inqulab which provide high nutrition value to reared insects. So it is concluded from the results that after feeding on wheat grains of cultivar Inqulab, the insect developmental parameters were better as compared to other tested cultivars.

**PRELIMINARY STUDIES ON DAMSELFLIES (ZYGOPTERA: ODONATA) OF NARA  
DESERT**

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The damselflies of Nara desert are studied for the first time. The material was collected from the Village Muhammad Akram and Achiro Thar localities of Nara desert during October 2011. Following species were identified: *Agriocnemis pygmaea* Rambur, *Ischnura forcipata* Morton, *I. aurora* Baruer, *Pseudagrion laidlawi* Fraser, *Rhodischnura nursei* Morton, *Lestes thoracicus* Laidlaw, *L. umbrinus* Selys, *Cydonolestes cyanea* Selys.

**PRELIMINARY STUDIES ON DRAGONFLIES ANIOSPTERA: ODONATA) OF NARA  
DESERT**

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The dragonflies of Nara desert are studied for the first time. The material was collected from the Village Muhammad Akram and Achiro Thar localities of Nara desert during October 2011. Following species were identified: *Brachydiplax sobrina* (Rambur), *Brachythemis* sp. *Crocothemis servilia* (Drury), *Diplacodes lefebvrei* (Rambur), *Neurothemis* sp. *Orthertrum purinosum neglectum* (Rambur), *O. Sabina* (Drury) and *Pantala flavescens* (F.).

**A CHECKLIST OF THE SPIDERS OF CHOLISTAN AND NEIGHBORING AREAS**

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The present survey conducted on the spiders of Cholistan desert, Pakistan during 2001-2003. Spiders were collected by hand-picking and jarring method. Identification of sampled Spiders was made on the basis of morphological characteristics. There diversity and abundance of different species was analyzed by using Shannon diversity indices. Spiders collected during study period from different localities of Cholistan, belonging to 10 families, 32 genera and 62 species. Family Lycosidae with (68%) spiders of whole data was most dominant family. This information of data will be critical for different evaluation programmes of I. P. M.

**IMPACT OF MATING ON LONGEVITY AND FECUNDITY OF MALES AND FEMALES  
OF *DYSDERCUS CINGULATUS* (HEMIPTERA: PYRRHOCORIDAE)**

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Cotton is a natural fiber material used throughout the world. In Pakistan cotton yield for the last few years has been reduced which may be due to the heavy pest infestations or other hidden reasons such as weather variations, manure problems, irrigation and may be incorrect timings of cultivation. Present study focuses on the biology and mating strategies of Red Cotton Bug a major pest of cotton crop, commonly known as cotton stainer. The results of the present study may be used to determine the weak stages of life cycle of this pest to control or at least to reduce the infestation level. The observations on fecundity and longevity were carried out on sex starved pairs of *Dysdercus cingulatus* at laboratory conditions. It includes recording the size of mated and unmated females with life time fecundity (total number of eggs produced throughout their life). Also longevity (total life span) of mated (males, females) and unmated (males, females) was determined. Moreover, the egg-laying processes of mated and unmated females were studied. The mated females live longer than unmated females a reason to believe that males may be transferring some proteinous nutrients to females at mating. Also, the mated males live less longer than the

unmated males a confirmation for transferring some vital resources required for enhanced longevity other than the sperm and seminal fluids.

**EFFECT OF WEATHER ON FORAGING FLIGHTS OF BUMBLEBEES (APIDAE: HYMENOPTERA) IN NORTHERN, PAKISTAN**

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Foraging behavior of bumblebees is under the influence of different ecological variables like temperature, humidity, rainfall, altitude etc. To study the effect of these variables, foraging activity of bumblebees was observed from March to September within 100m radius during the bloom of the target crop/ plant for one hour. Canonical correspondence analysis (CCA) was applied on different variables which highlighted the influence of each variable on the distribution of bumblebees. CCA of bumblebees abundance (first two axis) accounted for 75.61% of variance, of which 14.18% was explained by the different variables which clearly depicted that wind have profound effect on the foraging activity of bumblebees particularly *B. avinoviellus* and *B. biroi* at Naltar which was under the influence of winds throughout the year. Similarly, altitude showed marked influence on *B. lucorum* and *B. marrussinus* in particular especially at Hunza, however, flight activities of *B. asiaticus*, *B. rufofaciatus*, *B. avinoviellus*, *B. kashmirensis*, *B. himalayanus* and *B. haemorrhidalis* were comparatively more affected by rains especially at Doarian and Sangum which received heavy showers throughout the year as compare to the rest of sites.

**POPULATION PREFERENCE OF WHITEFLY (*BEMISIA TABACI*) ON COTTON**

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The present project was conducted to determine the effect of weather factors towards the resistance/ susceptibility of ten transgenic and traditional cultivars of cotton against whitefly and to compare the population on transgenic (Bt) and non transgenic cotton varieties. The experiment was conducted at Ayub Agricultural Research Institute (AARI), Faisalabad (Punjab), Pakistan and the data was collected per week basis. The results exhibited that maximum population of whitefly (1.71/leaf) was recorded on V5 (CRSM-2007) and the minimum population (1.15/leaf) was recorded on V8 (FH-941). Whitefly showed two population weeks throughout the season. Whereas the mid of July and the 3<sup>rd</sup> week of September showed population peaks. Whitefly was more on Bt varieties (1.59/per leaf) as compared to Non-Bt varieties (1.42/per leaf). Among all weather factors, relative humidity and rainfall were negatively correlated with whitefly population while

temperature was positively correlated. These results confirmed that whitefly population causes more damage to transgenic varieties than conventional, so further research will be needed to know the behavioral preference of this pest.

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#### POPULATION DYNAMICS OF *Aedes albopictus* IN URBAN AND RURAL AREAS

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The evaluation of population stability of *Aedes albopictus* in natural conditions (undisturbed environment) triggered the distribution and relative abundance due to temperature, relative humidity and precipitation. The population *Aedes albopictus* monitored in discarded tires, bamboo stems, man-made cement tanks, stagnant water, sewerage water and septic tanks in urban and rural areas. The relative abundance and occurrence of *Aedes albopictus* in urban and rural localities significantly affected by changed environment, thus showing varying complexity in population number at different breeding sites. Discarded tires in botanical garden and public parks represented as the potential breeding sources due to nearby vegetation growth followed by bamboo stumps and stagnant pools in urban vicinity. No or less population catchments observed in the rural masses as dominated with two genera *Culex* and *Anopheles*. These two genera preferred stagnant water, sewerage water and septic tanks to inhabit. It was determined that population of *Aedes albopictus* fluctuates due to climatic conditions. It is closely associated with human environment and potentially found in the urban settings preferably used automobile tires.

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#### PREDATOR-PREY RELATIONS OF COCCINELLIDS AND DIFFERENT APHID SPECIES OCCURRING IN *BRASSICA* CROP FIELDS

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Biological diversity within ecosystems, including agro-ecosystems, provides a wide range of biological functions and there are some indications that more diverse agricultural ecosystems may enhance natural control of pests. Family *Brassicaceae* includes a number of species that have considerable nutritional and economic values. The present study has met the objective of exploring and evaluating arthropod population interactions and suspected synergies for the crop plants in terms of pest reductions. Data was collected with the help of hand net and by hand picking from selected quadrates of 1m<sup>2</sup>. A total of 9067 specimens, 8946 from the crop plants and 121 from the weed plants associated to crop, belonging to 81 species were sampled from *Brassica*-weed complex, of which 1096 were coccinellids along with other predators, 3963 aphids along with other pest/prey and 1729 were found to be omnivores. Cruciferous crops were highly vulnerable to pests like many aphids and some lepidopteran species. Maximum Coccinellids (predator) peaks were observed during March and April because they develop well at 22-25°C and due to the presence of

their preferred prey (aphids). Predators dominated the weed plants while prey-pest populations dominated the crop plants throughout the cropping season. Therefore, the study evidenced the synergetic effects of phyto-morphic diversity helping pests control on Crucifers.

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#### **EFFECT OF ARTIFICIAL DIETS ON FECUNDITY OF LADYBIRD BEETLES**

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The present study was conducted in the laboratory aiming to improve techniques for mass production of Coccinellid ladybird beetle for use as biological control agent. Fecundity was assessed in the rearing units (RU). Effects of two artificial diets were investigated and compared with natural diet (nymphs/ adults of aphids) under controlled conditions ( $26\pm 2^{\circ}\text{C}$ ). It was recorded that there was a greater quantity of eggs on paper and walls of the RU's. For the diet 1, more eggs (10.0%) were recorded as compare to diet 2. The diet ingredients Casein (30.0%) with yeast (20.0%), honey (10.0%), distilled water (30.0%) and vitamin solution (10.0%) were used as diet of adults' of ladybird beetles.

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#### **DISTRIBUTION OF SLANT FACED GRASSHOPPER (ACRIDINAE: ORTHOPTERA) FROM SINDH**

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The slant faced grasshoppers are very important from economic point of view they cause significant damage to agricultural field and range land in Pakistan. During the present study a total of 5 species namely *Truxalis exmia exmia* Eichwald, *Truxalis grandis fitzgeraldi* Dirsh, *Acrida exaltata* (Walk), *Phlaeoba tenebrosa* (Walk) and *Duroniella laticornis* (Kr) were recorded. The most abundant species is *Truxalis exmia exmia* its occurrence has been reported at all districts of Sindh while the *Phlaeoba tenebrosa* has been reported only from the Dadu, Sukkur and Kashmir districts of Sindh. In addition to this morphological characters of these 5 species are also discussed.

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#### **ON THE OCCURRENCE OF PHANEROPTERINAE ( TETTIGONIDEA : ORTHOPTERA) FROM SINDH**

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Phaneropterinae are Phytophagous insect and considered important pests of agricultural crops, forests, fruits culture, grapevine and berry shrubs in the world including Pakistan. At the present extensive survey has been carried out in Sindh during the year 2011-2012 and 6 species

namely: *Phaneroptera falcata* Pada, *Ph. quadripunctata* Brunner, *Pseudanerota robusta* B.Bienko, *Anisotima dispar* B.Bienko, *Trigonocorypha unicolor* Stoll, and *Barbitistes constrictus* Brunner belonging to 5 genera representative of 4 tribes viz: Phaneropterini, Ducetini, Trigonocoryphini, and Barbitistini of Phaneropterinae were collected from different agricultural field and its distribution has been discussed at district level. This study is financially supported by Pakistan Science Foundation Islamabad under research project PSF/RES/S-SU/BIO (423)

#### IDENTIFICATION AND DOCUMENTATION OF WASP IN GILGIT-BALTISTAN

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Wasps are important pesticides and are well established not only in Pakistan but also in the world. Almost every insect pest species has at least one wasp species that preys upon it or parasitizes it, making wasps critically important in natural control of their numbers, or natural bio control. Parasitic wasps are increasingly used in agricultural pest control as they prey mostly on pest insects and have little impact on crops. The present study was conducted to identify and document the various species of wasp which are present in three districts of Gilgit-Baltistan during the month of August and September 2010. Gilgit-Baltistan has strength in wasp biodiversity, during the present study total 300 wasps has been collected by trapping and killing method. Out of three hundred 90 species were randomly selected for identification and documentation and from each localities (18) samples were selected. During this investigation six reported species of wasp has been identified from the three district of GB. Five reported species were identified from district Gilgit namely, *Vespa tropica* Linnaeus and *Vespula germanica*, *Vespa orientalis* Linnaeus, *Polistes gallicus* Linnaeus and *Polistes indicus* Stofa. Two species were identified from district Ghizer namely; *Polistes (Polistes) associus* Kohl and *Polistes gallicus* Linnaeus and one specie were identified from district Hunza-Nagar was *Vespula germanica* Fabricius. The present study has revealed that the identified species of wasp belongs from two families' vaspinea and polistinea.

#### DIVERSITY AND ABUNDANCE OF SPIDER FAUNA IN OAT FIELD FROM NANKANA SAHIB, PUNJAB, PAKISTAN

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The study was conducted from November, 2010 through March, 2011 in an oat (*Avena sativa*) field at Sangla Hill (31.16°N, 73.91°E) Nankana Sahib, Punjab, Pakistan. The collection was done by pitfall trapping for five consecutive days each month that resulted in the capture of 253 specimens belonging to seven families, 12 genera and 25 species. Lycosidae was the most dominant family, comprising 90.90% of the total catch followed by Oxyopidae (4.29%), Salticidae (2.73%) and others (Gnaphosidae, Oecobidae, Sparassidae and Thomisidae) (1.95%). Numerically the most abundant species was *Pardosa birmanica* Simon, 1884 (30.9%) followed by *Lycosa mackenziei* Gravelly, 1924 (13.28%). The immature and sub-adults were significantly higher

(42.68%) than females (32.80%) and males (24.50%). Highest number of spiders was recorded during November whereas it was lowest in December. Species richness was high in November however; evenness was high during February and March. Abundance of spiders was significantly higher on edge as compared to margin and center of the field.

**SYSTEMATIC AND ECOLOGICAL STUDY OF *SCHIZODACTYLUS MINOR* (ANDER 1938) (ORTHOPTERA: SCHIZODACTYLIDAE) FROM SINDH**

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The genus *Schizodactylus* Brulle comprises on the seven species throughout the world. Of these *S.minor* (Drury) and *S.minor* (Ander) occur along the shores of River Indus Sindh Pakistan. Earlier, Ander (1938) described single male of *S.minor* from River Ganga (India). At the present *S.minor* is recorded for the first time and female is described for the first time in world. Present study deals with peculiar morphological characters, amazing burrow habitat and carnivorous behavior of this insect. In addition to this variation in different body components was also illustrated. It was also observed that during burrow formation *S.minor* damaged the under ground roots of the plants this behavior has been observed for the first time. The depth of adult burrow recorded 64.26±6.91 cm in depth, however, the shorter burrow was recorded only 23.2±4.36 cm depth due to wet soil and the low altitude. Nothing was published on the *S.minor* before this. Current study was initiated for the first time from this region.

**STUDIES ON THE TRIBE ACROTYLINI (ACRIDIDAE: ORTHOPTERA) FROM PAKISTAN**

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Grasshoppers are important to carry out research study for the aim of insects systematic. During present work five species and subspecies of the tribe Acrotlyini were examined namely *Acrotylushumbertianus* (Saussure), *A. insubricusinsubricus* (Scopoli) *A. patruelis* (Herrich-Schaffer) *A. longipeslongipes* (Charpentier) and *A. longipessubfasciatus*. Although there is shortage of information about the genitalia of these grasshoppers. Where as some information is present in the literature on morphology of species of the genus *Acrotylus* (Fieber). Indeed, my studies highlight the diagnostic characters of Phallic complex and epiphallus that have useful value for the purpose of accurate identification. Epiphallus with moderately wide bridge, narrow ancorae and bilobate lophi. Spermatheca is a coiled duct of ectodermal origin. It is of varying sizes and shapes. The spermatheca usually dilates to form a sac like structure, for storing the sperms which enter during copulation. On other hand the present study will make the unique support for new research workers in future. These insects has been collected from the agricultural lands, deserted and semi-deserted areas, rocky areas having the scattered vegetation of the grasses; herbs and shrubs. The

members of this tribe are widely distributed throughout the world. Occurrence of previously recorded species has been confirmed and their distribution has been extended to new localities.

**GENETIC DIVERSITY OF SIX-SPOTTED ZIGZAG LADYBIRD (*CHEILOMENES  
SEXMACULATA*, COLEOPTERA) POPULATION FROM VARIOUS AGRO-  
ECOSYSTEMS OF PUNJAB, PAKISTAN**

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Random Amplified Polymorphic DNA (RAPD) markers were used to investigate the genetic structure of different populations of six-spotted zigzag ladybird (*Cheilomenes sexmaculata*) from various agro-ecosystems of Punjab, Pakistan. The six-spotted zigzag ladybird is a valuable predator against important insect pests of the cropland. The percentage of polymorphic bands (90.05%), Shannon Index ( $H' = 0.152$ ) and Nei's average gene diversity ( $I_N = 0.278$ ) of *C. sexmaculata* at species level were rather high when compared with other coleopteran species. 37% of polymorphic loci showed statistically significant differences in their allelic frequencies. A genetic distance D of 0.15 indicated that these populations showed some degree of isolation. Overall, such genetic data is considered to be useful in designing conservation plans aimed at local preservation of differentiated genetic resources that, in the future, could potentially result in ecologically and behaviorally differentiated populations.

**STUDIES ON THE TRIBE ACROTYLINI (ACRIDIDAE: ORTHOPTERA) FROM  
PAKISTAN**

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members of this tribe are widely distributed throughout the world. Occurrence of previously recorded species has been confirmed and their distribution has been extended to new localities.

**SYSTEMATIC STUDIES AND HOST SPECIFICITY OF *SCELIO*(HYMENOPTERA: SCELIONDAE) FROM SINDH**

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*Scelio*L. has world-wide distribution and available host data suggests that they are exclusively parasitoids of orthoptera eggs in many countries of world including Pakistan. In many parts of world *Scelio* implemented as important biological control agents. At the present three species of *Scelio* namely *Sceliohieroglyphi* (Timb.), *S.aegypticus* Priesner and *S.mauritanicus* Risbec were recorded from three host species of grasshoppers viz: *Hieroglypgusperpolita* (Uvarov), *H.oryzivorous* Carl and *H.nigrorepletus* I.Bolivar from Pakistan. A detail account was presented on the general morphology, distribution pattern and host association of *Scelio* and comparison was also carried out with already described host species on world-wide basis. In addition to this, one new record and one new host of *Scelio* was also described from Sindh province. Furthermore, this information has been gathered for the first time from this region. This study is financially supported by Higher Education Commission Islamabad under research project (HEC No.20-1762 / R&D/10)

**BUTTERFLY FAUNA OF KOHAT, PAKISTAN**

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The present research was conducted to explore butterflies fauna of Kohat, Pakistan during September-December 2008. During survey, 21 species were identified belong to three different families including, Nymphalidae, Papilionidae and Pieridae. Majority species were related to families Nymphalidae and Pieridae. In Nymphalidae included (8 species): Indian fritillary, *Argynnis hyperbius* Linnaeus; peacock pansy, *Junonia almanac* Linnaeus; painted lady, *Cynthia cardui* (Linnaeus); common castor, *Ariadne merione* (Cramer); blue pansy, *Junonia orithya* Linnaeus; common leopard, *Phalantha phalantha* (Subfamily: Nymphalinae); white edged rock brown, *Hipparchia parisatis* (Kollar) (Subfamily: Satyrinae) were identified. In Papilionidae included (2 species): lime butterfly, *Papilio demoleus* Linnaeus and common mormon, *Papiliopolyte* Linnaeus were identified. In Pieridae included (12 species): green-veined white, *Pieris napi* (Linnaeus); Murree green-veined white, *Pieris ajaka* Moore; small cabbage white, *Pieris rapae* Linnaeus; large cabbage white, *Pieris brassicae* Linnaeus; pioneer white butterfly, *Belenoi aurota* Bingham (Subfamily: Pierinae); common brimstone, *Genopteryx rhamni* (Linnaeus); common grass yellow, *Eumera hecab* (Linnaeus); lemon emigrant, *Catopsilia pomona* Fabricius; little orange tip, *Colotis etrida* Boisduval; yellow orange tip, *Ixias pyrene* Linnaeus (Subfamily: Coliadinae); blue spot arab, *Colotis protractus* Butler; dark clouded yellow, *Coliascroceus* (Geoffroy) (Subfamily: Coliaclinae). The wingspans of collected butterflies, minimum was 25 mm of *C. setrida* and maximum was 100 mm of *P. demoleus* and *P. polytes*.

**INCIDENCES OF *SCELIO* SPECIES ON THE EGG-PODS OF THREE *HIEROGLYPHUS* SPECIES FROM PAKISTAN**

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*Scelio* species are important parasites of grasshopper and locust's eggs in many countries of world including Pakistan. This genus is considered as largest within the subfamily Scelioninae, with an estimated round about 500 species. At the present three species of *Scelio* namely: *S.hieroglyphi* (Timberlake), *S.aegyptiacus* Priesner and *S.mauritanicus* Risbec were collected from the egg-pods of three host species of *Hieroglyphus* i.e *H. perpolita* (Uvarov), *H. nigrorepletus* Boliver and *H. oryzivorous* Carl from Pakistan during the year 2011. At the present out of 1357 egg-pods 770 were found severely effected by *Scelios* species overall parasitism ratio was reported significantly highest 23.36% on *H.nigrorepletus* followed by 19.38% and 14.0% on *H.oryzivorus* and *H.perpolita* respectively. However un-effected egg-pods were counted 587 with 43.26%. The eggs of *Scelios* species are very small in size. If a female of each parasite therefore, deposits the eggs in a host egg, it is very difficult to distinguish the parasite eggs from the yolk of the host eggs. In addition to this, present study suggested that large sized egg-pods are partially parasitized as grasshopper nymph also emerge along with the parasites, where as in pods with fewer eggs nothing except the parasite emerge and are called fully parasailed freshly laid egg-pods are preferred over old during parasitization. Present study strongly recommended that *Scelios* species play a significant role (as bio-control agent) in the regulation of Orthopteran populations in the field and are implicated in suppressing numbers of numerous pest species of locusts and grasshoppers. This study is financially supported by Higher Education Commission Islamabad under research project (HEC No.20-1762 / R&D/10)

**STUDIES ON THE TRIBE ACROTYLINI (ACRIDIDAE: ORTHOPTERA) FROM PAKISTAN**

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Grasshoppers are important to carry out research study for the aim of insects systematic. During present work five species and subspecies of the tribe Acrotlyini were examined namely *Acrotylushumbertianus* (Saussure), *A. insubricusinsubricus* (Scopoli) *A. patruelis* (Herrich-Schaffer) *A. longipeslongipes* (Charpentier) and *A. longipessubfasciatus*. Although there is shortage of information about the genitalia of these grasshoppers. Where as some information is present in the literature on morphology of species of the genus *Acrotylus* (Fieber). Indeed, my studies highlight the diagnostic characters of Phallic complex and epiphallus that have useful value for the purpose of accurate identification. Epiphallus with moderately wide bridge, narrow anchorae and bilobate lophi. Spermatheca is a coiled duct of ectodermal origin. It is of varying sizes and shapes. The spermatheca usually dilates to form a sac like structure, for storing the sperms which enter during copulation. On other hand the present study will make the unique support for new research workers in future. These insects has been collected from the agricultural lands, deserted and semi-deserted areas, rocky areas having the scattered vegetation of the grasses; herbs and shrubs. The

members of this tribe are widely distributed throughout the world. Occurrence of previously recorded species has been confirmed and their distribution has been extended to new localities.

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### EXPLORING SPIDER FAUNA OF PESHAWAR, FATA, PAKISTAN

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The spiders are known as poisonous arthropods but they have unforgettable role as biological control agent. The present research was conducted during May 2009 to September 2010 to explore the spider fauna of FR Peshawar. Spiders were collected and identified with the help of available literature, keys and already identified specimens housed. The total recorded spider species were 23 belong to the families Clubionidae (1 sp.), Sprassidae (1 sp.), Scytodidae (1 sp.), Araneidae (2 spp.), Pholicidae (2 spp.), Thomisidae (3 spp.), Salticidae (2 spp.), Gnaphosidae (2 spp.) and Lycosidae (9 spp.). However, the largest spider collected is huntsman, *Isopoda tuhodnigra* (Barrion) with total body length  $15.80 \pm 0.83$  mm (range: 15-18 mm). Moreover, the smallest spider is wolf spider *Pardosa birmanica* (Simon) with total body length  $4.20 \pm 1.30$  mm (range: 3-6 mm). The daddy long leg, *Crossopriza lyoni* (Blackwall), has the longest legs with first leg length  $49.70 \pm 3.71$  mm (range: 30-45 mm) and last leg length  $41.50 \pm 3.13$  mm (range: 29-46 mm). Furthermore, jumping spider *Plexippus paykullii* (Audouin) has the smallest legs with first leg length  $5.8 \pm 0.83$  mm (range: 5-7 mm) and last leg  $4.50 \pm 0.83$  mm (range: 4-6 mm). Most of the species recorded have brown and grayish in colour. The crab spiders, *Thomisus pugilis* (Stoliczka), *Thomisus spectabilis* (Doleschall), *Diaea evanida* (Thorell) are the colourful species belonging to family Thomisidae.

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### EXPLORING THE DRAGONFLY (ODONATA: ANISOPTERA) FAUNA OF DISTRICT LOWER DIR, PAKISTAN

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Dragonflies are of large, intermediate to small size having different colours and variable morphological characters. They also carry ornamental value. A collection of dragonflies was made during May-July 2011, from District Lower Dir. As a whole 318 dragonfly were collected from different areas. Among them 11 species of dragonfly were identified under three families i.e. Libellulidae, Gomphidae and Cordulegasteridae of order Odonata. These are *Rhodothemis rufa* (Rambur), *Orthetrum cancellatum* (Linnaeus), *Diplacodes lefebvrei* Rambur, *Diplacodes trivialis* Rambur, *Orthetrum pruinosum neglectum* (Rambur), *Orthetrum triangulare triangulare* (Selys), *Sympetrum decoloratum* Selys, *Orthetrum Sabina* (Drury) and *Pantala flavescens* (Fabricius), *Onychogomphus bistrigatus* Selys and *Cordulegaster brevistigma brevistigma* Selys. It is concluded that there is a diversity to explain dragonfly fauna from District Lower Dir.

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**EXPLORING THE LADYBIRD BEETLES FAUNA OF HAZARA UNIVERSITY,  
GARDEN CAMPUS, MANSEHRA, PAKISTAN**

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The Coccinellids commonly known as ladybird beetles have great economic importance and interest in agriculture and forestry, since adults and larvae of the most species are predators of herbivorous pests such as aphids, psyllids, mealy bugs and scale insects as employ in biological control. On this regard a survey to explore lady bird beetles fauna of Hazara University, Garden campus, Mansehra was carried out during March to May, 2011. The study area was divided into 3 sites: A: Residential area; B: Administration area; C: Main campus. Samples were collected from a wide variety of terrestrial habitat throughout in each locality to ensure that the overall landscape of that locality was represented. Visual searches were done and the specimens were collected by hand picking. Constant field visits were carried out and a total of 300 specimens were collected. The specimens were identified in National Agricultural Research Council (NARC) Islamabad with the help of available literature, key and already identified species which is placed in museum of NARC. Seven predatory species of ladybird beetles were identified belonging to a single subfamily, Coccinellinae. The species were present in the following order; seven-spotted ladybug, *Coccinella septempunctata* (Linnaeus) (220), adonis ladybird, *Hippodamia variegata* (Goeze) (32), fifteen-spotted ladybird, *Harmonia dimidiata* (Fabricius) (24), transverse ladybird, *Coccinella transversalis* (Fabricius) (10), *Oenopia sauzeti* (Mulsant) (9), *Adalia tetraspilota* (Hope) (4), six spotted zigzag ladybird, *Menochilus sexmaculatus* (Fabricius) (2). These species were found on different host plants. The most encountered species found was *C. septempunctata*, whose 220 specimens were collected. The least encountered specie to be found was *M. sexmaculatus*, whose only 2 specimens were collected. Seven species in the same study area with just three different study sites and the separation of species between sites suggests that this region may have a diverse and rich fauna of coccinellid beetles. Similar surveys on large scale are recommended in this region to fully evaluate the predatory Coccinellid fauna of the Hazara division.

**REVISION OF SOUTH AND CENTRAL ASIAN SPECIES OF GENUS *HALYS*  
FABRICIUS (PENTATOMIDAE: PENTATOMINAE: HALYINI) WITH DESCRIPTION  
OF THREE NEW SPECIES FROM PAKISTAN**

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*Halys* is a cosmopolitan genus of tribe halyini found in almost all oriental region of the world. They are polyphagous, feed on a number of food plants and plants of commercial importance such as Shesham, Babur and Mulberry trees. Present study includes eight species from oriental region of South and Central Asia; *H. fabricii* Fabricius, *H. nilgiriensis* Distan, *H. shaista* Ghauri, *H. serrigera* Westwood and *H. sulcatus*, including three new species *H. hyderabadensis* sp.n, *H. mulbariensis* sp.n, *H. noakotensis* sp.n, one from Punjab and two from Sindh, Pakistan respectively. The new species are described on the basis of external morphology and the

description of internal and external male with special references to scent gland apparatus. The new species are compared with their closely allied species, and key of eight species including new species is given here. All three new species are new record in the existing literature.

**BIOLOGICAL OBSERVATION OF IMMATURE STAGES OF *CHRYSOPERLA CARNEA* FEEDING ON *CORCYRA CEPHALONICA* EGGS AT TWO TEMPERATURE LEVELS**

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Biology of immature stages of *Chrysoperla carnea*, feeding on *Corcyra cephalonica* eggs at two different temperature (24±1°C) and (32±1°C) with 65±5% relative humidity, were studied. The results indicate that incubation period was 4.9±0.22 days and 3.0±0.62 days. Developmental duration of first, second and third instars were 3.6±0.11, 3.3±0.27, 4.9±0.37 days and 2.4±0.24, 2.0±0.60, and 2.8±0.21 days respectively. The pupal stage lasted for 9.2±0.32 and 6.8±0.13 days. Biological cycle of immature stages were 26.7±0.3 and 17.0±0.56 days respectively with 74.0% and 36.0% survival rate from egg to adult emergence at two different temperature levels respectively. The results indicate that developmental cycle and survival rate of immature stages were significantly different at two different temperature levels.

**EFFECT ON DEVELOPMENT AND PREY PREFERENCE OF EPISYRPHUS BALTEATUS ON TWO SPECIES OF APHIDS**

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*Episyrphus balteatus* is a major species of family Syrphidae (Diptera) Adult is pollen and nectar feeder while at its larval stage it is an important predator of aphids. Study on its life cycle and prey preference was carried out at 26C°±5C° temperature fed by two species of aphids *Myzus persicae* and *Brevicoryne brassicae*. The life cycle duration from newly hatched larval to the adult stages varied when fed with the above two aphid species as 10.25 and 12.25 days, respectively. The average pupal stage duration was 8.25 and 8.50 days, respectively. The survival rate of adults was 28.5% and 23.5% respectively. The results revealed that the developmental period of *Episyrphus balteatus* was less when fed on *Myzus persicae* while less survival rate of the syrphid predator when it fed upon *Brevicoryne brassicae*. It was concluded that *Myzus persicae* was preferred food item of *Episyrphus balteatus*. Temperature had also effect on the duration of the syrphid's life cycle which was observed as reduced during the month of relatively higher temperature.

**ARMY WORM MANAGEMENT IN COTTON THROUGH INSECTICIDES UNDER THE AGRO ECOLOGICAL CONDITIONS OF DERA GHAZI KHAN**

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Cotton (*Gossypium hirsutum* L.) is cash crop of the country. It contributes 26% of the total gross domestic production of the agriculture of the country. Insects pest of cotton cause a significant reduction in seed cotton yield production. Army worm (*Spodoptera litura* L.) is an economic pest of cotton in Dera Ghazi Khan. It causes significant damage o cotton lint by hindering the flow of photosynthates from leaves to bolls. Farmers use to spray various insecticides in cotton for army worm management in Dera Ghazi Khan. However, they are not aware of the efficacy and profitability of these insecticides. A study was, therefore, conducted at the farm of Adaptive Research Farm, Dera Ghazi Khan during Kharif, 2011 to evaluate the performance of different insecticides for the management of armyworm in cotton. These insecticides include: Coragen [Chlorantraniliprole 20 % SC], Runner [Methoxyfenozide 240 SC], Match [Leufenuron 5.2% w/w], Radiant [Spintoram 120 % SC] and Belt [Flubemiamide 40 % SC]. These insecticides were sprayed @ 125,500,500,100 and 63 ml/ha, respectively. All the insecticides were compared with check plot in terms of seed cotton yield and its yield contributing parameters. It was found that Match performed the best performance. It was economical, too. So it is suggested that this aforementioned insecticide should be used for army worm management in cotton under the agro ecological conditions of Dera Ghazi Khan.

**INTRAGUILD PREDATION BETWEEN *OXYOPES JAVANUS* (OXYOPIDAE: ARANEAE) AND *LYCOSA TERRESTRIS* (LYCOSIDAE: ARANEAE)**

ABIDA BUTT AND SUNDAS NASEER

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Interference between predator species frequently decreases predation rates for shared prey. Such interference can also occur between conspecific predators. Therefore present study was designed to understand the importance of predator interactions in biological control. For this purpose two spider species; *Lycosa terrestris* and *Oxyopes javanus* were selected due to their high abundance in the agricultural ecosystem of Punjab. Inter and intraspecific. interactions were studied in the presence and absence of the extraguild prey *Bemisia argentifolli* at various densities of food and predators. Polynomial logistic-equation ~reported a-type I functional response of *L. terrestris* and *O. javanus* to the number of extraguild prey and type II functional response to the proportion of extraguild prey offered. Data showed a pronounced evidence of cannibalistic activity in *L. terrestris* and *O. javanus*. Rate of cannibalism increased in both spider species with the increase in conspecific density and in the absence of food, the. Intraguild predation showed asymmetry in the favor of *L. terrestris*. This study indicates that food availability and density of both predators is important in determining their behavior in the. laboratory and field.

**EFFECT OF COLD STORAGE ON THE FITNESS OF ZIGZAG BEETLE *MENOCHILUS SEXMACULATUS* (FABRICIUS)**

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Zigzag beetle *Menochilus sexmaculatus* (Coleoptera; Coccinellidae) is one of the important generalist predators used widely against aphids. Apart from being aphidophagous, it has a vast host range including psyllids, whiteflies, mealybugs, leaf and planthoppers, mites, and early instar lepidopteran larvae. Use of cold storage as an aid to mass-rearing has proved to be a valuable tool not only in mass production but also in the delivery of biological agents to the release sites. Here we examine the effect of short term (7, 14, 21 and 28 days) cold storage at 0, 5 and 10°C on survival, fecundity and predation in field collected populations of zigzag beetle. Results showed that survival of both male and female beetles decreased slightly as the storage duration increased at 0°C. The ladybirds showed more than 90% survival both at 5 and 10°C. With a shortened preoviposition period, reproductive capacity of individuals remained unaffected. Cold storage of ladybird beetles for short periods can be done effectively to deal with any unexpected problem in rearing conditions.

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**DISTRIBUTION OF ANOPHELES MOSQUITOES IN KARACHI-PAKISTAN**

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Anopheles mosquitoes are universally called malaria mosquito, about 460 different anopheles species are globally found, whereas it came into existence over 150 million years ago. Although their life span is very short, but 60 anopheles species are responsible to transmit malaria to the human throughout the world. The distribution of Anopheles mosquitoes was studied by collections that were carried out three times per month in five different districts of Karachi-Pakistan during January 2003 to December 2004. Of the five districts sampled, twenty five areas of each district were visited in two years studies. In night collection performed indoor, between 7 PM to 7 AM by netting, using aspirator and light trap method (CDC) and also by spraying insecticide in the room. Different methods were applied in each house. There was a high aggregation of anophelines with different areas, more than 70% of the total anophelines being collected from the houses and only 30% of the different places sampled. At least 65% anopheline mosquitoes were collected from the congested houses. After collection, all specimens were brought to the laboratory and Anopheles morphologically identified according to Gillies and DeMeillon keys. Male and Females were counted and stored in a refrigerator for further examination. Overall, five anopheline mosquitoes species were identified, among these *Anopheles culicifacies* predominating during the first year (50.59%) and second year (42.07%), the second highest rate of anopheline was *An.stephensi* 31.16% and 38.46% during 2003 and 2004 respectively. Intra district collection showed variation in mosquito distribution. *An. pulcherimus* showed the lowest rate of incidence 3.94% and 2.29% during 2003 & 2004 respectively. Present investigation of vectors play a vital role in the transmission of diseases, it is obvious that this research will provide useful information to health care workers and vector control and malaria eradication program.

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**EFFECT OF DIFFERENT INFESTATION LEVELS OF *CHILO INFUSCATELLUS* (SNELLEN) ON QUANTITY AND QUALITY PARAMETERS OF SUGARCANE**

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Population dynamics of sugarcane stem borer on commercial varieties of sugarcane were evaluated. The adults of *Chilo infuscatellus* (Snellen) emerged from over wintering larvae during fourth week of February. The population of adults of *C. infuscatellus* remained low in early vegetative growth stage of sugarcane crop. The number of moth! trap increased gradually as the growth of the crop advanced and the maximum population of 37.4±2.20 was recorded during August. When mean number of 18.98 moth! trap were collected. The population of *C. infuscatellus* was declined after August and 28.6±2.42, 14.6±1.74 moths were captured! trap in month of September and October, respectively. The infestation of sugarcane stem borers was inversely proportional to the quantity and quality of sugarcane. Maximum cane weight of 13.5±0.21 and 12.1±0.05 kg were recorded in CPF-237 and Thatta-10 varieties at 0% infestation level, while minimum at 20% infestation (9.3±0.12 and 8.5±0.12 kg), respectively. Highest sugar recovery (11.0±0.08 and 10.2±0.11 %) was observed at 0% infestation while lowest (9.3±0.12 and 8.5±0.12%) at 20% infestation in both the varieties.

**SOME NEW RECORDS OF ACRIDID (ACRIDIDAE: ACRIDOIDEA: ORTHOPTERA) OF THE PUNJAB PROVINCE OF PAKISTAN**

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Abstract: A total of 58 species of short homed grasshoppers were collected from the various districts of the Punjab of the Pakistan. The following species *Dericorys tibialis* (Pallas), *Choroedocus illustris* (Walker), *Cataloipus cognatus* (Walker), *Gelastorhinus semipictus* (Walker) are recorded for the first time from this area.

**REDESCRIPTION OF *PERICALLIA RICINI* (F) (LEPIDOPTERA: ARCTIIDAE) RECORD- FROM COASTAL AREA OF SINDH**

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Hubner in 1881 discovered *Pericallia ricini*. Walker in 1855 and Moore in 1882 redescribed same species on the basis of its morphological character only. This species is widely distributed. It is first time that this species redescribed from coastal area of Sindh on the basis of head appendages, venations of fore and hind wings, & male genitalia. The identification characters are: Head and thorax brown except yellow spot on collar, abdomen crimson except reddish brown 8-spots on dorsum and 7-lateral patches. Eyes large, frons broadly rounded, palpi large, anteriorly

porected, slightly passing fronts with 2nd segment about 2Y:zx the length of 3rd segment, proboscis short slightly coiled. Fore wings moderate, apically narrowed, veins R3 and I~ moderately stalked, further stalked with R2 anastomosing with Rs and originate from upper angle of cell, M3 originates below lower angle of cell, Hind wings small, broad, anterior margin convex, apically broadly rounded, yellow except large patches on costal margin and sub-apical margin and 5-small apical spots, veins Rs and MI anastomosing and originate from upper angle of cell, M2 originates just above lower angle of cell, M3 and CUI anastomosing and originate from lower angle of cell, Tegumen large, pitcher-shaped, sclerotized, saccus sclerotized with broad base, laterally produced into broad lobe, juxta plate-like, broad at base, gradually narrowed at distad, uncus narrowed with outer and inner margin sinuated aedeagus lobe very large with small spine-like bunches of cornuti. This species is most closely related to *Pericallia imperialis* Kollar in having fore wings with vein Rs directly originates from upper angle of cell, in males tegumen with prominent dorsal lobe, gnathos short reduced, but it can easily be separated from same in having eyes large, frons convexly produced, proboscis reduced, maxillary palpi with 3rd segment anteriorly directed, in males tegumen pitcher-shaped, tegumen broad, parameres inwardly highly curved apically bilobed, juxta distally dome-shaped, membranous conjunctiva with many small bunches of spine-like cornuti. This species is recorded from Coastal area of sindh i.e. Karachi and Thatta in between the range of 21m above sea level. The population is very high during July and August and very less recorded in December and January. The temperature varies during summer 36°C and in winter 21°C, while average annual temperature is 27°C. Amount of Precipitation is between 50-60mm or sometimes to about 100 mm. Average relative humidity (mean) is at 1200 UTC 46%.

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#### MOSQUITO SURVEILLANCE IN LAHORE DURING OCTOBER-NOVEMBER, 2011

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For collection of eggs, larvae and adults of *Aedes aegypti* and *A. albopictus*, 412 houses and 267 containers in different localities of Lahore were examined, after Dengue outbreak in Lahore. List of the localities and the type of the containers examined are described. Ninety-five percent samples contained *A. aegypti* and the remaining five percent *A. albopictus*, indicating that Dengue Haemorrhagic Fever is mostly caused by *A. aegypti*, with House Index (2.66) and Container Index (2.99).

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#### EFFECT OF HONEYBEE (*APIS MELLIFERA L.*) POLLINATION ON SUNFLOWER (*HELIANTHUS ANNUUS L.*) CULTIVAR PARSUN-2

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WAGHCHOURE AND RASHID MAHMOOD  
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A field study was conducted to determine the role of honeybee on sunflower cultivar Parsun-2 during pollination spring crop 2009 at National Agricultural Research Centre Islamabad. Three plots were selected at random, containing 20 plants in each. Three types of treatments were applied (1) free visits of bees and other pollinators, (2) plants caged with bees and (3) plants caged

without bees. Plants where bees had access produced comparatively more seed than plants where bees and other pollinators were excluded. Both the free visits of bees and other pollinators and caged plants with bees increased the number of filled seed per head and 100 seed weight (90.7%, 5.36 gm; 84.45%, 5.17 gm respectively) as compared to plants caged without bees (65.63%, 4.21 gm). It is concluded that use of honeybees for sunflower yield is useful for pollination. Bees should be moved in sunflower field at the onset of flowering.

**STUDY ON FEEDING BEHAVIOR WITH SPECIAL PREFERENCE TO REARING TECHNIQUES OF HOUSE FLIES (*MUSCA DOMESTICA*) IN LABORATORY**

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An easy and trouble-free rearing scheme was developed; moreover a concise appraisal is specified on various physical techniques in reproduction of house flies (*Musca domestica*). The rearing was carried out in the glass cages of 23 inch high, 30 inch long and 18 inch wide. The length wise sides of the cage was covered with glass sheets and the bottom of the cage was also made up of a glass sheet, while, on one side, cage was equipped with a long sleeve made up of white muslin cloth, which was used to fulfill the cleaning and feeding necessities. On the opposite end of the muslin cloth side, a transparent glass was fitted to observe the activities of the adult and larvae house flies, and to study the feeding behavior of the insects. During the rearing phase, comparative study of rearing medium was also carried out. This technique was particularly supportive for the study of insect behaviours. Rearing was done in the glass cage which was so helpful to study the behavior of several flies at the same time. In the present study feeding preference of house flies were also studied. For this purpose 10 recipes having various compositions of ingredients were provided to the house flies and each recipe was marked its number from 1 to 10. visits of house flies were counted very carefully.

**FEEDING PREFERENCES OF *HETEROTERMES INDICOLA* (WASMANN) (ISOPTERA: RHINOTERMITIDAE) TO COMMERCIAL WOOD SPECIES**

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Ten species of wood were tested for their natural resistance and to reveal feeding preferences of *Heterotermes indicola* (Wasmann) under no choice and choice laboratory and field trials. The impact of drying temperature (60°C, 70°C, 80°C, 90°C & 100°C) was studied. The amount of wood consumed in general, increased with increase in drying temperature indicating that heat contributed to the loss of natural resistance components of the woods. In no choice laboratory experiments, *C. deodara* and *D. sissoo* were found highly resistant and *P. euramericana* highly palatable. When a choice was given and the wood was offered in combination of two, *H. indicola* repeated its instinct, easily identified the most preferred wood and consumed it more. Consequently *H. indicola* showed maximum feeding on *P. euramericana* and the minimum on *C. deodara* and *D. sissoo*. The mean feeding propensity was significantly different ( $P < 0.000$ ). Based on the feeding propensity, the woods are arranged in descending order of preference: *Populus euramericana* >

*Mangifera indica* > *Pinus roxburghii* > *Acacia arabica* > *Morus alba* > *Abies pindrow* > *Tectona grandis* > *Azadiracta. indica* > *Dalbergia. sissou* > *Cedrus. deodara*. Although *H. indicola* fed aggressively on *A. pindrow*, the wooden blocks had undesirable effect on the survival of the species, manifesting toxic nature of the wood.

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**A PRELIMINARY STUDY OF MOSQUITOES FROM AQUATIC HABITATS AT THREE VILLAGES OF SWAT RANIZAI SUB DIVISION MALAKAND**

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Survey of mosquito larval habitats was conducted to determine species composition, relative abundance and habitat preference of mosquitoes of Swat Ranizai of Malakand District. The survey was conducted from April 2007 to August 2007 and then from Oct to Dec 2007. Mosquito larvae were collected from different temporary and permanent breeding sites such as streams, springs, rock pools, ponds, drains and rice fields selected in three villages viz Thana, Dheri allahdand and Zormanda in Swat Ranizai subdivision of Malakand district. Fifteen species belonging to five genera *Culex*, *Anopheles*, *Aedes*, *Culiseta* and *Armigeres* were identified. Genera *Culex* and *Anphelese* were represented by six species each. The species belonging to *Culex* were *Cx. quinquefacitus*, *Cx. tritaenorrhynchus*, *Cx. bitaenorrhynchus*, *Cx. theileri*, *Cx. mimeticus*, *Cx. vishnui* while *Anopheles* species were *An. stephensi*, *An. fluviatilis* *An. maculatus*, *An. culicifacies* *An. subpictus* *An. lindesayi*. The remaining three species were identified as *Aedes vittatus*, *Culiseta longiareolata* and *Armigeres subalbatus*. *Cx. quinquefacitus* (79.51%) and *An.stephensi* (6.27%) were dominant and constant and recorded in most of the months and majority of habitats Subdominant species were *Cx. tritaenorrhynchus*, *Cx. theileri*, *Cx. mimeticus* and *An. maculatus* which were and moderate in distribution. The density of the rest of the species was placed in satellite and distribution as moderate, infrequent and sporadic. These species were collected in two or three of the studied months.

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## SECTION – IV

## PARASITOLOGY

**FIRST RECORD OF DEVELOPMENTAL STAGES OF TWO SPECIES OF ACANTHOCEPHALA IN FISH OF KARACHI COAST, PAKISTAN**

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There is no previous record in Pakistan about the study of developmental stages of acanthocephalan parasites in fishes. During the present studies developmental stages of two species of genus *Serrasentis* are identified in the visceral mesenteries of fish *Pseudosciaena diacanthus* of Karachi coast. For detail studies visceral mesenteries were pressed and flattened between two glass slides, fixed in AFA solution, stained with Mayer's carmalum, dehydrated in graded series of alcohols, cleared in Xylol and Clove oil, mounted permanently in Canada Balsam, photograph were taken with a Nikon (Optiphot-2) photomicroscope. The adults of acanthocephalan species *Serrasentis mujibi* and *Serrasentis longus* are already known from the fishes of Karachi coast but development of these species were not described previously. Here the developmental stages including embryos and various stages of acanthella and cystacanth are identified and reported here.

**BRACHYLECITHUM HECKMANNI SP.N. (TREMATODA: DICROCOELIIDAE) FROM THE HOST EGRETTA ALBA (LINNAEUS) GREAT EGRET OR LARGE EGRET SYN. ARDEA ALBA IN SINDH, PAKISTAN.**SANJOTA N. DAS<sup>1</sup> AND RAFIA R. GHAZI<sup>2</sup><sup>1</sup>*Department of Zoology, University of Karachi, Karachi 75270.*<sup>2</sup>*Ex Director Vertebrate Pest Control Laboratory, Southern zone Agricultural Research Centre, Karachi University Campus, Karachi- 75270.*

*Egretta alba* Great Egret or Large Egret were purchased from the Empress market in Karachi city. The birds were anaesthetized autopsied and examined for helminth parasitic infections. The worms were mounted permanently according to standard procedures. A detail study was conducted and a new species *Brachylecithum heckmanni* is proposed belonging to the genus *Brachylecithum* from the small intestine of the *Egretta alba* (Linnaeus) Great Egret or Large Egret syn. *Ardea alba* in Sindh, Pakistan. The Genus is characterized by having: Body long, slender, flattened or cylindrical. Tegument unspined or covered with spines or conical papillae. Suckers in anterior third of body; ventral sucker may be slightly larger than oral. Pharynx rather small. Esophagus short. Caeca terminate far from posterior extremity. Testes tandem or somewhat diagonal, distant from or close to one another. Cirrus-sac elongate, may reach anterior border of ventral sucker. Genital pore median, usually at level of intestinal bifurcation, somewhat anterior or posterior to it. Ovary median, usually separated by uterine coils from posterior testis. Uterine coils occupy entire hind body, also extra-caecal. Vitellarium in two relatively short bands composed of few large follicles, in middle third of body. Excretory vesicle Y-shaped, with long stem, which may reach close to level of vitellarium.

**IN VITRO VALIDATION OF TREMATOCIDAL ACTIVITY OF *CYBOPOGON JWARANCUSA* AGAINST THE *PARAMPHISTOMUM CERVI***

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The parasitic trematode infection causes a great economic loss to livestock industry. Conventionally, chemotherapy is used to cure the infection. But, resistance development in trematodes against anthelmintic drugs leads to develop alternative sources of treatment to cure the condition. Medicinal plants possess anthelmintic activity which can be considered as alternative to chemical treatment in livestock industry especially in large ruminants. Keeping in view, present study was designed to screen out and evaluate, and standardize the extracts of *Cymbopogon jwarancusa*, commonly known as khavi. Different concentrations of crude methanolic extracts were used to determine *In vitro* trematocidal activity. Time and dose dependent trematocidal effects on live *Paramphistomum cervi* worms were observed and LC50=0.67 mg/m exhibited pronounced inhibitory effects. The present study demonstrated that plant *Cymbopogon jwarancusa* showed the significant ( $p<0.05$ ) trematocidal activity than controls that may justify their traditional use in ethno-veterinary medicine.

**FIRST RECORD OF GENUS *STRONGYLOIDES* GRASSI, 1879 (NEMATODA: STRONGYLOIDAE) IN PAKISTAN**

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Nematodes belonging to the genus *Strongyloides* Grassi, 1879 were collected from the bird Black Coot, *Fulica atra* (Gruiformes: Rallidae). The host birds were collected from different water bodies of Sindh Province, Pakistan. A total of 1663 ♀ nematodes were collected from the intestines and gizzards of 69 hosts. These specimens have close resemblance with *Stroglyoides avium* Cram, 1929 and identified as such. However, this genus is being reported for the first time from Pakistan.

***RAILLIETINA (RAILLIETINA) GALERITAE* (SKRJABIN, 1914) KHAN AND HABIBULLAH, 1967 RECOVERED FROM THE COMMON HOST *PASSER DOMESTICUS*.**

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Seventy males and forty five females of *Passer domesticus* were purchased from the birds empress market out of seventy fifteen males and twelve females were found infected from the helminth parasitic infections, the trematodes were mounted according to the slandered procedures,

The Genus is characterized by having: numerous proglottides. Rostellum with double circle of hammer-shaped hooks. Sucker margins with several circles of minute hooks. Testes usually numerous; cirrus pouch small, usually not reaching excretory stems, rarely crossing them. Genital pores unilateral or irregularly alternating. Ovary bilobed or not, median or somewhat poral; Vitelline gland compact, postovarian. Seminal receptacle present. Egg capsules containing one to several eggs; capsules often grouped or surrounded by modified parenchyma.

### ARGULOSIS, A PARASITIC INFECTION IN TWO FRESHWATER ORNAMENTAL FISHES

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Two imported ornamental fishes, black moor a variety of goldfish, *Carassius auratus* L. and koi a variety of *Cyprinus carpio* L. were obtained from pet shops in Lahore. These fishes were examined for parasitic infection, argulosis. The causative agent, *Argulus foliaceus* was recovered from fins and skin of the fishes. Black moor (n=16, mean total length = 8.15±1.59cm; mean weight = 9.63±6.54g) showed 12.50% infection (mean intensity 4.5). Koi (n=22, mean length=9.95±2.5cm; mean weight=11.4±1.5g) had 13.63% infection (mean intensity=6.0). The infected fishes showed typical clinical signs; poor growth, debility, faded skin color, lesions on skin and rubbing against walls of aquarium. This is a typical example of introduction of fish pathogens from one country to other county along with their host (in this case through import of live fish). Though the infection level is low in both these fish species, yet it is an alarming and threatening situation for the local fish species and aquatic ecosystem and also to fish health management experts. Hence, strict regulations on import of healthy and disease free live fish are required to be implemented at fish landing sites, such as international airports in the country.

### LOSSES CAUSED BY PLANT-PARASITIC NEMATODES ON VEGETABLE CROPS.

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During 2007 and 2009, plant-parasitic nematode population densities were determined from 325 root and soil samples collected from vegetable growing areas in Pakistan. Yield losses associated with nematode presence were quantified on 19 vegetable crops including bitter melon (*Momordica charantia*), cabbage (*Brassica oleracea*), carrot (*Daucus carota*), chilies (*Capsicum annuum*), coriander (*Coriandrum sativum*), cowpea (*Vigna sinensis*), cucumber (*Cucumis sativus*), eggplant (*Solanum melongena*), lettuce, (*Lactuca sativa*), Melon (*Cucumis melo*), mustard (*Raphanus sativus*), okra (*Hibiscus esculentum*), potato (*Solanum tuberosum*), pumpkin (*Cucurbita argyrosperma*), spinach (*Spinacea oleracea*), sponge gourd (*Luffa cylindrical*) squash (*Cucurbita pepo*), tomato (*Lycopersicum Esculentum*) and watermelon (*Citrullus lanatus*). The most abundant plant-parasitic nematodes detected, in order of decreasing frequency of infestation (percentage of

samples), were *Meloidogyne incognita* (90%), *Pratylenchus penetrans* (30.2%), *Tylenchorhynchus clarus* (29%), *Hoplolaimus columbus* (15%), *Paratrichodorus minor* (7.5%), *Xiphinema americanum* (7.1%), *M. javanica* (7%), *Belonolaimus longicaudatus* (5.6%), *Longidorus africanus* (5%), and *Helicotylenchus dihystra* (3.2%). We observed ca 23% yield losses ranged from 2% for cabbage to 45% for squash, which is 35%, 80%, and 46% higher compared to developed countries, USA, and India, respectively. The main reason for more losses in Pakistan might be related to incognizant growers about the presence of nematodes and the damage they cause. Another reason might be non-availability of resistant crop cultivars and nematicides. Another possibility is that on small-sized farms with varied cropping histories and inattention to the pest-host-status one result appears to be a larger list of available nematode species, compared to farming practices in USA where only one or two nematode species become dominant. This study provides important information for extension specialists and creates awareness among growers about these hidden crop enemies. It also suggests the need for improved management measures to avoid crop losses.

#### STUDY OF ECTO- AND ENDO-PARASITES IN RING-NECKED PHEASANTS AT JALLO PARK, LAHORE

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The present work was undertaken to assess the prevalence of ecto- and endo-parasites of ring-necked pheasants (*Phasianus colchicus*) at Jallo Park, Lahore, Pakistan. For this purpose, 40 birds (21 males and 19 females) were randomly examined. Ecto-parasites were collected, preserved, stained and identified. Among selected birds 20 (95.24%) male and 16 (84.21%) female were infested with different species of ecto-parasites. A total of 204 specimens of lice belonging to five species namely *Cuclutogaster heterographus* (n=102; 70 females, 26 males, 5 nymph), *Goniocotes gallinae* (n=30; 25, 5 nymph), *Goniocote chrysocephalus* (n=32; 23 females, 9 nymph), *Lipeurus caponis* (n=29; 24 females, 1 males, 4 nymph), *Menacanthus stramineus* (n=11; 3 females, 8 nymph) were identified. The dominant lice species was *Cuclutogaster hetrographus* with relative abundance of 50% followed by *Goniocotes chrysocephalus* (15.68%), *Goniocotes gallinae* (14.70%), *Lipeurus caponis* (14.23%) and *Menacanthus stramineus* (5.39%). For endo-parasitic study, faecal materials of 50 birds (29 males and 21 females) were collected and endo-parasites were identified. Among examined birds, 20 (68.96%) males and 12 (57.14%) females were infested with endo-parasites. Eggs of various helminthes were also identified. Total of 21550 eggs of endo-parasites of five different types of species namely, *Ascaridia* spp. (n=700), *Capillaria* spp. (n=1550), *Eimeria* spp. (n=17500), *Heterakis isolonchae* (n=1600) and *Syngamus trachea* (200) were observed. The dominant species of endo-parasites were *Eimeria* spp. with relative abundance of 79.73% followed by *Heterakis isolonchae* (7.29%), *Capillaria* spp. (7.06%), *Ascaridia* spp. (3.19%) and *Syngamus trachea* (0.91%). From this study, it was concluded that it is the need of time for extensive study on ecto- and endo-parasites of wild pheasants, so that, these can be preserved from diseases caused by these agents which ultimately affect the health and reproductive performance of these birds.

**DETECTION OF SOME NEMATODES OF ZONOTIC IMPORTANCE IN PET DOGS  
BY VARIOUS TECHNIQUES**

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There is a wide range of nematode species of zoonotic potential that can infect pet dogs. The present study was conducted to detect nematode infestation in pet dogs at Lahore. A total of 300 fecal samples were examined from January 2011 to June 2011 by various techniques. Two types of nematodes were identified (*Toxocaracanis* and *Ancylostomacanium*). The prevalence of *T.canis* was 11.33% and 8.33% for *A.caninum* by Direct Smear Method. By Flotation technique, prevalence of *T.canis* was 14.33% and 12.3% for *A.caninum* and by Sedimentation technique, the prevalence of *T.canis* and *A.caninum* was 11.66% and 10% respectively. To investigate the effect of physical factors on prevalence of nematodes, month-wise mean temperature and mean monthly relative humidity were also noted from January 2011 to June 2011. The results of study revealed that temperature has a positive relationship with the prevalence of infection, as peak number of *T.canis* and *A.caninum* infections were observed in the month of June when the temperature was maximum while the prevalence of both species was lowest in January when the temperature was minimum. The percent prevalence of both types of nematodes did not show any direct relation with relative humidity. The results of the study were analysed by ANOVA. As pet dogs are the reservoir of transmission of diseases to humans specially children, so there is a need to control nematode infections by establishing hygienic conditions and regular clinical examination of pet dogs.

**SCREENING OF DIFFERENT OKRA CULTIVARS FOR THEIR RESISTANCE AND  
SUSCEPTIBILITY AGAINST ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA***

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The root-knot nematode, *Meloidogyne incognita*, is one of the major limiting factors affecting plant growth and yield causing an estimated \$100 billion loss per year worldwide. Synthetic pesticides, though instantaneously effective, are usually prohibitively expensive, not readily available particularly for the resource-poor farmers in the country and cause a lot of hazards to both man and livestock and inflict injury to the environment. Notable among these alternatives is the use of resistant plants which is cheap and eco-friendly. In the present studies, twelve okra (*Abelmoschus esculentus* L) cultivars were evaluated for their resistance against *M. incognita* under green house conditions. Ten-day old okra plants of different cultivars were inoculated with 5000

J2s of *M. incognita*. Six weeks after inoculation the plants were uprooted and examined for galls, number of egg masses, root/shoot lengths and weights and reproduction factors. All the cultivars caused reduction in various growth parameters to varying levels over their respective controls. The response of cultivars was assessed on the basis of root-knot galling index. None of the cultivars was found immune, highly resistant or resistant. The cultivars Sanam, Dikshah, Sabz Pari, Arka Anomika, Ikra-1, Ikra-2, PMS-55, PMS Beauty and Super Star were found moderately resistant to *M. incognita*. Two cultivars viz. Selection-31 and Okra Sindha were moderately susceptible and the cultivar Punjab Selection was found highly susceptible.

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**NEMATOCIDAL ACTIVITY OF *CEDRELLA TOONA* AGAINST ABOMASAL NEMATODES OF SMALL RUMINANTS**

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Among the helminth parasites, nematodes are major constraint to livestock productivity around the world. Conventionally, chemotherapy is used to cure and control the infection. But the problems such as development of resistance, being expensive, residual toxicity and inaccessibility of these drugs, are associated with this mode of treatment. Ethnoveterinary medicines, which are safe, inexpensive and easily available, present a good substitute to cope with these problems. In this regard, *in vitro* screening of an ethnoveterinary plant *Cedrella toona*, collected from the Pothwar region, was performed to test nematocidal activity on adult worms acquired from the abomasa of small ruminants. Live worms were incubated in different concentrations of crude methanolic plant extract. A dose and time dependent efficacy was observed and LC<sub>50</sub> = 4.56mg/ml was determined. Present study indicated that *Cedrella toona* posses the nematocidal activity, suggesting the *in vivo* analysis for use as ethno-veterinary medicine.

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**STUDY OF ECTO- AND ENDO-PARASITES OF PEAFOWLS CAPTIVE AT JALLO WILDLIFE PARK, LAHORE (PAKISTAN)**

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This study was designed to find the ecto- and endo-parasites of peafowls kept at Jallo Wildlife Park, Lahore, Pakistan. Sixty Indian peafowls (*Pavo cristatus*) consisting of 21 adult peahens, 16 adult peacocks, 11 juvenile peahens and 12 juvenile peacocks were thoroughly examined for the presence of ecto-parasites. Similarly, for endo-parasitic study, faecal materials of 71 peafowls (24 adult peahens, 26 adult peacocks, 8 juvenile peahens and 13 juvenile peacocks) were examined. One thousand two hundred and nine lice (628 female, 571 male, 97 nymph) were collected and identified from all age groups of peafowls. Relative abundance of female, male lice and nymph was 51.58%, 40.14% and 8.38% respectively. All birds were infested with ecto-parasites having 11 types of chewing lice (ecto-parasites) namely, *A. minuta* (n=109; 44 female, 65

male), *A. phaeostoma* (n=10; 5 female, 5 male), *C. tausi* (n=137; 74 female, 63 male), *C. thoracicum* (n=37; 14 female, 20 male), *G. mayuri* (n=26, 15 female, 11 male), *G. perviceps* (n=18, 11 female, 7 male), *G. rectangulatus* (n=182; 76 female, 106 male), *G. gigas* (n=145; 66 female, 79 male), *G. pavonis* (n=465; 248 female, 161 male, 56 nymph), *L. pavo* (n=71, 14 female, 20 male, 37 nymph) and *M. stramineus* (n=96; 58 female, 34 male, 4 nymph). The relative abundance of dominant species was *G. pavonis* (35.88%), followed by *G. rectangulatus* with (14.04%), *G. gigas* (11.19%), *C. tausi* (10.57%), *A. minuta* (8.41%), *M. stramineus* (7.41%), *L. pavo* (5.48%), *C. thoracicum* (2.85%) and *A. phaeostoma* (0.77%). The fecal materials of 67 (94.37%) birds were loaded with the eggs of five types of helminths namely *Ascaridia spp.* (5700 EPG), *Capillaria spp.* (725 EPG), segment of cestodes (21275 EPG), *Heterakis spp.* (6550 EPG), *Strongyloides spp.* (3350 EPG) and a protozoan endoparasite namely *Eimeria spp.* (30325 EPG). Eggs of *Eimeria spp.* were dominant with 30325 EPG. From this study, it is clear that it is utmost important to carry out survey in the area to estimate the magnitude of disease problems caused by ecto- and endoparasites in these wild birds so that necessary action could be taken to prevent the disease outbreaks.

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#### MOLECULAR EPIDEMIOLOGY OF CUTANEOUS LEISHMANIASIS IN KOHAT, KARAK AND HANGU DISTRICTS, KHYBER PAKHTUNKHWA, PAKISTAN

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Cutaneous leishmaniasis (CL) is endemic in all the four provinces of Pakistan particularly Khyber Pakhtunkhwa province. Migration of large number of Internally Displaced People (IDPs) to Kohat and Hangu districts has resulted in increased prevalence in recent years. Moreover, *Leishmania* species prevailing in this part of Pakistan needed identification using molecular biological tools. House to house surveys were performed in Kohat, Karak and Hangu and 3590 tissue samples were collected from cutaneous leishmaniasis (CL) suspected patients. The samples were processed through microscopy, culture and different primer based PCRs followed by Restriction Fragment Length Polymorphism and subsequent sequencing. The overall prevalence of CL in these study districts using PCR was 2.54%. The highest degree of prevalence was recorded in Karak that was 3.17% followed by Kohat 2.70% and Hangu 1.85%. Statistical analysis showed that children of age group 0-15 year were significantly more susceptible to CL due to low level of immunity. Although no correlation was found on gender basis. Presence of domestic animals, wall and roof type, presence of internally displaced people (IDPs) were associated with increased risk of cutaneous leishmaniasis. Kinetoplastic DNA- PCR was the most sensitive (98%) of all the assays while specificity (76.2%) was lower than the ribosomal and Internally transcribed spacer 1 primers having 100% specificity. The ITS1-PCR-RFLP analysis using *Haemophilis* III enzyme confirmed *L. tropica* in 241/275 ITS1 PCR amplified products and only 25 of the total samples were confirmed as *L. major*. Sequencing analysis-using ITS1 gene confirmed *L. tropica* in 40 samples

representing different villages of study district. Molecular epidemiology of study districts concluded CL to be highly prevalent in Southern districts of Khyber Pakhtunkhwa and *L.tropica* is first time confirmed to be prevailing leishmania species. Further studies are required to investigate the role of sand flies and animal reservoirs in the identified endemic foci of study districts.

#### ASSESSMENT OF THE DAMAGE CAUSED BY *MELOIDOGYNE INCOGNITA* ON CUCUMBER AT DIFFERENT INOCULUM LEVELS

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Among the most damaging root-knot nematode species, *Meloidogyne incognita* is one of the major constraints to cucumber (*Cucumis sativa* L.) in vegetable-growing areas in Pakistan and other countries of the world. The relationship between a geometric series of six initial population densities (Pi) of *M. incognita* (0, 500, 1000, 2000, 4000 and 8000 J2s) per Kg of soil and growth parameters and nematodes infestations was investigated on cucumber cv "Royal Sluis". Observations were recorded 45 days after inoculation with the nematode. It was found that all the inoculum levels reduced the shoot and root lengths, and fresh and dry weights. Increasing the nematode inoculum level increased the number of galls, egg masses and nematode population build up. The reductions in growth parameters and nematode infestations were found to be directly proportional to the inoculum level. On the other hand an inverse relationship was observed between the nematode build up and inoculum levels. Similarly, age of plants had negative correlations with shoot length, root length, shoot weight and reproduction factor at each inoculum level showing that the magnitude of damaging effects of nematode densities decreased as the age of plants increased at the time of inoculation. However, there was a positive correlation between plant ages and number of galls and egg masses.

#### FREQUENCY OF INFESTATION OF HELMINTH PARASITES IN DONKEYS, *EQUUS ASINUS* IN PESHAWAR, PAKISTAN

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The donkey, *Equus asinus* Linnaeus is very common animal in the world used as heavy load carrier. The general prevalence and population of intestinal helminthes parasites of working *E. asinus* were conducted during September 2010-January 2011 in Peshawar. For this purpose, 162 fecal samples were examined for the infestation. In *E. asinus*, helminthes parasites were *Parascarium equirum* (66.66%), *Strongylus* sp. (58.2%), *Dictylocalus arnified* (30.86%), *Cyathostoma* (11.11%), *Triodontosoma* (20.98%), *Habronema* (6.17%), *Dicrocoelium lanceatum* (8.64%), *Apophalus donicus* (6.17%), *Delafondia vulgaris larvae* (4.93%), *Trochostrongylus axei* (3.70%),

*Oxyuri sequi* (2.46%). *Anoplo cephalo* was not reported and 10 samples were free of these parasites. This study shows that working *E. asinus* in Pakistan are infected with middle range of intestinal helminthes parasites and are representatives of the important pathogenic parasites found in equines worldwide. It can be concluded that good management practices, education and awareness of the equines holders is of prime importance in reducing helminthes infestation in *E. asinus* to utilize its maximum efficiencies.

#### INCIDENCE AND PREVALENCE OF GASTROINTESTINAL NEMATODES IN DOMIESTIC SHEEP, *OVIS ARIES* LINNAEUS IN MALAKAND DISTRICT, PAKISTAN

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Domestic sheep, *Ovis aries* Linnaeus are an important part of the global agricultural economy, raised for meat, milk, important for wool, and as model organisms for science. *Ovis aries* also has an important role in Abrahamic faiths. Eid al-Adha is a major festival in Islam in which domestic sheep are sacrificed in large scale every year. A study on incidence and prevalence of gastrointestinal nematodes in 260 *O. aries* was carried out in Malakand district from October 2010 to January 2011. Gastrointestinal nematodes cause losses through lowered fertility, reduced work capacity, a reduction in food intake and lower weight gains, treatment costs, and mortality in heavily parasitized animals. Faecal egg counts were recorded during the laboratory diagnoses of the samples in VRI, Peshawar. Simple test tube flotation method was used for qualitative study of nematode eggs in faecal samples. During the diagnoses 212 sheep were positive for 6 different species of gastrointestinal nematodes and 48 were negative. *Haemonchus contortus* (75.38%) was found to be predominant of gastrointestinal nematode parasites, *Trichostrongylus* spp. (35.38%) was the next most prevalent species, others, including: *S. papillosus*, (25.00%), *N. spathiger* (8.46%), *O. circumcincta* (6.15%), *T. ovis* (4.23%) were found. The results show that major nematodes belonging to genera *Haemonchus*, *Trichostrongylus*, *Strongyloides*, *Nematodirus*, *Ostertagia* and *Trichuris* were prevalent in the areas of Malakand district. The results of this study could be used to design a programme to minimize and control gastrointestinal nematode infections in sheep.

#### EFFECT OF *CANNABIS SATIVA* AND *ZANTHOXYLUM ALATUM* ON THE HATCHABILITY AND MORTALITY OF ROOT-KNOT NEMATODES (*MELOIDOGYNE INCOGNITA*)

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Plant extracts have the advantages of cheapness, and ready availability over the conventional nematicides. Their environmental safety in an environmentally conscious world also holds promise for their acceptability and use by resource-poor farmers. The objective of the present

study was to assess the activity of aqueous extracts of *Cannabis sativa* and *Zanthoxylum alatum* on hatching and mortality of *Meloidogyne incognita*. The extracts of both the plants were tested at different concentrations viz. S: 1, S: 5, S: 10, S: 25, S: 50 and S: 100. The plants had significant effects on larval mortality and hatching inhibition. Mortality and hatching inhibition caused by *C. sativa* were significantly higher than that of *Z. alatum*. Similarly concentrations also had significant effects on juvenile mortality and hatching inhibition. Maximum mortality and hatching inhibition were caused at 'S' and minimum at 'S: 100'. Mortality and hatching inhibition decreased as the concentration decreased and were found directly proportional to concentration of the extract. Time duration also affected mortality and hatching inhibition significantly. As the duration increased, mortality and hatching inhibition also increased.

#### IDENTIFICATION OF PREVALENT TICKS AND THEIR COMPARATIVE STUDY IN COWS AND BUFFALOES IN LOWER DIR, PAKISTAN

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Mammalian livestock is the source of milk and other dairy products, their skin used in leather industry. The blood and bone of animals are also used as fertilizer to increase crop yields. For the present research, a survey was conducted during March-June 2011 in Lower Dir. Among ecto-parasites, ticks have been recognized as the notorious threat due to severe irritation, allergy and toxicosis and of diseases like babesiosis, theileriosis and anaplasmosis etc. During present survey, 731 livestock were examined from different areas of Lower Dir. Ticks were identified for their genera, in parasitology laboratory, VRI, Peshawar with the help of identified specimen, available literature and using proper keys. The cows, *Bos taurus* (Bojanus) 440 and buffaloes, *Bubalus bubalis* (Linnaeus) 198 were found infested by different tick genera. In the present study, 7 genera of ticks were identified, which are, Lone star tick, *Amblyoma sp* (Koch) (4.58%) Cattle tick *Boophilus sp* (Curtice) (8.42%) Rocky Mountain wood tick, *Dermacentor sp* (Koch) (6.92%) Rabbit tick, *Haemaphysalis sp* (Koch) (25.27%) Bont legged tick, *Hyalomma sp* (Koch) (9.06%) Black legged Tick, *Ixodes sp* (Latreille) (4.26%) Brown dog tick, *Rhipicephalus sp* (Koch) (22.28%). However it is concluded that in cows and buffaloes *Ixodes sp* have caused minimum infestation while *Haemaphysalis sp* have caused maximum infestation and high ticks' infestation were found in femals adults' cows and buffaloes compared with males' young cows and buffaloes. The high infestation was due to housing system and grazing. Further investigation, good management practices, education and awareness of the livestock holders are of prime importance in reducing ticks infestation on animals.

#### PATHOLOGICAL CHANGES IN THE INTESTINE OF CHICKEN (*GALLUS DOMESTICUS*) NATURALLY INFESTED WITH CESTODE PARASITES IN SINDH

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The chicken (*Gallus domesticus*) is one of the common domesticated poultry animals. It is an important source of protein in the fonn of eggs as well as meat, and constitutes about 30% of all

protein consumed world wide. In developing countries most of the families in villages keep chicken flocks in free range scavenging system. Their supplementary feed consists of many house hold wastes, insects and seeds. Scavenging habits of chicken provide fair chances of acquiring parasitic infections from their environment, which results in the economic losses in the form of retarded growth, reduced egg production and mortalities. The present study was undertaken to study the histopathology caused by the cestode parasites in chicken intestine. During present investigation desi chickens (*Gallus domesticus*) were brought from the market of Hyderabad city. The chickens were dissected and their intestines were carefully removed and examined for helminthes. The parasites collected from the intestine were preserved, stained and mounted by using standard techniques. Tissue samples from infected intestine were fixed in Bouin's fluid for 24 hours. Using routine histological techniques, 6-8 micron thick sections were prepared and stained with hematoxiline and eosin. These serial sections were mounted in Canada Balsam. The slides were observed under light microscope. During study the chicken were found infected with *Cotugnia digonophora*, *Choanotaenia infundibulum* and *Raillietina cestocilluscetodes*. Microscopic observations revealed severe intestinal tissue damage, which included architectural disintegration, erosion, fusion and shortening of the villi. Atrophy and vacuolation of muscular layer. Hyperplasia, fusion and disintegration of the glands were also observed. These changes definitely affect the vital activities of the chicken.

#### OCCURRENCE OF HELMINTHES PARASITE IN SHEEP, *OVIS ARIES* LINNAEUS IN PESHAWAR DISTRICT, PAKISTAN

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Sheep, *Ovis aries* Linnaeus is raised for fleece, meat (lamb, hogget or mutton), milk, important for wool, pelts use in agriculture, as dairy animals and as model organisms for science. Sheep are also occasionally sacrificed to commemorate important events in Islamic cultures. A survey, occurrence of helminthes parasite in sheep in Peshawar district was conducted during September-December 2010. Helminthes Parasites have been shown to adversely affect on milk production, reduce breeding efficiency, reduce weight gains, decrease hair quality, reduce feed efficiency and negatively affect the immune system by decreasing the animal's ability to fight off other health problems. In this survey from 356 sheep, *O. aries* the fecal samples were collected from the different areas of Peshawar. The laboratory diagnoses of the samples were conducted in VRI, Peshawar. During the diagnoses 274 sheep were positive for different internal helminthes parasite and 82 were negative. In the present study 8 species of internal helminthes parasites were identified. During study 1 specie of cestodes with 1.68% of *Moniezia expansa* and 1 specie of trematodes with 0.56% *Fasciola hepatica* were identified. There are 6 species of nematodes were reported. Among nematodes with 53.37% of *Haemonchus contortus*, the high worm load was observed. The other nematodes *Trichostrongylus vitrinus* 12.92%, *Nematodirus spathiger* 1.68%, *Strongyloides papillosus* 3.93%, *Ostertagia sp.* 2.24% and *Trichuris ovis* 0.56% were also identified. It is concluded that there was low to moderate parasitic infestation were found in *O. aries*. Further research is required to prevent parasitic infestation in *O. aries*.

**IDENTIFICATION OF THE PREVALENT TICKS (IXODID) IN BUFFALOES, COWS,  
GOATS AND SHEEP IN PESHAWAR, PAKISTAN**

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A study to investigate hard tick (Ixodid) infestation in district Peshawar was carried out during March-July 2011. Ticks were identified for their genera level, in Parasitology Laboratory, Veterinary research Institute (VRI), Peshawar. Among ecto-parasites, ticks have been recognized as the notorious threat due to severe irritation, allergy and toxicosis and of diseases like babesiosis, theileriosis and anaplasmosis etc. The effect of age, status of body condition, post treatment effect of acaricides, housing and grazing systems on tick infestation was also recorded. Out of 710 farm animals studied, 310 cows, *Bos taurus* (Bojanus), 230 buffaloes, *Bubalus bubalis* (Linnaeus), 75 sheep, *Ovis aries* (Linnaeus) and 95 goats, *Capra hircus* (Linnaeus) were studied for tick infestation. In the present study, 5 genera of tick were identified which were (i) *Haemaphysalis* (Koch) (27.40%) (ii) *Rhipicephalus* (Koch) (21.92%) (iii) *Boophilus* (Curtice) (11.89%) (iv) *Amblyoma* (Koch) (10.02%) (v) *Ixodes* (Latreille) (7.35%). However, it is concluded that in cows, buffaloes, sheep and goats *Ixodes* have caused minimum infestation while *Haemaphysalis* have caused maximum infestation in cows and buffaloes compared with sheep and goats. Appropriate control measures for ticks need to be employed in the study area for economical animal production.

**INCIDENCE OF HELMINTHES PARASITES IN PHEASANTS OF DHUDIAL  
PHEASANTARY, MANSEHRA**

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In present survey during February to March, 2011. Presence of helminthes parasites in pheasant. Were study 34 representative faecal samples were collected from 34 cages of pheasant. All the samples were brought to the Parasitology Laboratory at Veterinary Research Institute, Peshawar. All the samples were processed and examined for helminthes ova under microscope. Among these samples from 34 cages of pheasants, 22 were found positive for different internal parasites and 12 negative). Out of 22 positive samples in the present study, 06 species of the helminthes parasites were identified through ova detection belonging to the phylum Platyhelminthes, class nematoda and cestoda. The ova of the helminth's parasites identified in the beats in all the six (6) types of pheasant's species were as follow. Phylum Platyhelminthes, class nematoda, and cestoda. Which are *Raillietina* (Cestoda), *Capillaria* (Nematoda), *Echinuria uncinata* (Nematoda), *Heterakis* (Nematoda) *Trichostrongylus tenuis* (Nematoda) *Hymenolepis setigera* (Cestoda). Among the identified species, nematodes were the most common and most important helminth species in the birds found, and the majority of which causes pathological damage to the host. Though a few species of the nematodes may occur in other parts of the body, the majority are found/live in the gastro intestinal tract.

**NEW NEMATODES SPECIES (RHABDOCHONIDAE) ISOLATE FROM PISCINE HOST  
IN PAKISTAN**

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Two new species of rhabdochonid nematodes (based on male specimens), *Rhabdochonaspinispiculum* n. sp. and *Rhabdochonabowispicula* n. sp. are described from the intestine of freshwater fish, *Tor putitora* (Hamilton, 1822) from upper Bolan river basin in western Pakistan. The former species is characterized largely by prostome, with 8 anterior teeth, two small, lateral, bifurcate deirids, the length ratio of muscular and glandular portions of esophagus ((1:1.64-1.71), postequatorial excretory pore, right spicule with conspicuous spine projecting outwardly at posterior tip, specular ratio (1: 5.22-5.84), arrangement of caudal papillae (8 preanal, 5 postanal), and by the shape of the tail tip (bluntly pointed). The later species is characterized mostly by a small body (2.99-3.4 mm long), 8 anterior prostomal teeth, the length ratio of muscular and glandular portions of esophagus (1:1.45-1.69), bifurcate deirids, postequatorial excretory pore, left spicule with prominent long spine at distal tip, ratio of spicules (1:4.8-4.97), arrangements of caudal papillae (10 preanal and 5 postanal), and by pointed tail with striations on dorsal side. The present new nematodes species distinguish from each other and also from all their congeners reported previously in differential diagnostic features stated above.

**SURVEY OF RICE FIELDS FOR PLANT PARASITIC NEMATODES IN AND AROUND  
DISTRICT SHEIKHUPURA**

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The presence and population of plant parasitic nematodes associated with rice cultivation was determined at the rice cultivation zone of Local Government Area of District Sheikhupura, Pakistan, through a survey. Samples of plant roots, soil and shoot were randomly collected for the presence of nematodes while extraction was conducted using Sieving and Modified Baermann Funnel technique. The data was recorded on the basis of relative density, inoculum density and nematode prevalence. The results showed the presence of six genera of nematodes: *Heterodera*, *Hirschmaniella*, *Meloidogyne* and *Pratylenchus* in the roots and soil and *Aphelenchoides* and *Ditylenchus* from shoots. There was a significant ( $P < 0.05$ ) difference in the distribution percentage of the different nematode population with *Hirschmaniella* recording a 70% distribution. The relative density and inoculum density of *Hirschmaniella* was recorded as 30% and 18% respectively. These results further demonstrate that the significant presence of *Hirschmaniella* in the studied area could pose a potential problem to rice cultivation.

**EFFECT OF RADIANT ON THE SECOND STAGE JUVENILE MORTALITY AND EGG HATCHING OF ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA***

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Efficacy of Radiant (bioproduct) in various combinations was assessed on the second stage juvenile mortality and egg hatching of root-knot nematode, *Meloidogyne incognita* under in vitro conditions. The concentrations of 1%, 0.75%, 0.50%, 0.25% of Radiant were tested at 24, 48, 72 hours time interval. Mortality of juveniles of *M. incognita* was affected by all concentrations and exposure time in all treatments. Radiant at 1% and 0.75% concentrations had more effect on mortality (50.0) after 72 hours from its 0.5% (48.2) and 0.25% (40.2) concentrations. Egg hatching of *M. incognita* was inversely proportional to the concentrations of Radiant at all time intervals. Egg hatching of *M. incognita* was significantly reduced in 1% (14.6), 0.75% (23.8), 0.5 % (31.0) and 0.25% (39.6) concentrations of Radiant after 72 hours of exposure as compared to control (49.00). Our findings suggest that Radiant have the ability to regulate nematode population and may serve as an alternative to nematicides.

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**SUSCEPTIBILITY OF TOMATO PLANTS AT DIFFERENT POPULATION DENSITIES OF *MELOIDOGYNE INCOGNITA***

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Nematode reproduction and plant growth variables were compared on susceptible tomato line round-41 at various densities of *Meloidogyne incognita* viz. 0, 250, 500, 1000, 1500 eggs. The results revealed that there was increasing damage potential to tomato plants by increasing inoculum level as it reduced the plant growth in terms of foliage length and total plant fresh weight. Nematode reproduction on tomato roots also varied as the inoculum level increased. The regression analysis revealed inverse relationship between root weight and foliage length by  $r = -0.99$  and between gall index and total fresh weight ( $r = 0.98$ ). Direct relationship was observed in case of root length and foliage length ( $r = 0.97$ ) and no of galls and egg masses ( $r = 0.99$ ).

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**COMBINED EFFECT OF PLANT GROWTH PROMOTING RHIZOBACTERIA AND ENTOMOPATHOGENIC NEMATODES FOR THE MANAGEMENT OF *M. INCOGNITA* IN EGGPLANT**

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Root knot nematodes have become a threat for vegetable production, reducing marketable yields both quality and quantity. The present study was conducted to determine the combine effect

of entomopathogenic nematodes, plant growth promoting rhizobacterium for the management of *Meloidogyne incognita*. The results revealed that *S. felti* was most effective in reducing no of galls, no of females, no of egg masses and reproduction factor in screening experiment. *Steinernema felti*, when applied with PGPR, gave minimum no of galls, no of females, no of egg masses and final population. It also gives maximum shoot, root length and shoot weight in combine effect experiment. From the findings of this investigation it may suggested that EPN and PGPR can be successfully used for the management of root knot nematodes.

#### REPRODUCTIVE POTENTIAL OF ENTOMOPATHOGENIC NEMATODES ON WAX MOTH *GALLERIA MELLONELLA*

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Entomopathogenic nematodes are successfully being used for the management of insect pests of many crops in world. Local species, *Heterorhynchus indica*, *H. bacteriophora*, *Steinernema feltiae*, *S. carpocapsae* and *S. krussae* species of entomopathogenic nematodes of both families Steinernematidae and Heterorhynchidae were assessed for their reproductive potential on wax moth in laboratory. All six species varied in their reproductive potential. Out of six species, the maximum reproductive potential was observed in *S. feltiae* and *H. bacteriophora* followed by *H. indica* whereas reproductive potential was less in *S. krussae* and *S. carpocapsae*. Reproductive mean ranges from 7614.9 to 978.3. Maximum nematodes were recovered from *S. feltiae* followed by local species, *H. bacteriophora*, *H. indica*, *S. carpocapsae* and *S. feltiae*.

#### NEMATODES ASSOCIATED WITH GUAVA (*PSIDIUM GUAJAVA* L.) IN KARACHI DISTRICT, SINDH

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Guava (*Psidium guajava* L.) is a common fruit of Sindh, Pakistan. It is primarily grown in three provinces of Pakistan namely Punjab, Sindh and Khyber Pakhtoonkhwa. Guavas are useful source of nicotinic acid, calcium, phosphorus and soluble fibres. It is a tropical tree that adopts itself to most soil conditions and climate. There are two seasons of guava fruit; winter and summer. In Pakistan its production has increased from 19,000 tons in 1958 to 552,000 tons in the year 2008, an annual growth rate of 6.9 percent. The major exports of guava from Pakistan are to U.A.E., U.K., Canada, Qatar and Saudi-Arabia. During the period from March 2011 to November 2011, an intensive survey of phytoparasitic nematodes associated with ten localities was conducted. A total of 120 composite soil and root samples were collected from a depth of 5-30 cm using a steel blade trowel, sealed in clean labeled plastic bags and directly transplanted to the laboratory. Prior to nematode extraction, each soil sample of 250 cm<sup>3</sup> was processed for extraction of soil nematodes by sieving through 100, 200, 300  $\mu$ m screens respectively, followed by centrifugal flotation technique (Jenkins, 1964). Extracted nematodes were examined under a stereoscopic microscope

and identified to the genus. The most predominant nematode species were *Meloidogyne* Goeldi, 1892 spp.; *Pratylenchus* Filipjev, 1936 sp. and *Rotylenchulus* Linford and Oliveira, 1940 sp.

**PARAMONOSTOMUM BILQEESAE N.SP. (TREMATODA: NOTOCOTYLIDAE) FROM COMMON TEAL (*ANAS CRECCA* L.) IN SINDH**

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A new trematode, *Paramonostomum bilqeesae* recovered from the bird common teal, *Anas crecca* L. is described here. The present species differs from *P. macrovesiculum* in shape of body and cirrus sac, in the absence of pharynx, shape of the ovary and in having a different host. The differences in the diagnostic features of the present and previously described species of the genus *Paramonostomum* substantiate the statement that the specimens under study are new to science and named as *Paramonostomum bilqeesae*. This genus is being reported for the second time from Sindh, Pakistan.

**CATHUCOTYLE ARABIANSIS N.SP. (MONOGENEA: GOTOCOTYLIDAE LEBEDEV, 1984) FROM FISH *SCOMBEROMORUS GUTTATUS* OF KARACHI COAST, PAKISTAN**

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*Cathucotyle arabiansis* a new species is described from the gills of fish *Scomberomorus guttatus* of Karachi coast, Pakistan. The present specimens are compared with all existing species of the genus, which are different in several diagnostic characteristics, such as, aseptate oral sucker. Pharynx diverticulate, sub-spherical. Testes 120-130 in number. Male copulatory organ armed with spines ensheated by thick-walled pouch. Single rounded vagina, horseshoe-shaped ovary, clamps consisting of marginal sclerites, 3 pairs of connecting and median sclerites.

**INTERACTION OF *MELOIDOGYNE INCOGNITA* AND *FUSARIUM OXYSPORUM* F.SP *LYCOPERSICAE* FOR TOMATO WILT**

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Tomato is an important horticultural crop of Pakistan. Its production is threatened by number of biotic and abiotic factors. Among these *Fusarium* wilt is an important yield limiting factor. Effect of *Meloidogyne incognita* and *Fusarium oxysporum* f.sp *lycopersicae* were studied in pot and field trials alone or in combination with each other. Data were recorded on root and shoot weight, shoot length, root length, number of leaves, galling index, egg masses, number of females

per 1g of root system. In pot maximum number of galls were observed in roots of tomato treated with *M. incognita* alone followed by the combined treatment of *M. incognita* and *F. oxysporum* f.sp *lycopersicae*. Growth parameters were adversely affected by the combine treatment of *M. incognita* and *F. oxysporum* f.sp *lycopersicae* in terms of root and shoot parameters. Wilting of the most of the plants occurred after 17 days similar results were also observed in microplots as mortality of the plants were maximum in *M. incognita* and *F. oxysporum* f.sp *lycopersicae* treated plants as compared to alone treatments. This study will be helpful to assess the interaction of *M. incognita* and *F. oxysporum* f.sp *lycopersicae*.

#### **LOBULAR DEGENERATION AND ATROPHY IN LIVER OF GOAT WITH FASCIOLIASIS**

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Study of tissue damage in liver of goat with fascioliasis indicated severe tissue alteration leading to degeneration of parenchymatous constituent and increase in its connective tissue. Lobulation of live is disturbed and its degeneration and atrophy occurred. Lobules lost the normal architecture and hexagonal shape become elongated linear mass with dilatation of sinusoids. This conditions are reported here in chronic case of fascioliasis in goat.

#### **DIVERSITY OF HELMINTHS PARASITIC FAUNA IN RAT AND MICE PESTS OF AGRICULTURAL AND ZONOTIC IMPORTANCE IN DISTRICT SWAT, PAKISTAN**

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During investigation on intestinal helminth of rodents' pests of agricultural and zoonotic importance (July 2011 to October 2011) a total of 110 rats (*Rattus rattus*) and 29 mice (*Mus musculus*) were collected from different crop fields from higher and lower altitudes of upper Swat, K.P.K, Pakistan. The voucher specimens were preserved in 5% formalin while most of the rats were dissected and the viscera were preserved in 2% formalin. The preserved specimens were investigated for helminth infections. Of the collected specimens 60 rats and 15 mice were examined. 27(45%) rats and 10 (66.6%) mice were found to be infected with one or more than one type of helminths infection. Nematodes, cestodes and trematodes were found. 14 (42.2%), 8 (24.2%), 5 (15.1%) and 5 (50%) 4(40%), 1 (10%) nematodes, cestodes and trematodes were found in rats and mice respectively. It was concluded that rats and mice are the potential reservoirs of helminth parasites, zoologically important and threat to farmers health, working in the crop fields.

**STYLET BEARING NEMATODES AND BACTERIA ASSOCIATED WITH JAMUN  
(*SYZGIUM CUMINI* L.) IN LOWER SINDH, PAKISTAN**

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Jamun (*Syzgium cumini* L.) is a very common, large, evergreen beautiful tree of Indian subcontinent. The fruit appears in May - June. Jamun is useful in many ways; its fruit serves as fodder. for cattle especially during drought. U mipe is used to make vinegar. The seed is rich in protein and carbohydrates. It contains traces of calcium. The juice is criminating, diuretic and has soothing effect on human digestive system. Jamun juice is also used for preparing beverages. Diabetic patients can take Jamun fruit regularly during season of its availability. A total of seventy-two soil and root samples were collected from lower Sindh to access the major nematodes and bacteria associated with Jamun. The most prevalent nematodes were *Filenchus* (Andrassy, 1954) Meyl, 1961 species and *Helicotylenchus* Steiner, 1941 species, while the most prevalent bacteria were *Pseudomonas* spp. and *Bacillus* spp.

**A NEW TREMATODE OF GEGUS *MEHRAORCHIS* SRIVASTAVA, 1934  
(TREMATODA; LECITHODENDRIIDAE (LUHE, 1901) ODHNER, 1910) PARASITE OF  
FROG *RANA TIGRINAIN* KARACHI**

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An undescribable species of genus *Mehraorchis* (Srivastava, 1934) as *M. mujibi* recovered from the intestine of *Rana tigrina*. Only a single species of this genus reported previously as *M. ranarum* (Srivastava, 1934) recorded by Bhutta and Khan, 1975 from *R. Ilgrina* in Lahore. The same species is also recorded by Bilqeess and Kaikabad, 1976 from *R. cyanophlyclis* in Karachi. Description of *M. mujibl* based upon globular body shape with narrower anterior region. Oral sucker large and terminal. Pharynx well-developed followed by esophagus which is of moderate length. Caeca wide reaching to posterior region.

**A NEW SPECIES *PLEUROGENOIDES BISEMINALIS* (TREMATODA:  
LECITHODENDRIIDAE (LUHE, 1901) ODHNER, 1910) FROM *RANA TIGRINA*  
KARACHI.**

NASIRA KHATON,<sup>1</sup> A.G. RIZWANA,<sup>1</sup> F.M. BILQEES<sup>1</sup> AND ASMATULLAH KAKAR<sup>2</sup>

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During the period of study, a new species of the genus *Pleurogenoides* (Travassos, 1911) as *P. biseminalis* . It is recovered from the intestine of *Rana tigrina* which is different from the

previously described species. It is characterized by its body size. Oral sucker is large and tenninal while ventral sucker is small, postovarian and posterior to cirms pouch. Pharynx well developed and esophagus short caeca broad, preacetabular in mid body region. Only one species of the same genus is reported from Pakistan as *P. gastroporus* (Lube, 1901) by Bhutta and Khan, 1975 from the intestine of *Rana tigrina*.

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**LOXOGENE MAGNAPHARYNX.SP. (TREMATODA: LECITHODENDRIIDAE (LUBE, 1901) ODHNER, 1910) FROM RANA TIGRINA IN KARACHI**

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A new species *Loxogene magnapharynx* belonging to family Lecithodendriidae is reported from the intestine of frog *Rana ligri*!!!. Only one report of this genus *Loxogene sindhensis* (Bilqeess and Khan, 2003) from *Rana cyanophlyctis* in Karachi published from Pakistan. Now it is the second report of the same genus which is characterized by the presence of sub-globular, aspinulate body with somewhat equal anterior and posterior region. Oral sucker terminal whereas ventral sucker intracaecal, pretesticular and postovarian in position. Pharynx well-developed followed by short esophagus. Testes tWo rounded and equal in size. Cirrus pouch thin-walled. Ovary is also rounded. Uterine coils in hind body region extending to fore body in a form of profuse mass.

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**CUCULLANUS PSEUDOANNULLATUS REHANA AND BILQEES, 1976: SCANNING ELECTRON MICROSCOPIC OBSERVATIONS.**

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Rehana and Bilqeess, 1986 redescribed *Cucullanus annulatus* (Margolis, 1960) Rehana and Bilqeess, 1976 as *C. pseudoannulatus* from the same fish that is *Mystus caucasius* of Kalri lake, Sindh, Pakistan due to number of specific differences. In order to observe more details, scanning electron microscopic study of some male specimens was carried out. Specimens for S.E.M. were prepared according to the standard procedures through the curtesy qf Centralized Science Lab6raotry, University of Karachi, Karachi University Campus, Karachi. The anterior extremity is rather straight instead of being bent dorsally. The mouth is bounded, by two large lateral lips, the mouth~ opening is slit like, inside the mouth is a row of. several pointed teeth like structures, these teeth appear to be quite strong which indicates a firm grip of the parasite to the host intestine for sucking the nutrients. There are two p9irs of lateral internal smaller mouth papillae and the outer surface is provided with three comparatively larger head papillae. The caudal region is curved ventrally. Spicules are unequal in size, strongly alate and roughly pointed. Pre anal caudal papillae are three pairs quite larger in size, while the post anal papillae are quite numerous and smaller in size. The tail tip is pointed, it appears that the anal region is covered with numerous scale -like structures, not reported earlier.

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***CENTRORHYNCHUS SCANENSE* LUNDSTROM, 1942 (ACANTHOCEPHALA:  
CENTRORHYNCHINAE) IN INDIAN LITTLE BUTTON QUAIL FROM KARACHI,  
SINDH, PAKISTAN**

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In a survey of Acanthocephala from birds of Sindh, Pakistan six female worms were collected from intestine of five Indian little button quail (*Turnix sylvatica* Desfontaines) from Karachi, Sindh, Pakistan. On detail study the worms were identified as *Centrorhynchus scanense* Lundstrom, 1942. This species is being reported for the first time from Pakistan. The female worms had elongate body measuring 10.4-10.6 by 2.04-2.12 millimeter. Proboscis measuring 0.72-0.73 by 0.54-0.55, divided into 2 parts by constriction at the level of receptacle attachment. Proboscis armature consists of 20-22 longitudinal rows having 8-12 hooks each. Hooks rows 16-17 in the anterior half and 4-5 in the posterior half. The hooks in posterior half are rootless, the hooks of the anterior half 0.038-0.039 by 0.11, while those of posterior half measure 0.0034 by 0.10. Neck short and cylindrical. Proboscis receptacle somewhat cylindrical double walled measuring 0.90 by 0.52-0.54. Lemnisci longer than proboscis receptacle, the left measuring 0.25-0.76 by 0.072-0.075; and the right measuring 0.72-0.74 by 0.073-0.075. Eggs oval measuring 0.078 0.079 by 0.021-0.022. Genital pore subterminal. This is the seventh species of the genus *Centrorhynchus* Lihé, 1911 reported from Pakistan, the earlier recorded species are viz. *C. fasciatum* (Westrumb, 1821) Bilqees and Khan, 2005; *C. bilqeesae* Ghazi *et al.*, 2005; *C. gibsoni* Khan *et al.*, 2002; *C. amini* Khan *et al.*, 2010; *C. nickoli*; Khan *et al.*, 2001 and *C. sindhensis* Khan *et al.*, 2002.

***POLYMORPHUS FATIMAAEKHAN ET AL. 2008 (ACANTHOCEPHALA:  
POLYMORPHIDAE) IN KITE (ELANUS CAERULENS DESFONTAINES FROM  
THATTA, SINDH, PAKISTAN***

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Acanthocephalan parasites of birds have little been studied in Pakistan. During a survey of Acanthocephala of birds of Sindh, two female and a single male specimen were recovered from the intestine of a kite (*Elanus caeruleus* . Its fontaines collected from Thatta, Sindh, Pakistan. The worms were fixed in F .A.A. (a solution of formalin, acetic acid and 50 percent alcohol in the ratio of 5:3:92) and stained in Mayer's carmalum and mounted permanently in Canada balsam. On detail examination of the specimens it was revealed that they belonged to a known species of genus *Polymorphus* Lihé, 1911 namely *P. fatimae* Khan *et al.*, 2008 recorded from crow (*Corvus splendens* Vieillot) from Hyderabad, Sindh, Pakistan. Minute differences were observed in the length of the neck and lemnisci. The present species is being recorded from a different host and locality.

## SECTION - V

### FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY, MARINE BIOLOGY

#### POPULATION ESTIMATION AND DISTRIBUTION OF WHITE CRESTED KALIJ PHEASANT (*LOPHURA LEUCOMELANOS HAMILTONII*) IN GARHI DUPATTA MUZAFFARABAD, AZAD JAMMU AND KASHMIR, PAKISTAN

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A study was conducted in Ghari Dopatta and its surrounding areas to investigate the distribution pattern and population status of Kalij pheasant during April to December 2010. For this purpose, call count and line transect methods were used. A total population of 123 birds was estimated in 14 km<sup>2</sup> of total area surveyed with the highest population density (14.29 birds/km<sup>2</sup>) recorded at locality Kandlaan and the lowest population density at Baandi (5.38 birds/km<sup>2</sup>). The overall population density was recorded as 8.73 birds/km<sup>2</sup>. Highest estimated population density (9.76 birds/km<sup>2</sup>) was noted at the elevation ranges of 1236-1555 m, while minimum (6.43 birds/km<sup>2</sup>) was recorded at 1865-2180 m altitudinal range. The dominant vegetation of the study area was composed of *Pinus wallachiana*, *Pinus roxburghii*, *Viburnum foetene*, *Berberis lyceum* and *Plectranthus rugosus*. Forest damage, hunting, poaching and over grazing by livestock were major threats to conservation of Kalij pheasant in the area. Result would be helpful to identify the potential habitat of Kalij pheasant and provided a base line data for the further scientific research on this species.

#### POPULATION AND DIET OF MIGRATORY COMMON STARLING (*STERNUS VULGARIS*) IN DISTRICT SIALKOT, PAKISTAN (2010-11)

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Common starling (*Sturnus vulgaris*) is a winter visitor to different areas of Pakistan, however, information is lacking about its winter activity in the country. The current study investigated its arrival and departure dates, population and diet composition in district Sialkot. Results showed that the species arrived in the study area during first week of November 2010 while its departure was recorded in the last week of February 2011. Its average population density in study area was 26.29 birds per km<sup>2</sup> whereas its population was variable in different study sites and during different months, with highest population in January 2011 (39 birds/ km<sup>2</sup>). Sizes of feeding flocks rarely exceeded 50 birds while size of roosting flock was up to 500 birds per flock. Foraging

observations in the field showed that it mainly consumed wheat, lentil, *Trifolium* and *Brassica* crops, among vegetables, pea and potato fields were visited more in irrigated form than non-irrigated ones. The bird species was also found to visit non-cultivated fields, rice mills and garbage sites. For night time roosting, common starling selected dense leafy vegetation like dense patches of bamboos, sugarcane crop fields, and dense patches of *Eucalyptus* in the area. During November (2010), December (2010) and early January (2011), common starlings were observed to feed on wheat sprouting and lentil grains and also found to catch insects from these crops. Analysis of gizzard contents showed invertebrates, on average, 41.68% while seeds and other plant matter collectively constituted 58.03%. Among invertebrates were mostly insects including coleopterans, hymenopterans and members of hemiptera. Seeds and other plant matter consisted of freshly plucked seedlings or sprouting of wheat crop and partially as well as completely undigested wheat, lentil and rice grains.

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**DISTRIBUTION PATTERN AND GENERAL HABITAT ASSESSMENT OF  
HERPETOFAUNA IN PIR LASORHA NATIONAL PARK AND SURROUNDING AREAS  
OF DISTRICT KOTLI, AZAD JAMMU AND KASHMIR (PAKISTAN)**

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A study was conducted to explore the distribution and general habitat assessment of herpetofauna in the Pir Lasorha National Park (PLNP) and surrounding areas of District Kotli (Azad Jammu & Kashmir) from June to September 2010. Visual encounter method was used to identify and collect the specimens. A total of 13 herpetofaunal species were recorded belonging to two orders (Squamata and Anura) and six families. Order Squamata was represented by 5 families; Elapidae (*Bungarus caerulus*), Colubridae (*Boiga trigonata*, *Amphiesma stotatum*, *Amphiesma platyceps* and *Ptyas mucosus*), Varanidae (*Varanus bengalensis*), Agamidae (*Agama aculeate*, *Laudakia agorensis* and *Calotes versicolor*) and Gekkonidae (*Hemidactylus flaviviridis* and *Indogecko rohtasfortai*). Order Anura was represented by Bufonidae family having two species (*Bufo stomaticus* and *Bufo melanostictus*). *Boiga trigonata* was found in thick forest, plantations and grasslands with thick bushes. *Bungarus caerulus* was found in marginal vegetation along rice fields in holes and cervices in ground. *Amphiesma stotatum* was common in low land and tropical forests. *Amphiesma platyceps* was common in flowing water and stream while *Ptyas mucosus* was found in grasslands, cultivated fields, gardens and forests. Poisonous snake of the study area was *Bungarus caerulus*. *Bufo stomaticus* and *Bufo melano stictus* were nocturnal and found in holes and cervices among stones near water. *Varanus bengalensis* was common in cultivated areas and barren badlands. *Laudakia agorensis* was found in cervices among stones and rocks. *Hemidactylus flaviviridis*, *Calotes versicolor* were nocturnal and found in gardens and bushes. *Indogecko rohtasfortai* was found on trees and shrubs. *Agama aculeate* was found inside the thatch of huts in villages. The terrestrial and aquatic ecosystem of the study area was favoring the species diversity. The increasing of human impact on the existing natural resources in the study area, has threatened the diversity of wildlife species, and the population of some herpetofaunal species (especially frog species) has declined.

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**POPULATION AND FORAGING BEHAVIOR OF MIGRATORY DUCKS AT KHABEKI LAKE, DISTRICT KHUSHAB**

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Wetlands of Pakistan host millions of migratory birds annually. Out of world's seven migratory birds' flyways, Pakistan is situated along Indus Flyway (Green Route or Fly way # 4). A number of country's important wetlands are located along this flyway including Khabeki Lake. It is a part of Uchali Wetlands Complex, a Ramsar site of Pakistan. It is located in the north central Punjab. It is the second largest lake in the salt range with little marsh vegetation and completely surrounded by agricultural land. The objectives of present study were estimation of population of migratory birds at Khabeki Lake, comparison of richness and abundance of migratory birds with previous available data and observation of foraging behavior of migratory birds at Khabeki Lake. The lake was visited twice a month during the study period. Surveys were under taken during morning and evening hours. Total count of the bird species were undertaken from a vantage point at selected sampling units of the study site. Number of individuals (n) of the birds were counted thrice and then their average was calculated and recorded during each survey. Secondary information was collected from locals and published literature. Few important indices of richness and abundance such as Shanon-Weiner diversity index, similarity index and census index were calculated.

**STATUS AND DISTRIBUTION OF GALIFORMS IN AZAD JAMMU AND KASHMIR**

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A study was conducted to gather information about the distribution, population, presence and habitat utilization of galliforms of Azad Jammu and Kashmir during 2006-2010. Data were collected through field surveys using flushing and count call methods as well as from secondary sources *i.e.*, records of wildlife department. The diversity index was calculated using Shannon-Wiener index. Results revealed that Azad Jammu and Kashmir harbors fourteen species of galliforms with diversity index of  $H' = 2.1862$ . A total of which 9 species were common in their restricted habitat including *Lophora leucomelanos* ( $p_i = 0.317$ ), *Alectoris chukar* ( $p_i = 0.125$ ), *Pavo cristatus* ( $p_i = 0.125$ ), *Lerwa lerwa* ( $p_i = 0.094$ ), *Pucrasia macrolopha* ( $p_i = 0.077$ ), *Tetraogallus himalayensis* ( $p_i = 0.062$ ), *Francolinus francolinus* ( $p_i = 0.16$ ), *Gallus gallus* ( $p_i = 0.007$ ), and *Coturnix Coturnix* ( $p_i = 0.033$ ). Three species were noted as rare including *Coturnix coromandelica* ( $p_i = 0.034$ ), *Francolinus pondicerianus* ( $p_i = 0.014$ ) and *Lophophorus impejanus* ( $p_i = 0.046$ ) while two species were recorded as vulnerable including *Catreus wallichii* ( $p_i = 0.036$ ) and *Tragopan melanocephalus* ( $p_i = 0.016$ ). *Alectoris Chukar* was the most widespread bird distributed from the high altitude (1300-16500 ft) in northern most reaches of Neelum Valley, though *Gallus gallus* and *Pavo cristatus* having limited distribution and were recorded only in Deva Vatala National Park. The destruction of habitat (through overgrazing, grass cutting, lopping, erosion, fuel wood

collection, timber harvesting, medicinal plant collection, mushroom collection, landslides and encroachment) hunting and unawareness about the economic and ecological importance of species in the area are the major threats to the existing galliforms.

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**RANGING BEHAVIOR OF HIMALAYAN GREY LANGUR (*SEMNOPITHECUS AJAX*)  
IN MACHIARA NATIONAL PARK, AZAD JAMMU AND KASHMIR, PAKISTAN**

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Home range and daily travelling behavior of Himalayan grey langur (*Semnopithecus ajax*) was studied in Machiara National Park (34°-31 N and 73°-37 E), Azad Jammu and Kashmir (Pakistan). For this study, three focal groups, including one uni-male bisexual troop (UMBS), one multi-male bisexual troop (MMBS) and one all-male band (AMB) were selected and observed during all seasons of the year 2007. Area covered (home range) and daily traveled distance by each focal group was recorded using Global Positioning System (GPS) and analyzed in ArcGIS 9.2. The GIS analyses indicated that, the average home range size of UMBS and MMBS was 2.354(±0.923) km<sup>2</sup> (235.4 ha) and 3.275 (±0.554) km<sup>2</sup> (327.5 ha) respectively, while, the average home range of AMB was 3.519 (±0.996) km<sup>2</sup> (351.9 ha). Similarly, the average daily path length of UMBS, MMBS and AMB was recorded as 1.228 (±0.276), 175.3 (±0.342) and 1.839 (±0.696) kilometers, respectively. A numbers of factors, including troop size, troop composition, food availability, weather conditions, and human interference, were found affecting home range and daily travelling of langur. Larger troops were using larger home ranges and vice versa. Food availability (numbers of consumable plant species) was negatively correlated with the home range in UMBS ( $r = -0.8125$ ,  $p = 0.0013$ ) and AMB ( $r = -0.7034$ ,  $p = 0.0107$ ). However, the home range of MMBS troop was not significantly correlated with food availability ( $r = -0.2683$ ,  $p = 0.3991$ ). The percentage time spent on food consumption was not affecting the home range; however, it was negatively correlated with the daily travelling length. Seasonality and weather conditions also affected the ranging behaviour; usually larger home ranges were occupied in winter season than summer. Human disturbance, altitudinal movements and home range fluctuations were also observed during different months. It was concluded that, AMB utilized larger home range than bisexual troops and several environmental factors were influencing the ranging behavior of Himalayan langur.

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**STUDY OF ICHTHYO FAUNA OF RIVER POONCH**

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Present study was conducted to analyze the overall status of ichthyo-fauna from different localities of River Poonch (freshwater reservoirs). River Poonch is the reservoir of many

endangered and threatened fish species of the world. Sixteen different species of fish were caught from different nullahas which are the tributaries of river Poonch. Most of the captured fishes are indigenous fishes namely; *Aspidoparia mora*; *Barilius pakistanicus*; *Schistura alepidota*; *Labeo dyocheilus pakistanicus*; *Mastacembelus armatus*; *Puntius ticto*; *Schistura nalbanti*; *Puntius sophore*; *Tor putitora*; *Glyptothorax pectinopterus*; *Glyptothorax plagiostomus*; *Glyptothorax stock*; *Crossocheilus latius*; *Ompok pabda*; *Garra gotyla* and *Botia lohachata*. Statistically values for richness of river Poonch was significantly high. Keeping in view the importance of river Poonch, its fauna and last resort for endangered fishes like mahseer, the government of AJK on advice of the Department of wildlife & fisheries, has declared “River Poonch Mahseer National Park”, the country’s first ever aquatic national park.

**DISTRIBUTION AND STATUS OF CHEER PHEASANT (*CATREUS WALLICHII*) IN AZAD JAMMU AND KASHMIR, PAKISTAN**

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Cheer pheasant (*Catreus wallichii*) or “Riyar” was distributed in Hazara District NWFP, Rawalpindi District Punjab and some parts of Azad Jammu and Kashmir, now it has been extirpated from most of its range in Pakistan; however, it is still surviving in some isolated pockets in Azad Kashmir. Three major sites, where cheer pheasant has been recently surveyed, are Pir-Chinase Range (Niazpura, Lowassi) district Muzaffarabad, Qazi Nag Game Reserve in Jhelum Valley, district Hattian and Phalla Game Reserve in Bagh District. A reconnaissance survey was conducted in March to May 2011 to determine the potential of these sites as a possible focal point to start cheers Pheasant Conservation activities. A total of 579 birds have been estimated during this study in AJ&K, distributed in 28 sub-sites. Highest population (n=434) was recorded in Qazi Nag, followed by Phalla (117) and Pir Chinase (28) Game Reserves. Habitat deteriorating factors include anthropogenic activities like fuel wood collection, agricultural activities and over grazing were observed in the study area. Study revealed that the Azad Jammu and Kashmir is the potential habitat and best site for conservation of the cheer pheasant.

**POPULATION DYNAMICS AND HABITAT UTILIZATION OF WESTERN HORNED TRAGOPAN (*TRAGOPAN MELANOCEPHALUS*) IN MACHIARA NATIONAL PARK, MUZAFFARABAD AZAD JAMMU AND KASHMIR, PAKISTAN**

MIR MUHAMMAD SALEEM<sup>1</sup>, MUHAMMAD SIDDIQUE AWAN<sup>1</sup>, USMAN ALI<sup>2</sup>, RIAZ AZIZ MINHAS<sup>1</sup>

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Western Horned Tragopan (*Tragopan melanocephalus*) is a threatened species distributed in Machiara National Park, Azad Jammu and Kashmir. Current study aimed to investigate the population dynamics and habitat utilization of Western Horned Tragopan in Machiara National Park. The study was conducted from December 2009 to November 2010. The study area was

divided into three main zones with 9 localities. Using call count method, a total of 61 birds were estimated in Machiara National Park with the highest population density (10.67 birds/km<sup>2</sup>) recorded at locality Raveri followed by Kuldaber (7.81 birds/km<sup>2</sup>), Roshan Wala Nulla (6.93 birds/km<sup>2</sup>) and Kuthiali (6.89 birds/km<sup>2</sup>) while minimum (6.03 birds/km<sup>2</sup>) population density was noted at Mali locality. Habitat was analyzed using quadrat method and importance value was calculated of each plant species and compared with the population density of Western Horned Tragopan. The dominant plant species were *Pinus wallichiana*, *Quercus dilatata* and *Abies pindrow*, among trees, *Viburnum grandiflorum* and *Rosa lavigata* among shrubs and *Dryopteris remosa*, *Skimmia laureola*, *Adiantum incisum*, *Phytolacca lethenia*, *Bergenia ciliata*, *Viola biflora* and *Taraxcum officinale* among herbs in the study area. Population density varied with changing altitudinal range, highest population density (2.17 birds/km<sup>2</sup>) was noted at the elevation range of 2586-2790 m followed by 1.6 birds/km<sup>2</sup> (3195-3396 m) and 0.90 bird/km<sup>2</sup> (2790-2992 m), whereas, minimum population density (0.89 birds/km<sup>2</sup>) was recorded at the altitudinal range of 2992-3195 m. Forest cutting, livestock grazing, fodder collection and encroachment were the major factors contributed toward the habitat degradation of Western Horned Tragopan and thus are threat to its survival. These factors should be controlled by improving law enforcement and generating awareness among local people to conserve this threatened species.

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**STUDY OF ETHNO-CARNIVORE RELATIONSHIP IN DHIRKOT, DISTRICT BAGH,  
AZAD JAMMU AND KASHMIR (PAKISTAN)**

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Human-carnivore conflict is the major issues for the carnivore conservation in Azad Jammu and Kashmir. Due to the habitat degradation of the wildlife, the natural prey of carnivore species is declined resulting into the increased depredation of livestock which in turn causes the human-carnivore conflicts. This paper presents the results of the study of the human-carnivore relationship in Tehsil Dhirkot, Azad Jammu and Kashmir, Pakistan during 2009-2010. Study was aimed to explore the extent of depredation (injuries and deaths) of livestock and human beings by different carnivore species, estimation of the economic loss of the local villagers, perceptions of the locals and retaliatory killings of carnivores during the last three years. A sum total of 150 affectees were interviewed using structured questionnaires and discussions were made with the local community during 40 surveys conducted in 20 villages of eight union councils of the study area. Three main carnivore species were found to be responsible for livestock killing and human injury. Common Leopard was responsible for the majority (70.8%) of such livestock killing; mainly goats (20%) and donkeys (18%). Jackals were responsible for about 80% of the poultry killings. These livestock depredation by carnivore species resulted into a financial loss of about US\$ 23529.412 during 2009-2010. About four persons were also injured during the leopard attacks at Surang, Hill, Munhasa and Sohawa. In response, four leopards were killed by the local community at Ghaziabad, Narakot, Surang and Rangla. Most (85%) of the people dislike predators and about 90% respondents recommended that predators should be conserved in zoos and wildlife parks while 10% respondents were in favor of predator conservation in natural habitat. The study revealed that leopards along with other carnivore species are widely involved in depredation of livestock and

have been heavily persecuted by the rural communities. This issue is the major threat to the conservation of large carnivore species in the area.

**BAT'S ROOSTING SITES: A PRELIMINARY ASSESSMENT FROM TWO VILLAGES (SARD CHEENA & YAQOOBI), DISTRICT SWABI, KHYBER PUKHTUNKHAWA, PAKISTAN**

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The bat fauna of the District Swabi with special focus on two villages (Sard Cheena & Yaqoobi) Khyber Pukhtunkhawa (KPK) is poorly known. The present study was designed to investigate the roosting sites and the population of bats in the buildings of urban area. The area was divided into three habitat types 1) old/historical buildings, 2) new buildings, and 3) farm houses. Survey was done at dawn and dusk. Bats were captured with the hand net and identified. A total of 55 buildings were reported to contain the active bat colonies. Two species of bats; Greater yellow bat (*Scotophilus heathii*) and Lesser mouse-eared bat (*Myotis blythii*) were common in the two villages of district Swabi. Old buildings were found to be more favorite places for bat roosting as compared to new buildings and farm houses. In conclusion, the constructions of the old/historical buildings were seen to contain spaces/crevices inside the roof and walls, which act as bats roosting sites and also provide shelter from predators, but the population of bats in the farm houses is 2<sup>nd</sup> high due to the presence of insects as food source.

**DISTRIBUTION AND ROOSTING HABITS OF SOME MICROBATS AFFECTING THE ANTHROPOGENIC SUBHABITATS IN RAWALPINDI DISTRICT**

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This study was aimed at knowing the microchiropteran fauna of urban, suburban and rural human settlements of Rawalpindi district. The study extended from March 2010 to November 2010. A total of 50 specimens of bats was collected with hand nets; 26 specimens belonged to *Scotophilus heathii*, 15 to *Pipistrellus pipistrellus*, five to *Pipistrellus javanicus*, three to *Pipistrellus tenuis* and one to *Rhinolophus lepidus*. Of the 50 bats, 29(58%) specimens were captured from urban localities, 14(28%) from sub-urban areas and seven (14%) from rural areas. The roosting sites are important components of ecological niches of bats. Five (10%) specimens were taken from cracks in the structure of a nullah bridge. Of the remaining 45 specimens, 35(70%) were taken from crevices in buildings, 9(18%) from cavities in trees and a lone (2%) specimen of *Scotophilus heathii* was taken from a fissure in a rock near Rawal Lake. Excepting *R. lepidus*, the microbats of this study seem to have adapted to use roosting sites in and around human settlements as their natural habitat succumbed to anthropogenic alterations.

**EVALUATION OF *tris*-CITRIC EGG YOLK, SKIM MILK AND EGG YOLK-CITRATE EXTENDERS FOR THE LIQUID STORAGE OF URIAL (*OVIS VIGNEI PUNJABIENSIS*) SPERMATOZOA**

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Artificial insemination is used to ensure long term survival of wildlife species and restoration of genetic variability in small wild and captive populations. The intensity of cold shock damage to spermatozoa is species specific and largely affected by the type of semen extender during storage. Therefore, the present study was designed to compare *tris*-citric egg yolk (TCY), skim milk (SKM) and egg yolk-citrate (EYC) extenders for the liquid storage of urial spermatozoa at 5°C. Three adult urial rams (*Ovis vignei punjabiensis*) of similar age group kept at PMAS Arid Agriculture University, Rawalpindi Semen were used in the study. Semen was collected with electric ejaculator in falcon tube. Semen ejaculates were split into three aliquots, diluted with extender (37°C), cooled from 37°C to 5°C in 2 hours and stored for three days at 5°C. Sperm motility (%; visually), live/dead ratio and sperm viability (%; with dual staining test using Trypan-blue Giemsa), functional and structural plasma membrane integrity (%; with supravital hypo-osmotic swelling test) were studied at 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> day of storage. Sperm motility, live/dead ratio, sperm viability and structural plasma membrane integrity was similar (P>0.05) in all experimental extenders at 1<sup>st</sup> day of storage. At 2<sup>nd</sup> day of storage, sperm motility, live/dead ratio, sperm viability and structural plasma membrane integrity remained higher (P<0.05) in TCY extender compared to EYC extender. However, the values of aforementioned parameters remained higher (P<0.05) in TCY extender compared to SKM and EYC extenders at 3<sup>rd</sup> day of storage. In conclusion, *tris*-citric egg yolk extender is more capable than skim milk and egg yolk-citrate extenders for liquid storage of urial spermatozoa at 5°C for three days.

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#### **STRUCTURAL FEATURES OF THE BACULA OF SOME MICROCHIROPTERAN BATS OF POTHWAR REGION**

AMBER KHALID, AMJAD RASHID KAYANI, MUHAMMAD SAJID NADEEM,  
MUHAMMAD MUSHTAQ, RABIA ZAMAN AND NAEEM AHKTAR.

Baculum is a bone found in the phallus of many species of mammals. The structure of baculum varies from species to species. This study provides information on the morphology of the baculum of five species of microchiropteran bats, viz., *Pipistrellus pipistrellus*, *Pipistrellus tenuis*, *Pipistrellus ceylonicus*, *Pipistrellus javanicus* and *Scotophilus heathi*, taken from Pothwar. Statistical analysis revealed that three of the five morphological features of the bacula were significantly different in the bats of this study; the three features were the basal width, distance between the two distal ends of the fork, and the total length of the body.

#### **THE CRANIAL MORPHOMETRY OF DIFFERENT MICROCHIROPTERAN BATS OF POTHWAR REGION**

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Bats are flying mammals that belong to Order chiroptera which is divided into two suborders Megachroptera (mainly Frugivorous) and Microchroptera (predominately insectivorous). Worldwide approximately 1100 species are reported. Pakistan bat fauna is diverse representing 46 genera, 8 families with 50 species of which 46 are microbats. The present study had been designed to use cranial morphometry in assessing the variation in microchiropteran bats species and to determine the character of taxonomic importance. From study area, Pothwar plateau, 8 species of microbats were captured belonged to 4 families and 5 genera. Species named *Pipistrellus pipistrellus*, *Pipistrellus tenuis*, *Pipistrellus ceylonicus*, *Pipistrellus javanicus*,

*Rinopoma microphyllum*, *Scotophilus heathi*, *Taphozous nudiventris* and *Megaderma lyra*. Non significant difference was found between male and female individuals of all species, significant difference was found in all cranial measurements among all species, even among the species belonging to same genus *Pipistrellus*. The data obtained and analyzed in this study provides base line information about cranium of microbats of Pothwar region. The comparison of different cranial measurements and character of taxonomic importance will help to identify the microbats species both in field and laboratory.

**A STUDY OF PROTEIN SPARING EFFECT AND THE EFFICIENCY OF DIFFERENT FEED COMPOSITIONS ON THE GROWTH OF FINGERLINGS OF ROHU (*LABEO ROHITA*)**

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The effect of varying concentration of carbohydrate, lipids and proteins on the growth of fingerlings of Rohu (*Labeo rohita*) was studied. Three experimental feeds were given to three groups of fishes reared in cemented tanks for 45 days. One group of fishes (control group) was fed with hatchery formulated feed; and growth of control group was compared with the other experimental groups. In all the three experimental groups the protein level was kept below the required level, while carbohydrate and lipids level were kept above the required level to study their protein sparing effect, especially of the lipids. The growth of fishes was checked fortnightly. In three experimental feeds the protein, lipids and carbohydrates profile were; feed 01 (20.24%, 18.97%, 41.89%), feed 02 (20.94%, 24.56%, 37.61%) and feed 03 (20.37%, 29.28%, 34.68%), While for control group it was (36.49%, 19.94%, 30.16%). The mean FCR recorded for each tank was, control group  $4.7 \pm 0.53$ , feed 01 ( $3.86 \pm 0.14$ ), feed 02 ( $3.2 \pm 0.04$ ) and feed 03 ( $3.0 \pm 0.12$ ). Some important water parameters which influence the physiology of fishes were also recorded throughout the experimental period. Their mean values were; pH 7.21, Dissolved oxygen 6.8 ppm, temperature 22.55 °C, Alkalinity 221.83 ppm, Total hardness 218.91 ppm and Conductivity 438.91  $\mu$ S. It was concluded that the composition of feed 03 was relatively more suitable for fingerlings of *Labeo rohita* with lowest FCR and showed better protein sparing effect.

**ROLE AND MANAGEMENT OF DONKEY IN THE RURAL AREAS OF D.I.KHAN, KHYBER PAKHTOONKHAWA PAKISTAN**

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In this study a concise review is provided of the role and management of donkeys in the rural area of D.I. Khan, Pakistan. In this area donkeys are mostly used for the transport of drinking water, bricks and fire wood etc, the animals work intensively on an innutritious diet. The efforts of the veterinary teaching, health and research institutes and other private organizations helping in

care for donkeys are described. These institutions provide treatment, mobile veterinary services and awareness for donkey care and health. These institutions also provide trainings to the donkey owners and farmers on such subjects such as housing, management and healthcare etc.

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**ANALYSIS OF STOMACH CONTENTS OF FRESHWATER CATFISH,  
*EUTROPIICHTHYS VACHA* (HAMILTON, 1822)**

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This study was carried out from August to December 2011 to find out the diet of adult Catfish, *Eutropiichthys vacha*. A total of 216 fish stomachs were investigated to find out food items of this fish. Out of these, 153 fish stomachs had prey whereas remaining 63 stomachs were empty. 58.1% stomachs had small fishes, 36.8% stomachs had small aquatic and terrestrial insects and the remaining 5.2% had shrimp. In addition to this, phytoplanktons were also found as an important ingredient of the diet of this fish. Phytoplankton included *Lyngbya* and *Oscillatoria* (Cyanophyceae) and *Diatoma* (Bacillariophyceae). The study confirmed that there is no discontinuation of feeding in this fish from summer to winter as the average weight of stomach contents found same during this study. The structure of mouth, dentations, gills, and stomach were also studied as associated organs with food intake.

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**WATER QUALITY ASSESSMENT USING MACROINVERTEBRATES AS AN  
INDICATOR IN CHAKAR BARI LAKE 1 AT NALTAR WETLAND COMPLEX IN  
GILGIT BALTISTAN, PAKISTAN**

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The preliminary study aims to investigate the abundance, diversity and Community composition of benthic macroinvertebrates in one of the high altitude lake at Naltar Wetland Complex located in Gilgit-Baltistan, Pakistan. Beside macroinvertebrates collection some of the selected physio-chemical properties of the lake were also analyzed. Sampling was done from six different sites including inlet and outlet of the lake. Macroinvertebrates were collected using Kick net (500  $\mu$ m mesh size) while physio-chemical properties were analyzed using different probes such as Dissolved oxygen (DO) and temperature by DO meter, pH by pH meter and conductivity by using conductivity meter respectively. In this study a total of 2050 macroinvertebrates were collected with Chironomidae (86.5%) being the most abundant group of invertebrate community followed by Anisoptera (5.5%), Ephemeroptera (2.3%), Zygoptera (1.6%), Tricoptera (0.8%), Plecoptera (0.8%), Dysticidae (0.7%), Stratiomyidae (0.7%), Hydrophilidae (0.6%) Haliplidae (0.5%) and Turbellaria (0.05%) respectively. Sensitive group of macroinvertebrates such as Ephemeroptera, Plecoptera and Trichoptera were abundant in inlets as compared to outlets indicating good water quality at inlet. Analysis of physio-chemical properties revealed value for pH (7.7), high DO (8.6mg/l), low Temperature (6.2 °C) and high conductivity (169  $\mu$ S/cm) respectively. Physio-chemical and biological indicators indicated fairly good water quality although

long term research with intensive seasonal sampling is required to get the clear picture of overall lake ecosystem for the sustainable management of high altitude wetland in Gilgit-Baltistan.

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### A REVIEW OF SUPERFAMILY CERITHOIDEA (MOLLUSCA : GASTROPODA) FROM PAKISTAN

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Members of superfamily Cerithoidea are of common occurrence in shallow waters along the coast of Pakistan. A review of this superfamily revealed that 8 families belonging to this superfamily occur in Pakistan. Family Cerithidae was found to be most dominating represented by 16 species whereas family Cerithiopsidae was represented by 8 species and family Turritellidae contain 6 species. Family Litiopidae is a monotropic family. In the paper, ecology of some important members of this superfamily was described. *Cerithedia cingulata* was observed to be most common on mudflat and mangroves whereas *Batillaria sordida* and *Cerithium caeruleum* were common on rocky shores.

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### MACRO INVERTEBRATES AS AN INDICATOR OF WATER QUALITY ASSESSMENT IN CHAKAR BARI LAKE 2 AT NALTAR WETLAND COMPLEX IN GILGIT BALTISTAN, PAKISTAN

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Macroinvertebrates species diversity, abundance and community composition are important themes in aquatic ecology, and are often used to evaluate environmental stress resulting from a variety of anthropogenic disturbances. The aim of the present preliminary study was to investigate the water quality assessment using macro invertebrates and selected physico-chemical as an indicator in Chakar Bari Lake 2 at Naltar Wetland Complex. Samples were collected from five different sites including inlet of the lake. Sampling was done using standard methods such as Macroinvertebrates collection using kick net (500 µm mesh size) Dissolved oxygen (DO) and Temperature were analyzed by DO meter, pH by pH meter and conductivity by conductivity meter. A total of 1376 macro invertebrates were recorded comprising of Chironomidae (78.5%) being the most dominant taxa followed by Ceratopogonidae (10.9%), Trichoptera (1.7%), Haliplidae (1.7%) Zygoptera (1.5%), Plecoptera (1.3%), Ephemeroptera (1.2%), Anisoptera (0.9%), Hydrophilidae (0.7%), Stratiomyidae (0.5%), Dysticidae (0.5%) and Decapoda (0.3%) respectively. Sensitive group of macroinvertebrates such as Ephemeroptera, Plecoptera and Trichoptera abundance were higher in inlet as compared to other sites of the lake indicating better ecological health at inlet. Result of overall physico-chemical indicators of the lake were such as alkaline pH (mean 8.8), low temperature (mean 6.2°C), high DO (mean 8.7mg/l) and high conductivity (mean 160 µS/cm) observed during our sampling. Biological and Physico-chemical properties of the lake indicated fairly good water quality. However more intensive seasonal sampling is required to understand the

fluctuation of macroinvertebrates abundance and diversity with changing season and physico-chemical properties for the sustainable management of high altitude wetland for the livelihood of the mountain communities.

**SYSTEMATIC POSITION OF *CONRADIA EUTORNISCA* (MOLLUSCA:GASTROPODA)  
A RARE SPECIES DESCRIBED FROM KARACHI , PAKISTAN**

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*Fossarus eutornisus* was described from Karachi by Melvill (1918) as a member of Family Cerithiidae. Janssen *et al.* (2011) reported this species from Red Sea and placed it in genus *Conradia* A. Adam, 1860 (Family Skeneidae: Superfamily Trochoidea). Genus *Conradia* is generally placed in Family Planaxidae. This genus was placed by some authors in the Family Vanikoridae. The specimens collected from Red Sea differed from that collected from Karachi as it had seven spiral cords on body whorl instead of five. Closed examination of the specimens and comparing it with Melvill (1918) specimens it is evident that it is neither a fossarid or a vanikorid but a Trochoidean. The paper elaborates on this controversy and proposes placement of this species in the Family Trochidae.

**COMPARISON OF RICE POLISH AND MAIZE GLUTIN SUPPLEMENTATION IN  
AMMONIUM NITRATE FERTILIZED POND OF CARPS**

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The experiment was planned to observe the growth response of carps towards rice polish and maize gluten supplementation in ammonium nitrate fertilized earthen ponds stocked with Silver carp (*Hypophthalmichthys molitrix*), Rohu (*Labeo rohita*) and Mori (*Cirrhinus mrigala*) at the ratio of 1:2:1 respectively with a total number of 55 fishes. Pond 1 was treated with ammonium nitrate and rice polish while pond 2 was treated with ammonium nitrate and maize gluten at the rate of 0.3N/100g of wet body weight of fish on daily basis. Fertilizer was added on weekly basis while feed ingredients were given on daily basis. Total net fish production of pond 1 and pond 2 was 1104.2 and 1374.2 kg/ha/year. Pond 2, which was treated with ammonium nitrate and maize gluten showed 1.24 times greater fish production than pond 1 which was treated with ammonium nitrate and rice polish. Analysis of variance on gain in body weight shows that there was a highly significant difference in the increase in body weight of fishes in every week and there was a highly significant difference among species and pond as well. The physico-chemical parameters were measured on weekly basis. Total alkalinity, bicarbonates, total hardness, chlorides, total solids and planktonic biomass showed highly significant difference in both ponds.

**AN ASSESSMENT OF BYCATCH OF TUNA GILLNET FISHERIES OF PAKISTAN**

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Tuna is caught with pelagic gillnet in Pakistan. Gillnet being a passive but unselective gear entangles a number of non-target species. An assessment of the bycatch of the tuna gillnet was found to consist of Talang queenfish (*Scomberoides commersonianus*) which was dominant followed by dolphinfish (*Coryphaena hippurus*). The other species included in the bycatch were the Indo-Pacific sailfin (*Istiophorus platypterus*) and kingfish (*Scomberomorus commerson*). Among the sharks included in the bycatch were the thresher shark (*Alopias superciliosus*), silky shark (*Carcharhinus falciformis*), requiem sharks and mantas. The study also showed that turtles and dolphins also get entrapped in the gillnets especially if it is operated in neritic waters.

**DISTRIBUTION OF GIANT RIVER PRAWN *MACROBRACHIUM MALCOLMSONII* IN LOWER INDUS RIVER WITH NOTES ON ITS GROWTH**

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*Macrobrachium malcolmsonii* is the one of the commercially important species in Pakistan. Study was carried out from seven different localities of lower Indus River to clear the distribution and growth of *M. malcolmsonii* in lower reaches of Indus River. Study was carried out from April to June and shrimps were caught with the help of local fisherman. *Macrobrachium malcolmsonii* was observed at all the surveyed sites, its occurrence all at all sites indicate the amphidromy in the species, which calls for the further detailed study on its life cycle strategies. In total 94 individuals were used for the growth study. Total length (cm) and weight (gm) of shrimps was measured by digital caliper and balance respectively for length weight relationship (LWR). The size of male and female ranged from 12-26.2 mm and 11.2-21 mm respectively. The coefficient b values of LWR were near to isometric values in females ( $\approx 3.39$ ) and it indicate negative allometric for male (2.74) populations.

**INCREASING SHRIMP BYCATCH: A POTENTIAL THREAT FOR FISHERIES RESOURCES**

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Shrimp fishery is considered to be mainstay of fisheries of Pakistan. Because of excessive increase in fishing effort, the catch of shrimp per boat has decrease substantially during last decade. The shrimp fishermen, thus have switched over their fishing practices and now relying more on the

bycatch. Excessive trawling in coastal areas aiming to bycatch is seriously affecting the population of non-target species especially population of benthic invertebrates was observed to be most disturbed. The paper details the composition of bycatch of shrimp trawling, its seasonal variation and contribution of benthic invertebrates in bycatch. It was observed that not only surface dwelling benthic invertebrates are affected but also infauna is also seriously disturbed. The paper suggests measures to reduce bycatch in shrimp trawling operation.

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**ILLEGAL HUNTING OF INDIAN PANGOLIN (*MANIS CRASSICAUDATA*) IN POTOHAR REGION: A CASE-STUDY FROM CHAKRI AREA, RAWALPINDI, PAKISTAN**

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Indian pangolin (*Manis crassicaudata*), is a “Near Threatened” solitary mammal facing high risk of extermination in its wild habitat, where illegal hunting is one of the major intimidation to its survival. The current case study is a part of an ongoing HEC-funded research project and was carried out at “Chakri site”, located at 50 km away in the south-west of Rawalpindi city. The study area was thoroughly surveyed for occurrence of animal and its indirect signs like its burrows and faecal matter. In addition, local people, nomads and hunters were also interviewed. During surveys, six dead specimens of Indian pangolin including one dead body without scales; two complete skeletons along with outer covering of skin; one skeleton having vertebral column and pelvic girdle; one skeleton having vertebral column only and one with disarticulated bones of the animal, were recovered from the study area (Sihal), especially from around the nomads huts. The information generated revealed killing of overall at least 16 individuals of the species from September to December 2011 (4 months); Two separate hunters groups and nomads in the area were found involved in the killing and illegal trade of the species. One hunters group killed four individuals of the species and a scale jacket of the animal was also recovered from this group. Another group of hunters also killed four individuals. Nomads in the area were also involved in the illegal trapping and killing of the animal species, since six dead specimens were recovered from the vicinity of their huts. Moreover, one individual was thrown away by nomads after amputation of scales. One juvenile of the species was also live captured from the same area by our team. The killing of the animal species at large level during three months is a strong evidence of frequent illegal hunting of Indian pangolin in the study area. Nomads and local hunters are directly involved in capturing, killing and illegal trade of the animal species with selling price at the rate of 8000-10000/- rupees per animal. However, at the moment, the trade chain remains undetected. The study reveals that the particular animal species is worthy for its scales which have different uses; making necklaces for domestic animal to save them from evil spirits, use in traditional medicines by local Hakims and also manufacturing of bullet proof jackets. It is recommended that immediate conservation measures should be taken along with the strict implementation of wildlife laws, to save this vital species in the study area.

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**PROXIMATE BODY COMPOSITION AND GROWTH RESPONSE OF EXOTIC CARPS TOWARDS INORGANIC FERTILIZATION OF PONDS**

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Two ponds were stocked with Silver carp (*Hypophthalmichthys molitrix*), Grass carp (*Ctenopharyngodon idella*) and Common carp (*Cyprinus carpio*) at the ratio of 1:2:1 to monitor growth and flesh quality of carps. Pond 1 was treated with di-ammonium phosphate (DAP) and pond 2 with ammonium nitrate @ 0.3g N/100 gm of fish body weight daily. Fish growth parameters *i.e.* body weight and body length were recorded weekly. The physico-chemical parameters were analysed on weekly basis. The gain in weight 268.7 g was maximum for *Hypophthalmichthys molitrix* in the pond treated with di-ammonium phosphate while the gain in length 17.8 cm is maximum for *Cyprinus carpio* in pond treated with ammonium nitrate. Total net fish production of pond 1 and pond 2 was remained 608.975 and 618.335 kg/ha/year. The pond treated with ammonium nitrate showed 1.01 times more fish production than the pond treated with di-ammonium phosphate. At the end of the experiment the flesh quality of three said Chinese carps was observed. Maximum quantity of protein 18.50%, fat 5.90% and moisture content 55.05% were observed in *C. idella* while maximum quantity of carbohydrate 2.23% and ash content 26.39% was observed in *H. molitrix*. Proximate composition showed statistically non-significant ( $P>0.05$ ) results. Temperature, light penetration, dissolved oxygen, pH and planktonic biomass showed non-significant difference in both ponds.

**AVIAN URBAN ADAPTER OF RAWALPINDI CITY, DISTRICT PUNJAB, PAKISTAN**SAKHAWAT ALI,<sup>1</sup> SAJID MAHMOOD,<sup>2</sup> NAEEM AKHTAR ABBASI,<sup>3</sup> NAVEED AHMAD QURESHI<sup>1</sup> AND MUHAMMAD RAIS<sup>1</sup><sup>1</sup>*Department of Wildlife Management, PMAS-Arid Agriculture University, Rawalpindi*<sup>2</sup>*Department of Zoology, Hazara University, Mansehra, Pakistan*<sup>3</sup>*Department of Environmental Sciences, Quaid-e-Azam University, Islamabad*

Urbanization is leading cause of species endangerment, other than agriculture and interaction with non-native species. The present study designed to investigate the avian urban adapters of Rawalpindi city. For this purpose study area was divided into different types of habitats; *i.e.* Parklands, Towns, Metropolitans, Marshy areas and institutions. Total fifteen sites were selected for sampling, three parklands, four towns, three metropolitans, two marshes and three institutions. Data were collected during the month of March-2009 to February-2010. Observations were note down on early in the morning and one hour before evening. Both line transect and fixed radius point count methods were used for the census of species. Fourteen fixed length line transects and sixteen fixed radius point count stations were established in five habitat types *viz.* Parklands, Towns, Metropolitans, Marshy area and institution within the study area. The results shows that the frequency of birds species (abundance) in Parklands 27.94%, Towns 19.67%, Metropolitans 15.17%, Marshy area 14.75% and institution 22.44%. Total 62 bird's species were recorded from the study area that includes migratory and vagrant species. There are 15 birds' species having maximum abundance value; House sparrow (*Passer domesticus*) 19.13%, House crow (*corvus splendens*) 9.3%, Common myna (*Acredotheres tristis*) 7.3%, Indian kite (*Milvus migrans*) 5.06%, Bank myna (*Acredotheres ginginianus*) 4.3%, Little brown dove (*Streptopelia senegalensis*) 4.01%,

Red vented bulbul (*Pycnonotus cafer*) 3.9%, Indian ring dove (*Streptopelia decaocto*) 3.41%, Rock pigeon (*Columba livia*) 2.2%, Rose-ringed parakeet (*Psittacula krameri*) 2.13%, Brown rock chat (*Cercomela fusca*) 1.8%, Common starling (*Sturnus vulgaris*) 1.5%, Purple sunbird (*Nectarinia asiatica*) 1.1%, Hoopoe (*Upupa epops*) 1% and Sparrow hawk (*Accipiter badius*) 0.49% are declared as urban adapter of Rawalpindi city.

### COMPARATIVE MITOGENOMIC ANALYSIS OF FAMILY URSIDAE: A BIOINFORMATICS APPROACH

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In the recent era Bioinformatics facilitated evolutionary geneticist to sort out many missing links and patterns of inheritance. So we used computational approach to analyse the complete mitochondrial genome of bear family Ursidae. Out of the ten member species of this genus two are extinct (*Ursus spelaeus* and *Arctodus simus*) and six (*Ursus thibetanus*, *Helarctos malayanus*, *Ursus maritimus*, *Melursus ursinus*, *Tremarctos ornatus* and *Ailuropoda melanoleuca*) are in IUCN endangered species list leaving only two species (*Ursus americanus* and *Ursus arctos*) which are in safe range of population. The complete mitochondrial genome sequences of every species was retrieved from GenBank database and analysed for its nucleotide contents which revealed that all the species mitochondrial genome is rich in AT content. Phylogenetic analysis was conducted using MEGA5 to draw the evolutionary trees of genus. The positions of all thirty seven genes present on mitochondrial genome were identified. Homology analysis among the sequences was done by CLUSTALW. The deletions or insertions of nucleotides were independently identified in all genes of mitochondria. Tandem repeats across the genome were also identified using Tandem Repeat Finder. Amino acid sequence homology was identified by recognizing open reading frames with NCBI ORF finder. Then these ORFs were translated in silico and aligned by CLUSTALW. However even after extensive analysis of mitochondrial genome, ambiguities to trace evolutionary track of the genus remains a question because the trees generated exhibit different topology when individual gene on the mtDNA were analysed. This comprehensive mitogenomic analyses revealed the fact that phylogenetic trends cannot be predicted using mitochondrial genome only.

### MICROTETRAMERES TRAVASSOS, 1917 (FAMILY TROPISURIDAE) FROM THE HOST EGRETTA ALBA (LINNEAUS) GREAT EGRET OR LARGE EGRET SYN. ARDEA ALBA SINDH, PAKISTAN

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The eight birds *Egretta alba* (Linnaeus) Great Egret or Large Egret syn. *Ardea alba* were purchased from the birds empress market the birds were anesthetized / autopsied and examined for helminth parasitic infections, out of eight birds six were found infected of thirty five nematodes, these were recovered from the small intestine of the host. The specimens were fixed in steaming

70% alcohol and later stored in glycerin alcohol (1 part glycerin: 9 parts 70 % ethanol). The specimens were studied in detail and identified as belonging to the genus *Microtetrameres* sp. Travassos, 1917. These are Small, delicate worms, attenuated at both ends with caudal end more pointed than the anterior. Cephalic extremity provided with a small circular buccal cavity followed by a longitudinal vestibule which opens into the muscular esophagus. The small buccal cavity or the mouth opening is provided with minute pairs of cephalic papillae. Body cuticle is with fine transverse striations.

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#### **PHENOL INDUCED TOXICOLOGY IN THE GILLS OF *LABEO ROHITA* (ROHU)**

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Present study investigated the toxicity of phenol in the gills of *Labeo rohita*. The fish of both sexes were subjected to three sub-lethal concentration of phenol viz. 5,10 and 15 mg.L<sup>-1</sup> for 14 days. Fish were sacrificed after 14 day's period. Histopathological changes in the gills observed were aneurysm of lamellae, lamellar fusion, proliferation in the gill and filament, necrosis, dilation in blood vessels, blood congestion, intense lamellar vasodilation, lifting of epithelium, epithelium rupture, haemorrhage, weakening of gill epithelial and hyperplasia. Phenol was found to be toxic to *Labeo rohita* in both time and dose dependent regimes.

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#### **DESERT, DESERT LIFE AND DESERTIFICATION**

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Desert is some of the harshest places on earth with 25 cm rain or less a year. Hot deserts are found in Tropics and cold desert are found in high altitudes. Deserts lie between 15 and 30 North and South of equator. Desert cover 15% of land surface from Afro- Asian dry land extending 6000 miles. Thar and Cholistan are important deserts of Pakistan. Desert ecosystem is fragile and specialized meeting the vagaries of climate like precipitation, high temperature, low humidity, strong radiation and wind. Flora and Fauna have special adaptation to meet these challenges. Deserts are not wastelands with no socio\_ economic and cultural value. Great civilization and religions had their origin in desert including Islam. The rapid increase in population, phenomenal advancements in science and Technology leading to much anthropogenic intervention has lead to desertification becoming an alarming environmental problem. Degradation of soil, loss of vegetation and scarce forest, dwindling biodiversity and socio economic problems of deserts dwellers. Climate changes are also contributing to desertification. As early as 1984 a UN Report mentioned that 35% earthland surface was threatened by desertification. 50 million hectare in Africa, half of its total area is threatened.

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**COMMENSAL INVERTEBRATES FOUND IN ASSOCIATION WITH HERMIT CRAB  
*DIOGENES CUSTOS* FROM CLIFTON, KARACHI**

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*Diogenes custos* (Anomuran: Diogenidae) commonly occur on sandy shores at Clifton occupying a number of gastropodes shells. Shells of *Babylonia spirata* and *Neverita didyma* were found to be mostly occupied by the hermit crabs. During the investigation, it was found that this hermit crabs have commensal association with a number of invertebrates including slipper shell (*Crepidula walshii*), spionid polychaetes, sea anemone, bryozoan, sponges and barnacles. *Crepidula walshii* seems to be the most abundant fauna found in association with hermit crab. The paper describes various species found in association with the hermit crabs.

**BYCATCH COMPOSITION OF TUNA LONGLINING IN PAKISTAN**

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Large scale tuna longlining was practiced during last two decades in Pakistan, however, only limited scientific information about species composition and bycatch is available. An analysis of the data collected from tuna longlining vessel operating in Exclusive Economic Zone was made which revealed preponderance of dolphinfish (*Coryphaena hippurus*) in the bycatch. Other species commonly found the bycatch were *Istiophorus platypterus*, black marlin (*Makaria indica*), striped marlin (*Tetrapturus audax*), thresher shark (*Alopias superciliosus*), silky shark (*Carcharhinus falciformis*) and mako (*Isurus oxyrinchus*) and sickle pomfret (*Taractichthys steindachneri*). The paper also describes the seasonality of various bycatch species in longline gear.

**CHANGES IN WATER QUALITY OF RIVER CHENAB DUE TO POLLUTED WATER  
FROM URBAN AND INDUSTRIAL AREAS OF FAISALABAD**

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The water quality of most of Asian rivers and underground water especially in Pakistan do not meet the World Health Organization (WHO) standards. The natural purification system of water has been disturbed due to high level of pollution by mankind. The present project is planned to estimate the effect of pollution added by the drain containing waste water from urban and industrial areas of Faisalabad on the water productivity of the River Chenab through its definite length of 112 km. For this purpose, twelve locations were selected, from each location three samples were taken and seven chemical parameters of water were measured for each sample. Out

of 12 spots, 4 spots were before discharge of Faisalabad drain and other were after discharge of Faisalabad drain into the River Chenab. It has been found that, the water quality before the discharge of drain was normal but at spot where drain falls, the pollution level become toxic for flora and fauna. But as we go away from the spot of the pollution, toxicity level is diluted. Before drain discharge, CO<sub>2</sub>, O<sub>2</sub>, total alkalinity, hardness, total dissolved solids, and pH values were normal but after the discharge of Faisalabad wastewater into the River, the level of these parameters greatly reached to toxic level. Substantial decrease was reported in the concentration of CO<sub>2</sub> and dissolved O<sub>2</sub> just after receiving the drain wastes as the two essential gases for aquatic flora and fauna. Deficiency of dissolved oxygen in wastewater creates anaerobic conditions during which foul smelling compounds are generated creating environmental nuisance. There was significant increase in the total alkalinity, hardness, total dissolved solids, and pH leading to non-sustainable productivity of the water with minimum fish or other aquatic flora and fauna due to considerable difference in water quality parameters. The study thus brought forth the fact that water-chemical parameters that exceeded the allowed limits have a potentiality of ultimately polluting and rendering the water unfit for aquatic life and such wastewater discharge without treatment into main River should be banned and there must be an effluent treatment facility near to discharge point to control the ever increasing pollution load on the local environment. Currently, a constant monitoring program needs to be initiated to improve the present situation.

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#### HISTOPATHOLOGICAL EVALUATION AS A RELIABLE BIOMARKER FOR REVEALING ENVIRONMENTAL CONTAMINATION

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Histological alterations in gills, liver and intestinal organs of *Labeo rohita* and *Cyprinus carpio* from Bangla Shah Mohammad, Toba Tek Singh during August – September 2009 were investigated in Hematoxylin-eosin stained sections through light microscopy. Gills, liver and intestinal tissue samples of both fish having different weights and lengths were collected from control (Fish hatchery) and confirmed polluted site (Toba Tek Singh), for the presence of heavy metals. In an allied study, the same specimen of both fish were used to investigate the status of bioaccumulation of heavy metals which confirmed that liver, gills and intestine of *Labeo rohita* and *Cyprinus carpio*, have lead (Pb) and copper residues (Cu) in varying concentrations. Water analysis of both sites was done, which showed the marked differences in physiochemical properties of control and polluted sites. The pronounced histopathological changes observed in the gills of both fish collected from polluted site were degeneration, necrosis and disorganization of lamellae and hyperplasia of epithelial cells in comparison to control samples of both fish. While the liver sections of both fish showed aggregation, hypertrophy of hepatocytes, cytoplasmic degeneration, hemolysis between hepatocytes, focal necrosis and degeneration. In the intestinal tissue of both fish striking histopathological alterations including edema, atrophy in mucosal layer, hemorrhage between blood vessels, blood congestion and aggregations of inflammatory cells were observed. Whereas, these organs in control specimen of both fish exhibited normal structural patterns. The polluted water can affect almost all fishes but there are some variations in species susceptibility as in the present study *Labeo rohita* was more prone to alterations. Comparison of histopathology in each organ for both fish revealed that *Labeo rohita* and *Cyprinus carpio* showed equal degree of tissue damage in gills, while aggregation of hepatocytes in liver, and atrophy of mucosal layer in intestinal tissue of *Labeo rohita* was not found in *Cyprinus carpio*. It can be concluded that

histopathological evaluation is a useful and reliable biomarker for revealing environmental contamination and to study possible negative impact at structural level for the assessment of health or disease status of fish. The development of freshwater aquaculture in Pakistan urgently requires research and expertise on fish diseases and fish health protection through fish health monitoring which needs multiple diagnostic institution or laboratory for fish diseases assessment. It can provide more than ample material for research on fish diseases, their prevention and to monitor aquatic pollution, thus opening possibilities for incorporation of better health protection.

**EFFECTS OF FEEDING FREQUENCY ON THE GROWTH, FEED EFFICIENCY AND BODY CONDITION OF THE GOLDFISH (*CARASSIUS AURATUS*)**

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Feeding frequency play an important role for the maintenance of fish growth and health. Optimum feeding frequency results in considerable savings in feed cost. Excess or underfeeding in the aquarium causes detrimental effects on body which not only deteriorate the water quality but also reduces the body weight, poor food utilization and enhances the susceptibility to infection. For this study total 60 number of Goldfish (*Carassius auratus*) weighing on average 20 g, was divided equally into 4 aquaria having 100-L water holding capacity with 15 fish in each aquaria. There were 4 dietary treatments including control allocated a feed frequency of A(1), B(2), C(3), or D(4) meals/day (meals times was equally spaced in 24 hours). A standard commercially available Goldfish flake food (Osaka Green-1) was offered at an allowance of 3% body weight split equally among the allocated number of meals. The duration of this study was 12 weeks, during which water quality parameters were maintained within safe limits. The results showed that the final body weights and specific growth were significantly lower at D (4 times/day) and C (3 times/day) than B (2 times/day) and A (1 time/day) treatments ( $p < 0.05$ ). There were no significant differences FCR and survival rates ( $p > 0.05$ ). The results showed that weight gain, growth performance and feed efficiency of *Carassius auratus* were decreased with increased feeding frequency.

**BODY COMPOSITION OF FEATHER BACK *NOTOPTERUS NOTOPTERUS* (PARI) FROM THE HEAD BALOKI ON RIVER RAVI, PAKISTAN**

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Proximate compositions of fish is of paramount importance to evaluate it in regard to nutrient value and physiological condition. Biochemical composition of fish tissues are considerable interest for their specificity in relation to food values of fish and evaluating their physiological needs at different periods of life. Besides, protein, fish flesh also offers minerals, iodine, vitamins, fat etc. Knowledge of functional properties is important for utilizing the fish in the preparation of value added products. Proximate body composition is the analysis of water, fat,

protein and ash contents of fish. Carbohydrates and non-protein compounds are present in negligible amount and are usually ignored for routine analysis. The main purpose of this study was to investigate the body composition of the riverine fish (*Notopterus notopterus*). For this study 30 specimens having range of average body weight  $75.0 \pm 7.1$  and average body length  $21.7 \pm 0.8$ cm were collected from the Head Balloki on River Ravi, Pakistan. Each specimen was dried in the oven at 60°C and then ground in the piston mortar. The specimens were analyzed with the help of Near Infrared (NIR) Spectroscopy. The result showed that the moisture was (% wet body weight)  $76.2 \pm 3.5\%$ , fat  $6.1 \pm 1.3\%$ , protein  $74.8 \pm 0.6\%$ , fiber  $1.7 \pm 2.1\%$ , ash  $8.5 \pm 2.4\%$ , phosphorus  $1.3 \pm 0.3\%$ , dry Matter  $94.8 \pm 0.8\%$ . The mean values of body constituents, except for protein content (dry and wet body weight) differed significantly ( $P < 0.05$ ) which is comparable with commercially important fish species.

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**GROWTH PERFORMANCE AND MEAT QUALITY OF *LABEO ROHITA*, *CATLA CATLA* AND *CYPRINUS CARPIO*, UNDER THE INFLUENCE OF FERTILIZATION AND SUPPLEMENTARY FEED**

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A polyculture experiment was carried out to compare the growth performance and meat quality of *Labeo rohita*, *Catla catla* and *Cyprinus carpio*, under the influence of fertilization and supplementary feed in various combinations was conducted in earthen ponds having area of 0.02 ha with two replications for each treatment. After preliminary preparation of ponds, each pond was stocked with *Labeo rohita*, *Catla catla* and *Cyprinus carpio* in the ratio of 20:15:15, respectively. In this experiment, all the ponds received the same quantity of N but the sources were different. The amount of organic manure, fertilizer and supplementary feed was calculated on N-equivalence of 0.2g N/100g body weight of fish weekly for cow manure, nitrophos and daily basis for supplementary feed. In pond 1 100% nitrogen was taken from organic manure (cow manure) and in pond 2, 100% nitrogen was taken from inorganic fertilizers (nitrophos). In pond 3, 50% nitrogen was taken from cow manure and 50% from nitrophos whereas in pond 4, 50% nitrogen was taken from cow manure and 50% from supplementary feed. In case of pond 5, 50% nitrogen was taken from nitrophos and 50% from supplementary feed. Pond 6 received the 25% nitrogen from cow manure, 25% from nitrophos and 50% from supplementary feed under different treatments. The supplementary feed was formulated for treatments T<sub>4</sub>, T<sub>5</sub> and T<sub>6</sub> having 30% crude protein by using fish meal, rice polish, sunflower meal, maize gluten (30% C.P.), canola oil, vitamin and minerals premix. The following parameters viz., total body weight, total length, condition factor, specific growth rate (SGR), length-weight relationship, nitrogen conversion ratio (NCR), nitrogen incorporation efficiency (NIE), gross and net fish production, cost-benefit analysis, of these three fish species were recorded on monthly basis. At the final harvest, the proximate composition of fish meat sample was studied. From the above conclusion, manipulation of *Cyprinus carpio* along with the major carps and the provision of supplementary feed and fertilization enhanced the growth rate, production, meat quality in a semi intensive culture system. Furthermore it increased the effectiveness of liming application and the availability of nutrients to phytoplankton and zooplankton for the fish species in polyculture system which is helpful in reduction of the input costs.

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### REVIEW OF FISHES OF ORDER CYPRINDONTIFORMES FROM PAKISTAN

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A review of the fishes of Order Cyprinodontiformes occurring in Pakistan was made. Members belonging to three families Aplocheilidae, Cyprinodontidae and Poeciliidae are reported to occur in the area. In the freshwater green panchax (*Aplocheilus blockii*) and blue panchax (*Aplocheilus panchax*) seems to be most common whereas along the Pakistan coast euryhaline Arabian killifish (*Aphanius dispar dispar*) occurs in coastal lagoons and shallow waters. Exotic species such as gambusia, guppy, molly, mosquitofish, platyfish and swordtail are common aquarium fish in Pakistan. Some of the feral fishes of the family Poeciliidae have infested natural water bodies in Pakistan. The paper describes the importance of the members of Order Cyprinodontiformes especially their use in control of malaria and dengue fever.

### COMBINED EFFECT OF COWDUNG AND POULTRY MANURE DROPPINGS ON THE GROWING PERFORMANCE OF MAJOR CARPS

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A feeding trial was conducted in earthen ponds to observe the effect of organic fertilization on growth rate and production of major carps for 5 months in semi intensive culture system. Each pond was stocked with *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* @ 20:15:15, respectively. One of these ponds was kept as control (T<sub>0</sub>), whereas the other three were manured with cow manure (T<sub>1</sub>) at @ 10 kg / pond /day and poultry droppings (T<sub>2</sub>) @ 10kg /pond /day. While the treated pond (T<sub>3</sub>) was fertilized with cow dung and poultry droppings @ 10 kg/pond/day (5.5kg). Each treatment has tw replication. Supplementation of treated ponds with organic manure (Cow manure and poultry droppings) caused a marked increase in fish production of major carps as compared to other experimental ponds. Fish ponds fertilized with cow and poultry manure showed the best growth performance and production (P<0.05) as compared to other ponds. The growth performance of major carps in T<sub>1</sub> and T<sub>2</sub> were significantly higher than that of ponds without additives. Net fish yield of all these three fish species in T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> were recorded as 11.383, 27.170, 29.529 and 31.582 kg/pond/year, respectively. However the net fish production were computed to be 569.16, 1358.50, 1476.27 and 1576.22kg/ha/year in T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>, respectively. Pond fertilization with cow manure and poultry droppings showed 2.7 times greater net fish production as compared to that of other treated ponds. T<sub>2</sub> and T<sub>1</sub> showed 2.5 and 2.3 times greater fish production as compared to T<sub>0</sub>. The results of the study indicate that the use of organic manure including cow manure and poultry droppings significantly improved the growth rate as well as fish production of commercial carps. The reduced growth and production of fish in control ponds might be related to planktonic biomass, that showed positive and highly significant correlation in treated ponds. These results seem to confirm the applicability of this method for the assessment of growth and production of commercial carps under the semi-intensive culture system.

**COMMUNITY COMPOSITION OF ZOOPLANKTON REPORTED FROM COASTAL WATERS OF BALOCHISTAN**

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Zooplankton has an important role in marine ecosystem, mostly as consumers of microbial production, and by influencing the resources available to microbes through regenerating and excreting dissolved organic matter. Zooplankton are also good indicator of water quality conditions and environmental health. A survey on the distribution and abundance of zooplankton in coastal waters of Balochistan was carried out during winter at four different sites, namely, Miani Hor, Ormara, Astola Island and Jiwani. In total 17 different groups of zooplankton were identified. Highest abundance of zooplankton was recorded from station at Miani Hor followed by Astola Island, Ormara and Jiwani, respectively. The group copepod represents calanoids as the most abundant taxa. Herpactoid constitute the second abundant group, followed by cyclopoid. However, siponostomoid copepods occur only at Jiwani sites. The group malacostraca (amphipods) is the next abundant group observed at all four sites. The other groups of zooplankton observed includes the following in the descending order of abundance: euphausiids, isopods and mysids, respectively. Polycheate larvae were found in samples from three sites except Ormara. The present study reveals rich zooplankton population along Balochistan coast indicating good environmental conditions prevailing in these waters.

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**POPULATION STATUS, THREATS AND CONSERVATION STRATEGIES FOR PUNJAB URIAL IN KANHATI AND RAKH KHARIOT FOREST SALT RANGE DISTRICT KHUSHAB**

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Rakh khariat and kanhati area reserve forest of the Khushab district were surveyed for study of population status threats to population and conservation strategies in year 2010-2011. These two reserve forest patches are part of the Khushab scrub forest and are located in northern ridges of the Salt range. Area is mainly made up of the lime stone and sand stone layers. Kahu (*Olea ferruginea*) and Phuli (*Acacia modesta*) are two principal species of the area. Punjab Urial (*Ovis vignei punjabiensis*) is one of the important ungulates which is facing threat of extinction in area. Khabeki reserve forest (kanhati) and Rakh khariat are spread over an area of 5014 acres and 10,161 acres respectively. Population census of the Urial was carried by transect and quadrat methods. Vegetation and floral habitat of the Urial was also surveyed. Population survey revealed that Urial population is confined on both northern and southern side of the reserve forest. Khanu wala, Huriala Duma and akrandia are main spots where animals are frequently found. The population in the area was estimated to be twenty four (24) animals. Of the twenty four animals actually seen 16 were female and 8 were male. Most important threats recorded include lamb capture, illegal hunting, grazing, forest fires and heavy population pressure on forest resources. Lamb capture control and illegal hunting control are the two most important conservation actions along with the

active involvement of the local communities and forest and wildlife department. It was concluded that field level interventions along with vigilance and local communities' participation are the most effective tool in conservation of the Punjab Urial in the area.

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**INHIBITING THE POPULATION OF ROSE-RINGED PARAKEET (*PSITTACULA KRAMERI*) USING WIND POWERED HAWK EYE ROTATOR IN A FARMLAND OF FAISALABAD, PAKISTAN**

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The Rose-Ringed Parakeet (*Psittacula krameri*) is a serious avian pest amongst the cultivated crops and orchard fruits through Central Punjab, Pakistan. As such it causes a substantial damage and economic losses to a variety of crops. This paper presents study on inhibiting Parakeet's depredation at wheat and maize, by using the Wind Powered Hawk Eye Rotator (WPPER) carried out in a farmland agro-ecosystem of Faisalabad. Observations were recorded for two consecutive hours in the study period extending from April through May 2009. On control (without rotator) and employed (with rotator) for 20 minutes duration each. There was a considerable decrease in the number of parakeets for both maize and wheat which were also statistically significant with respect to time interval. It was evident that the application of mechanical repellents would prove beneficial to inhibit the Rose-Ringed Parakeet's depredation on not only the sampled crops but also for other food crops to minimize the economic losses and maintain the agro-ecosystem sustainability.

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**CHARACTERIZATION AND ESTIMATION OF LABORATORY-SCALE BIODEGRADATION POTENTIAL OF CRUDE OIL CONTAMINATED SOIL BACTERIA**

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Crude oil biodegradation has emerged as an important paradigm in the environmental toxicology. Various lines of evidence indicate substantial increase in methodologies to counteract environmental contamination. In the present study, bacterial flora of two oil field soils, Rajian & Missa Kaswal, from Punjab province was characterized and monitored for their crude oil biodegradation potential under laboratory conditions. Random soil sampling from various locations of waste pits was done. On the basis of morphological and biochemical tests, twenty oil degraders belonging to seven genera, *Pseudomonas*, *Bacillus*, *Klebsiella*, *Acinetobacter*, *Clostridium*, *Erwinia* and *Streptococcus* were characterized from Rajian oil field soil. These microbes were introduced separately in 22% crude oil contaminated garden soil for 30 days. Twenty-four isolates belonging to twelve genera, *Acinetobacter*, *Amphibacillus*, *Arthrobacter*, *Bacillus*, *Corynebacterium*, *Flavobacterium*, *Klebsiella*, *Lactobacillus*, *Listeria*, *Moraxella*, *Providencia* and *Pseudomonas* were characterized from Missa Kaswal oil field soil. Two consortia namely Consortium A (05 bacterial strains) and Consortium B (08 bacterial strains) were designed and applied on crude oil contaminated garden soil for 15 days. Microbial enumeration, reductions in weight and crude oil contents were recorded. Comparative findings demonstrated greater degradation of crude oil by members of *Pseudomonas* (20.20-13.34%), followed by *Bacillus* (16.68-8.40%), *Erwinia*

(11.55%), *Clostridium* (11.12%), *Klebsiella* (10.99-7.22%), *Acinetobacter* (9.68-9.20%) and *Staphylococcus* (5.99%) genera respectively. Effective removals of 13.38% and 15.84% crude oil contents were noted by Consortia A and B respectively. This study categorized *Pseudomonas*, *Acinetobacter*, *Bacillus*, *Corynebacterium* and *Klebsiella* as common microbial genera of both oil field soils and promising biodegradation of crude oil by microbial consortia in relatively shorter time.

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#### COMMUNITY COMPOSITION OF ZOOPLANKTON REPORTED FROM COASTAL WATERS OF BALOCHISTAN

PIRZADA J. A. SIDDIQUI\*, SEEMA SHAFIQUE, ZAIB-UN-NISA BURHAN, PERVAIZ IQBAL, AMJAD ALI, AND SHABIR ALI AMIR

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Zooplankton play an important role in marine ecosystem, mostly as consumers of microbial production, and by influencing the resources available to microbes through regenerating and excreting dissolved organic matter. Zooplankton are also good indicator of water quality conditions and habitat quality. A survey on the zooplankton distribution and abundance in coastal waters of Balochistan was carried out during winter at four different sites namely, Miani Hor, Ormara, Astola Island and Jiwani. Seventeen different groups of zooplankton were identified. Highest abundance of zooplankton was recorded at Miani Hor followed by Astola, Ormara and Jewani, respectively. In copepod group, calanoids were most abundant with herpactoid which was second abundant group, followed by cyclopoid. Siponostomoid only occur at Jewani sites. After copepods, malacostraca (amphipods) showed higher abundance at all four sites. This was followed by euphausiids, isopods and mysids, respectively. Polychaete larvae reported from three sites except Ormara.

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#### STUDY OF SOIL MACROFAUNA IN RELATION WITH SOME OF SELECTED SOIL PHYSIO-CHEMICAL PROPERTIES AT SUMAYAR-NAGAR IN DISTRICT HUNZA-NAGAR GILGIT-BALTISTAN, PAKISTAN

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Soil is one of the most essential and diverse natural habitat of biodiversity on earth. The Soil organisms (biota) carry out a wide range of processes that are important for soil health and fertility in both natural and managed agricultural soils. The total number of organisms, the diversity of species and the activity of the soil biota will fluctuate as the soil environment changes. The present preliminary study aims to investigate the abundance, diversity and community composition of Macrofauna in agricultural soils in Nagar-Sumayar in Hunza-Nagar district of Gilgit-Baltistan. Beside soil Macrofauna some of the selected physio-chemical properties of the soil were also investigated. For sampling the entire Sumayar-Nagar is divided into 9 clusters and total 54 soil pits were dug out with Quadrant size (25\*25\*15cm) and Macrofauna was picked and preserved in 4% formalin. Soil temperature was determined using Temperature probe, air humidity by humidity probe, and moisture determined by Gravimetric method. A total of 718 soil Macrofauna were

recorded from 14 orders, the most abundant taxa was Diptera comprising of (59%). Other major orders were Coleoptera (15%), Chilopoda (4.5%) Aranea (3%), Potworms (3%), Hemiptera (2.7%) Hymenoptera (2.7%) Oligochaeta (2%), unidentified Macrofauna (2.9%), Lepidoptera, Acari, Orthoptera and Homoptera (1%) respectively. pH of the soil varies from 7.7 to 8, soil temperature from 10-22°C and soil moisture from 14-20% in different clusters or areas of Nagar-Sumayar. Pearson's correlation indicated that pH was positively correlated ( $p < 0.01$ ) with Diptera larva while soil temperature with Diptera adult. Soil moisture was positively correlated with Lepidoptera and Aphidae and negatively correlated ( $p < 0.01$ ) with Orthoptera. Macrofauna abundance was positively correlated with soil pH. One way ANOVA showed that there were no significant differences of soil Macrofauna abundance among the various clusters. Highest soil quality was attributed to Daltho area and lowest was Yal area as determined by Macrofauna abundance. Further research need to be done with more intensive sampling to investigate the influence of seasons and other soil management practices on soil Macrofauna abundance and diversity.

#### **LENGTH-WEIGHT AND CONDITION FACTOR RELATIONSHIP OF TWO FISH SPECIES CAUGHT FROM THE RIVER INDUS, PAKISTAN**

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In the present study, 49 specimens of *Catla catla* and *Labeo rohita* of variable sizes ranging from 16 – 35cm total length and 5.52 – 558.35 g body weight were sampled from river Indus to investigate the parameters of weight-length and condition factor. Log transformed regressions were used to test the allometric growth. Each fish was measured and weighed. It was observed that growth and weight is almost proportional to the cube of its length. The value of the slope  $b$  coincides with the slope of that of an ideal fish. Condition factor (K) has positive influence with increasing length or weight. Regression parameters were found to be highly significant.

#### **PHYSICO-CHEMICAL AND BACTERIOLOGICAL ANALYSIS OF DRINKING WATER OF DANYORE VILLAGE, GILGIT-BALTISTAN**

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This study was conducted to determine the physico-chemical and bacteriological parameters of drinking water samples from Danyore village of Gilgit-Baltistan, where drinking water samples are not treated before it is consumed. The purpose was to ascertain the quality of water for human consumption from these sources. Samples were taken from ten different sampling points of the village and analyzed for the following parameters *i.e.* Color, Taste, Odour, pH, Turbidity, Temperature, Specific conductivity, Total Dissolved Solids, (TDS), level of Dissolved

Oxygen (LDO), Salinity, Total Alkalinity, Total Hardness as CaCO<sub>3</sub> and Total Coliform colonies by using different kits of water quality analysis. The data showed the variation of the investigated parameters in samples as follows: Temperature 5.6 - 22.5°C, pH 5.0 - 7.8, Conductivity (SP) 305.2 to 493.3 µs/cm, Turbidity 17.1-96.0 NTU, Total Dissolved Solids (TDS) 200-300 mg/l, Level of Dissolved Oxygen (LDO) 5.5-9.36 mg/l, Salinity 50-25 ppm, Total Alkalinity 60-90 mg/l, Total Hardness 160-190 mg/l. Upon water analysis Total Coliform/ml were observed. In channel Total Coliform were in higher than in tap water. The concentrations of some of the investigated parameters in the drinking water samples were within the permissible limits of the World Health Organization (WHO) drinking water quality guidelines and some others vary from those guidelines and not suitable for consumption.

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**USE OF GEOSPATIAL TECHNOLOGY FOR HABITAT EVALUATION OF WESTERN TRAGOPAN (*TRAGOPAN MELANOCEPHALUS*) IN SELECTED AREAS OF AJK, PAKISTAN**

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Western Tragopan (*Tragopan melanocephalus*) is endemic to Western Himalayan Ecoregion. It is the most westerly distributed pheasant species among the five Tragopans, occurring in Pakistan and India in the Himalayan region. It is restricted in its distribution in Pakistan; small populations survive in Duber, Palas, Kaghan, Pir Hasimar, Machiara and Pir Chinasi. Western Tragopan has large but fragmented distribution within western Himalayan moist temperate forests. It is globally 'vulnerable' because its sparsely distributed small populations are declining and becoming increasingly fragmented in the face of continuing forest loss and degradation throughout its restricted range. The geospatial technology, including Satellite Remote Sensing (SRS) and Geographic Information System (GIS) is found to be time and cost effective for modeling habitat and distribution of the species. Remote sensing coupled with ground based methods provides timely and accurate information on all the basic parameters used for habitat evaluation of the species. Western Tragopan, being a shy bird, has greater affinity towards vegetation that serves as shelter and food for it. Evaluation of potential habitat for Western Tragopan can be considered as one of the most important steps towards the conservation of this species. This study attempts to model Western Tragopan habitat suitability in Neelum and Muzaffarabad districts of AJ&K using remote sensing and GIS; and elaborates its procedure for enhancing extensive use of these efficient techniques for wildlife habitat evaluation. The potential distribution of Western Tragopan was mapped by incorporating topographic and environmental variables along with species sighting data into Habitat Suitability Model (MAXENT). Land cover and Normalized Difference Vegetation Index (NDVI) of the study area was developed from ALOS AVNIR-2 satellite images; Russian topographic sheets were used to extract topographic data (roads, settlements, drainage); Digital Elevation Model (DEM) was extracted from ASTER GDEM and consequently slope and aspect were derived; temperature and precipitation data were downloaded from WORDCLIM website and

species sighting data were collected from the field surveys conducted under Programme for Mountain Areas Conservation (PMAC). Land cover data, DEM, slope, aspect, NDVI, topographic data, temperature, precipitation and sighting datasets were processed in GIS to generate predictor variables, consequently used in the modeling software *i.e.*, MAXENT. An area of 11,112 ha (2% of the total study area) is considered highly suitable for the species, 30,248 ha area (5.3%) moderately suitable and 525,445 ha (92.7%) area is unsuitable for the species. The study will help in the *in-situ* conservation planning and management of the species in its distribution range.

#### **CHANGES IN THE BODY SURFACE AREA DURING DIFFERENT STAGES OF DEVELOPMENT IN BOYS FROM POTOHAR REGION: AN EXPLORATORY STUDY**

MADIHA ZAFAR<sup>1</sup>, SHAISTA ASLAM<sup>2</sup>, AFZAAL AHMED NASEEM<sup>1</sup>, MAZHAR QAYYUM<sup>1</sup>, NUSRAT JAHAN<sup>2</sup> AND SYED SHAKEEL RAZA RIZVI<sup>3</sup>

<sup>1</sup>Department of Zoology, PMAS Arid Agriculture University, Rawalpindi,

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<sup>3</sup>Pakistan Science Foundation, Islamabad

The body surface area (BSA) is the deliberate or premeditated surface of a body. The BSA is considered a better determinant of metabolic rate as compared to body mass index. The calculation of BSA is of prime importance in diagnostic and dose determination in endocrinopathies, anesthesia and certain pathological conditions such as cancer, burns etc. The present study is designed to determine the reference ranges of BSA in boys from prepuberty through puberty and adolescence in our local population. The height and weight of the boys were measured and other relevant information was collected through a questionnaire especially designed for the study. The BSA was calculated using formula described by Schlich et al. The present study demonstrates that there is a progressive increase in the mean height, weight, body mass index (BMI) and BSA of boys with advancing age. The increase in the height, weight, BMI and BSA were slow during prepuberty, early puberty and through adolescence to adulthood. On the other hand, the increase in height, weight, BMI and BSA was significant at mid and late puberty. In conclusion, the present investigation reports significant increases in BSA during mid to late puberty and warrants its determination for drug dosage in treatment of various diseases.

#### **IMPACT OF NON-CROP VEGETATION ON THE FREQUENCY OF INVERTEBRATE FAUNA IN THE AGROECOSYSTEM OF FAISALABAD DISTRICT**

NAUREEN RANA,<sup>1</sup> SHABANA NAZ,<sup>2</sup> TAHIRA RUBY,<sup>3</sup> MUHAMMAD ZAFAR IQBAL JANJUA<sup>1</sup>, MUHAMMAD NADEEM ABBAS<sup>1</sup> AND SAJIDA MUSHTAQ<sup>1</sup>

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The impact of non-crop vegetation on the frequency of invertebrate fauna in agroecosystem of Faisalabad district was studied by taking regular samples with the help of sweep net for a period of one year. A total 150 species of invertebrates were found harboring on five weeds and two tree

species viz., *Cnicus arvensis*, *Solanum nigrum*, *Vicia sativa*, *Dactyloctenium aegyptium*, and *melilotus indica*, *Delbergia sissoo*, *Mangifera* respectively. Of which 44 predators e.g. *Nemobius fasciatus*, *Mestiche vitticollis*, *Hippodamia convergens*, *Coccinella septumpunctata*, *Oxyopes macilentu*, 38 pests species e.g. *Diaferoma femorata*, *Camponatus spp.*, 56 herbivores, 4 scavengers, 2 omnivores and 2 parasite species were identified in the samples. Weed species were found harboring abundant and more diverse faunal population as compared to trees. We concluded that the non-crop vegetation not only enriches the diversity of invertebrates but also providing a reservoir of natural enemies that naturally regulate pest species and reducing pest load from crops and inturn increased crop yield.

**BIODIVERSITY, RELATIVE ABUNDANCE AND DYNAMICS OF  
MACROINVERTBRATES IN SUGARCANE AGROECOSYSTEM IN PUNJAB  
(PAKISTAN)**

MUHAMMAD NADEEM ABBAS,<sup>1</sup> SHAHNAZ AKHTAR RANA,<sup>1</sup> NAUREEN RANA,<sup>1</sup>  
MAHMOOD-UL-HASAN,<sup>1</sup> SHABANA NAZ<sup>2</sup> AND MUBARRA MUBEEN<sup>1</sup>

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The study on terrestrial macroinvertebrates in sugarcane agroecosystem was conducted in district Faisalabad, Pakistan. Species richness provides an enormously valuable measurement of species diversity when a complete catalogue of species in the community is attained. A total of 232 species was documented comprising of 178 species of insects in 79 families and 12 orders; 35 species of arachnids in 13 families; 16 species of Pulmonates in 10 families and only three species 3 species of isopods in 2 families. The diversity index showed the maximum diversity found on weeds ( $H' = 4.075$ ) than on sugarcane ( $H' = 3.673$ ) as well as significantly different ( $p = <0.001$ ), whereas the edge and center also showed significant difference ( $p = <0.001$ ). Findings indicate that weeds as well as the edges of the crops maintaining the diversity and abundance of macroinvertebrates in turn stabilizing natural ecosystem in sugarcane.

**BEAUTY OF SAMPLING, IF DONE PROPERLY**

MUHAMMAD SULEMAN

*Department of Microbiology Hazara University, Mansehra*

Sampling is a powerful tool used in research. It has a lot of advantages but also has some limitations. Statistical approaches including sampling methods are of particular importance for students of biological sciences because they have to deal with a great deal of variation in biological data. They need to be aware of the concepts of the relationship between sample and population, the importance of a representative sample, risks in using un-representative sample, estimation, margin of error, types of samples: probability vs convenient sampling, random sample, methods of taking a random sample (simple random sample, stratified, systematic sample, cluster sample, etc). Practical approaches involved in taking a random sample can be shared with the audience using a couple of examples. A field researcher needs to know how to use table of random numbers for random sampling. An investigator should have a clear concept of the requirement of an adequate sample

size. Media personnel commonly use  $1/\sqrt{n}$  as the margin of error in their opinion surveys; it is a conservative formula based on the adequate statistical formula. While planning a field study, the relationship between sample size and margin of error should be kept in mind. How to determine the required sample size will be demonstrated using a simple example. Sampling is a wonderful tool but it has to be used intelligently and carefully.

#### **PREVALENCE OF FISH DISEASES IN POTOHAR REGION OF PUNJAB, PAKISTAN**

SYED ZULFIQAR ALI ABIDI<sup>1</sup>, IFTIKHAR AHMAD QURESHI<sup>2</sup>, IFTIKHAR AHMAD<sup>1</sup>,  
MAZHAR QAYYUM<sup>3</sup> AND MARATAB ALI AWAN<sup>1</sup>

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Punjab is blessed with a variety of Aquatic resources which offer a tremendous scope for Fish culture activities. Aquaculture worldwide is going faster than most other sectors of the food industry because of increase in human population and growing food demand. Every effort is being made to get maximum per unit area fish yield which make the cultured fish species more vulnerable to various diseases. Present study is based on the recording of prevalence of fish diseases in Aquaculture Sector of Potohar Region *i.e.* Jhelum, Chakwal, Attock and Rawalpindi districts. In this area most of the Fish culture is practiced in mini dams which depend on rain water. Total of 374 cases of diseases were recorded during a period of 3 years (Jan,2009to Dec,2011) out of these 32.26-43.7% bacterial diseases, 11.76-22.59% crustacean, 11.29-11.6% protozoan, 8.4-12.9% fungal, 5.34-9.29% environmental, 4.2-12.21% helminthic, 3.23-6.72% nutritional, 1.61-5.34% viral, and 0.81-2.52% genetic diseases were found. Bacterial diseases were found more common rather than other diseases which may be associated with run off water.

#### **PALEOENVIRONMENTAL EXPLORATION OF MIDDLE MIOCENE OF POTWAR SIWALIKS OF PAKISTAN**

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Exploration of paleoenvironment of middle Miocene (Chinji Formation) of PotwarSiwaliks of Pakistan has been worked out. Methodological focus is on hypsodonty and mammalian community structure analyses of taxonomically resolved extinct taxa of large herbivorous mammals. The explored ecomorphic information suggests that there was prevalence of browsers, frugivores, browse dominated mixed feeders during Chinji (ca.14.2-11.2 Ma). This faunal assemblage corresponds to Bankas Formation of Nepal Siwaliks and Orleanian of Europe. Hypsodonty analysis of the mammalian fossilsportrays an ecosystem depicting the closed vegetation, humid and warm climatic inferences leading to exploration of tropical evergreen forest and savanna with abundance of C<sub>3</sub> grasses and first appearance of C<sub>4</sub> grasses. This study may contribute to the understanding of evolution of mammalian paleoecologic processes during middle Miocene of Pakistan.

**REARING AND BREEDING OF RED TYLAPIA AT FISH HATCHERY CHILYA  
THATTA, SINDH, PAKISTAN**

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*Directorate of Fisheries Sindh Inland Hyderabad, Livestock & Fisheries Department, Government  
of Sindh*

The present study was conducted to evaluate rearing and breeding of red tilapia in cemented tanks (15x6x3ft) at fish hatchery Chilya Thatta, Sindh for 150 days. Red tilapia fry (2.0 g) were imported from Thailand for rearing in the tanks. After acclimatization the fry were distributed randomly. The diet constituted 30% protein concentration was given to the fry at the rate of 5% body weight thrice a day. The basic ingredients in this diet were fish meal, mustered oil cake, rice bran, wheat bran, rice protein and wheat flour etc. The fry were hand fed thrice a day. The water quality parameters were water temperature 26-38°C, dissolved oxygen 5.2-7.5 mg/lit, pH 7.5 and hardness 70-185 ppm. The results showed that growth of fish fry was significantly high after 30 days. When attained 100-150 g in weight, it became mature for breeding after that the selected brooders were shifted into breeding hapa with 1:3 ratio (1 male and 3 female). Above 20000 eggs were collected from the mouth of reared Red tilapia brooders.

**DIVERSITY, PATTERN AND MULTIVARIATE ANALYSIS OF MOLLUSCS IN  
MANGROVE FORESTS PAKISTAN**

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NAUSHABA<sup>1</sup> AND MUHAMMAD UZAIR KHAN<sup>1</sup>

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The population distribution of molluscan fauna in the mangroves of Pakistan was examined using multivariate methods. Regular trips were arranged during the period Feb 2008- Dec 2009 for qualitative and quantitative sampling in five different locations of Pakistan coast i.e. Sandspit, Port Qasim, Kemari, Ketti Bunder and Sonmiani. 30 stands were sampled by using 1x1 m<sup>2</sup> quadrats. A total of 31 species were obtained from the sampling sites out of which 18 species were gastropoda while 13 species belong to bivalvia. The highest numbers of Gastropod species 23 (74.19%) were found at Sandspit mangrove forests whereas Kemari exhibited least number of species 2 (6.45%). The maximum number of bivalves were also recorded from Sandspit sampling area while at Ketti Bunder no molluscan species was found. The overall range of Gastropodes species diversity, evenness (equitability), richness and dominance were H= 0.661 to 2.527, J=0.806-0.954, d1= 0.182-1 and D=0.086-0.527 respectively while bivalves were ranged between H=0-2.220, J=0-0.965, d1=0-1.66 and D=0-0.277. Four main groups were disclosed by cluster techniques on the basis of composition of molluscan fauna of the habitats which could readily be superimposed on 2D ordination axis. It is obvious from the quantitative results that *Telescopium telescopium* and *Cerithedia cingulatus* members of family Potamididae are the dominant molluscs in mangrove forests. The distribution of these species showed aggregated pattern. This study will provide understanding about the molluscan fauna of mangroves in Pakistan.

**MONTHLY FLUCTUATIONS IN WINTER AND SUMMER AVIAN FAUNA OF  
MANGLA DAM, MIRPUR AZAD KHASHMIR**

BUSHRA NISAR KHAN<sup>1</sup>, ZULFIQAR ALI<sup>2</sup> AND FEHMEEDA BIBI<sup>3</sup>

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Wetlands are one of the most productive and resourceful areas which provide food, non-food aquatic resources and retains the ecological balance for the local residents. The present study was carried out to determine the avian diversity of Mangla Dam in comparison with different previous records and surveys. Mangla Dam is one of the largest man made earthen filled reservoir and favorite over wintering ground for migratory birds and is enlisted in the category of fresh water wetlands like Haleji Lake, Hub Dam and Tarbella Dam in Pakistan. As a matter of fact this internationally important over wintering site is most neglected wetland of Pakistan. This study was conducted with a total of twelve surveys of three days from January, 2011 to December, 2011, covering wetland area and its buffer zone of two kilometers. The point count method was used to determine to number of species and number of individual birds. All heard and observed birds were recorded in field diaries by use spotting scope and binocular. Of the avifauna listed (n=260 species), 177 species were migratory and 83 resident waterbirds were frequent at Mangla reservoir. Among the most dominant species were Common Pochard *Aythya ferina* (n=5,893), Little Cormorant *Phalacrocorax niger* (n=5,456), Coots *Fulica atra* (n=4,782), Mallard *Anas platyrhynchos* (n=4,702), Northern Pintail *Anas acuta* (n=4,062), Ruddy Shelduck *Tadorna ferruginea* (n=4,001), Great Cormorants *Phalacrocorax carbo* (n=3,996), Black Headed Gull *Larus ridibundus* (n=3,691) Euraisan Wigeon *Anas penelope* (n=3,387) and Bar-headed Goose *Anser indicus* (n=2238). A maximum total of 1,26,537 birds were observed at the site. Ever increasing human population, hunting, trapping, lack of awareness, deforestation in catchments area and agrochemical residues were the main problems in conserving the wetland and waterfowls at Mangla Dam. The research work demonstrated this important fresh water site must be treated according to international wetland standards to conserve the unique fauna of the migratory birds and to cope with major threats.

**EFFECT OF POLLUTION ON HEALTH STATUS OF FISHES IN THE RIVER CHENAB,  
AT HEAD TRIMMU AREA, DISTRICT JHANG, PAKISTAN**

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During January 2012, a survey to collect fishes in the river Chenab at head Trimmu pond area, Malkana Lake and three adjacent outlet canals was conducted. The fish fauna was collected for biological studies. Sixty different fish species were collected. These fish sample contained various commercial fish species such as *Channa*, *Labeo*, *Gibleon catla*, *Wallago attu*, *Bagarius*

*bagarius*, *Rita rita*, *Notpterus notopterus* etc and non commercial fish species; such as *Barilius*, *Botia*, *Colissa*, *Gagatta*, *Aspidoparia*, *Xenetodon* etc. Diseased fish specimens were observed in the sample. *Wallagu attu* population was infected with severely damaged skin and body parts. Specimens of *Channa marulius*, *Sperrata sarwari* and *Rita rita* were also found infected. Large scale mortality in non commercial species i.e., *Salmostoma*, *Barilus*, and *Aspidoparia* was also observed, which is attributed to aquatic pollution. In present paper, various factors affecting the health status of fish fauna in river Chenab have been discussed.

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### ISOLATION OF FUNGI FROM BLACK MOOR, A VARIETY OF GOLDFISH, *CARASSIUS AURATUS* L.

RABIA MUMTAZ AND ZAFAR IQBAL

*Department of Zoology, University of the Punjab, Lahore. P.code54590. Pakistan*

Sixteen black moor fishes were obtained from a pet shop in Lahore. These fishes were studied for fungal infections. Infected fishes showed clinical signs such as, eroded scales, partly eroded caudal fin, dorsal fin, pectoral fin and declourised skin. The material from various affected parts of the body of fish such as; skin, fins, gills and abdomen and the pet shop aquarium water were inoculated on three different media, MEA, SDA and PDA. The inoculated agar plates were incubated at 27-30°C. Fungal colonies of white, grayish black, green and reddish pink color were observed. Slides of isolated fungi were prepared and stained with trypanblue in lectophenol. Four species of fungi *Aspergillus* spp., *Mucor* spp., *Penicillium* spp. and *Alternaria* spp. were isolated. *Aspergillus* was the most prevalent fungi found in 13 fishes (86.6%) from gills, abdomen, caudal fins and pectoral fins. *Penicillium* spp. was recorded from 3 fishes (18.7%) from gills, pectoral fin and dorsal fin; *Alternaria* spp. and *Mucor* spp. were recovered from 4 fishes each (25%) from gills, abdomen, caudal fin and pectoral fins. Abdomen was the most affected area (30.76%) followed by gills and pectoral fins (23.7%) each, caudal fin (15.38%) and pectoral fin (7.69%). *Aspergillus* spp. was isolated from aquarium water obtained from pet shop in which the fish was maintained. The high prevalence of *Aspergillus* in black moor, corresponds to the contaminated aquarium water with this fungi. Great care is needed to be taken in management of fish aquariums in pet shops and other premises regarding feeding, water quality and health status of these ornamental fishes.

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### ISOLATION OF PATHOGENIC FUNGI FROM TWO TROPICAL ORNAMENTAL FISHES

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Two tropical ornamental fish species Koi, *Cyprinus carpio* L. and Shubunkin, *Carassius auratus* L. were obtained from pet shops in Lahore. These fishes were examined for fungal infection. Mean total length and mean weight of koi and shubunkin were  $9.9 \pm 1.5$ cm and  $11.4 \pm 2.5$ g and  $8.4 \pm 1.8$ cm and  $6.11 \pm 2.5$ g respectively. 50% koi and 66% shubunkin showed fungal infections on gills, caudal fin, anal fin, dorsal fin and skin. Infected fishes had clinical signs such as; eroded scales and fins, deep reddish gills and bulging eyes. Fungi isolated from infected areas were cultured on SDA, PDA, and MEA. The isolated fungi were incubated for 5-7 days at 30 °C.

White, black, grey and green colonies were observed. Slides were prepared by taking material from each colony and stained with trypanblue in lectophenol. Four fungi were isolated and identified as; *Aspergillus niger*, *Aspergillus flavus*, *Mucor* spp. and *Rhizopus* spp. Gills were the most affected area in both fishes (koi 68% and shubunkin 76%). *Aspergillus niger* was the most prevalent fungi (66%). The causes of fungal infection are discussed.

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#### **EFFECT OF INDUSTRIAL POLLUTION ON *LABEO ROHITA* INHABITING THE RIVER CHENAB**

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In Pakistan, the water pollution has become a serious problem due to discharge of untreated industrial effluents and domestic sewage, containing large quantities of toxic heavy metals, into the river water system that adversely affect the aquatic life. Therefore this study was carried out by involving *Labeo rohita* as an important inhabitant of the River Chenab of District Jhang, Pakistan. This river is affected by the effluents from leather, plastic, textile, printing, soap and sugar industries that are discharged into the main Paharang drain that falls into the River Chenab. This completely randomised study involved three polluted locations (Thatha Muhammad Shah, Pattan Draaj and Head Trimu) along the stretch of River Chenab in Jhang and a relatively less polluted site of Kot Khera as the control. Water and fish samples (108) were collected by involving nine fish samples of similar size (about 1000g) as replicates from four locations. The fish were dissected to collect kidneys for the estimation of their heavy metal, total oxidant and antioxidant status. Heavy metals in water and fish kidneys were determined by using Hitachi Polarized Zeeman Atomic Absorption Spectrophotometer (AAS, Z-8200, Japan). Total oxidant status (TOS) of kidneys was determined by using a novel automated measurement method and the total antioxidant status (TAS) was assessed by the automated colorimetric method. The data were statistically analysed by using ANOVA in Minitab software version 16 to see the effect of sampling sites on the metal profile, total oxidants and antioxidant status of fish kidneys. Tukey's post-hoc test was used to compare the means at  $P < 0.05$ . Results showed the significant differences ( $P < 0.01$ ) in metal profile of water that was sampled from four selected locations. The concentration of Pb, Cd, Ni and Zn were higher at the three sampling locations than the WHO maximum permissible levels of heavy metals for aquatic life. Conversely, the most metal levels except Ni at the control site were within the safe limits. There were significant differences in heavy metal profile and total oxidants of fish kidney sampled from selected locations ( $P < 0.01$ ). The concentration of heavy metals in fish kidneys at sampling sites exceeded the WHO standards of food fish and control fish except Zinc. Consequently the fish of this study area may cause metal related disorders in these fish and their consumers. Moreover the mean metal concentration in fish kidneys was several times higher than the corresponding samples of water. Non significant differences ( $P > 0.05$ ) were observed for total antioxidants of fish kidneys being sampled from the selected locations. It can be concluded that proper measures should be taken to avoid the contamination of the River Chenab by Main Paharang Drain which are responsible for the increased levels of metals and total oxidants which may be damaging to the fish health and other aquatic life.

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**MONTHLY FLUCTUATIONS IN WINTER AND SUMMER AVIAN FAUNA OF  
MANGLA DAM, MIRPUR AZAD KASHMIR**

BUSHRA NISAR KHAN<sup>1</sup>, ZULFIQAR ALI<sup>2</sup>, AND FEHMEEDA BIBI<sup>3</sup>

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Wetlands are one of the most productive and resourceful areas which provide food, non-food aquatic resources and retains the ecological balance for the local residents. The present study was carried out to determine the avian diversity of Mangla Dam in comparison with different previous records and surveys. Mangla Dam is one of the largest man made earthen filled reservoir and favorite over wintering ground for migratory birds and is enlisted in the category of fresh water wetlands like Haleji Lake, Hub Dam and Tarbella Dam in Pakistan. As a matter of fact this internationally important over wintering site is most neglected wetland of Pakistan. This study was conducted with a total of twelve surveys of three days from January, 2011 to December, 2011, covering wetland area and its buffer zone of two kilometers. The point count method was used to determine to number of species and number of individual birds. All heard and observed birds were recorded in field diaries by use spotting scope and binocular. Of the avifauna listed (n=260 species), 177 species were migratory and 83 resident waterbirds were frequent at Mangla reservoir. Among the most dominant species were Common Pochard *Aythya ferina* (n=5,893), Little Cormorant *Phalacrocorax niger* (n=5,456), Coots *Fulica atra* (n=4,782), Mallard *Anas platyrhynchos* (n=4,702), Northern Pintail *Anas acuta* (n=4,062), Ruddy Shelduck *Tadorna ferruginea* (n=4,001), Great Cormorants *Phalacrocorax carbo* (n=3,996), Black Headed Gull *Larus ridibundus* (n=3,691) Euraisan Wigeon *Anas penelope* (n=3,387) and Bar-headed Goose *Anser indicus* (n=2238). A maximum total of 1,26,537 birds were observed at the site. Ever increasing human population, hunting, trapping, lack of awareness, deforestation in catchments area and agrochemical residues were the main problems in conserving the wetland and waterfowls at Mangla Dam. The research work demonstrated this important fresh water site must be treated according to international wetland standards to conserve the unique fauna of the migratory birds and to cope with major threats.

**ILLEGAL HUNTING OF INDIAN PANGOLIN (*MANIS CRASSICAUDATA*) IN  
POTOHAR REGION: A CASE-STUDY FROM CHAKRI AREA, RAWALPINDI,  
PAKISTAN**

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MUHAMMAD RAIS, IFTIKHAR HUSSAIN, AND MAQSOOD ANWAR

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Indian pangolin (*Manis crassicaudata*), is a “Near Threatened” solitary mammal facing high risk of extermination in its wild habitat, where illegal hunting is one of the major intimidation to its survival. The current case study is a part of an ongoing HEC-funded research project and was carried out at “Chakri site”, located at 50 km away in the south-west of Rawalpindi city. The study

area was thoroughly surveyed for occurrence of animal and its indirect signs like its burrows and faecal matter. In addition, local people, nomads and hunters were also interviewed. During surveys, six dead specimens of Indian pangolin including one dead body without scales; two complete skeletons along with outer covering of skin; one skeleton having vertebral column and pelvic girdle; one skeleton having vertebral column only and one with disarticulated bones of the animal, were recovered from the study area (Sihal), especially from around the nomads huts. The information generated revealed killing of overall at least 16 individuals of the species from September to December 2011 (4 months); Two separate hunters groups and nomads in the area were found involved in the killing and illegal trade of the species. One hunters group killed four individuals of the species and a scale jacket of the animal was also recovered from this group. Another group of hunters also killed four individuals. Nomads in the area were also involved in the illegal trapping and killing of the animal species, since six dead specimens were recovered from the vicinity of their huts. Moreover, one individual was thrown away by nomads after amputation of scales. One juvenile of the species was also live captured from the same area by our team. The killing of the animal species at large level during three months is a strong evidence of frequent illegal hunting of Indian pangolin in the study area. Nomads and local hunters are directly involved in capturing, killing and illegal trade of the animal species with selling price at the rate of 8000-10000/- rupees per animal. However, at the moment, the trade chain remains undetected. The study reveals that the particular animal species is worthy for its scales which have different uses; making necklaces for domestic animal to save them from evil spirits, use in traditional medicines by local Hakims and also manufacturing of bullet proof jackets. It is recommended that immediate conservation measures should be taken along with the strict implementation of wildlife laws, to save this vital species in the study area.

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**HABITAT AND DIET COMPOSITION OF INDIAN PANGOLIN (*MANIS CRASSICAUDATA*) IN DISTRICT ATTOCK OF POTOHAR PLATEAU, PAKISTAN**

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Indian pangolin (*Manis crassicaudata*), the only member of Pholidota in Pakistan, is distributed locally in the country including the Potohar Plateau. The current study investigated its habitat characteristics and diet composition at two selected study sites in district Attock, from September 2010 to August 2011. Habitat analysis was carried out by quantification of trees, shrub and herbs species, by estimating feeding and sleeping burrows of the species and occurrence of ants colonies in selected sites. Trees were quantified by using "Point Centered Quarter" method while "Quadrat Method" was used for shrubs and herbs. Diet composition was investigated by faecal analysis (N = 50). Results showed that *Acacia modesta* had highest Importance Value Index (IVI=177.83) among different tree species, followed by *Capparis decidua* (IVI=107.37), *Ziziphus mauritiana* (IVI=103.75), *Salvadora oleoides* (IVI=39.21), *Prosopis juliflora* (IVI=30.48) and *Dalbergia sissoo* with the lowest (IVI=29.48) at both sites. Relative density of trees (33.33±14.45) was found higher in site-I than at site-II. Relative density, and frequency of shrubs species were higher at site-II while relative density, frequency and cover of herbs species were found greater at site-I. Physical parameters of feeding and living burrows (depth, and diameter) of the animal

species were also recorded. Faecal analysis showed body parts of ants (heads, legs, abdomens and egg shells), plant matters, stones, sand and clay. Ants body parts and clay were recovered from all samples analyzed, sand from 86%, plant matter 33%, egg shells 20% and stones from 10% samples. Clay recovered from the faecal samples constituted highest % V ( $50.84 \pm 2.76$ ), followed by ants body parts ( $30.3 \pm 4.73$ ), and sand ( $24.56 \pm 4.09$ ) while stones ( $0.20 \pm 0.14$ ) constituted the lowest volume. Identification of the prey items recovered from faecal samples with reference prey species indicated two ants species (*Camponotus confucii* and *Camponotus compressus*) and one termite species (*Odontotermis obesus*) consumed by Indian pangolin in the study area.

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**NATURAL RESOURCE USE AND STRESS ON LOCAL BIODIVERSITY WITH ECO-TOURISM DEVELOPMENT AT TAUNSA BARRAGE WILDLIFE SANCTUARY (TBWS), PAKISTAN**

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The biodiversity of an area influences many aspects of the lives of people who reside in it. The consumptive and productive cost of biodiversity is directly linked to public concerns in traditional communities. The basic purpose of conducting this study (2009-2011) was to determine the livelihood conditions of the people of three villages (Bait Qaim Wala, Allahwali Basti, Jannu) and their level of dependency on natural resources of Taunsa Barrage Wildlife Sanctuary, Pakistan. To study the socioeconomic status, Participatory Human Resource Interaction Appraisal and for biodiversity assessment, different census methods were used. The result shows that mega biodiversity that was observed n=82 species of flora and in fauna identified fish (n=21 species), amphibians (n=5 species), reptiles (n=6 species), mammals (n=14 species) and n=171 were birds species. The generally high illiteracy rate (17.8 percent) in the surroundings of TBWS was estimated. Overall the local community was dependent on wetland products as for use of fuel wood (77 percent), grazing (100 percent), fishing (90 percent), hunting, freshwater turtles trading (10 percent) and others 9 percent. The villagers of Bait Qaim Wala (75 percent) and Allahwali Basti (80 percent) were dependent on wetland products for their livelihoods while the resident of Jannu was mainly dependent on agriculture (80 percent). Mostly people (72 percent) live in joint family system; on the whole most of the villagers live in mud built houses (42 percent), and typical means of transportation in the area was still animal driven cart (25 percent); while according to income levels, 6 percent were ultra poor, 35 percent household extremely poor, 35 percent were non poor. Survey reveals that the monthly fuel wood consumption per house hold was 540 kilo gram. Almost all household (95 percent) had own livestock. Livestock was an extra source of income. As alternate resource development for livelihood was livestock (20 percent), and tourist facilities at taunsa barrage towards down stream could be on option. Therefore, it is important to reorganize the social sector and help local communities to fully benefit from the potential of ecotourism to generate additional income and employment opportunities. So it was concluded that community participation for the conservation and sustainable use of the natural resources in the wildlife sanctuary will gave a new strength to biodiversity and will maintain the function of the protected area.

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**POPULATION AND DIET OF MIGRATORY COMMON STARLING (*STERNUS VULGARIS*) IN DISTRICT SIALKOT, PAKISTAN (2010-11)**

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Common starling (*Sturnus vulgaris*) is a winter visitor to different areas of Pakistan, however, information is lacking about its winter activity in the country. The current study investigated its arrival and departure dates, population and diet composition in district Sialkot. Results showed that the species arrived in the study area during first week of November 2010 while its departure was recorded in the last week of February 2011. Its average population density in study area was 26.29 birds per km<sup>2</sup> whereas its population was variable in different study sites and during different months, with highest population in January 2011 (39 birds/ km<sup>2</sup>). Sizes of feeding flocks rarely exceeded 50 birds while size of roosting flock was up to 500 birds per flock. Foraging observations in the field showed that it mainly consumed wheat, lentil, *Trifolium* and *Brassica* crops, among vegetables, pea and potato fields were visited more in irrigated form than non-irrigated ones. The bird species was also found to visit non-cultivated fields, rice mills and garbage sites. For night time roosting, common starling selected dense leafy vegetation like dense patches of bamboos, sugarcane crop fields, and dense patches of *Eucalyptus* in the area. During November (2010), December (2010) and early January (2011), common starlings were observed to feed on wheat sprouting and lentil grains and also found to catch insects from these crops. Analysis of gizzard contents showed invertebrates, on average, 41.68% while seeds and other plant matter collectively constituted 58.03%. Among invertebrates were mostly insects including coleopterans, hymenopterans and members of hemiptera. Seeds and other plant matter consisted of freshly plucked seedlings or sprouting of wheat crop and partially as well as completely undigested wheat, lentil and rice grains.

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**DIET COMPOSITION OF ASIATIC JACKAL (*CANIS AUREUS*) IN MARGALLAH HILLS NATIONAL PARK ISLAMABAD, PAKISTAN**

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Asiatic jackal (*Canis aureus*) is a wild carnivore, categorized as "Near Threatened" in the country. The current study investigated its diet composition by scat analysis in Margallah Hills National Park, Islamabad. A total of 90 scat samples of the species were collected from three different selected sites and analyzed. Undigested food items recovered were segregated and identified by comparison with reference slides of the wild and domestic prey species in the same area. The medullary and scale patterns of hair recovered from scats were used to determine prey species consumed by the jackal. Analysis revealed, on average, 27 different food items including animal matter (46.47 % by volume), plant matter (25.08 %), soil (22.42%) and anthropogenic

material (5.35%). Animal matter included both wild prey (like rodents, and mongooses) whereas domestic prey species were poultry birds. Mammalian prey species were common in the diet and jackal also scavenged on wild boar and domestic livestock. Grasses, wheat, tomato, berries, grams, melon, water melon and orange were among the plant matter consumed by the jackal. Seasonal variation in diet composition of the species showed variation in the consumption of animal and plant matter but animal matter was dominant in all seasons. Prey species Richness (S) was found maximum (17) in summer and least (11) in autumn. Diversity Index (H') of the prey species was highest in summer (2.35) and least during autumn (2.12) season, while the Evenness Index (E) of the prey species was higher during winter (0.9) and least during summer season (0.82).

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**BURROW CHARACTERISTICS AND FOOD HOARDING OF THE LESSER  
BANDICOOT RAT (*BANDICOTA BENGALENSIS*) INHABITING AGRO-ECOSYSTEM  
OF CHAKWAL, PAKISTAN**

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In conformant to many other ecologies of south Asia, the lesser bandicoot rat (*Bandicota bengalensis*) is a major rodent pest in agriculture crops of Potohar Plateau, Pakistan. Beside damage to growing crops, underground food hoarding has also been documented across many ranges of its distribution. Since knowledge of food and shelter play significant role in devising strategies for effective management of a rodent pest population, the present study was therefore, conducted to explore characteristics of burrow system and food hoarding potential of the bandicoot rat inhabiting agro-ecosystem of district Chakwal, Potohar Plateau. There were excavated 30 burrow systems from randomly selected crop fields (8 in sorghum crop, 8 in groundnut, 5 in wheat and 9 burrows on crop fields' embankments) during their respective growth periods. Each burrow system was measured for: number and diameter of burrow openings, length, diameter and depth of main burrow tunnel, number of chambers and amount of hoarded materials. The results are given in Mean  $\pm$  SE and the values that do not share a letter are significantly different. The number of burrow openings were recorded as  $2.4 \pm 0.3^A$ ,  $2.0 \pm 0.3^{AB}$ ,  $2.8 \pm 0.4^A$ ,  $1.6 \pm 0.2^B$  in sorghum, groundnut, wheat and field embankments, respectively ( $F_{3, 26} = 3.90$ ,  $P = 0.02$ ). The diameters of burrow openings were measured to  $8.6 \pm 0.4^A$  cm in sorghum,  $9.0 \pm 0.3^A$  cm in groundnut,  $8.8 \pm 0.8^A$  cm in wheat and  $7.2 \pm 0.3^B$  cm in field embankments ( $F_{3, 26} = 4.28$ ,  $P = 0.014$ ). Length of main burrow tunnel was recorded as  $4.4 \pm 0.3^{AB}$  m in sorghum,  $3.5 \pm 0.2^B$  m in groundnut,  $2.1 \pm 0.3^C$  m in wheat and  $4.9 \pm 0.6^A$  m on field embankments ( $F_{3, 26} = 7.96$ ,  $P = 0.001$ ). The diameter of main burrow tunnel was measured to  $8.9 \pm 0.2^B$  cm in sorghum,  $10.6 \pm 0.4^A$  cm in groundnut,  $8.2 \pm 0.7^{BC}$  cm in wheat and  $7.6 \pm 0.4^C$  cm in field embankments ( $F_{3, 26} = 10.0$ ,  $P = 0.000$ ). Depths of the burrows were measured to  $15.2 \pm 1.5^A$  cm in sorghum,  $11.1 \pm 0.7^B$  cm in groundnut,  $14.5 \pm 1.8^A$  cm in wheat and  $7.0 \pm 0.3^C$  cm in field embankments ( $F_{3, 26} = 13.2$ ,  $P = 0.000$ ). Mean number of chambers recorded in burrows excavated from sorghum, groundnut, wheat and field embankments were  $1.9 \pm 0.4^A$ ,  $1.6 \pm 0.3^A$ ,  $2.0 \pm 0.3^A$  and  $1.7 \pm 0.2^A$ , respectively ( $F_{3, 26} = 0.36$ ,  $P = 0.781$ ). Hoarding of nesting and food materials was recorded in 28 (93.3%) burrows. The mean dry weight of the hoarded food material was  $14.4 \pm 1.1^{AB}$  g in sorghum,  $18.7 \pm 0.8^A$  g in groundnut,  $10.5 \pm 3.4^B$  g in wheat ( $F_{2, 16} = 4.60$ ,  $P = 0.026$ ). Since the hoarded material retrieved from burrows on field embankments contained only grasses therefore their weights were not measured. The combined data of all the 30 burrow systems revealed that mean number of burrow openings was 2.1

$\pm 0.2$  with diameter of  $8.3 \pm 0.2$  cm. Mean length of burrow tunnel was  $8.9 \pm 0.3$  m with mean diameter of  $3.9 \pm 0.3$  cm and mean depth of  $11.5 \pm 0.8$  cm. There were  $1.8 \pm 0.1$  numbers of chambers per burrow system and dry weight of hoarded food material was recorded  $14.7 \pm 1.2$  g per burrow system. The results help to infer that burrow building behavior of this rodent pest species is influenced, to some extent, by the prevailing field crops/vegetation.

#### **THE FEEDING HABITS AND GROWTH OF *TILAPIA RENDALLI* AT FLAG BOSHILO DAM, SOUTH AFRICA**

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Flag Boshielo Dam is an oligotrophic dam without any macrophytes, yet it supports a significant population of a macrophytes feeder, *Tilapia rendalli*. The diet of *T. rendalli* was investigated by examining the frequency of occurrence of different food items in the stomach of the fish over a period of twelve months. A size related dietary shift was evident. The diet of juvenile fish (<5 cm) was dominated by zooplankton and the diet of adult fish (>15 cm) was predominantly marginal vegetation, particularly *Cyperus sexangulasris* and *Panicum schinzi*. However, dietary overlaps between the different size groups were observed. The diversity of food items increased with fish size until the fish were 15 cm and thereafter declined as the fish predominately fed on marginal vegetation. Scales were used to determine the age of *T. rendalli*. Age at length data was fitted to the Von Bertalanffy growth model, which showed that males grew faster and attained a larger size than females. The growth of *T. rendalli* in Flag Boshielo Dam was comparable to those reported in other dams with macrophytes. It is therefore inferred here that food is not a limiting factor in the growth of fish in lentic ecosystems.

#### **ECOLOGICAL IMPACT ON THE STATUS OF MARSH CROCODILE (*CROCODYLUS PALUSTRIS*) IN CHOTIARI RESERVOIR (SANGHAR) SINDH-PAKISTAN**

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The Marsh Crocodiles perform an integral role in maintaining the structure and function of fresh water ecosystem since they are the top predator and keystone species within their natural environment. The Crocodylians which are well known and wide spread occupants of tropical and subtropical habitats. Historically they were heavily hunted for their valuable hides and numbers were severely reduced almost worldwide. In recent years, through combinations of trade controls and innovative applied conservation measures, Crocodiles are thriving and numbers are back to carrying capacity throughout much of their range. The Marsh Crocodiles are bio indicator which really focused attention on the major issues of the pollution which are directly concerned with the human health and life by using the polluted water. Present study on the Bio-ecological status and distribution of Marsh Crocodile or Muggier (*Crocodylus palustris*) was carried out from January 2006 to June 2009. 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. A study on some physico-chemical parameters were also carried out at several sites to know the ecological status and water quality of species.

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**THE PRECARIOUS STATUS OF MARSH CROCODILES (*CROCODYLUS PALUSTRIS*) AND THEIR FUTURE SURVIVAL IN CHOTIARI RESERVOIR**

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The Marsh Crocodiles are often the largest predators and scavengers in their ecosystem, and as such, they contribute greatly to keeping rivers and lakes clean, and maintaining a good variety of fish. As a 'key-stone' species, their presence helps to provide the stability and a natural balance by regulating some prey species such as rats and snakes. The Marsh Crocodiles also eat a number of predatory fish allowing other fish species to flourish. They also eat dead or dying animals, recycle nutrients and keep channels and waterways open during the drier months. 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 km<sup>2</sup>. The reservoir has been constructed on the east side of the Nara Canal. In the flood season the Ranto canal has filled the Chotiari Reservoir. The study on the precarious status and future survival of the Marsh Crocodiles (*Crocodylus palustris*) was carried out from November 2007 to September 2009 during which Crocodiles were recorded from Chotiari Reservoir and its surroundings with an estimated population of Marsh Crocodiles. The major threats have been faced to Marsh Crocodile (*Crocodylus palustris*) obsessed the construction of irrigation networks and illegal skin trading, hunting, environmental changes, habitat destruction have been changed the status and considered as an Endangered Species (CITES) in Pakistan. Through anthropogenic activities and some extent reduced water release up-stream of their habitat.

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**INSULTS OF URBAN POLLUTANTS TO RIVER RAVI AFFECT GROWTH OF THREE INHABITANT FISH SPECIES ADVERSELY**

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River Ravi, Pakistan resumes a wastewater carrier role, while passing through the city, Lahore. Growth profiles of *Catla (C) catla*, *Cirrhinus (C) mrigala* and *Labeo (L) rohita* netted from three downstream polluted sites (Shahdera=B; Sunder=C and Head balloki=D) and compared with those collected from a less polluted upstream site (Siphon =A) during low and high flow seasons in this study. Weights and total lengths of sampled specimens were not significantly different ( $P>0.05$ ) in different seasons and sites. Condition factor (K) in *C. mrigala* was estimated as 0.97 to 1.05 g/cm<sup>3</sup>, 1.03 to 1.18 g/cm<sup>3</sup> for *L. rohita* and for *C. catla* it was 1.19 to 1.27 g/cm<sup>3</sup>. Growth coefficient (b) measured highest with values of 3.19 and 3.16 for *C. mrigala*, 3.21 and 3.17 for *L. rohita* and 3.16 and 3.11 for *C. catla* sampled from the upstream location A, while lowest

values of the parameter viz, 3.08 and 3.07 for *C. mrigala*, 3.08 and 3.06 for *L. rohita* and 3.03 and 3.01 for *C. catla* appeared at site C during high and low flow seasons, respectively. The 'b' results ( $P < 0.001$ ) represented positive allometric growth pattern with lower values during low than the high flow season for the sampled species. Reduction in 'b' value in downstream polluted sites indicated adverse effects of aquatic pollution on fish growth.

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#### NUTRITIONAL COMPARISON OF THREE FARMED FISH SPECIES RAISED UNDER THE SAME CONDITIONS

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Proximate composition (Crude protein, fat carbohydrate, ash and moisture contents) of three farmed fish, were determined. Nine specimen of each species, *Cirrhinus (C) mrigala* (364-898 g), *Labeo (L) rohita* (332-878 g), *Catla (C) catla* (376-882 g) were collected from Punjab University Research Farm. Weight and length were not significantly different ( $P > 0.05$ ) among sampled species. However, *L. rohita* was highest in crude protein (19.30 %) content but lowest in fat (1.71 %), carbohydrates (1.85 %) and moisture (75.96 %). Whereas *C. catla* was highest in fat (2.16 %), moisture (77.26 %) and ash (1.36 %) contents but lowest in crude protein (16.86 %). The ash (0.96 %) were lowest while carbohydrates (2.82 %) were highest in *C. mrigala*. In conclusion, *L. rohita* showed significantly higher protein and lowest fat and moisture contents than *C. catla* and *C. mrigala* and is declared nutritionally better than the other two species when cultivated together.

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#### METALS' PROFILE OF RIVER RAVI WATER RECEIVING THE LAHORE ORIGIN EFFLUENTS

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To assess the impact of urban and industrial effluents, concentrations of Cd, Cr, Cu, Fe, Pb, Zn, Mn, Ni and Hg in river Ravi before and after passing through the Lahore were studied. Water samples (triplicate) were collected from three polluted downstream sites (Shahdera=B, Sunder=C and Balloki headworks=D) during high and low flow season and compared with less polluted upstream site (Siphon=A). All metals concentration increased up to site C, then the metals' concentration more or less stabilized in water sampled at site D and rather showed a small recovery as compared to third sampling point. The trend of mean metals' concentration was significantly higher during low flow than high flow at all sites. The metals' concentrations ranged from 0.07 mg/l to 0.10 mg/l cadmium, 2.26 mg/l to 3.71 mg/l chromium, 3.38 mg/l to 3.77 mg/l copper, 37.01 mg/l to 42.53 mg/l iron, 0.94 mg/l to 1.14 mg/l lead, 22.12 mg/l to 25.16 mg/l zinc, 4.29 mg/l to 6.52 mg/l manganese, 1.20 mg/l to 1.53 mg/l nickel and 0.87 mg/l to 1.19 mg/l mercury content, when the date of both the flow seasons was pooled. The mean mercury concentrations approached 0.14 mg/l and 0.12 mg/l at site A which increased to 107% and 25 % at site B, 1700% and 1317 % at site C, and 1185% and 1177 % at site D during low and high flows, respectively. It was found that urban sewage and industrial discharges created worst water qualities during low flow of the river. The high metal load in the river water is of immediate concern for riverine fish consumers. Recreational and irrigative use of such waters also poses health risks.

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**METALS CONTENTS OF LAHORE ORIGIN EFFLUENTS INFLUENCED SEDIMENT OF RIVER RAVI**

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The present study was conducted to investigate concentrations of Cd, Cr, Cu, Fe, Pb, Zn, Mn, Ni and Hg in sediment of river Ravi, Pakistan. River bed sediment (triplicate) samples were collected from three polluted downstream sites (Shahdera=B, Sunder=C and Balloki headworks=D) during high and low flow seasons and compared with less polluted upstream site (Siphon=A). All metals' concentration increased up to site C, and then stabilized more or less in sediment sampled at site D and rather showed a small recovery as compared to third study area. The trend of mean metals concentration was significantly higher during low flow than high flow at all sites. About 917% and 876 % (cadmium), 461 and 319 % (chromium), 300 and 303 % (iron), 254 and 286 % (lead), 463 and 548 % (zinc), 170 and 123 % (manganese), 723 and 431 % (copper), 853 and 874 % (nickel), and 1699 and 1442 % (mercury) are indicative of urban pollution increases at site C than A during low and high flow respectively. This information provides a useful reference of heavy and other metals contamination of the river Ravi for river management.

**FEEDING HABITS OF *HEMIRAMPHUS ARCHIPELAGICUS* COLLETTE AND PARIN, 1978 (FAMILY: HEMIRAMPHIDAE) FROM KARACHI COAST**

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Feeding habits of *Hemiramphus archipelagicus* (Hemiramphidae), Collette and Parin, 1978 in Karachi coast, were studied based on the analysis of stomach contents. Monthly samples were taken from September, 2010 to December, 2011 from Karachi Fish Harbour. Stomach contents of 333 fishes (male n = 200, female n = 133) were examined. Eleven food categories were identified: Bivalvia, Mollusca, Cirripedia, Amphipoda, Isopoda, Brachyura, Crustacea, Polychaeta, Pisces eggs, unidentified animal materials, plant materials and unidentified materials. Of these prey categories, the results showed that *H. archipelagicus* is an omnivorous predator and diet depends upon the availability of local prey species, especially in intertidal areas. The proportions of food items in the diet composition of the *H. archipelagicus* in the 145-185 mm and 195-235 mm length classes were found roughly equal.

**SEASONAL VARIATION IN LENGTH-WEIGHT RELATIONSHIP AND RELATIVE CONDITION FACTOR OF *SARDINELLA GIBBOSA* OF BALOCHISTAN COAST**

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The present study describes the length-weight relationships (LWR) as well as the condition factor of Sardine fish (*Sardinella gibbosa*) from the Balochistan coast. The fish were collected

monthly from fisherman during the month of August ,2010 to July, 2011. Log transformed regression was used to determine the growth of specimens (n=351). The regression coefficient 'b' of the relationship has a value b=3 which is when compared with an isometric slope b=3 show that growth of *Sardinella* is positively allometric. It was concluded that body proportion changed as fish grew in size. Result showed highest mean length of fish of 19.5±0.68 cm in winter season and lowest of 14.5±0.95 cm in spring, the highest and mean weight of 80±4.266 cm and 45.5±2.65 respectively, were recorded in spring. The condition factor (k) average to 1.29 ±0.18. the mean condition factor was highest in summer 1.49±0.078 and the lowest also in summer 1.05±0.007. The relationships between condition factor and log weight and condition factor and total length were highly significant.

#### COMPARISON OF GENETIC DIVERSITY AND PHYLOGENETIC ANALYSIS OF THE PHEASANTS SPECIES OF THE NORTHERN PAKISTAN

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Pheasants belong to family Phasianidae and order Galliformes. During the present research, genetic diversity (GD) and phylogenetic analysis of different pheasant's species of the northern Pakistan have been conducted. For this purpose, total genomic-DNA was isolated from blood for Polymerase Chain Reaction (PCR) using ten Simple Sequence Repeat (SSR) primers. The results of genetic diversity revealed low to medium level of DNA based affinity in terms of GD (4–57%) among them. Maximum GD of 57% was estimated when compared the Himalayan monal female, *Lophophorus impejanus* (Latham) with blue-peacock male, *Pavo cristatus* (L.) which followed by GD of 54% was compared the white crested-kalij male, *Lophura leucomelana* (Latham) with western-tragopan female, *Tragopan melanocephalus* (Gray). Two comparisons, male *L. impejanus* with female cheer-pheasant, *Catreus willachii* (Hardwicke) and male *T. melanocephalus* with female *C. willachii* showed minimum GD 4%. Phylogeny of the species was analyzed through constructing a dendrogram on PCR based amplification of DNA-data showed that the pheasant-genotype sorted into three main, A, B and C. All the male and females of each species clustered into their own groups, i.e., koklas pheasant, *Pucrasia macrolopha* (L.) male and female clustered into group A; *L. impejanus*, *T. melanocephalus* and *P. cristatus* clustered into group B and *L. leucomelana* male and female were clustered in group C. It is concluded that the male and female *C. willachii* were sorted into different clusters, i.e., groups C and B, respectively, which need further scientific elaborations.

#### EXPLORING THE FISH FAUNA OF UPPER SWAT RIVER, NORTHERN PAKISTAN

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The Swat River is a historical River. Swat River with a peculiar combination of fish fauna. A survey of fish fauna of River Swat at Khwaza Khela to Qandil was conducted during April to

September (2010). In this collection 9 species of fishes were identified, that belong to three orders, Cypriniformes, Salmoniformes, Siluriformis and 4 families, Cyprinidae, Sisoridae, Balitoridae and Salmonidae. Among the families, the family Cyprinidae was the richest and represented by 4 species viz: *Crossocheilus diplocheilus*, *Schizothorax richardsonii*, *Cyprinus carpio* and *Carassius auratus*. Family Balitoridae was represented by 3 species viz: *Schistora alipidota*, *Triplophysa choprai* and *Triplophysa naziri*, where as Family Salmonidae by *Oncorhynchus mykiss* and Family Sisoridae by *Glyptosternum reticulatum* were represented by single species each. Comparison of size of the collected fish species revealed that *Oncorhynchus mykiss* was the largest with 20 cm, whereas *Triplophysa choprai* was the smallest with 8.2 cm. It is concluded that the great diversity of fish species is found in Swat River with great variation in their morphology. More research is required to explore the fish fauna of Swat River.

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**EFFECTS OF MARBLE INDUSTRIAL POLLUTION ON WATER QUALITY AND FISH POPULATION OF RIVER BARANDU IN DISTRICT BUNER, PAKISTAN**

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The studies were conducted to investigate the effects of marble industrial pollution on water quality and fish fauna of River Barandu in district Buner. The water and fish samples were collected on monthly basis from different sites i.e, upstream, industrial site and downstream from December 2010 to March 2011. The physiochemical parameters of water are: temperature (10.80-19.47 °C), pH (7.64-8.76), electrical conductivity (68.39-93.21 µS/cm), alkalinity (140.30-461.50 mg/L), hardness (188.77-513.07 mg/L), dissolved oxygen contents (4.64-7.83 mg/L), total dissolved solids (259.5-378.3 mg/L), total suspended solids (24.43-2929.80 mg/L) and Calcium (1.83-176.80 mg/L). All parameters are within the permissible limit except TSS (41.75-2269.75 mg/L) which is above the recommended value of 50-150. Total number of fish were collected at upstream 63, industrial site 23 and downstream 35. It is further investigated that the species *Tor putitora* was not found at industrial site and downstream and *Cirrhinus mrigala* was absent at industrial site only. It is concluded from this study that marble industrial pollution has effected fish population in River Barandu of district Buner. The marble industry should treat the effluents before dumping in the river and it is also recommended that further studies should be required to evaluate the direct effects of the marble effluents on fish fauna of district Buner.

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**CAMALLANUS BOOMKERI NEW SPECIES FROM THE FRESH WATER SNAKEHEADS (CHANNIDAE: PERCIFORMES) IN PANJGUR, BALOCHISTAN, PAKISTAN**

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A new species of camallanidnematode (Nematoda: Camallanidae) is described from the swim bladder of freshwater Ray-finned fish, *Channaorientalis* (Bloch and Schneider, 1801) caught off the Gwarku River at Panjgur (Balochistan). The present new species *Camallanus boomkeri*.

sp. differs distinctly from other congeneric species parasitizing freshwater fishes in South Asia and world other regions, mainly in the structure of the buccal capsule, longitudinal ridges (teeth) and the female caudal end. The new species could be distinguished by body measurements, in the nature of tridents, position of nerve ring and excretory pore, position and length of spicules, number and arrangement of anal papillae, vulva position and by shape of tail end.

### EXPLORING THE PHEASANTS OF DHODIAL PHEASANTRY, MANSEHRA, PAKISTAN

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The pheasants belong to subfamily Phasianinae of the family Phasianidae in order Galliformes. They have moderately large size. A survey of pheasants of Dhodial Pheasantry was conducted during October 2010 to January 2011. In this survey, 33 species (4 sub species) of pheasants were reported, out of which 6 are already identified, that belong to the same order and family i.e., Galliformes and Phasianidae, respectively. Genus *Lophura* was the richest genus and represented by 8 species (4 subspecies); *Lophura imperialis*, *L. edwardsi*, *L. leucomelana hamiltoni*, *L. leucomelana leucomelana*, *L. nycthemera*, *L. diardi*, *L. ignita nobilis*, *L. ignita rufa*. Genus *Syrmaticus* was represented by 4 species; *Syrmaticus reevesi*, *S. ellioti*, *S. humiae humiae*, *S. mikado*. Genus *Tragopan*, *Phasianus*, *Crossoptilon*, *Gallus*, *Chrysolophus* and *Pavo* were represented by 2 species each which are: *Tragopan satyr*, *T. temmincki*, *Phasianus colchicus*, *P. versicolor*, *Crossoptilon auritum*, *C. manchuricum*, *Gallus gallus*, *G. sonnerati*, *Chrysolophus pictus*, *C. amherstiae*, *Pavo cristatus* and *P. muticus* respectively. Genus *Lophophorus*, *Catreus*, and *Polyplectron* were represented by single species each which are: *Lophophorus impeyanus*, *Catreus wallichi* and *Polyplectron bicalcaratum* respectively. In addition 1 hybrid specie: yellow golden pheasant belongs to *Chrysolophus* while 2 hybrids; purple neck peafowl and black shoulder peafowl (*P. cristatus negripennis*), and 2 mutative species: *P. cristatus* albino and *P. cristatus* mutation pied belong to *Pavo* and 1 hybrid male of *Syrmaticus ellioti* and *S. humiae*. Some pheasants missing their male or female and it is necessary to import them.

### DIVERSITY AND DENSITY OF INVERTEBRATE GROUND FAUNA OF AGROECOSYSTEMS OF FAISALABAD

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For this study, twelve fields of sugarcane and wheat located around Faisalabad were sampled randomly using pitfall traps. Specimens collected were preserved and identified using different keys. Among invertebrates, 4526 specimens were collected from sugarcane field and 8877 from wheat field. All the collected specimens were grouped into 11 orders, 23 families, 34 genera and 40 species. Maximum density was recorded in order Collembola (Springtails), family Onychiuridae, Genus *Tullbergia* and species *Tullbergia granulate* while maximum diversity was recorded in Order Coleoptera (Beetles), family Clubionidae (Spiders), Genus *Clubiona* (Spiders).

Collembola was most abundant in sugarcane field than that of wheat field while Isopodes (Pillbugs) were most abundant in wheat field than that of sugarcane field.

### A SURVEY OF PHEASANTS SUFFERING FROM RESPIRATORY DISEASES IN DHODIAL PHEASANTRY, MANSEHRA PAKISTAN

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This study was conducted in Dhodial Pheasantry, Mansehra from November 2010 to February 2011. The purpose of study was to identify the common respiratory diseases in Dhodial Pheasantry. In Mansehra, it is very cold weather and humidity increase therefore; several respiratory diseases of pheasants were exploited. Sometimes, these infection leads to serious disease. All respiratory disease can be identified by its external symptoms. During study time 18 special cases which are (2.4%) of total pheasants (697) of Dhodial Pheasantry were studied in which one pair (0.27%) of Siamese fire back, *Lopura diardi* (Bonaparte, 1856) were infected by chronic respiratory disease (CRD), also four (0.55%) male lady amherest's pheasants, *Crysolophus amherstia* (Linnaeus, 1758) one black shoulder peacock, one peahen, which are 0.27 % and two (0.41%) ring nicked pheasant, *Phasianus colchicus* (Linne, 1758) were infected by chronic respiratory disease and slight fungal infection, Three cheer pheasants, *Caterus walichi* (Hardwicke, 1827) which are (0.41%) were infected by conjunctivitis. Three postmortems were conducted which showed other diseases which are not concern with respiratory diseases. The number of total pheasants died with respiratory disease in 2008, 2009 and 2010 are 30, 12 and 26, respectively. The present study shows that common respiratory diseases in Dhodial Pheasantry are chronic respiratory disease and conjunctivitis, infected birds should be kept in separate cages therefore, it may prevent the spreading of disease.

### CONSERVATION STATUS OF COMMON LEOPARD (*PANTHERA PARDUS*) IN PIR LASORA NATIONAL PARK KOTLI, AZAD JAMMU AND KASHMIR

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Common Leopard (*Panthera pardus*) is listed as critically endangered in Pakistan. Present study was conducted in Pir Lasora National Park from August 2009 to January 2011 with the aims to assess distribution range, human leopard conflicts and their effects on its conservation. To collect these information leopard signs (Pugmarks, scrapes, scats etc.) and questionnaire survey were used. Leopard killed 72 domestic animals during the study period. Goats dominated the kills (46%) followed by the dogs (32%), sheep (17%) and cow (4 %). Most killing were reported during month of June (18%) followed by May (15%) and July (10%). Most of the animals were killed at night (48%) followed by evening (26%), morning (10%) and daytime (16%). Intensified predation on

livestock in summer season differs from patterns of leopard predation elsewhere but reinforces the pattern that large carnivores take more livestock when native preys are most difficult to find and kill. Attacks on human beings were also reported which are increasing day by day. Linear measurements of front and hind pugmarks and strides were classified which indicated that at least 3-5 individuals of leopard were present in the study area (1580 ha). Killing of 12 leopards were reported from 1999-2010, with 3 leopards were killed during 2010. Biotic pressure in the form of wood cutting, forest fire and fodder collection was very high in the study area. Although majority of the respondents didn't believe that leopard is a useful animal, after awareness program 50% people were in favour of leopard existence with 30% negative and 20% unknown attitudes. Main reasons for the livestock depredation was scarce natural prey for the leopards in the park and in summer free grazing of livestock become their easy prey. Increased livestock depredation rate around the villages was poor herding practices. Better awareness about livestock husbandry practices and compensation scheme for killed livestock would be a promising way of reducing livestock losses and conservation of common leopard.

**NEW REMAINS OF *CAEMENTODON OETTINGENAE* (ELASMOTHERIINI, RHINOCEROTIDAE) FROM THE MIDDLE MIOCENE OF PAKISTAN**

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MUHAMMAD AKHTAR

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New material attributed to *Caementodon oettingenae* from the Chinji Formation of the Siwaliks is described and discussed. The identification of the newly discovered dental material is based on the morphometric features of the species. The described specimens reflect brachydonty. The new material provides knowledge on the systematics and contributes our understanding on the anatomic features of the species.

**FOOD COMPOSITION OF COMMON LEOPARD (*PANTHERA PARDUS*) IN MACHIARA NATIONAL PARK, AZAD JAMMU AND KASHMIR**

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MUHAMMAD ALI NAWAZ, TARIQ MAHMOOD AND MUHAMMAD RAIS

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Common Leopard (*Panthera pardus*) is listed as critically endangered in Pakistan. The present was conducted to investigate food habit of common leopard in Machiara National Park, Azad Jammu & Kashmir. For this purpose, leopard scats were collected, analyzed and prey species determined by matching the hairs found in the scats with reference hairs of potential prey species in the park. Twenty three prey species were identified from leopard scats including seven species of large mammals, 11 meso-mammals and five small mammals. Domestic livestock dominated providing 40.43% of their diet followed by meso mammals (29.75%), small mammals (18.97%) and wild ungulates (5.13%). Among domestic animals, dog (12.82%) dominated followed by goat (12.31%) and sheep (11.2%). Among meso-mammals major prey species were Palm civet (5.64%), Yellow-throated marten (5.13%) and red fox (4.1%). Small mammals i.e. rodents comprised 18.97% of the diet in which field mouse (6.15%) dominated, followed by house rat (5.13%) and Murex

vole (4.1%). In terms of biomass consumed, livestock dominated by contributing 45.84 % of the diet with goat as major food (16.18%). Livestock, wild ungulates, meso mammals and small mammals were not significantly different in their diet in summer and winter seasons. Common leopard killed 65 domestic animals during the study period in which goat were maximum (37%), followed by dog (26%), sheep (20%), cow (8%) and mule (3%). Most of domestic animals (66%) were killed in open areas including pastures, forest and agriculture land while 34% killings occurred inside the corral. Most animals were killed at night (51 %), 26% in the evening 17% in the morning and 6% during day time Majority of local people (61 %) showed negative attitude towards leopard while 24% were positive. Decline in wild prey base has turned leopard to feeding on domestic species resulting in higher human-leopard conflict. Adequate natural prey availability is essential for effective conservation of leopard populations in the park.

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#### **DENTAL MORPHOLOGICAL STUDIES OF *DEINOTHERIUM* FROM EARLY LATE MIOCENE OF LAVA, PAKISTAN**

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In this paper, the first discovery of complete cheek teeth of a deinotherium from Lava, Chakwal district, Punjab, Pakistan, has been reported. The material collected from Chinji formation includes damaged mandibular rami along with premolars and molars of both the left and right side. On the basis of large size of molars as well as the presence of a continuous longitudinal ridge in P3, which connects the protoconid with hypoconid, the specimen is being identified as *Deinotherium indicum*.

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#### **POPULATION AND HABITAT OF HIMALAYAN IBEX (*CAPRA IBEX*) IN NAGAR VALLEY, DISTRICT HUNZA-NAGAR**

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Himalayan or Siberian Ibex (*Capra ibex sibirica*) is the most common species of Caprinae which is widely distributed in relatively dry mountains of inner Himalayas, Karakoram and Hindu Kush in Pakistan. In Himalayas, this ibex is found well above tree line in more propitious rocky mountains. The present study was conducted from September 2010 to June 2011 to determine current population density and habitat status of Himalayan ibex in Nagar Valley district Hunza Nagar (36.1706N and 75.0339E) between 1776m and 3988m elevation. Population density was determined through direct observations using the point count method. For habitat use and preference, vegetation survey of study area was conducted by using quadrat method, in addition to noting physical features of habitat. Threats to Himalayan Ibex were also assessed. A total of 458 ibex (194 females, 156 males, 54 yearlings, 74 kids) in winter and 456 ibex (188 females, 147 males, 54 yearlings, 73 kids) in spring were observed in four study sites. Overall population density of ibex in study area was 0.33/km<sup>2</sup> in winter and 0.32/km<sup>2</sup> in spring. Population density was highest (1.57/km<sup>2</sup> in winter) at Sikanderabad site and lowest (0.22/km<sup>2</sup> in spring) at Bar valley site. A total of 47 plant species were recorded in ibex habitat including; three species of trees eight

shrubs, four grasses and 34 herbs. Dominant plant species were *Juniperus macropoda*, *Juniperus excelsa*, *Betula utilis* and *Salix* spp., *Rosa webbiana*, *Hippophae rhamnoides*, *Tamarix gallica*, *Sophora mollis*, *Berberis lyceum*, *Artemisia maritima*, *Ephedra* spp, *Indigofera* spp and *Poa bulbos*. As many as 30.7% of ibex were sighted on steep rugged, 15.3% on smooth slopes cliffy areas and others in grassland, barren areas, forest and snow covered areas. Majority (77.1%) of Ibex were observed at altitudes between 2500-.4000m. The major threats to Himalayan ibex in Nagar Valley included the loss/degradation of habitat due to biotic pressure by the local population i.e. livestock grazing, fuel wood collection, grass cutting for stall feeding, illegal hunting and tourism related activities.

**TISSUE DAMAGE IN FISH SKIN OF *OTOLITHES ARGENTEUS* INFECTED WITH  
*OODINIUM* SP. FROM KARACHI COAST**

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Tissue damage observations are made on the skin infection of the fish *Otolithus argenteus* infected with *Oodinium* dinoflagellate. 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 colour film. This infection severely damaged the skin tissue, common findings were erosion of outer surface, dislocation of muscle fiber, and shrinkage and atrophy which produced large spaces in between the muscles fibers. Hyaline degeneration of muscles fiber was also prominent in some sections. Muscle fibers were severely damaged and fragmented

**FIRST RECORD OF RUSSELL'S CHAIN VIPER (*DABOIA RUSSELLII RUSSELLII*) IN  
DEVA VAT ALA NATIONAL PARK: AN ADDITION TO THE HERPETOFAUNA OF  
AZAD JAMMU AND KASHMIR**

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Russell's chain viper (*Daboia russelii russelii*) is found in the Indus valley from Karachi to Rawalpindi at low altitudes in Pakistan and considered rare. Existing literature does not show presence of this viper species in Azad Jammu and Kashmir territory. A study was conducted to document herpetofauna from August, 2009 to March, 2010 in Deva Vatala National Park, Azad Jammu and Kashmir. For this purpose, study area was divided in four sites i.e. Chumb, Deva, Barmala and Vatala. Visual encounter survey method was used to explore the distribution and general habitat of herpetofauna. Sighting record information and threats to Russell's chain viper were also determined through questionnaire survey. Two live individuals were directly sighted at Chumb, one dead individual at Deva and crawling signs were observed at Barmala and Vatala sites.

Photographs of the sighted snake were taken and got identified. Field guides were used to identify the sighted individuals. Presence of Russell's chain viper was confirmed at all four sites in Deva Vatala National Park (32°51.592-55.327 NL to 74°16.854-24.550 E). This was the first documented record of Russell's chain viper from Deva Vatala National Park. Russell's chain viper was distributed in the study area within about 8.8 km<sup>2</sup> area. It was found in sub-tropical forest in the park where dominant vegetation was; *Butea monosperma Lamk*, *Dalbergia sissoo*, *Acacia nilotica*, *Mangifera indica*, *Trichodesma indica*, *Saccharum spontaneum*, *Cyperus spp.*, *Lantana camara*, *Ziziphus jujuba*, *Dodonea viscosa* and *Carissa opaca*. Questionnaire survey determined that 85% of local people were against the viper, whereas only 15% were in its favour. It also came out during the study that this viper is ruthlessly killed by local people, mainly out of fear.

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**ROLE OF ZIKRI SECT IN THE MARINE FISHERIES DEVELOPMENT OF PAKISTAN AND THEIR BELIEVES, LOCATION, POPULATION, MAJOR ECONOMIC ACTIVITY, ETC.**

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Zikri is one of extreme sect in Islam, what is now considered as a non-Muslim sect all over the world like QadianijAhmedijLahori sects. They do believe that "Imam Mehdi" will be their prophet, whenever, he will be born and do not accept Hazrat Mohammad Salal Laho Elahi wasalm as a Prophet of Allah. Their worship places are very close to Islamic Mosque. It is found that they are thickly populated along the Balochistan coasts, namely Ormara, Pasni, Gawadar and Jiuwny (Iran Border). However, along the Sindh coast they are living in their own community Family villages and relatively small in numbers. The only one village is present from Badin (Indian Border Coast) to Karachi Coast that is situated at Landhi. Where there are 5 villages from Kemari to Sonera Beach (border of Sindh & Balochistan Coast). Survey reveals, that about 50,000 Zikri's are living across the Karachi Coast in the 5 villages, where the average number of houses per village is 1000. On the contrary, in Balochistan they are also living away from the coastal zones i.e Kalat, Khzdar, etc. but in Sindh Province their residence is stickly confined into the Coastal Areas. It is also calculated that more than 10,00,000 Zakri are living in the Baluchistan Province. Their major economic activity of Zakri's is fishing. About 75% of Zikri in Pakistan are involved into the Fishing activities. At the last, but not least, in this paper, it is discussed that why this study is necessary to conduct. The Zakri do not marriage with Muslim. And Muslim also don't like to take food with Zakri at one table. Dasterkhwan, we know, in the boat there is not enough space and time for separate Dasterkhwan. Even Muslims do not take water into the glasses of Zikri's, etc. Hence, these type of social problems are leading to the economics loss. Therefore, the authors attempt to do this study. The 100 interviews reveal that Zikri's believe, honor, recite, learn the holly Quran and advice to their childred to do so. Their every workshop place (ZIKIR GAH) is containing dozens of the Holly Quaran. They accept Quran but very against to offer the Nemaz. Moreover, 95% of the Zikri's are illiterate. Even they can not read Urdu news papers, in turn, can not read the translation of the Quran. It is recommended that they can easily be converted into Muslim if some tell them that Quran is heavy emphasizing to offer the Nemaz

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**POPULATION AND HABITAT OF INDIAN PEAFOWL (*PAVO CRISTATUS*) IN DEV  
A VATALANATIONAL PARK, AZAD JAMMU AND KASHMIR**

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Indian Peafowl (*Pavo cristatus*), also known as blue or common peafowl, is the largest bird of family Phasianidae. In Pakistan it is distributed in north-eastern border of the Punjab, south-eastern Sindh and Deva Vatala National Park in Azad Jammu c.1 Kashmir. The present study was conducted in Deva Vatala National Park to determine population density and habitat status of Peafowl from September 2010 to July 2011. For this purpose, t11 park was divided in to Deva and Vatala Ranges which were further sub-divided in to three habitat types *i.e.* forest areas, cultivated areas and open areas. Data on population density was recorded though direct observations using line transect method and habitat was analyzed through vegetation survey of its habitat using quadrat method. Threats to Indian Peafowl were assessed through questionnaire survey. Overall population density of peafowl in the study mea was 10 birds/km<sup>2</sup>. Population density in open, forest and cultivated areas was 18, 10 and 6 birds/km<sup>2</sup> at Deva and 4, 19 and 4 birds/km<sup>2</sup> at Vatala, respectively which was significantly different among the habitat types. A total of 54 plant species were recorded in peafowl habitat including; 11 trees, 8 shrubs, 22 herbs and 13 grasses. Dominant tree species were *Dalbergia sissoo*, *Magnifera indica*, *Acacia nilotica*, *Acacia modesta* and *Zizyphus jujuba*. Major shrubs included *Adhatoda zeylanica*, *Lantana camara*, *Dodonea viscosa*, *Gymnospora royleana* and *Carissa opaca*. Dominant herbs were *Parthenium hysterophorus*, *Ageratum housfonianum*, *Opuntia dillenii*, *Aerva persica*, *Acroptilon repens*, etc and grasses such as *Saccharum bengalensis*, *Trith;wn aestivum*, *Cynodon dactylon*, *Dasmotachya bipinatta*, *Heteropogan contortus*, *Sorghum helpens*, etc. Major threats to peafowl population reported by respondents in the study area included; habitat degradation (35%), hunting (21 %), disturbance by human activities (24%) and livestock grazing pressure (14%), in addition to predation and water scarcity.

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**EFFECT OF DRY POULTRY WASTE ON THE PHYSICO-CHEMICAL AND FISH  
GROWTH PARAMETERS AT CARP FISH HATCHERY (DISTRICT BADIN), SINDH,  
PAKISTAN**

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To test the effectiveness of introducing dry poultry manure/waste and to study its effect on water quality, growth and survival of exotic carp, *Cyprinus carpio*, a six month study was conducted from March to August 2010 at carp fish hatchery district Badin, Sindh. The poultry manure was maintained in four management regimes: 500, 1000 and 1500 g and control were designated as treatment I, II, III and IV respectively. All treatments were replicated thrice. The physico-chemical parameters like temperature, pH, salinity, total dissolved solids, conductivity and dissolved oxygen of the cisterns were recorded fortnightly with the help of digital portable water quality multiparameter. The significantly ( $p > 0.05$ ) lowest pH and temperature ranges were found (7.9-8.87 and 30.0-34.2°C respectively) from treatment I followed by treatment II (8.1-8.97 and

31.8-35.2°C respectively) and highest values of pH and temperature were recorded from treatment III (8.2-9.1 and 31.5-35.7°C respectively). Salinity and TDS values in different treatments showed slight variations. The significantly lowest conductivity was noted from treatment I (1220-1786  $\mu\text{S}/\text{cm}$ ) followed by treatment II (1300-1890  $\mu\text{S}/\text{cm}$ ) while highest values were obtained from treatment III (1520-2100  $\mu\text{S}/\text{cm}$ ). The dissolved oxygen values were recorded highest from low dose of manure (4.5-4.9 mg/L) while recorded low with the increasing dose of poultry manure. The results of the growth parameters of the experimental fish showed better growth in terms of mean weight gain, percentage weight gain, specific growth rate, survival rate and production from treatment I followed by treatment II and lowest from treatment III. It was concluded that the treatment I with 500 g poultry manure was found to be suitable for both water quality and fish growth parameters.

#### BAT DIVERSITY AND DISTRIBUTION IN PROTECTED AREAS OF PAKISTAN

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Bats are the least studied group of small mammals in Pakistan. We studied their diversity and distribution Margalla Hills National Park (MHNP), Chinji National Park (CNP) and Lal Suhanra National Park (LSNP). Additionally, we studied them in the agroecosystems of central Punjab (CP). We recorded 192 bats that belonged to twelve species, seven genera and four families. Bat diversity ( $H'$ ) values ranged from 1.82 (at MHNP) where nine species were recorded to 1.37 (at CP) where six bat species were captured. *Scotophilus heathii* was recorded from all the sub-areas. *Pipistrellus tenuis* was recorded only from MHNP, *Rhinolophus blassi* from CP while *Rhinopoma hardwickii* and *Scotoecus pallidus* were recorded only from LSNP. *Taphozous perforates*, *Scotophilus kuhlii*, *Pipistrellus Javanicus*, *P. tenuis*, *P. pipistrellus* and *Hypsugo savii* showed and extension in their range while *Rhinopoma microphyllum*, *Megaderma lyra*, *Hippociderous fulvus* and *H. cineraceus* were not recorded during this study.

#### ECOLOGICAL ASSESSMENT OF NARMADA RIVER WITH SPECIAL REFERENCE TO DIVERSITY OF MOLLUSCANS

ZAHOR PIR,<sup>1</sup> SHAIENDRA SHARMA,<sup>2</sup> L. K. MUDGAL,<sup>1</sup> IMTIYAZ TALI,<sup>1</sup> AND ANIS SIDDIQUE<sup>2</sup>

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The molluscans are helpful in purification of water in their capacity to act as scavengers. Narmada river is one of the most important river of India, which covers 98,796 sq. km of total water shed area. Narmada is considered to be life line and west flowing river of state Madhya Pradesh. Limnological study was carried out for a period of eight months from Aug 2009- March 2010 in selected stations of Narmada river. In present study various species of molluscans

belonging to class gastropoda and pelecypoda were recorded. Among gastropods the *Vivipara benglansis* was dominant followed by *Bellamya benglansis* in both stations. Among pelecypods *Perreysia caerulea* was dominant through out the study period. Highest Shannon and Weiver index was observed in station I and lowest in station II. The value of correlation coefficient indicates that there was a moderate positive correlation between the pelecypods and transparency, alkalinity, while the gastropods show significant positive correlation with transparency. A moderate negative correlation was between molluscan diversity and BOD, pH, temperature. The result of the present study emphasizes the importance of conserving the world's freshwater molluscan population, which are declining at an alarming rate through habitat destruction and pollution.

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#### IMPACTS OF URBANIZATION ON DIVERSITY AND DISTRIBUTION OF AVIFAUNA IN LAHORE CITY, PAKISTAN

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Present study was conducted in different parts of Lahore city to evaluate the impact of urbanization on avian diversity. For this purpose, we selected seven diverse habitats viz, Anarkali (Urban site), Botanical Garden University of the Punjab (dense vegetation patch; roosting site), Canal Road (Tree plantation area), Farm land (semi- urban Agricultural Area), Jallo Park (Park vegetation), Shera Kot and Mehmood Booti (solid waste dumping site). A total of 44914 birds were counted, out of which three dominant species viz; *Corvus splendens* (Common Crow), *Milvus migrans* (Kite) and *Acridotheres sp.* (Mynas) comprised of 87.35% of the total avifauna. Common crow, Kite and Mynas consist of 45.23, 24.93 and 17.19 percent of the total abundance data and were present on all sampling sites. Highest species richness (23) was recorded at Jallo Park. Highest diversity indices (Shannon 1.936; and Simpson 0.807) and evenness (0.698) were recorded at the farmland area. Generally, lower richness level was observed in urban site. However, solid waste dumping sites and roosting site showed comparatively lower diversity but highest relative abundance. The results of present study indicated that higher relative abundance of Crow, Kites and Myna is inversely related to the bird species richness, which is indicating that these dominant species are competing for resources with other less abundant. Open solid waste dumping and improper waste disposal practices are favouring the high relative abundance of crow, kite and myna. There is dire need to manage the solid waste problem in Lahore city because it is creating major ecological threat to the integrity of avian assemblage.

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#### PHYSICAL FEATURES OF TWO SANDY BEACHES (CLIFTON AND SANDSPIT) AT KARACHI

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Two sandy beaches: Sands pit (24°48'N, 66°59'E) and Clifton (24°48'N, 67°0'E) of Karachi are discussed for the physical parameters which determine the properties of interstitial environment of these shores. Sandspit is an open beach with small tidal difference and great wave exposure, the

beach is steeply sloping with greater wave action during the monsoon, whereas Clifton is a semi protected very flat and composed of fine sand, wave exposure is moderate and beach slopes very gently. Salinity of sea near shore shows very little fluctuation at both sites. The highest salinity reaches upto 40‰ at Clifton during summer. Stable burrows of macrofauna are found at Clifton while absences of these burrows are noted at Sandspit.

#### **DIVERSITY AND DISTRIBUTION OF EARTHWORMS IN URBAN AREAS OF LAHORE, PAKISTAN**

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Earthworms are one of the most important indicators of soil pollution and play a vital role in maintaining the soil fertility. Distribution of earthworms varies on the basis of physical and chemical properties of soil as well as pollutants. Present study was designed to evaluate the impact of urbanization on the diversity and distribution of earthworm assemblage in different localities of Lahore city, Pakistan. A total of 190 earthworms belonging to six species of genus *Pheritima* were collected from nine different sites located along the canal road with different intensity of urbanization. Maximum number of species were recorded from agricultural area located in University of the Punjab, whereas, minimum number from urban area Fatah Garh. *Pheretimahawayana* was the most dominant species and accounted for 42.6% of the total earthworm fauna. Highest Shannon ( $H'$ :1.386) and Simpson ( $D$ : 0.75) diversity were calculated from ThokarNiazBaig and minimum ( $H'$ : 0.586) and ( $D$ : 0.396) from FatahGarh sampling site. The results of present study are indicating the earthworm diversity and richness is determined by multiple factors such as urbanization, organic matter, soil type, moisture content and pollutants.

#### **STUDY OF CYSTIC ECHINOCOCCOSIS AMONG THE SURGERY PATIENTS OF KHYBER TEACHING HOSPITAL (KTH), PESHAWAR**

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The present study examined the prevalence of hydatid cyst disease among the surgery patients of Khyber teaching hospital (KTH), Peshawar. A nine months survey was conducted from January to September 2011. Out of the 6929 patients admitted in all the surgical wards of KTH, 49 (0.7%) patients were found to be positive for the hydatid cyst disease, including both local Pakistanis and Afghan refugees. The disease was found to be more prevalent (24.5%) among age group from 50- 59 years with the highest age at being 80 years and it went on increasing as age increased. It might be due to the reason that the hydatid cyst disease takes many years to develop completely. The frequency of hydatid cyst disease was significantly higher in females (63.2%) than in males (36.7%). The most affected organ was the liver and the most common symptom was

abdominal pain. Out of total, 69% patients are those who were infected due to an intimate association with the domestic animals. Hydatid cyst disease was more prevalent in rural areas (72%) than in urban ones (28%). Yearly comparison of cyst patients in KTH of 2010 and 2011 was also carried out. The results have demonstrated that prevalence of cyst disease in 2010 was 0.6% while in 2011 (uptil September) it was 0.7% showing a slight increase. Our study excavated some important factors playing a key role in the prevalence of hydatid cyst disease in our area. Unhygienic conditions, intimate contact with domestic animals, uncontrolled slaughtering of sheep & goats, free wandering of the dogs in our localities, improper disposal of dead animals, lack of knowledge and awareness about the disease are some of the highlighted issues for the prevalence. Surgical treatments have been suggested to most of the patients as infected individuals are normally careless in early diagnostics of echinococcosis. Similarly many individuals related with the profession of sheep husbandry or veterinary services are found to be a victim of this disease. Hydatid disease is not contagious and is not transmitted by person-to-person contact. The offices of the public administration have a responsibility to inform citizens about the control of street animals, environmental health, and food hygiene.

#### **ELEPHAS FROM THE UPPER SIWALIKS OF PAKISTAN: NEW COLLECTION**

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The material presented here has two species of genus *Elephas*, *Elephas namadicus* and *Elephas planifrons*. The new material excavated from two localities i.e. Sardhok of the Gujrat District and Kundal of the Mianwali District, Punjab, Pakistan. In the Pliocene and Pleistocene sediments of Pakistan, the proboscidean fossils are widespread and the fossil material is discovered from the sediments belonging to this age.

#### **ETHNO-MEDICINAL USES OF PLANT RESOURCES IN GAHKUCH, DISTRICT GHIZER, GILGIT-BALTISTAN, PAKISTAN**

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The ethno-medicinal survey was carried out to collect information regarding the various traditional uses; especially the medicinal uses of plant species used during summer 2010 in village Gahkuch, Ghizer. Total of 45 species belonging to 24 families were reported as locally used for various medicinal purposes. The mostly used plant species as medicines are *Hippophae rhamnoides*, *Coriandrum sativum*, *Capris sipinosa*, *Berberis rhamnoides*, *Datura stromonium*, *Ephedra jeradiana*, *Medicago sativa*, *Meliolotus officinal's*, *Artemisia meritima*, *Plantago major* etc. Majority of the recipes are prepared in the form of decoction from freshly collected plant parts. Mostly medicines were taken orally. They use medicinal plants for asthma, cough, tonic, abdominal pain, anthelmintic, carminative, snakebite, jaundice, diarrhea, dysentery, hair tonic and cancer etc. As most of the people in the area still depend on herbal medicines to cure different diseases.

Besides the use of plants to treat different human diseases many live stocks are also being treated by herbal medicines like *Artemisia*, *Hippopheae rhamnoides*, *Berberis rhamnoides* and *Solanum nigrum*. Other uses of medicinal plants are as food / fodder, fuel and timber. People aged 50 to 75 have more knowledge about medicinal uses as compared others. The major threats to the medicinal plants are natural disasters, over grazing and cutting for fuel.

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**NEW RECORDS OF *HARMOTHOE* AND *PARAHALOSYDNOPSIS* (POLYCHAETA: ANNELIDA) FROM THE COAST OF KARACHI**

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Three species of scale worms belonging to family Polynoidae Malmgren, 1867 are reported for the first time from Pakistan. These worms were collected from intertidal rocky shores and sandy beaches in Karachi. The three species are: *Harmothoe hirsuta* Johnson, 1897; *H. liaoi* Barnich, Fiege and Sun, 2004 and *Parahalosydropsis arabica* Wehe, 2006 Three species of the genus *Harmothoe*; that is *H. dictyophora* (Grube, 1878), *H. goreensis* Augener, 1918, and *H. imbricata* (Linnaeus, 1767); have already been recorded from Karachi while a single species of the genus *Parahalosydropsis*, namely *H. tubicola* (Day, 1973) is also known to occur in the coastal waters of Karachi. Diagnostic characters of all the three new records are given and illustrated.

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**MAJOR THREATS TO NALTAR WETLANDS COMPLEX AND PROTECTIVE MEASURES FOR ASSOCIATED BIODIVERSITY**

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The country supports over an area of about 1.3 million hectares of wetlands including about 225 significant wetland resources. Nineteen of these have been internationally recognized by the Ramsar Convention Bureau. In case of high altitude wetlands, Hindukush–Himalayan Mountains possess an unlimited number of wetlands, some of these have been explored but most of these are still unexplored. Wetlands are the world's most productive ecosystem which, support high concentrations of birds, mammals, reptiles, amphibians, fish and invertebrate species. Wetlands of Gilgit-Baltistan are welcome sites for migratory birds. Highly productive ecosystems capture energy and provide feeding and breeding grounds for animals. Wetlands support an enormous diversity of plants and animals, including a large number of threatened and endangered species. In the past 50 years the rate of wetland loss has increased dramatically, and is still continuing. Naltar wetland Complex comprises of five small and medium size lakes in 2 sq km area. All these lakes are fed by melting of glaciers in form of spring and streams. The general climate of the Naltar valley is dry temperate characterized by the severe and prolonged winters and short but pleasant summer. This area provides an excellent habitat for wild life in the form of alpine lands, sub alpine scrub and temperate forests. The main threats to the Naltar wetland complex are excessive use of pastures, deforestation, water pollution, Spreading of solid waste, Air pollution, Ill manage tourists, lack of law enforcement, Introduction of exotic fishes, lack of scientific research. Policy measure

for optimum utilization of wetlands resources are mainly based on five principles set by Ramsar Convention Bureau like (1) incentives for local and indigenous people's involvement and wise use (2) trust among stakeholders (3) flexibility (4) knowledge exchange and capacity building, and (5) continuity of resources and effort.

### **HUMAN-WILDLIFE CONFLICT IN DANYORE, SULTANABAD AND MUHAMMADABAD VILLAGES OF CKNP GILGIT-BALTISTAN, PAKISTAN**

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The human wildlife conflict is very common over all in the world. Due to human wild life conflict many of the live stock is loss. As a result local people killed precious wild life. Because local people depend on livestock for their food, milk, wool and income. In this study the intensity of livestock depredation by snow leopard (*Uncia Uncia*) Tibetan Wolf (*Canis lupus chanku*) and Lynx (*isbellina*) in three villages of Gilgit district namely Danyore, Muhammad Abad, and Sultan Abad villages of central Khunjerab national park was find out. The most important Karakoram highway is passing through these villages of CKNP. According to our study 109 live stock were lost during five year from 2007-2011. Total income loss was Rs. 1018000. The snow leopard killed 56 goats which was about 73%, while sheep 21 in number which was 27%. Wolf involved in hunting of goats 57% which was 4 in number, sheep 43% and were 3 in number. Lynx hunted 13 goats which was 52% and sheep 12 in number which was 48%. Among all predators snow leopard was most dangerous predator which killed 71% live stock, lynx is second predator which killed 23% live stock and wolf killed 6% of live stock. Among all live stock, goats were more victims which were 73% and next was sheep with 27%. Beside cattle, donkeys were present in pasture but low in number. This animal was used for transport purposes. Recently one donkey was killed in pasture due to slap. According to 26 people snow leopard was rare, and 8 responded as this was common and 5 people responded as absent. According to 11 people Wolf was common 19 people said rare and 8 people responded as absent. For lynx 10 people said common, 20 said rare and 10 people responded as absent.

### **BURROW MORPHOLOGY OF GHOST CRAB (*OCYPODE ROTUNDATA*) ALONG THE COAST OF KARACHI**

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Burrow morphology of *Ocypode rotundata* was studied on the sandy beaches of Karachi coastline with the aim to identify their significance and relationship to the shore environment. Mostly small burrows occurred near the sea shore and largest size burrows were observed at upper tidal zone and extended up to dry or splash zone. Variations in diameter, length, and in morphology of the burrows together with a decrease in burrow abundance from the foreshore to the back edge of the shore differentiate the shore in sub environments. Significant seasonal difference in burrow density was also observed with low densities of burrows in winter. The low burrow densities were

probably related to a delay crab activities due to low temperatures during winter season. Burrow of *O. rotundata* usually had a single opening oriented towards the sea, mostly straight single tube and had depth range between 60 cm to 150 cm. Strong correlation ( $r^2=94.6$ ) was observed between carapace length and burrow diameter of the living crabs which results that burrow diameter can be good observational technique to estimate crab size. The sediment properties (porosity, organic and grain size) were also analyzed to identify the relationship of crabs with habitat.

**BACTERIOLOGICAL ANALYSIS OF FISHES AND WATER ALONG WITH PHYSICO-CHEMICAL PARAMETERS OF BLUSHBAR HATCHERY, ASTORE GILGIT BALTISTAN PAKISTAN**

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A study was conducted to investigate the bacteriological quality of water and fishes (Rainbow trout and brown trout) of Blushbar Hatchery Astore, Gilgit Baltistan, Pakistan along with physico-chemical parameters in October 2011. A total of 24 fish were collected from 08 ponds of Blushbar hatchery and were analyzed for bacterial contamination on its skin and abdomen respectively. Major bacterial communities found on its skin and abdomen was composed of *Escherichia Coli*, *Pseudomonas*, *Salmonella*, *shigella* and *Proteous vulgaris* respectively. 16 water samples were collected from the same ponds and analyzed for bacterial contamination. The bacterial community of the water was composed of 05 major species of bacteria, *Escherichia Coli*, *Pseudomonas*, *Salmonella*, *shigella* and *Proteous vulgaris* respectively. Also physico-chemical parameters like pH, Temperature, and Dissolved oxygen were assessed on the spot by using different digital probes. Analysis of the results for all samples revealed values for pH (6.5 to 8.0), temperature (6.1°C to 7.9°C), dissolved oxygen (8.90 mg/l to 12.44 mg/l) and turbidity (-5 to -6) respectively. Further seasonal sampling and analysis should be done on this hatchery to determine any additional microbial species and their effects on the health of fishes in order to increase the production to meet the demands of growing population.

**OBTAINING WATER FROM THE HILL SFEW WELLS BY CLOSE SIPHON SYSTEM FOR FJSH SHRIMP FARMING IN PAKISTAN**

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The authors are having rich experience of visiting the remote area of Pakistan. During these visits they observed serious freshwater scarcity at the area like Quetta (Blochistan province), mithi, Omer Coat, Sonehra Beach, Rahri Goath (Sindh Province). Losses due to water scarcity are also documented in this paper. The author further observed that the very high hills are present in the

above mentioned areas. During the visit of Deplo (Mithi, Tharparker) the author founds few well that are having the water table at 200 feet. Extraction of water from these well is not a job of single man. When the authors were standing at this well they saw/found that few villages are present at the bottom of hill. When the surface distance of these village from instant well was measured, it was observed that this well is 20 Km away from these villages. The height of well was measure by the "Level instrument of survey" as compared to level of villages. It is found that the surface of instant well is 300 higher than the instant village (of bottom hill). At that point the author found/think that water could be pulled-up from the instant well for the distanced village that are situated the distance of 20 Km away from the \Coell under question. When we subtract 300 from 200 we will get 100. Hence "100 feet of depth power" can easily..pulled-up the water from 200 feet (well) by the close siphon system. Later a practical was done to do so at Rahri Coastal Village, of Karachi. The experiment was proved very successful. The material and methods is also noted down in this paper. On the ground of this experiment, it is recommended that people can obtain water from well with close siphon system into the hilly area without the use of any kind of man made energy. Once close siphon stats, we can not stop it. Temporarily stopping or breaking of siphon is a very risky job, specially, when the arms of close siphon is in having the length in kilometers. Definitely, at the outer end of siphon one will have to make a cemented tank to stock water for other uses. By exercising this project, one will receive water from the tube-well not for days, but for years continuously. Obviously, one is receiving water for years he will have to make a very "large tank" to stock water. The authors believe that we can use this "Large tank" for fish/shrimp farming into to the water scarcity areas. These "large tanks" will not only support to the fish/shrimp farming but will also develop a rout for dozens of other industry. In turn, what will reduce unemployment and give revenue to the Country. These benefits are mentioned with details in this paper.

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**BACTERIOLOGICAL ANALYSIS OF BROWN TROUT AND WATER ALONG WITH  
PHYSICO-CHEMICAL PARAMETERS OF NALTAR HATCHERY, GILGIT  
BALTISTAN PAKISTAN**

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A study was conducted to investigate the bacteriological quality of water and brown trout of Naltar Hatchery Gilgit Baltistan, Pakistan along with physico-chemical parameters in October 2011. A total of 16 fish were collected from 04 ponds of Naltar hatchery and were analyzed for bacterial contamination on its skin and abdomen respectively. Major bacterial communities found on its skin and abdomen was composed of *Escherichia Coli*, *Pseudomonas*, *Salmonella*, *shigella* and *Proteous vulgaris* respectively. 12 water samples were collected from the same ponds and analyzed for bacterial contamination. The bacterial community of the water was composed of 05 major species of bacteria, *Escherichia Coli*, *Pseudomonas*, *Salmonella*, *shigella* and *Proteous vulgaris* respectively. Also physico-chemical parameters like pH, Temperature, and Dissolved oxygen were assessed on the spot by using different s digital probes. Analysis of the results for all samples revealed values for pH (6.5 to 7.5), temperature (6.4°C to 7.7° C), dissolved oxygen (8.94

mg/l to 12.32 mg/l) and turbidity (-5 to -8) respectively. Further seasonal sampling and analysis should be done on this hatchery to determine any additional microbial species and their effects on the health of fishes in order to increase the production to meet the demands of growing population.

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### IDENTIFICATION OF SPECIES OF BRANCHIOMYCES CAUSING DAMAGE TO FISHES IN CULTURE PONDS OF PESHAWAR REGION

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Two species of *Branchiomyces* that is *Branchiomyces sanguinis* and *Branchiomyces demigrans* were studied in carp fishes (Rohu, Mori, Grass carp and Silver carp) from June to November 2010 at Public sector fish farm (CH&TC) and Private fish farm owned by Miss Fatiha Anjum. To culture spp. of *Branchiomyces* PDA media was used. Two species viz *B. sanguinis* and *B. demigrans* were recorded from carp culture ponds. Silver carps were found more susceptible to *Branchiomyces* infection which shows that silver carp is more sensitive to environmental hazard. The *Branchiomyces sanguinis* was found more common in culture ponds of Peshawar region. The private fish farm was having great mortality due to *Branchiomyces* as compared to the public fish farm. Two parameters that is water temperature and water pH were also measured during fish sample collection. The fish samples were collected with different intervals and the total duration of sampling was six months. To know about a representative value for all the months, their mean value was determined. The mean of water temperature for Public fish farm (CH&TC) at morning and evening time during June to November 2010 were 25.34°C and 28.67°C respectively. While the mean of water temperature for Private fish farm at morning and evening time during June to November 2010 were 24.83°C and 27.83°C respectively. Mean pH recorded for public sector fish farms were 7.76 and Mean pH recorded for private sector fish farm were 6.65.

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### COMMUNITY BIOGAS PLANTS: A PROMISING FUTURE ENERGY SOURCE FOR RURAL PUNJAB

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Energy is the most expensive and limited resource for the world especially for the developing countries like Pakistan. Nowadays public and governmental interest is increasing towards renewable energy sources due to current energy crisis in the country. Community biogas plants can be a good source of energy for the >70% rural population of the region. In order to estimate the kitchen energy needs and cattle manure availability (raw material for biogas plant) a preliminary survey was conducted in the Nankana Sahib and Sheikhpura districts of Punjab province. Survey conducted in three villages at random. The results showed that community biogas plants in villages can produce enough biogas to meet the kitchen energy requirements as abundant quantity of cattle manure is available to feed biogas plant. More than 91% people showed interest

to have biogas plant instead of current sources of kitchen energy. Additionally it can provide good quality fertilizer, will reduce environmental pollution (methane and nitrogen), can reduce cost on other energy sources and ultimately can help in poverty alleviation in rural Punjab.

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**BREEDING HABITS OF RED JUNGLE FOWL (*GALLUS GALLUS*) IN DEVA VATALA NATIONAL PARK, AZAD JAMMU AND KASHMIR**

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Red jungle fowl (*Gallus gallus*) is widely distributed in Asia, however, in Pakistan it is only found in DevaVatala National Park, Azad Jammu and Kaslunir. This national Park is located at 32°54' 50"N and 74°21' 65"E between 306 and 411m elevation. The present study investigated the breeding habits of Red jungle fowl in Devavatala National Park from August-2010 to July-2011. For data collection 10 study sites, five each in Vatala and Deva Ranges were selected in potential habitat of Red jungle fowl. The breeding season at Vatala Range started from March, with peak breeding months of April and May having breeding calls intensity of  $5 \pm 0.44$  and  $5.9 \pm 0.82$ , respectively. Breeding season at Deva Range started in February, with peak breeding months are February, March and April, having breeding calls frequency of  $9.1 \pm 1.74$ ,  $11.4 \pm 3.21$  and  $8.3 \pm 1.99$  calls/minute, respectively. Difference between first call to second call was 14.16 seconds in breeding season and 50-60 seconds or even more in non-breeding season. The harem sizes at Vatala and Deva Range was  $2.8 \pm 0.83$  and  $3.2 \pm 0.93$  females with one male, respectively. The harem sizes varied during breeding and non-breeding seasons; Two active nests were located having 7 eggs and 4 eggs in each. Nests were built on the ground mostly on sloppy areas under vegetation cover. Nesting material used was down feathers and dry leaves. The depth of nest ranges from 2.5-4 inches. The mean number of chicks recorded with a hen was 3.16 and 3.50 at Vatala and Deva Ranges, respectively. Red jungle fowl inhabited dense forest vegetation in the study area. It is concluded that the breeding season of Red jungle fowl is from February to July in this park. Egg poaching is major threat to population of Red jungle fowl in the study area.

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**THE LARVAL DEVELOPMENT OF *METAPLAX INDICA* H.MILNE EDWARDS, 1852 AND *METAPLAX DISTINCTA* H. MILNE EDWARDS, 1852 (DECAPODA: BRACHYURA: VARUNIDAE) UNDER LABORATORY CONDITIONS**

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The ovigerous females of *Metaplex indica* and *Metaplex distincta* captured from Buleji and Sandspith (back waters) on February 03, 2007 and March 24, 2007, respectively and were kept under the laboratory conditions. On February 06, and April 02, 2007 larvae were hatched, respectively. Three zoeal stages of *Metaplex indica* and zoea I of *Metaplex distincta* were obtained at room temperature (27- 34°C), salinity 35 - 41 ppt and pH 7.5 - 7.9. The larvae are described, illustrated and compared with the larval account of *Mdistincta* and *M elegans* given by Krishnan and Kannupandi, (1989) and Pasupathi and Kannupandi, (1988), respectively.

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**STEGOLOPHODON CAUTLEYI FROM PADRI (DHOK PATHAN FORMATION)  
MIDDLE SIWALIKS, JHELUM, PUNJAB, PAKISTAN**

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AKBAR KHAN<sup>1</sup> ZAFFAR HAMEED BHATTI<sup>1</sup> AND SOBIA MUSHTAQ<sup>1</sup>

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Fossil remains of *Stegolophodon* have been described and discussed from the Padri outcrops of the Dhok Pathan Formation, district Jehlum, Punjab, Pakistan. The molar pattern of the studied specimens clearly resembles to the species *Stegolophodon cautleyi* and species found in the Late Miocene of the Siwaliks.

**FARMING AND BIOLOGICAL ASPECTS OF OSTRICH (*STRUTHIO CAMELUS*)**

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The project undertaken includes some of the important considerations of *Struthio camelus* in captivity, its behavior and ecology. The selected parameters of the study are different activities of the animal, such as, feeding and water needs, sexual behavior, egg laying and artificial incubation. This project is an attempt made to bring together the information about this unique bird, that is, the largest and most heavy flightless bird on Earth. The data was collected during a time period of six months, that is, July to December. The study carried out on different age group flocks at the farms of Pakistan Ostrich Company: Mengal goth and Cheeku farm. The farm was visited in the morning to collect the data needed for study of their feeding and breeding behavior. From the studies conducted at the farm it was concluded that the animal has a good tolerance for cold and humid conditions which is one of the reason Ostriches are found in different countries including Pakistan. Ostriches are not selective in feeding; they can browse on any object found on ground, so the rearing environment of Ostriches is kept free from impurities. Ostriches have a silent behavior, they are not shy neither aggressive in normal conditions but mature males become aggressive during breeding seasons.

**OCCURRENCE OF LACUSTRINE ROTIFERA IN FRESHWATER LAKES OF SINDH**

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Rotifera is a diverse group in zooplankton. They constitute natural feed for most of-aquatic vertebrates and invertebrates at their early life stages. Rotifer fauna of four lakes including Manchar, Keenjhar, Haleji and Chotiari Reservoir were studied during 2009. Total eighteen stations were fixed for the collection of water and zooplankton samples. Rotifer samples were collected using a plankton sampler having 55 µ mesh size. Water quality was measured by standard methods. A total of 8 rotifer species were identified from these water bodies. The most dominant genus was *Brachionus* (18 species) whereas other dominant genera included *Lecane* (15 species),

*Monostyla* (10 species) and *Keratella* (6 species). Among these species there are 30 species which are recorded for the first time from Pakistan. This will contribute to the existing rotifer record in Pakistan. The water chemistry showed the quality of Keenjhar, Chotiari and Haleji lakes in better condition while in case of Manchhar Lake salinity, Hardness, Chlorides and TDS were two to three folds higher than the limits suggested by WHO. Trophic State Index (TSI) calculated revealed that all lakes studied fall in the category of eutrophic water bodies. The degree of trophic state however, shows the Manchhar Lake at the highest.

### IMPACT OF SOCIAL VARIABLE ON ECONOMIC PERFORMANCE OF MARINE FISHERIES INDUSTRY

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Honoring commitment, speaking truth, etc are go tools to promote any business. Some of these tools are declining from our society. We are ranked among the world top corrupt nationals. There are numbers of other social ariables that are analyzed in this paper. It is worked out that the improvement in these variables we can increase the total landing of country. For controlling theft on fishing boat one can increase numbers of security guards, to increase interest of employee one can give share to the employee in the total profit, but instead of these steps the landing is decline. The authors concluded that we should send our fisheries employee for 4 month training in the Tubliieghi amat at, Wrai Wind, Lahore, Pakistan. The salary of security guard may be giving to a moulana, who may permanently appoint in a boat for motivation to the employee according to the preaching ofIslam to work hard.

### TEMPORAL AND SEASONAL ASSEMBLAGE OF *POTAMIDES CINGULATUS* (GMELIN) FOUND IN THE MANGROVE CREEK AREA OF KARACHI, PAKISTAN

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An annual study on *Potamides cingulatus* assemblages with environmental and biological parameters was carried out during low tide from two sites in Korangi Phitti mangrove creek area and Sandspit back waters along the coast of Karachi from January to December 1999. Environmental factors in mangrove creek area included water temperature, salinity, pH, and Eh (mv), and the biotic parameters; fiddler crab burrows and pneumatophores (or roots) were also studied. Density of *Potamides cingulatus* ranged between 4 to 664 individual m<sup>2</sup>. Significant temporal variations were observed in density between sites ( $F_{1, 83} = 56.99$ ,  $P < 0.05$ ), and were not significant with seasons and sites nested in seasons. The total mean seasonal distribution showed high density of *Potamides cingulatus* during pre-monsoon and post-monsoon respectively. The total *Potamides cingulatus* densities were significant and positively correlated with the sites, root density, pH water and volume of sediments.

**FUNGAL DISEASES OF SOME FRESHWATER ORNAMENTAL FISHES IN PUNJAB**

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An investigation was conducted on fungal diseases of three imported freshwater ornamental fishes goldfish (*Carassius auratus*), Platy (*Xiphophorus maculatus*) and guppy (*Poecilia reticulata*). A total of 130 fish specimens were dissected, the fungal infection rate in these fish was, goldfish (84.3%), Platy (78.5%) and Guppy (45.1%). Different body parts of fish, gills, head, fins and skin were infected. The fungus was isolated from the infected areas on the fish and cultured on different media, MEA, SDA, PDA. The fungus was allowed to grow for five to seven days at 28°C. After the growth the fungus was observed. Five genera of fungi were identified. *Aspergillus* sp., *Penicillium* sp., *Blastomyces* sp., *Alternaria* sp. and *Mucor* sp. *Blastomyces* sp. was associated with eroded gills of goldfish, *Aspergillus* sp. and *Penicillium* sp. with cotton wool like appearance at the site of infection on body of infected fish. *Blastomyces* sp. attacked the gills of Platy, *Mucor* sp., *Aspergillus* sp. and *Alternaria* sp. found on bloody spots at site of infection on caudal and pelvic fin of Platy. While *Aspergillus* sp. and *Mucor* sp. found on abdomen, caudal fin, pelvic fin and gills of the Guppy. Incidence of *Aspergillus* sp. was (76.2%), *Penicillium* sp. (15.1%), *Blastomyces* sp. (7.9%), *Alternaria* sp. (7.6%) and *Mucor* sp. (3.2%). Incidence of *Aspergillus* sp. was highest in infected fish sample. Reasons for fungal attacked on fish are rough handling of fishes may cause their outer skin peel off thus making it vulnerable to attack of fungus. Dead fish in aquarium can also lead to spreading of fungus in aquarium. Poor water qualities also facilitate spreading diseases. If the temperature is kept too low it may lead to fungal attack also. Cleaning and management of aquarium and maintaining water quality may help in control of fungal infection.

**SPECIES DIVERSITY, SEASONALITY AND BIOMASS OF PENAEID SHRIMPS FROM THE COMMERCIAL LANDING OF SONMIANI BAY LAGOON, BALOCHISTAN, PAKISTAN**ZUNAIRA AMANAT <sup>1</sup> AND NAUREEN AZIZ QURESHI <sup>2</sup><sup>1</sup> *Centre of Excellence in Marine Biology, University of Karachi, Karachi 75270, Pakistan (presently Federal Urdu University of Arts, Science and Technology, Karachi)*<sup>2</sup> *Department of Zoology, Government College University Faisalabad, Allama Iqbal Road, 38000 Faisalabad Emails: [zunaira\\_am@yahoo.com](mailto:zunaira_am@yahoo.com); [naureenaziz.queshi@gmail.com](mailto:naureenaziz.queshi@gmail.com)*

Shrimps contribute to foreign exchange with high profit margins of Pakistan. Sonmiani is an important shrimp fishing center on the coast of Balochistan, which is situated some 90 km. away from Karachi. A considerable numbers of commercially important penaeid shrimp species are abundant in the Sonmiani lagoon due to the plenty of food and water conditions that are favourable for growth of shrimps. The species composition, seasonality, diversity, biomass and size distribution of the penaeid shrimps were studied, based on the shrimp landing data observation from January 2005 to June 2007 obtained from the mole holders or middle men companies in Sonmiani Bay. Total thirteen species were identified in different percent composition, however all species were not persistently present through out the study period only six species were found to be abundant namely: *Penaeus indicus* (28.60%) was the most abundant species followed by the *Penaeus merguensis* (21.97%), *Parapenaeopsis stylifera* (21.91%), *Metapenaeus affinis* (16.95%), *Metapenaeus Stebbingi* (5.84%) and *Penaeus monodon* (0.62%). The species composition varied

with the months and the biomass distribution of shrimp catch as well showed a seasonal pattern in lagoon water.

**MORPHOMETRIC RELATIONSHIP AND SEXUAL DIMORPHISM IN FOUR SPECIES OF FIDDLER CRABS (*UCA*) FOUND ALONG THE COAST OF PAKISTAN**

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Fiddler crabs are notorious for their extreme sexual dimorphism, with female crabs equipped with two isomorphic small chelipeds, and adult males possess dimorphic chelipeds. In male, one of the chelae is enlarged for advantage in intra-sexual competition and mate-guarding activities. In present study, morphometric relations and sexual dimorphism in four species of fiddler crabs inhabiting coastal areas were investigated. The male of *Uca* spp. can either have the left or the right cheliped enlarged mostly defined as dextral (right handed male) or sinistral (left handed male) male crabs. The percent distribution of dextral hypertrophied clawed male was high in *Uca (Tubuca) urvillei* and was nearly equal to the sinistral males of *Uca (Paraleptuca) annulipes*. In *Uca (Paraleptuca) sindensis* and *Uca (Paraleptuca) chlorophthalmus* the percent distribution of dextral and sinistral males were nearly equal. The mean sizes of carapace width (CW) in male and female crabs were significantly different for three species, *U. sindensis*, *U. chlorophthalmus* and *U. annulipes*. The CW of the male and female crabs of *U. urvillei* were not significantly different but showed difference in the mean size of CW of males ( $14.8 \pm 3.9$  mm and  $15.1 \pm 4.4$  mm) and females ( $13.8 \pm 4.3$  mm and  $14.3 \pm 4.6$  mm). Generally, the relative growth showed higher value of the slope 'b' for the male crabs compared to female crabs with positively allometric growth. The relationship between the carapace length and carapace width showed nearly isometric growth for both, male and female crabs of all four *Uca* species. Positive relationship were observed between the weight of male fiddler crabs and the weight of enlarge cheliped of *Uca (Tubuca) urvillei* and *Uca (Paraleptuca) chlorophthalmus* but was isometric for the *Uca (Paraleptuca) sindensis* and *Uca (Paraleptuca) annulipes*.

**DISTRIBUTION OF FISH FAUNA WITH IMPACT OF WATER QUALITY IN RIVER NEELUM AND ITS TRIBUTARIES IN GUREZ, NEELUM VALLEY AZAD JAMMU AND KASHMIR, PAKISTAN**

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Water quality has important effects on fish distribution worldwide. Inappropriate means of transportation, harsh weather condition and firing between Pakistan and Indian armies hinder Neelum valley for researcher particularly from Pakistan and worldwide. The present study aimed to investigate 17 physical and chemical parameters of River Neelum and its associated tributaries and their impact on fish distribution in remote areas of the upper Neelum Valley. Six river sites and 15

tributaries were selected for study along with mixed region of these tributaries with River Neelum. Results revealed that water quality parameters were according to the limit of World Health Organization (WHO) for surface water quality. A significant correlation ( $p < 0.05$ ) was noted between Electrical conductance (EC), Total Dissolved Solids (TDS), pH, while highly significant correlation ( $p < 0.01$ ) was noted between TDS and Total Suspended Solid (TSS). A highly significant correlation ( $p < 0.01$ ) was noted between fish distribution and river water temperature while significant correlation ( $p < 0.05$ ) was recorded between fish distribution and TSS and EC in tributaries water. Highest fish distribution was noted at Surgan and Hanthi tributaries and Sharda site of river Neelum. Local fish industry should be established particularly at Surgan, Hanthi and Tao Butt (Gaggai) tributaries to enhance local income and hence reduce pressure on natural resources.

#### **WATER QUALITY AND FISH FAUNA OF ZALZAL LAKE, CHIKAR, MUZAFFARABAD AZAD JAMMU AND KASHMIR**

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Limnology is one of the preferred fields of study of human beings, through which he is benefited by various ways. Azad Jammu and Kashmir was shaken badly by the earthquake of October 2005 that resulted huge land slide. Such large landslides blocked the water course at Chikkar and resultantly form a lake commonly known as Zalzal Lake. The present work aimed to investigate physio-chemical and biological parameters of the water of Zalzal Lake, including temperature, turbidity, Total Dissolved Solids (TDS), pH, chloride, dissolved oxygen (DO), alkalinity, calcium, magnesium and nitrate. Result revealed that the mean values of temperature ( $19.9 \pm 0.17$  °C), EC ( $169 \pm 1.56$   $\mu$ S/cm), turbidity ( $12.74 \pm 4.0$  NTU), TDS ( $296.05 \pm 0.05$  mgL<sup>-1</sup>), alkalinity ( $1.36 \pm 0.116$  mgL<sup>-1</sup>), chloride ( $24.1 \pm 0.670$  mgL<sup>-1</sup>), calcium ( $57.94 \pm 0.55$  mgL<sup>-1</sup>), magnesium ( $1.43 \pm 0.04$  mgL<sup>-1</sup>), nitrates ( $6.64 \pm 1.72$  mgL<sup>-1</sup>), pH ( $7.76 \pm 0.09$  mgL<sup>-1</sup>) and DO ( $9.48 \pm 0.11$  mgL<sup>-1</sup>) were according to the standards set by World Health Organization for the surface water quality. Fish fauna was represented by only one species, *Schizothorax plagiostomus* which is coldwater fish found in the area. Pollution free water of the lake could support fish industry and some other fish species should be introduced in the lake.

#### **FIRST RECORD OF CYNOPTERUS BRACHYOTIS FROM PAKISTAN**

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*Cynopterus sphinx* has not been recorded from the country. We recorded it from a cave in Tura Gata (N 34° 26.832' E 71° 48.964') in Heroshah of district Malakand (KPK) from where. Fourteen bats were captured. All of them were identified as *Cynopterus sphinx* in the field. But

later investigations showed that forearm length, a species specific character, of two females was smaller than the forearm length of *Cynopterus sphinx* reported in literature. Body weight (n =2), external body (n= 2) and cranial measurements (n =1) were of these females were recorded while one of them was preserved at BatLab, Department of Zoology and Fisheries, University of Agriculture, Faisalabad. These preliminary finding suggest that Pakistan has five species of fruit bats including *Cynopterus brachyotis* which is new country record.

### SEASONAL FISHERIES FROM SONMIANI BAY LAGOON, BALOCHISTAN

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Sonmiani (Miani Hor) is an important fishing centre on the coast of Pakistan, contributing to employment, income generation and export revenues. Sonmiani bay is bestowed with a variety of fisheries resources i.e. finfish and shellfish etc. The landings catch data of various fisheries products were collected monthly from the local companies present in Sonmiani village from December 2004 to June 2007. Finfish production is mainly for domestic consumption as well as export, while shellfish such as shrimp, lobsters and crabs are important export species. Squids (loligo and sepiia) and jelly fish data were also collected in various months during this study period. The seasonality was observed in catch landing and especially controlled by the environmental conditions mainly temperature. The present study is aimed to document the seasonality of fisheries in the Sonmiani bay.

### FISHERIES AND FISH DIVERSITY ALONG MEKRAN COAST

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Fisheries and fish diversity was assessed along Mekran coast during baseline survey at Sonmaini, Ormara, Astola Island and Jiwani conducted in January 2011. These coastal areas are not only economically important (coastal population largely depend on fisheries resources for their livelihood) but also extremely significant ecologically. These areas include ecological niches (creak areas having mangrove forest, rocky areas with narrow continental shelf and clear open sea), and supports number marine life forms including ichthyo-fauna ranging from small clupeids (Anchovies/Sardines) to large predatory fishes (Mackerel and Shark). Preliminary surveys were made to record fishing activities of and fish species caught by commercial fishing boats. Fish landing sites/markets were also surveyed. Efforts were also made to record catch by recreational fishing activities in each area and information from Balochistan Fisheries Department. The information revealed that the fishing activities are carried out throughout the year except for summer monsoon season (May-September) due to harsh weather condition in the Arabian Sea

which poses limitation to the fisheries operation. Various type of fishing crafts ranging from small canoe, 6-8 meters length over all (LOA) to a large Gillnetters (15-25 meters LOA) equipped with a variety of fishing gears ranging from simple hook-n-line gear (locally known as Dori) to modern type of Purse-seine net (locally known as Katra) and very popular gillnet (locally known as Ruch) that can be more than 1000 meters long depending upon the boat size. A total of 96 fish species belonging to 36 families were recorded from Miani Hor, 75 species belonging to 35 families from Astola Island, 64 species with 34 families from Ormara and 83 species belonging to 37 families from Jiwani area. Balochistan Fisheries Department categorized fish landing in 39 major fish species including Shark (*Carcharhinus* spp), Queen-fish (*Scomberoides commersonianus*), Lobster (*Panulirus* spp), Spanish Mackerel (*Scomberomorus commerson*), Indian Mackerel, Pomphret, Tuna (*Thunnus* spp), Catfish (*Arrius* spp), Barracuda (*Sphyraena* spp), Dolphin-fish (*Coryphaena hippurus*, *C. equiselis*), Grunter (*Pomadasys* spp), Hair-tail (*Lepturacanthus salvala*), Marlin (*Makaira indica*), Mulllets (*Mugil cephalus*, *Valamugil* spp, *Liza* spp), Travelly, Cobians and Cuttlefish (*sepia* sp.) that are commercially important species. The data was assessed with respect to fisheries trend and its price value.

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**POPULATION STUDY OF PHEASANT TAILED JACANA, *HYDROPHASIANUS*  
*CHIORURGUS* IN PUNJAB, PAKISTAN**

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Pheasant Tailed Jacana, *Hydrophasianus chiorurgus* is only beautiful bird maintain its presence in abandoned wetlands of Punjab during summer Season. This study was designed to study the population status and its future potentials to estimate the threats and to propose the measures for its conservation if required. Literature suggested sites were selected and schedule of visits month and year wise was developed and sites were visited accordingly, data gathered was analyzed statistically. The population distribution appears, between 298 and 347 highest (83-96) at Marala and lowest (21-57) at Baluki, while density found highest (3/km<sup>2</sup>) and lowest (0.3/ km<sup>2</sup>), being the migratory bird March was observed as start of arrival and October was observed as completion of departure from the study areas. Female to male ratio appears 1:0.75 while juvenile to adult ratio observed 1:0.35 are sign of population stability. The physical factors of the habitat such as temperature, pH, conductivity, turbidity, DO, CO<sub>2</sub>, Chlorides, Calcium, Magnesium, Sodium, Sulphate, alkalinity, hardness and dissolved salts were recorded and their relation with Pheasant Tailed Jacana were studied and ranges found appropriate. Similarly biotic factors like algae, protozoan, Rotifers, Cladocerans, and Copepods in plankton studies were recorded to find their optimal range in the favor of this species. In the phytosociological studies of the habitat distribution and frequency of rooted vegetation species were recorded to understand their association with the species, which shows 31 species distributed in 4 vegetation types with in the habitats lies in study areas, the studies on biology of this bird were focused on food and feeding, food consumption which revealed that 56.3% of its food is consists of plant matter and 27.3% on animal matter. Breeding biology studies suggests the clutch size remains between 1-4 eggs, and most of the fertile females laid 2 consecutive clutches to hand over two different males for hatching and chick rearing, incubation calculated of 25-29 days hatchability found around 80%. Defensive responses, egg

laying and chick care were observed to understand general behaviour of the species. The results of this study suggested that major threats to Pheasant Tailed Jacana are the commercial activities with in its habitat and immediate adjacent to them, which could be regulated for its sustainability.

#### **HABITAT AND MORPHOLOGY OF *CYNOPTERUS SPHINX* RECORDED FROM MALAKAND, PAKISTAN**

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Eleven *Cynopterus sphinx* were captured from a cave in Heroshah (N 34 26.770 E 71 49.090) and another from a cave in Brah (N 34 29.915 E 71 46.822). The cave at Heroshah is 32 m long, 2m wide and 2 m high used for chromite mining while the cave at Brah is 11 m long, 0.8 m wide and 1.2 m high is abandoned after chromite mining nowadays used a dump for garbage for locals. The body weight (n = 12), external body (n = 12) and cranial measurements of (n = 10) were recorded and compared with those recorded in literature.

#### **CHARACTERIZATION OF SHEEP BREEDS OF GILGIT – BALTISTAN**

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Present study was carried out in six different districts of Gilgit Baltistan of Pakistan. The aim of this study was to Phenotypically characterize and document the sheep breeds of Gilgit Baltistan. The data were collected on 245 Sheep (91 males and 154 females) in early June 2008 from Ghezir, Gilgit, Hunza Nagar, Baltistan, Diamer and Astore. The parameters under study included morphological characteristics, growth traits and Somatometric measurements. In Ghezir two types of sheep, thin tailed and fat tailed Koh-e-Ghezir and Batherathi respectively were studied. Traits like, body weight, body length, body height, heart girth, face length, horns length, width of horns, neck length, forelimb length and hind limb length, body width, and body height, tail length, were recorded for this study. The difference between breeds for adult body weight was non significant for males while highly significant for females. The difference in body length, fore limb length, body height, height at shoulder and face length, tail length was significant in males as well as in females among the breeds of Gilgit Baltistan. The horn length and Ear length were significant in males. The wool analysis showed that the wool diameter ranged from  $28.43 \pm 5.16 \mu$  (Gilgiti) to  $36.72 \pm 13.92 \mu$  (Diameri). In addition to the study of phenotypic characteristics, blood samples were collected from the sheep under study for future DNA studies in order to establish breed status of sheep of Gilgit-Baltistan.

**STATUS, DISTRIBUTION AND POPULATION OF HIMALAYAN IBEX IN SHISPER  
NULLAH HUNZA, PAKISTAN**ADINA BAIG\*<sup>1</sup>, BABAR KHAN<sup>2</sup> AND ARSHAD ALI SHEDAYI<sup>1</sup><sup>1</sup> *Department of Biological Sciences, Karakoram International University, Gilgit, Pakistan*<sup>2</sup> *World Wide Fund for Nature Pakistan, Gilgit 15100 Pakistan*

The study was conducted in Shisper nullah, Hassanabad Hunza during May, July and November 2011 to establish baseline information on distribution, population and conservation of Himalayan ibex (*Capra ibex sirbirica*). For ease and accuracy, study area was divided into five plots namely Tongi Basa, Faraj-e-Harai, Daltar, Dodar-e-Harai and Shishper, each roughly covering an area of 2.5–3.0 km<sup>2</sup>. Fixed point direct counting method was used to record information. Each plot was scanned for wildlife using 10 x 50 power binocular (PENTAX XCF; Pentax Co., Philippine) from fixed vantage points early in the morning and late in afternoon when animals were more active in feeding and drinking. Following Wegge (1997) and Schaller (1987) each herd seen was classified into different sex and age classes viz., male, female, adult ( $\geq 2$  years age), yearling ( $\geq 6$  &  $< 12$  months), kid ( $\leq 6$  months) and trophy size males ( $\geq 7$  years old). Global Positioning System (GPS GARMIN III) was used to record location and altitude of the vantage points. A total of 47 animals, including 10 male, 17 female and 10 yearlings were recorded during first survey (May 2011). Four out of 10 in the group were trophy size males. In the following summer (July) survey, a total of 40 ibex were sighted at the same points inclusive of 4 male, 13 female, 3 yearlings and 20 kids but none of the males in group was of trophy size. In winter (Nov) survey, a total of 71 animals including 31 male, 17 female, 7 yearling and 16 kids were seen. Out of the total, almost 23 males were of trophy size. As a whole, maximum number of ibex (28) was recorded from Dodar-e-Harai during winter survey, with largest number of trophy size animals (11/17 males). Study revealed a male to female ratio of almost 1:1, female to kid ratio of 1:1 and total males to trophy size of 2:1. Trophy size animals were not appeared in the summer survey possibly due to upward migration of animals for foraging with the melting of snow at higher elevations. Although population statistics of H. ibex in Shisper is not discouraging but presence of animal carcasses (trophies) and livestock at medium and lower elevations reflect hunting and grazing pressures. Himalayan ibex is abundant and majority of the herds are of pre-reproductive age indicating viability of the population in the area. However, further research and conservation measures are suggested for better management of the species and its habitat in the study area

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**SECTION - VI****POSTER SESSION****CHARACTERIZATION AND OPTIMIZATION OF PHYTASE FROM YEASTS ISOLATED FROM INDUSTRIAL WASTEWATER**

MAZHAR HUSSAIN AND ABDUL REHMAN

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Phytase is an enzyme which is of significant importance in poultry, health-care and environmental perspectives. In this study 13 yeasts isolates were screened for phytase activity and 9 were found to be phytase producing and verified by solid plate assay, turbidity assay and enzyme assay. Three yeasts isolates were further selected based on their high turbidity in broth turbidity assay, zone of hydrolysis in solid plate assay and enzyme activity (%). Yeast Isolates YR and 21A showed maximum enzyme activity of 64% and 59%, respectively. Growth curves were also determined. All yeast isolates showed less growth in phytic acid supplemented medium as sole phosphorous source as compared to  $\text{KH}_2\text{PO}_4$  supplemented medium. Enzyme was characterized for its optimum temperature and pH activity. Enzyme activity in the presence of various metal ions was also determined.

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**BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF CADMIUM RESISTANT BACTERIA FROM INDUSTRIAL WASTEWATER**

ZAMAN KHAN AND ABDUL REHMAN

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Heavy metals are the most abundant pollutants in the sewage and in wastewater and are one of the main causes of water and soil pollution. Heavy metal pollution is badly affecting human health as it gets entry into human food chain and drinking water. It is important to establish an efficient and low cost method for the removal of toxic metal ions. Microorganisms with the ability to grow in the presence of heavy metals and with a significant metal uptake have a potential use in bioremediation of polluted waters. The present research work aims at the isolation, growth and tolerance to cadmium toxic ions of microorganism from industrial wastewaters of different industrial areas of Pakistan. Samples were collected from water reservoirs receiving industrial effluents from different areas of Pakistan. Screening was done and three bacterial isolates that showed maximum tolerance against Cadmium were selected. Minimum inhibitory concentration (MIC) of these isolates against different metal ions was also determined. Two isolates were able to grow at 3000  $\mu\text{g/ml}$  of  $\text{CdCl}_2$  and one bacterial isolate was able to resist cadmium up to 2500  $\mu\text{g/ml}$ . Bacterial isolates were characterized as *Klebsiella pneumoniae*, *Salmonella* sp., and *Alcaligenes* sp. on the basis of molecular and biochemical tests.

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**METAL RESISTANCE IN YEASTS ISOLATED FROM INDUSTRIAL WASTEWATER**

SIDRA ILYAS AND ABDUL REHMAN

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Heavy metal pollution is badly affecting human health as it gets entry into human food chain and drinking water. It is important to establish an efficient and low cost method for the removal of toxic metal ions. Microorganisms with the ability to grow in the presence of heavy metals and with a significant metal uptake have a potential use in bioremediation of polluted waters. The present research work aims at the isolation, growth and tolerance to toxic metal ions of microorganism from industrial wastewaters of Sheikhpura. Samples were collected from water reservoirs receiving industrial effluents from different areas of Pakistan. Screening was done and two yeast isolates that showed maximum tolerance against metal ions were selected. Minimum inhibitory concentration (MIC) of these isolates against different metal ions was also determined. Two isolates were able to grow at 3000 µg/ml of Cd, Pb, Cu, Cr and As. Yeast isolates were characterized on the basis of 18S rRNA as *Candida tropicalis* and *Pichiakudriavzevii*, respectively. Level of glutathione in the presence and without presence of metal ions in yeast was determined. PAGE was also performed to know the protein behavior in the presence of metal ions. These metal resistant yeasts can be used to remove such pollutants from the environment.

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**SCREENING AND CHARACTERIZATION OF CHROMATE REDUCTASE IN BACTERIA ISOLATED FROM INDUSTRIAL WASTEWATER**

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The widespread use of chromium compounds by industries has led to large quantities of this element being released to the environment. Only hexavalent chromium (Cr(VI)) and trivalent chromium Cr(III) are ecologically important because they are the more stable oxidation states. Being mutagenic, carcinogenic and teratogenic, Cr (VI) is approx. 100-fold more toxic than Cr (III). A large number of micro-organisms such as bacteria, yeast and fungi are being used to detoxify Cr (VI) into less toxic Cr (III). The present study aimed at isolation, biochemical and physiological characterization, growth curve, temperature, pH and metal ion effect of chromium resistant bacteria. Wastewater samples were collected from Sheikhpura industries and metal resistant bacteria were isolated and Gram staining was done and their results showed that these were Gram positive cocci which had a Minimum Inhibitory Concentration (MIC) of 2500 µg/ml. Their optimum temperature was 37°C and optimum pH for the growth of two isolates was 7 and one isolate showed its optimum growth at pH of 8. Diphenylcarbazide assay was performed for chromate reductase which is mainly responsible for the reduction of Cr (VI) into less poisonous Cr (III). These bacterial isolates can be used to remove such pollutants from the metal-polluted environment.

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**BAGASSE AS A CHEAP CARBON SOURCE FOR THE PRODUCTION OF BIOPLASTIC USING BACTERIA**

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Polyhydroxyalkanoates (PHA) is a biopolymer generally called as bioplastic, easily degradable plastic and environment friendly. PHA is an extra storage material of some bacterial strains in minimal nutrient conditions, *Pseudomonas*, *Ralstonia eutropha*, and *Enterobacter* species are important for PHA production. *Pseudomonas* has been reported as a good PHA producing organism. Different carbon sources like glucose, molasses, and bagasse and plant oils have been reported and used for PHA production. Bagasse was used as cheap carbon source for PHA production using *Pseudomonas*. Accumulation of PHA was observed in *Pseudomonas* by sudanblack staining and it was identified by Nile blue fluorescence on agar plates. By time profiling of *Pseudomonas* in PHA accumulating media it was observed that after 48 hours there is the accumulation of PHA. DNA was extracted and *phaC synthase* gene was amplified.

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**CHROMATE RESISTANCE AND EXOPOLYSACCHARIDE PRODUCTION BY STAPHYLOCOCCUS AUREUS ISOLATED FROM TANNERY EFFLUENT**

MUHAMMAD USAMA MARGHOOB AND RIDA BATOOL

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Soil sample was collected from a tannery of Sialkot city, Pakistan. Serial dilutions were made and spreaded over L agar plates, incubated at 37°C for 24 hours. Morphologically different colonies were selected and purified on chromium supplemented media at variable concentrations (250, 500, 1000 µg ml<sup>-1</sup>) of K<sub>2</sub>CrO<sub>4</sub>. Bacterial colonies which resisted 500 µg/ml of K<sub>2</sub>CrO<sub>4</sub> concentration were selected. Selected bacterial isolates were biochemically and morphologically characterized. As the colonies were Gram +ve cocci and were catalase and Dnase +ve which confirmed them as *S. aureus*. These strains were found Cr<sup>+6</sup> resistant. So, their metal resistivity against other heavy metals (e.g., Ni, Cu, Pb, Co) was also determined. Strains were sensitive to antibiotics ampicillin, gentamycin and tetracycline and showed a little resistance against chloramphenicol and erythromycin (10 µg ml<sup>-1</sup>). All strains showed EPS production ability when grown on EPS screening media. EPS structural gene of one strain was amplified.

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**BACTERIAL BIOPLASTIC PRODUCTION USING ORGANIC WASTEWATER**

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Broader usage of biodegradable plastics in packaging and disposable products as a solution to environmental problems would heavily depend on further reduction of costs and the discovery of cheap carbon sources as a substrate for bioplastic production. As the first step in our pursuit of eventual usage of industrial wastewater as nutrients for microorganisms to synthesize environmental-friendly bioplastic, we investigated the usage of carbon sources for the production of polyhydroxyalkanoates (PHA) by selected strain of microorganism. Strains were isolated

from wastewater. Biochemical analysis followed by ribotyping showed that PHA producing strains belong to *Pseudomonas* and *Stenotrophomonas* genera. On the basis of screening results strains were selected for optimization and time profiling experiments with two different carbon sources; glucose and paper mill wastewater. Strains were used separately and as a mixed culture to produce PHA. Highest PHA produced was about 74% after 48 hours of incubation at 37°C from mixed culture of both strains.

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**SCREENING OF ANTIBACTERIAL COMPOUNDS PRODUCED BY *PSEUDOMONAS*  
AND *BACILLUS SP* UNDER DIFFERENT NUTRIENT CONDITIONS**

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A wide range of bacteria produce different types of antimicrobial compounds which are the parts of their defense mechanism. These antimicrobial compounds include antibiotics and antimicrobial peptides *i.e.* Bacteriocins. The aim of our study is to isolate bacteriocin producing strains and to check their activity against human pathogens *S. aureus* and Methicillin Resistant *S. aureus* (MRSA). For the above mentioned purpose bacterial strains were isolated from various environmental samples and they were screened for their bacteriocin activity against *S. aureus* and MRSA. Two strains belonging to *Pseudomonas* and *Bacillus* were selected for further studies. The growth of these strains and their maximum bacteriocin production were tested in Nutrient Broth, Tryptic Soy Broth and Brain Heart Infusion Broth. In order to determine the time of maximum bacteriocin activity time profiling and growth curve studies were also done. The antimicrobial activity of the selected strains were also checked by preparing its extract with organic solvent, ethyl acetate. PCR studies for bacteriocin producing gene were also done in case of *Bacillus* strain.

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**OPTIMIZATION OF BIOPLASTIC PRODUCTION BY *EXIGUOBACTERIUM SP.* USING  
MOLASES AS CARBON SOURCE**

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Two bacterial strains *Exiguobacterium sp.* and LFSM2 were screened for PHA production by Sudan Black Staining. Strains exhibited PHA production ability. The two strains were optimized for PHA production using different carbon sources as Glucose ( as control carbon source), sodium gluconate and molasses containing 0.117g/100 ml carbohydrate content as estimated by phenol-sulfuric acid method, at 37 °C and Ph 6.8. Two different concentrations of the three carbon sources were used as 0.1 % and 2%. Sodium hypochlorite- chloroform method was used for PHA extraction. *Exiguobacterium sp.* produced PHA in glucose optimally at 2% glucose as 0.236g/100 ml biomass, 0.018g/100ml or 6.521% PHA at 78<sup>th</sup> hour, in sodium gluconate as 0.056g/100 ml biomass, 0.015 g/100ml or 26.31% PHA at 32<sup>th</sup> hour in 2% concentration. The same strain showed exponential growth in molasses at 78<sup>th</sup> hour in 2 % concentration as 0.540g/100 ml biomass, 0.072g/100ml or 13.3% PHA. The strain LFSM2 produced PHA in glucose optimally at 2% concentration as 0.224g/100 ml biomass, 0.058g/100ml or 25.89% PHA at 130<sup>th</sup> hour whereas in sodium gluconate at 130<sup>th</sup> hour, 2% concentration as 0.187g/100ml biomass, 0.011g/100

ml or 4.910PHA. In molasses, Bioplastic production was recorded optimal in 0.1% concentration.

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**TIME PROFILING AND TEMPERATURE STABILITY OF BACTERIOICIN PRODUCE  
BY *BACILLUS CEREUS* AND *BREVUNDIMONAS***

HINA SADIQ AND NAZIA JAMIL

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Bacteriocin producing strains were isolated from a soil sample. The effect of bacteriocin was tested against Gram Positive test organisms. The bactericidal effect of *Bacillus cereus*, *Brevundimonas* and a *Bacillus* strain were observed to be at 1250, 625 and 312 arbitrary units (AU/mL) respectively. Moreover, time profiling as well as temperature stability of bacteriocin was determined using two different growth media which showed that high titers of bacteriocin were produced in log phase and more stability was observed at low temperatures. *Cerein 7B* structural gene of *Bacillus cereus* was amplified using BLIS7B-7 and BLIS7B-8 primers and sent for sequencing.

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**IN-VITRO ANTIOXIDANT EFFECT OF *CAMELLIA SINENSIS* ON HUMAN CELL  
LINES**

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Free radicals put in to more than one hundred disorders in humans. Environmental pollutants, radiation, chemicals, toxins, deep fried and spicy foods as well as physical stress contribute in their production. They cause depletion of immune system antioxidants, change in gene expression and induce abnormal proteins. Recently there has been an upsurge of attention in the therapeutic potentials of medicinal plants. Numerous natural antioxidants have already been isolated from different varieties of plant material such as leaf vegetables, fruits, seeds, cereals and algae. Their protective mechanism can be explained by the capacity of the antioxidants compounds e.g. phenolics, flavonoids and polypropanoids in the plants and plant products themselves to scavenge free radicals, due to their proton donating ability. Research methodology for present study involved isolation and extraction of plant extracts, screening for phytochemical components, antioxidant and antimicrobial activity and its effect on living cells using lymphocytes as model. *Camellia sinensis* (green tea) extracts were prepared in three different solvents viz. aqua, methanol and ethanol. Initially, phytochemical screening involved determination of ascorbic acid, phenolic, flavonoids, flavonol, iridoids and phenylpropanoid glycosides contents. Antioxidant activity determination includes scavenging assays e.g. DPPH assay and ABTS assay, HPLC, hydrogen peroxide scavenging assay and reducing power assay. The antibacterial activity was evaluated by agar well diffusion method. *In-vitro* antioxidant actions were determined by the activities of catalase (ELISA kit method), superoxide dismutase, lipid peroxidation and total protein contents on lymphocyte cell lines. *Camellia sinensis* showed high contents of ascorbic acid, phenols, flavonoids, and flavonol. It is also a good scavenger of oxidants as evident by DPPH, ABTS and reducing power assay. *In-vitro* antioxidant results also showed positive effects. In addition, promising antimicrobial activities were also observed. Among extraction solvent methanol showed the highest

activity. Experimental plant is potential source of natural antioxidants and antimicrobial activities. *In-vitro* experimental trial showed strong antioxidant repair mechanism of plant against oxidative stress.

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#### **DIABETES MELLITUS - AN EXTRA-HEPATIC MANIFESTATION OF HEPATITIS C IN PAKISTAN**

SARWAT REHMAN<sup>L</sup>, FOUZIA QAMAR<sup>1</sup>, MANZOOR HUSSAIN<sup>1</sup>, MUHAMMAD IQBAL<sup>1</sup>  
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Infection with HCV has serious consequences apparent not only in the form of disturbed liver function profile but also associated with various extra-hepatic manifestations contributing to many unrelated diseases. Infection by Hepatitis C has been proposed, as being capable of triggering the development of diabetes mellitus among hepatitis C positive patients. We have designed a study to evaluate a possible relationship between different biochemical and molecular factors leading to the development of diabetes in patients visiting different hospitals in Pakistan.

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#### **REDUCTION OF HEXAVALENT CHROMIUM AND BIOSORPTION STUDIES BY USING BACTERIA ISOLATED FROM TANNERY EFFLUENT**

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Eight chromium resistant bacterial strains were isolated from the waste water of tannery. These strains were isolated on chromium supplemented L-agar plates. Bacterial strains isolated from waste water of tannery were subjected to morphological, physiological and biochemical characterization. All the bacterial strains were gram negative cocci and non-spore formers only. They showed gram positive behaviour. Most of the strains showed resistance to studied heavy metals (mercury, copper, cobalt, lead zinc) but found sensitive to antibiotics (ampicillin, erythromycin, tetracycline, chloramphenicol and gentamycin). Ribotyping analysis of A<sub>8</sub> strain showed that it belongs to the genera *Cellulosimicrobium sp.* All the isolated strains were checked for their reduction potential in stationary and shaken culture media at different initial concentration of Cr (VI). As A<sub>8</sub> exhibited best and exceptional results among all the isolated strains, hence it was selected for biosorption studies. Four different materials sand, PVC, stone and rubber tubing were used as solid support for biofilm formation of chromium resistant bacterial strain. Maximum reduction (96%) was exhibited by sand coated with bacterial strain after 96 hours. Fluorescent microscopy revealed that sand was best coated with bacterial biofilm. Fourier transforms infrared (FTIR) spectroscopy was carried out to find out the change in the distribution of functional groups of chromium resistant bacterial strain isolated from waste water of tannery industry under stress and non-stress conditions. Cell-free enzyme assay was performed to check the reduction of Cr (VI) *in vitro*.

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**CELLULOSE DEGRADING YEASTS ISOLATED FROM LOCAL ENVIRONMENT**

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Cellulose degradation with the help of microbial enzyme is significant in environment rich in plant organic matter. This study was carried out to isolate cellulose degrading yeasts from local environment. All isolates (designated as A2, B5, W2 and W3) showed optimum growth at 37°C and at pH range of 6-8. The dominant form of the enzyme was found to be extracellular and yeast isolates showed maximum enzyme production after 5 days of incubation. The optimum temperature for the activity of cellulase determined was 40°C for A2, B5 and W2 isolates and 90°C for W3 isolate. The optimum pH for cellulase activity was 9 for A2 isolate, 7 for B5 and W3 isolates and 8 for W2 isolate. Cellulase activity of A2 was increased (6%) in the presence of Zn<sup>+2</sup> while other metals have slight inhibitory effect on enzyme activity. Similarly enzyme activity of B5 was enhanced 1.1% and 7.5% in the presence of Mn<sup>+2</sup> and K<sup>+</sup> and other metals have inhibitory effect. In case of W2 isolate no metal has positive effect on enzyme activity. Cellulase activity of W3 was increased 4.2%, 6.5%, 7.2% and 15.2% by Ca<sup>+2</sup>, Mg<sup>+2</sup>, Mn<sup>+2</sup> and K<sup>+</sup>, respectively. The two other enzymes pyruvate decarboxylase and aldehyde dehydrogenase which convert pyruvate ultimately to ethanol were also assayed.

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**LIVER FUNCTION PROFILE IN THE PATIENTS SUFFERING FROM DENGUE VIRAL INFECTION**AISHA RIAZ<sup>1</sup>, FOUZIA QAMAR<sup>1</sup>, MANZOOR HUSSAIN<sup>1</sup>, MUHAMMAD IQBAL<sup>1</sup> AND  
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Abnormal liver function is a serious consequence of dengue virus infection, which is overlooked in majority of cases. To predict the level of dengue infection seriousness and risk, liver function tests should be taken into account for the proper management that will lead to accurate prognosis. The main objective of the current research work is to evaluate the degree of involvement of the vital organ i.e liver in the dengue viral infection. The goal was achieved by measuring the levels of different enzymes predicting the degree of liver damage, as dengue being the causative agent.

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**SCREENING OF BIOSURFACTANT PRODUCING BACTERIA AND THEIR ROLE IN OIL BIODEGRADATION**

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Biosurfactants are surface active compounds produced by the microorganisms. They have potential to solubilise hydrocarbon contaminants and make them available for microbial

degradation. In the present study, Biosurfactant producing bacterial strains isolated from oil contaminated soil and oil from drilling wells and screened them for biosurfactant production by using different screening tests. All bacterial strains were further characterized morphologically (colony morphology, gram staining, spore staining, capsule staining and motility test), biochemically (Catalase, oxidase, Urease, Nitrate reduction, Lactose fermentation, Oxidation fermentation, Methyl red, Citrate utilization, Indole test, Casein hydrolysis, Starch hydrolysis, Lipid hydrolysis, Gelatin hydrolysis etc.), physiologically (pH, Temperature, Antibiotic, heavy metals, antimicrobial activity) and genetically (plasmid isolation, Plasmid detection and 16S rRNA sequencing). Strains which showed positive drop collapse and stable emulsion formation were selected as positive strains for biosurfactant production. On the basis of 16SrRNA gene sequencing analysis strains S5-1 identified as *Acinetobacter* (100% homology) S1-8 as *Enterobacter* (99% homology) and S7-1 as *Pseudomonas Stutzeri* (100% homology). They showed resistance to different metals and antibiotics and showed antimicrobial activities against pathogenic strains. On plasmid isolation clear bands of plasmids were observed in all strains. The complete decolorization of redox indicator 2, 6 dichlorophenol indophenol (DCPIP) at different concentrations of oil (1%, 1.5%, 2%, and 2.5%) was observed in case of S5-1, S1-8 and S7-1 which showed their ability to degrade oil. Biosurfactant producing strains have showed appreciable oil degrading ability which can be exploited for bioremediation of oil contaminated sites and can be helpful in oil spill removal in soil and aquifers which have very harmful effects on environment.

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**ISOLATION AND IDENTIFICATION OF *EISENIA FETIDA* ASSOCIATED  
*PSEUDOMONAS AERUGINOSA* SPP. AND ITS CONTROL**

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Earthworm plays a foremost role in the proper functioning of the soil ecosystem. It acts as forager and helps in recycling of dead and decayed plant material by feeding on them. Earthworm increases the soil fertility and is often referred to as a farmer's friend. In current research it was observed that *Pseudomonas aeruginosa* associated with the earthworm. These pathogens penetrate into the body, cause diseases to the host and leads to the opportunistic infection. *E. foetida* collected from the local soil of the Azad Jammu and Kashmir, Muzaffarabad and *P. aeruginosa* spp. were isolated in Biotechnology lab, Department of Zoology. The bacterial strain was confirmed through morphological and biochemical tests. In order to control the pathogenicity of *P. aeruginosa*, we used chloroform and isoamylalcohol extracts of medicinal plant including *Cinnamomum zylanicum* (Cinnamon; Dalchini), *Cuminum cyminum* (Cumin; Zeera), *Syzygium aromaticum* (Clove; Loang), *Curcuma long Linn* (Turmeric powder), *Trachyspermum ammi* (Carom seeds; Ajwain) as well as the n-Hexane, chloroform, ethanolic, methanol and ethyl acetate extracts of *Momordica charantia* (both seeds and green parts Bitter gard) were investigated. On the other hand various antibiotics were also investigated. Antibiotic sensitivity showed that isolated *P. aeruginosa* was highly sensitive to Streptomycin, Gentamycin and Ciproflaxin (45-55mm), whereas Tobramycin and Tetracyclin had moderate effect (20 and 25mm). In case of medicinal

plants and *M. charantia*, the ethanol and methanol extracts of green parts of *M. charantia* and isoamylalcohol extract of medicinal plants specified the significant zone of inhibition such as 30mm, 28mm, and 17-33mm, respectively. It was concluded that the extracts of these medicinal plants and organometallic compound have considerable effect on the bacterial pathogens. Isolation and purification of different phytochemicals may further yield significant antibacterial agents.

### ALTERED PHYSICO-CHEMICAL PARAMETERS OF RIVER RAVI, SEGMENT SUBJECTED TO URBAN POLLUTANTS

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To study the impact of Lahore industrial and sewage effluents at different flow seasons on water quality of the river Ravi. water samples were collected from three polluted downstream sites (B; Shahdera, C; Sunder and D; Balloki headworks) in two seasons (high and low flow) and compared with less polluted upstream site (A; Siphon). All parameters showed higher values during low flow than the high flow season, except temperature, dissolved oxygen and total suspended solids. All parameters showed highly significant differences between seasons and along stream sites. Highest temperature (24.23 °C), total dissolved solids (692 mg/l), total suspended solids (802 mg/l), nitrite (5.80 mg/l), nitrate (8.30 mg/l), phosphate (7.24 mg/l), chloride (232.98 mg/l), ammonia (1.08 mg/l) and sulphate (821.33 mg/l) were measured at site C. The river appeared to be polluted as indicated by the high values of total suspended solid (908 mg/l) and sulphate (963 mg/l) in comparison to the respective permissible limits of 150 and 600 mg/l being suggested by National Environmental Quality Standard for safer drinking water. The results suggest that the water quality of river Ravi is adversely affected and impaired by the discharge of domestic, agricultural and industrial wastes, the usual practice throughout Pakistan. The data generated from this study provides base line information for potential remediation and other management decisions.

### DETERMINATION OF THE SEQUENCE ENVIRONMENT AROUND METHYLATED AND ACETYLATED RESIDUES IN MAMMALIAN PROTEINS, *IN SILICO*

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Bioinformatics has become a basic need for life sciences and is very helpful to study biological problems. Post-translational modifications are investigated *in silico* with the help of the bioinformatics tool MAPRes (Mining Association Patterns among preferred amino acids residues in the vicinity of amino acids targeted for post translation modification). This tool mines association pattern around modified and non-modified residues. In this work association rules were mined for acetylated and methylated mammalian proteins. Acetylation and methylation of histones and transcriptional factors play an important regulatory role in developmental processes, proliferation and differentiation. The data was retrieved from dbPTM. Furthermore to validate the mined association patterns around modified and non-modified residues MeMo, MASA and BPBMMS

were utilized for methylated Arg and Lys residues and Pail, EnsemblePail and PredMod for acetylated and non-acetylated Lys residues. The mined association ruled showed: Highest preference of Gly in Arg methylation, Ser/Gln in Lys methylation, His/Lys in acetylated Lys and Ser/Leu in non-acetylated Lys. The prediction methods showed a high conformity level, ranging from 13%-72% for methylated and 47%-70% for acetylated proteins. This work will be helpful for determining methyl-transferases and acetyl-transferases sequence specificity in mammalian proteins, which may lead to drug development in different pathologies.

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#### **DNA BASED CHARACTERIZATION OF CROW SPECIES OF DISTRICT MANSEHRA, PAKISTAN**

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Crows are large passerine birds of genus *Corvus* in family Corvidae ranging in size from large to medium small and are the most intelligent among birds. Present research was initiated for DNA based characterization of crow species of District Mansehra, Pakistan. During present study three species of crows, i.e., *Corvus macrorhynchos* Wagler, *Corvus splendens* Vieillot and *Corvus monedula* Linnaeus were found in District Mansehra. Crows were trapped for blood sampling and protocol for the isolation of total genomic DNA from crow's blood was optimized. The quality and quantity of isolated DNA was checked through gel electrophoresis. The PCR amplification of DNA was done with five RAPD primers. All the amplification profiles were observed and genetic distance was estimated. The genetic diversity estimates from RAPD primer GLC-19 ranged from 70-100%. The RAPD primer GLB-14 and GLC-11 showed genetic distance range from 70-80%. Results of RAPD primers GLC-15 and GLC-16 gave the genetic distance in range of 20-100%. The average genetic distance estimates ranged from 50-90%. Phylogenetic relationship was elaborated through dendrogram which grouped the *C. macrorhynchos* and *C. splendens* in the same cluster while *C. monedula* into separate one. From the results it is concluded that *C. macrorhynchos* and *C. splendens* are much related to each other genetically than *C. monedula*. The study also revealed the RAPD markers as effective for such types of studies where an overall picture of genome is required. The genetic analysis of those newly discovered species whose morphological characters are confusing to classify them properly, is recommended for the purpose of classification.

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#### **NEST ARCHITECTURAL PATTERNS BY DIFFERENT WASP SPECIES AND THEIR SOCIAL BEHAVIOUR WITH REFERENCE TO PARENTAL CARE**

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In the present study, nest architectural patterns, elemental analysis, social behaviour and parental care were carried out in three wasp species: the hornet wasp, *Vespa velutina* (Lepeletier); the paper wasp, *Polistes flavus* (Cresson) and the mud dauber wasp, *Sceliphron formosum* (Smith) from the different localities of the Mansehra. *Vespa velutina* nest was completely closed acceptance

opening for entry or exit with 1-10 steps' layers with hexagonal cells inside the nest. Nest of *P. flavus* were found among bunches of leaves of trees with 1-5 steps' layers with hexagonal cells same as in *V. velutina*. Nest of the *S. formosum* were pitcher shaped found in muddy place consists of 1-10 cells. Elemental analysis by XRD of three hymenoptera species nests showed Ca with the highest while K with the lowest amounts. Elements in nests with descending order were in *V. velutina*: Ca>K>Al>Mg>Si; *P. flavus*: Ca>Mg>Si>Al and *S. formosum*: Si>Ca>K>Al>Mg. Social behaviour of wasps showed strong foraging, defensive behaviours, pseudo-attack, subsequent erratic flight, wing buzzing, mandibular pecking, abdominal pumping and abdominal twisting with highly developed parental care. If disturb, they try to protect their larvae in their nest. When they felt their nest was no longer safe, they shifted their larvae from the nest to another place picked with their limbs.

#### PHOTOFERMENTATIVE HYDROGEN PRODUCTION FROM STARCH MANUFACTURING WASTES

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Efforts are continuously under way to replace rapidly depleting non-renewable fossil fuels with biofuel. In this regard H<sub>2</sub> is considered an ideal biofuel while practical sustainable means of its production are still under concern. Different photosynthetic bacteria, especially purple non sulfur bacteria have the potential capacity to utilize a variety of substrates including dark fermentation residual effluents and many other wastes rich in sugars and organic acids. In present study one of the bacterial isolate designated as SS-7 was reported for 580ml of H<sub>2</sub>L<sup>-1</sup> of culture from potato starch after optimizing conditions in nitrogen deficient medium under incandescent light illumination of 100W at 30±2.0°C with initial medium pH 7. The strain was later identified as *Rhodospseudomonas palustris*-SS as a result of blast from NCBI with accession number JQ315814. Nearly complete conversion of substrate to hydrogen and carbon dioxide by capturing energy from sunlight or artificial light.

#### COMPARISON OF PROTEIN PROFILE FROM LIVER, MUSCLE AND BLOOD OF BROILER CHICKEN BY (SDS-PAGE)

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The present study is aimed to compare the protein profile of muscle, liver and blood proteins from the broiler chicken in nine different weight's *i.e.* 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75 and 3.00 was compared through SDS-PAGE. These proteins of different chicken samples were compared to identify the relationship between protein profile and weight of chicken. One gm sample of muscle, liver each and 1 ml of blood serum was processed by Laemmli method (1970). The qualitative analysis revealed that eighteen protein subunits were common in muscle samples of different weight categories having molecular weights ranging from (118 KDa to 13

KDa), while in case of liver samples fourteen protein subunits having molecular weights from (118 KDa to 11 KDa) were present there were eighteen protein subunits in blood samples with molecular weights (132 KDa to 11 KDa). Whereas wide variation was observed in case of exclusive protein subunits for muscle, liver and blood samples in chicken. There were 59, 46 and 43 different exclusive protein subunits in muscle, liver and blood samples in different weight categories respectively. The variation was observation in total number of protein subunits in chicken samples with increase and decrease in different weight categories as an indicative of protein synthesis and protein turn over activities. No obvious trend in number of protein subunits was observed. This might be due to various control parameters which are responsible for final growth expression. This in turn is the result of interactions between nutritional, environmental and genetic factors interacting with endocrine secretions.

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#### **URBAN POLLUTANTS AFFECT NEGATIVELY THE FATTY ACID PROFILES OF ROHU FROM RIVER RAVI**

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The water quality of the river Ravi that flows through Lahore in Pakistan, has been deteriorated tremendously due to the urban sewage and industrial effluents. The change in water quality can affect the health and nutritional composition of different fish species that are harvested from this river. This completely randomized study compared the fatty acid profiles of *Labeo (L) rohita* (Rohu) collected from three known (Low (Siphon) =A, moderate (Shahdera) = B and high (Sunder) = C) polluted sites of the river Ravi during low and high flow seasons. The saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) were significantly ( $P < 0.001$ ) different among the sampling sites in both seasons. Total PUFA were higher at site A (10.91 % and 12.18 %) than sites B (9.85 % and 10.76 %) and C (5.46 % and 9.28 %) during low and high flow season, respectively. Palmitic (32.26-37.46 %), stearic (5.58-9.44 %) and myristic (3.16-8.92 %) acids were main SFA whereas oleic (12.34-19.91 %) and palmitoleic (5.89-11.56 %) acids were the major MUFA and the linoleic ( $\omega 6 = 2.57$ -5.13 %) and  $\alpha$ -linolenic ( $\omega 3 = 0.97$ -2.51 %) acids were the predominant PUFA. The site A fish specimens contained the highest levels of Omega-6 and Omega-3 PUFA. This study signifies a change in the fatty acid composition of *L. rohita* fish in response to the pollution of different sites of the same river water system. This change in fatty acid composition of fish implies that the river water pollution had been affecting the nutritional quality of fish and subsequently the health of fish consuming communities. Results of this study are suggestive for opposing the general local myth advocating beneficial and health promoting nature of riverine (and thus polluted) fish as compared to pond reared fish. Further studies are needed to examine the nutritionally influenced health aspects of the fish consumers. Drastic and moderate decrease of health promoting unsaturated fatty acid in the meat of fishes capturing during the low and high flow seasons clearly demonstrate the changes as function of the pollutants. Likewise fatty acids profiles' of downstream localities further testify the involvement of the water pollutants in this regards.

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**ENVELOPE 2 PROTEIN PHOSPHORYLATION SITES S75 & 277 OF HEPATITIS C  
VIRUS GENOTYPE 1A AND INTERFERON RESISTANCE: A SEQUENCE  
ALIGNMENT APPROACH**

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Hepatitis C virus (HCV) is a major cause of liver disease affecting ~200 million individuals worldwide. Pegylated interferon-alpha (IFN- $\alpha$ ) alone and its combinations are currently being used to eradicate this pathogen. Due to high rate of mutations, resistance to this therapy is increasing periodically. Our investigation of the interferon resistance of HCV focused on E2, an outer protein of the viral envelope that may participate in virus binding to target cells and also induces neutralizing antibodies. Envelope protein E2 interacts with double stranded RNA-dependent protein kinase (PKR) through an element homologous to the phosphorylation site of PKR and its target, eukaryotic translation initiation factor-2 $\alpha$  (eIF2 $\alpha$ ). E2 possibly inhibits the kinase activity of PKR and blocks its inhibitory effect on protein synthesis and cell growth. This interaction of E2 and PKR may be an important mechanism through which HCV circumvent the antiviral effect of interferon. Mammalian expression vector was designed for envelope E2 gene of HCV. Phosphorylation sites were predicted by using NetPhos 2.0 software. Protein Structure Analysis was designed by using I-TASSER. Discovery Studio and SWISS PDBViewer were applied to visualize 3D protein structure of the putative sites. PDB structure was built up by using the server I-TASEER (Ab-initio protein structure predictor). After this analysis two phosphorylation sites (S75 and S277) were found to be most reliable sites. An online server NetSurfP was used to find the surface accessibility of local envelope sequence, as the phosphorylation sites should be exposed on the surface of proteins. This E2-PKR interaction may account for the intrinsic IFN resistance of HCV. A hybrid in-silico and wet lab. approach of motif prediction, evolutionary and structural analyses has pointed out seriner 75 and 277 of the HCV E2 gene as a promising candidate for the sites of serine phosphorylation. It is proposed that serine phosphorylation of HCV E2 gene has a significant role in interferon resistance. The aim of the present study is to establish a correlation between phosphorylation of envelope protein and PKR protein and its further role in the resistance of interferon in case of HCV treatment.

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**HOST AND VIRUS RELATED FACTORS EFFECTING RATE OF RESPONSE  
TOWARDS CHRONIC HEPATITIS C STANDARD TREATMENT**

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Current standard therapy commonly followed for chronic hepatitis C in Pakistan is interferon alpha plus ribavirin combination therapy (IFN  $\alpha$ /ribavirin). Another improved form of therapy consists of pegylated interferon plus ribavirin (PegIFN/ribavirin). Later has raised rate of sustained virological response than standard IFN  $\alpha$ /ribavirin therapy. Aim of this study was to analyze rate of treatment response [sustained virological response (SVR), end of treatment response (ETR), rapid virological response (RVR)] in patients following standard treatment IFN  $\alpha$ /ribavirin and in patients following pegylated interferon treatment. Serum samples of 136 patients following

standard IFN  $\alpha$ /ribavirin and 50 patients following PegIFN/ribavirin were collected. After RNA extraction genotyping was done and then real time PCR was used for quantification of HCV viral load before treatment, after 4 weeks, after 12 weeks, after 24 weeks and finally after 6 months follow up period. 86 patients out of total 136 patients following standard IFN  $\alpha$ /ribavirin therapy completed treatment and 69% of them achieved ETR. On the other hand 50 patients following PegIFN/ribavirin treatment completed treatment and 80% of them achieved ETR. 64 out of 86 patients following standard IFN  $\alpha$ /ribavirin therapy completed follow up period and 53.9% of them achieved SVR. 45 out of 50 patients following PegIFN/ribavirin treatment completed follow up and 70% achieved SVR. Our study with reference to previous studies confirms younger age and rapid virological response as important factors for the achievement of sustained virological response. Our study further established that male patients have a greater chance of achieving SVR responses.

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**CONTINUED CIRCULATION OF DENGUE VIRUS SEROTYPE 2 (SUBTYPE 4) AND DENGUE VIRUS SEROTYPE 3 (SUBTYPE 3) IN PUNJAB, PAKISTAN: A CAPSID-PREMEMBRANE NUCLEOTIDE SEQUENCE BASED GENETIC CHARACTERIZATION OF DENGUE VIRUS ISOLATED FROM DIFFERENT OUTBREAKS**

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Since the first reported outbreak of dengue hemorrhagic fever in Pakistan, several cases have been reported in the region. Dengue virus serotype 3 (DEN-3) was first documented in 2005 outbreak in Karachi. Reports show that serotype 3 is prevalent in Lahore since 2008. Serotype 2 (DEN-2) is the major circulating serotype in Pakistan as it is documented since 1994. We have conducted a detailed study of outbreaks of dengue virus infection that occurred in years 2007, 2008, 2009 and 2011 in Lahore by using molecular techniques such as nested-PCR and nucleotide sequencing of the C-prM gene junction of dengue virus. Phylogeny of the studied sequences were inferred using UPGMA and Neighbor-joining methods. Through the analysis of 114 serum samples collected over the period of three years (2007-2009), a total 20 patients were infected with dengue virus (17.5%). In recent outbreak of year 2011, a total of 494 blood samples were serotyped out of which 76% were positive for dengue virus. Concurrent infections with serotype 2 and 3 remained around 3% in studied period of 2007-2009 and 2011. *C-prM* gene sequencing revealed genotype 4 of serotype 2 and genotype 3 of serotype 3 through the studied years. Our study showed that serotype DEN-2 was dominant serotype in dengue virus infections during the period 2007-2009 and 2011. The other serotype present was serotype DEN-3. Genotypes of serotype DEN-2 and serotype DEN-3 were subtype IV and subtype III, respectively.

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**EXPRESSION OF HCV CORE GENE IN HUH7 CELL LINE: A TOOL FOR GENE EXPRESSION PROFILING**

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Infection with hepatitis C virus (HCV) is a major cause of acute and chronic liver disease. The high prevalence of the virus, the insidious course of the disease and the poor prognosis for

long-term persistent infection make this pathogen a serious medical and socioeconomical problem. Especially in underdeveloped countries including Pakistan with limited health facilities it leads to chronic infection that accounts for 60%-90% of all primary liver malignancies. Molecular events that develop hepatocellular carcinoma (HCC) during HCV infection are feebly identified. In this regard, HCV core protein plays an important role in the development of HCC. To date cell culture system is considered as a reliable system to study the viral life cycle and helpful in the development of antiviral drugs. In the current study an expression vector of HCV core gene is constructed which is successfully expressing HCV core protein in Huh7 cell line. Initially we cloned the PCR amplified complete core gene into TA cloning vector, for this RNA was extracted from serum of patient who never received anti-viral treatment before. Core gene was amplified and cloned into TA cloning vector and sequenced. The sequences were submitted to NCBI [Accession number GQ451336]. Successful cloning of core gene into TA vector was confirmed by utilizing Colony PCR, restriction analysis and sequencing. Then taking the core/TA vector as template core gene was amplified using primers having restriction sites for enzymes *Hind*III and *Eco*R 1 in the inner set of sense and antisense primers at 5' site respectively. Amplified product was digested with restriction enzymes and subcloned into pcDNA3.1 (+). Successful cloning was confirmed by Colony PCR, restriction analysis and sequencing. Then the linearized pcDNA/core vector was used to transfect the Huh7 cell line and selection of single cell clone was made on G418 selection marker. Expression of core gene was confirmed by RT-PCR, Real Time PCR and western blotting. In conclusion, the developed system is efficiently expressing core gene of HCV genotype 1a isolate. This may be helpful to explore the molecular events that are affected by core protein in cell and to study role of core protein in viral life cycle that are very useful to understand HCV genomic replication.

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**CHARACTERIZATION OF COMPLEXITY OF ENVELOPE PROTEIN HYPER  
VARIABLE REGION 1 IN PATIENTS RESPONDING VERY EARLY TO THE ALPHA  
INTERFERON AND RIBAVIRIN COMBINATION THERAPY**

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Hepatitis C virus roots a chronic liver disease. The current approved treatment strategy includes administration of alpha interferon and ribavirin in combination for 24-48 weeks. One of the predictor of sustained virological response is an early virological response to treatment characterized as rapid response. Hyper variable region 1 (HVR1) of E2 protein has been reported to be responsible for viral entry and at the same time acts as a target for neutralizing antibodies. Any mutation in this region would therefore be important for virus interaction with target cell and viral persistence. We therefore investigated E2 HVR1 sequences of thirty one clones of six pretreatment samples subjected to combination therapy. Three of the patients were rapid responders (R1, R2 and R3) and two were breakthrough responders (BT1 and BT2). Our study revealed that no specific pattern of nucleotide variations were associated with rapid virological response. Heterogeneity was equally high and random in all samples regardless of their treatment response. Our study further confirmed that although hyper variable region 1 is quite variable region it still maintains its physiochemical properties. It also indicated that average composition of hydrophilic and basic amino acids were comparatively lower in rapid responders than other samples affecting probable interaction of virus with target cells. A central non antigenic region was constant among the

breakthrough responders but differed in length significantly among rapid responders reflecting the adaptive nature of HVR1 to the immune response.

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