

PROCEEDINGS OF PAKISTAN CONGRESS OF ZOOLOGY

(Proc. Pakistan Congr. Zool.)

Volume 33

2013

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Pakistan Museum of Natural History, Islamabad hosted the 33rd Pakistan Congress of Zoology (International).

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33rd PAKISTAN CONGRESS OF ZOOLOGY (INTERNATIONAL)
PAKISTAN MUSEUM OF NATURAL HISTORY, ISLAMABAD

April 2 – 4, 2013

PROGRAMME

TUESDAY, APRIL 2, 2013

08:30 AM REGISTRATION
09:30 AM Inauguration: Recitation from the Holy Quran
09:35 AM Welcome Address BY Director General, PMNH
09:45 AM Address by Secretary General, Zoological Society of Pakistan
10:00 AM Address by the President, Zoological Society of Pakistan
10:05 AM Address by Vice Chancellor, PMAS-AAUR
10:15 AM Address by Chairman, Pakistan Science Foundation
10:25 AM Distribution of Medals and Awards
11:00 AM Address by the Chief Guest, Secretary MoST
11:15 AM Vote of Thanks
11:20 AM Refreshment

12:05 PM - JOINT SESSION I: Plenary Lectures

Chairperson: Dr. Manzoor H. Soomro

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

- Speakers: 1. **Dr. Shahid Mahmood Baig**
Head, Health Biotechnology Division, NIBGE, Faisalabad
Prevention of genetic diseases in Pakistani population
2. **Dr. Richard Bishof**
Determination of photographic detectability and implication for camera trap studies targeting multiple carnivores.
3. **Dr. Manzoor Hussain Soomro and Dr. Muhammad Rafique**
Pakistan Science Foundation, Islamabad and Pakistan Museum of Natural History, Islamabad.
Role of UNESCO's Man and Biosphere programme in Conservation of Natural Habitats and Biodiversity.
- 01:00 PM Lunch and Prayer

7:30 PM - JOINT SESSION II: Plenary Lectures

Chairperson: Prof. Dr. M. Afzal kazmi
Co-chairperson: Dr. Abdul Majeed Cheema

- Speakers: 1. **Dr. Nusrat Jahan**
Chairperson, Department of Zoology, GC University, Lahore.
Advanced trends for the control of dengue vectors in Pakistan
2. **Dr. Mirza Habib Ali,**
Islamabad
Funding Opportunities under Natural Sciences Linkage Programme of PSF

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

	Chairperson:	Prof. Dr. Mazhar Qayyum
	Co-chairperson:	Dr. Muhammad Naeem
02:15 PM	Paper reading	
04:30 PM	Tea Time	

SESSION II

	Chairperson:	Prof. Dr. Bushra Mirza
	Co-chairperson:	Dr. Irfan Zia Qureshi
05:00 PM	Paper reading	
06:25 PM	Prayer	

SESSION III

	Chairperson:	Prof. Dr. Shamsuddin Shaikh
	Co-chairperson:	Dr. Bushra Muneer
06:40 PM	Paper reading	

HALL – 2

SECTION II: PEST AND PEST CONTROL

SESSION I

Chairperson: Prof. Dr. Naheed Ali
Co-chairperson: Dr. Riffat Sultana
02:00 PM Paper reading
04:30 PM Tea Time

SESSION II

Chairperson: Prof. Dr. Imtiaz Ahmad
Co-chairperson: Dr. Abid Farid
05:00 PM Paper reading
06:25 PM Prayer

SESSION III

Chairperson: Prof. Dr. Mushtaq A. Saleem
Co-chairperson: Dr. Muhammad Hamid Bashir
06:40 PM Paper reading
07:30 PM Executive Council Meeting
08:30 PM Dinner

HALL – 3

SECTION IV: PARASITOLOGY

SESSION I

Chairperson: Prof. Dr. Asmatullah Kakar
Co-chairperson: Dr. Syeda Azra Qamar
02:15 PM Paper reading
04:30 PM Tea Time

SESSION II

Chairperson: Prof. Dr. Akram Shah
Co-chairperson: Dr. Noor un Nisa
05:00 PM Paper reading
06:30 PM Prayer

WEDNESDAY, APRIL 3, 2013

09:00 AM - JOINT SESSION III: (Plenary Lectures)

Chairman: Prof. Dr. A.R. Shakoori
Co-chairman: Prof. Dr. Imtiaz Ahmad

- Speaker
1. **Mr. Abdul Aziz Khan,**
Islamabad
Bioeconomic impacts of vertebrate detriogens in agro-forestry systems of Pakistan

 2. **Prof. Dr. Abdul Majeed Cheema,**
University of Lahore, Lahore
Thyroid: Phylogeny and function

 3. **Dr. Syed Azhar Hasan,**
Ex-Director General, Pakistan Museum of Natural History, Islamabad
High altitude butterflies of Pakistan and their responses to climate change

HALL – 1

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

SESSION IV

	Chairperson:	Dr. M. Afzal Ghauri
	Co-chairperson:	Dr. Sarwat Jahan
10:30 AM	Paper reading	
11:00 PM	Tea Break	

SESSION V

	Chairperson:	Prof. Dr. Javaid Iqbal Qazi
	Co-chairperson:	Dr. Bilal Haider Abbasi
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. Amina Zubieri
05:00 PM Paper reading
08:00 PM General Body Meeting
08:00 PM Dinner

HALL – 2

SECTION III: PEST AND PEST CONTROL

SESSION IV

Chairperson: Mr. Abdul Aziz Khan
Co-chairperson: Mr. Shahid Munir
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. Samina Qamar
02:00 PM Paper reading
04:30 PM Tea Break

SESSION II

	Chairperson:	Dr. Syed Azhar Ali
	Co-chairperson:	Dr. Shafique Saeed
05:00 PM	Paper reading	
08:00 PM	General Body Meeting	
08:00 PM	Dinner	

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

	Chairperson:	Dr. Abdul Aleem Chaudhry
	Co-chairperson:	Dr. Muhammad Ali Nawaz
10:30 AM	Paper reading	
11:00 AM	Tea Break	

SESSION III

	Chairperson:	Dr. Abdul Aleem Chaudhry
	Co-chairperson:	Dr. Farkhanda Manzoor Duggal
11:30 AM	Paper reading	
01:00 PM	Lunch Break and Prayer Break (Zuhar)	

SESSION IV

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

SESSION V

	Chairperson:	Prof. Dr. Pirzada Jamal Siddiqui
	Co-chairperson:	Dr. Javed Mustaquim
05:00 PM	Paper reading	

07:00 PM - JOINT SESSION IV: (Plenary Lectures)

Chairman: Dr. Shahzad A. Mufti
Co-chairman: Prof. Dr. M. Suleman

- Speaker
1. **Dr. Abdul Aleem Chaudhary,**
Ex-Director General, Wildlife & Parks (Punjab), Lahore
Biodiversity and its status in Pakistan
 2. **Prof. Dr. Imtiaz Ahmad,**
M.A.H. Qadri Biological Research Centre, University of Karachi, Karachi
Control of Dengue fever in Pakistan

THURSDAY, APRIL 4, 2013

09:00 AM - JOINT SESSION V: (Plenary Lectures)

Chairman: Prof. Dr. A.R. Shakoori
Co-chairman: Prof. Dr. Shamsuddin Shaikh

- Speaker
1. **Prof. Dr. Perwaiz Iqbal,**
Department of Biological & Biomedical Sciences, The Aga Khan, Karachi
Dietary patterns and coronary heart diseases.
 2. **Prof. Dr. Syed Akram Shah,**
Department of Zoology, University of Peshawar, Peshawar
Current status of cutaneous leishmaniasis.
 3. **Prof. Dr. Pirzada Jamal Siddiqui,**
Director, Center of Excellence in Marine Biology, University of Karachi
Status of recent knowledge on marine cetacean abundance and diversity in Pakistani waters

11:00 AM Tea Break

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

	Chairperson:	Prof. Dr. Shamim Akhtar
	Co-chairperson:	Dr. Sajid Malik
10:30 AM	Paper reading	
11:00 AM	Tea Break	

SESSION IX

	Chairperson:	Prof. Dr. Muhammad Shahbab
	Co-chairperson:	Dr. Firdous Kazmi
11:30 AM	Paper reading	
01:00 PM	Lunch Break	

SESSION X

	Chairperson:	Prof. Dr. Samina Jalali
	Co-chairperson:	Dr. Qamar Javed
02:00 AM	Paper reading	

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. Nasreen Memon
	Co-chairperson:	Dr. Muhammad Athar Rafi
11:30 AM	Paper reading	

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

SESSION IIIV

	Chairperson:	Prof. Dr. Muhammad Akhtar
	Co-chairperson:	Dr. Ghazala Roohi
11:30 AM	Paper reading	
01:00 PM	Lunch Break (Zuhar)	

HALL – 3

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

SESSION VI

	Chairperson:	Prof. Dr. N.T. Narejo
	Co-chairperson:	Dr. Zafar Iqbal
10:30 AM	Paper reading	
11:00 AM	Tea Break	

SESSION VII

	Chairperson:	Dr. Muhammad Rafique
	Co-chairperson:	Dr. Zaigham Hassan
11:30 AM	Paper reading	
01:00 PM	Lunch Break (Zuhar)	

SESSION VIII

	Chairperson:	Prof. Dr. M. Naeem Khan
	Co-chairperson:	Prof. Dr. Ali Muhammad Yousafzai
02:00 PM	Paper reading	
04:00 PM	Concluding Ceremony Recitation	
04:05 PM	Congress Report by President ZSP	
04:15 PM	Award Ceremony	
05:00 PM	Concluding Remarks by the Chief Guest	
05:25 PM	Vote of Thanks	
05:30 PM	Refreshments	

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

1. Muzaffar Ahmad Gold Medal 2013

Fourteenth Muzaffar Ahmad Gold Medal 2011 was received by Ms. Maryam Umer for obtaining first position in the M.Sc. Zoology examination of the University of the Punjab.

2. Ahmed Mohiuddin Memorial Gold Medal 2013

Ninth Ahmed Mohiuddin Memorial Gold Medal 2013 was given to Ms. Bakhtawar Soomro, who obtained first position in the M.Sc. Zoology examination of the University of Sindh, Jamshoro.



Ms. Bakhtawar Soomro

3. Afsar Mian Gold Medal 2013

Fourth Afsar Mian Gold Medal 2013 was given to Ms. Maria Barkaat who obtained first position in the M.Sc. Biology/Zoology examination of the Arid Agriculture University, Rawalpindi.



Ms. Maria Barkaat

4. Muhammad Afzal Hussain Memorial Gold 2013

Fourteenth Muhammad Afzal Hussain Memorial Gold 2013 was given to Ms. Saira Bano for obtaining first position in Parasitology for her M.Sc. Zoology examination of the University of Karachi.



Ms. Saira Bano

5. Mujib Memorial Gold Medal 2013

Seventeenth Mujib Memorial Gold Medal 2013 was given to Ms. Aatika Sikandar, who obtained first position in the M.Sc. Zoology examination of the University of Sindh, Jamshoro.



Ms. Aatika Sikandar

6. Prof. Dr. S.N.H. Naqvi Gold Medal 2013

Eighth Prof. Dr. S.N.H. Naqvi Gold Medal 2013 was given to Dr. Uzma Mehboob for his Ph.D. degree in Zoology specializing in the field of Toxicology from University of Karachi, Karachi.



Dr. Uzma Mehboob

7. **M.A.H. Qadri Memorial Gold Medal 2013**

Eighth M.A.H. Qadri Memorial Gold Medal 2013 was given to Dr. Muti-ur-Rehman for his Ph.D. degree in Zoology specializing in the field of Parasitology from University of Karachi, Karachi.



Dr. Muti-ur-Rehman

SOME NEW RUMINANT FOSSILS FROM DHOK BUN AMIR KHATOON OF THE LOWER SIWALIKS, PUNJAB, PAKISTAN

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Abstract. – The new ruminant remains from the Middle Miocene outcrops nearby Dhok Bun Amir Khatoon (DBAK) of northern Pakistan, have been described and discussed. This paper deals with the study of the following identified macromammals from Dhok Bun Amir Khatoon: *Miotragocerus*, *Gazella*, *Hyotherium*, *Conohyus* and *Gaindatherium*. They point to an age of the Middle Miocene. The new material from the DBAK reinforces the idea that a variety of ruminants were present in the outcrops of the Lower Siwaliks during the Middle Miocene. The analysis of the recovered dental characters was allowed individual variations and contributes knowledge regarding the Siwalik Middle Miocene ruminants.

Keywords: Vertebrate, Mammalia, Artiodactyla, Miocene, Siwaliks.

INTRODUCTION

The studied material comes from the sediments of Dhok Bun Amir Khatoon (72° 55' 45.4 E, 32° 47' 26.4 N) of the Chakwal district, Punjab, Pakistan (Fig. 1). The DBAK (Dhok Bun Amir Khatoon) area consists of an almost unbroken geological record spanning from 18.4 to 4.5 Ma and interpret all five of the Siwalik formations (Kamlial, Chinji, Nagri, Dhok Pathan and Soan (Johnson *et al.*, 1982). Cheema (2003) and Khan *et al.* (2008) illustrate the DBAK fauna in the middle part of the Chinji Formation, comprising both macro- and microvertebrate assemblages.

Dhok Bun Amir Khatoon is inadequately recognized fossil site of the Siwaliks which had got the attention of the researchers towards itself when few fossils were discovered during the involuntary work for erection of a dam. Earlier workers (Pilgrim, 1910, 1911; Matthew, 1929; Colbert, 1933, 1935) neither gave any attention to this site nor mentioned it in their faunal list. Generally, the Siwalik group in the DBAK area is composed of five lithostratigraphic units,

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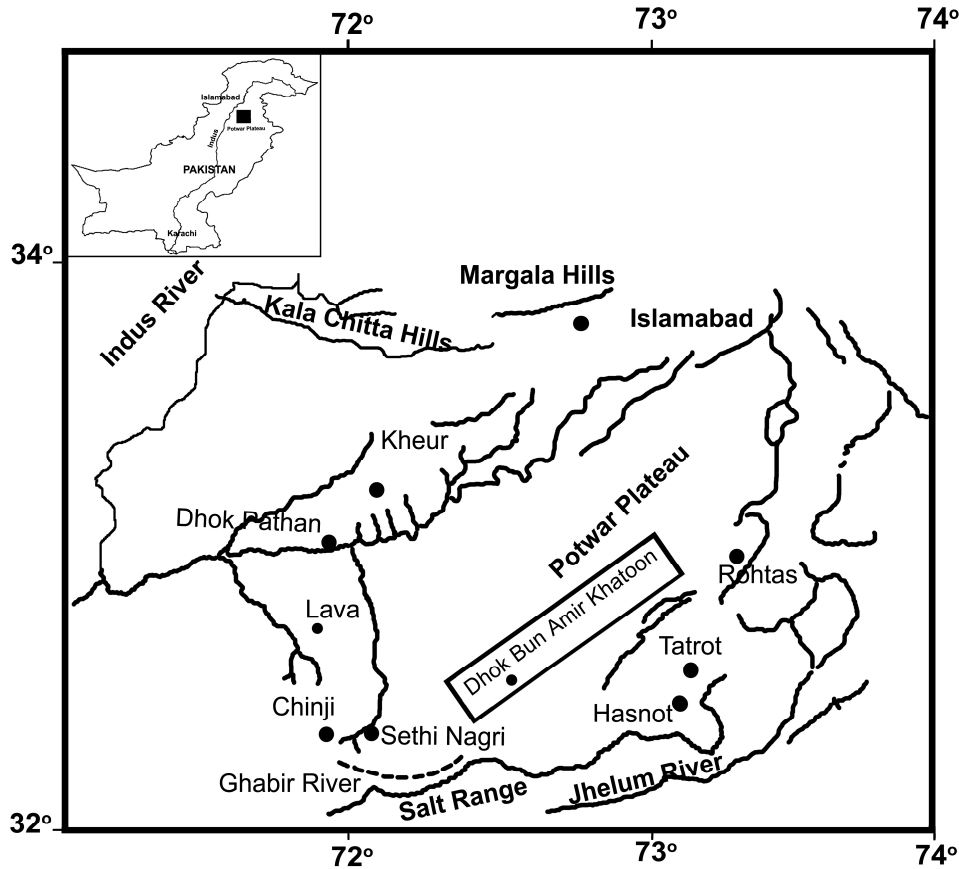


Fig. 1. Map of Pakistan with an enlargement of the Chakwal area and the location of Dhok Bin Amir Khatoon where the described material has been found.

which are Kamliyal, Chinji, Nagri, Dhok Pathan and Soan. The overall lithological composition of these component formations are fairly identical with those described for their type sections however they are relatively less thick and contain more mud stones. Geological analysis of DBAK pointed out that these deposits show fossil rich sediments covering 6-7 km and are up to 30-40 m thick. These silt sediments are wealthy in a variety of animal remains.

The studied specimens were collected from the outcrops belong to the Chinji Formation. The Chinji Formation is composed of red brown mud stone (Behrensmeyer, 1987; Barry *et al.*, 1982, 2002). The deposits consist of shales,

siltstones and sand stones. The sandstones are medium to coarse grained and thick bedded to massive. The locality represents lateral facies associations and pedogenesis within the fine grained fossil-bearing floodplain deposits that are characteristic of fluvial depositional environment. This is dominantly composed of bright red and brown orange siltstones inter bedded with soft, ash grey sandstones. The sediments were probably deposited in a fluvial environment leaving behind the fine-grained and fossil-bearing flood plains (Barry *et al.*, 2002). Fluvial depositional environment is also represented by lithofacies and characteristic faunal composition. The lithostratigraphy of the area was described in detail by many researchers (Behrensmeyer *et al.*, 1995; Barry *et al.*, 2002).

The Middle Miocene outcrops of Dhok Bun Amir Khatoon contain a significant volume of ruminant remains and consequently, a special attention is paid to the fossils remains of ruminants. The material provides additional evidence for the middle Miocene/earliest upper Miocene ruminant fauna of Pakistan.

Abbreviations

PUPC, Punjab University Palaeontological Collection, Lahore, Punjab, Pakistan; PC-GCUF, Palaeontological Collection of Government College University Faisalabad, Punjab, Pakistan; Ma, Million years ago.

MATERIALS AND METHODS

The outcrops nearby the Dhok Bun Amir Khatoon village of the Chakwal district, northern Pakistan (Chinji Formation) have been thoroughly investigated in order to obtain the ruminant fossils. Surface collection was the primary method to collect the fossils however the embedded material was excavated with the help of geological hammers, chisels and fine needles. In the laboratory, the fine needles and brushes were used to remove sediments. The measurements are expressed in millimeters and taken with the help of digital caliper.

The morphological and metrical characters of the specimens are described and their systematic determination is discussed. The catalogue number of the specimens consists of series *i.e.*, yearly catalogue number and serial catalogue number, so figures on the specimen represent the collection year (numerator) and serial number (denominator) of that year (*e.g.* 09/12). Uppercase letters stand for upper dentition (*e.g.* M) and lower case for lower dentition (*e.g.* m). The

terminology of the tooth crown elements and manners of measurements follow Gentry and Hooker (1988).

SYSTEMATIC PALAEOLOGY

Order Cetartiodactyla Montgelard, Catzefflis and Douzery, 1997

Infraorder Pecora Linnaeus, 1758 sensu Webb and Taylor, 1980

Family Bovidae Gray, 1821

Subfamily Bovinae Gray, 1821

Genus *MIOTRAGOCERUS* STROMER, 1928

Miotragocerus cf. *gluten* (Pilgrim, 1937)

New material

PUPC 69/731, rM2; PUPC 13/54, lM2; PUPC 68/353, lM2; PUPC 13/55, lM2.

Description and comparison

The cusps in upper molars are in oblique form lingually and the lingual lobes are less constricted (Fig. 2A, B). The enamel is shiny and rugose. The labial notch is V shaped. The styles and columns are strong labially. The mesostyle, metastyle and parastyle are well developed in upper molars. The metastyle and parastyle are almost equal in height. The metaconus and paraconus ribs are present. The paraconus rib is connected to the parastyle basally. The entostyle is absent. The pre-fossette is narrower than the post-fossette. The lower molars are narrow crowned (Fig. 2C, D). The apices of metaconid and entoconid are more conical than hypoconid and protoconid. The protoconid is slightly wider than hypoconid. The post-proto-cristid and post-hypo-cristid are equally strong. The metaconid rib is stronger than entoconid rib. The ectostylid is present. The metaconid and the paraconid stylids are equal in height. The anterior transverse flange is present. The fossettes are narrow.

The upper molars are characterized by quadrate shape, subhypsodonty and developed parastyle. The lower molars are characterized by brachydonty, strongly arched teeth, strong mesostylid labially, definitely convex non smooth lingual wall, shiny enamel, presence of the anterior transverse flange, deep fossettes of trigonid and talonid. The external features of the dental specimens are archetypal of Miocene boselaphines often; the conflicting styles of the teeth make their addition in boselaphines. The studied upper molars are well

accentuated distinguished than those of *Tragoportax* (Spassov and Geraads, 2004). Morphometrically, the teeth show the typical resemblance with *Miotragocerus* and match (Fig. 2A-D; Table I) with the type specimens of *Miotragocerus gluten* (Pilgrim, 1937; Spassov and Geraads, 2004; Khan *et al.*, 2010).



Fig. 2. *Miotragocerus* cf. *gluten*: **A**, PUPC 69/731-rM2; **B**, PUPC 13/54-IM2; **C**, PUPC 68/353-lm2; **D**, PUPC 13/55- lm2. cf. *Gazella* sp.: **E**, PUPC 67/83, right mandible fragment with m2-3. cf. *Hyotherium*: **F**, PUPC 13/48, partial probably lower molar. cf. *Conohyus sindiensis*: **G**, PUPC 13/47-IM2. Views are occlusal (Aa, Ba, Ca, Da, Ea, Fa, Ga), lingual (Ab, Bb, Cb, Db, Eb) and labial (Ac, Bc, Cc, Dc, Ec).cf. *Gaindatherium browni*: **H**, PUPC 13/56-i2 (a, lingual; b, labial; c, medial; d, lateral). Scale bar 10 mm.

Subfamily Gazellinae Coues, 1889

Genus *GAZELLA* Blainville, 1816

cf. *Gazella* sp.

New material

PUPC 67/83, right mandible fragment with m2-3.

Description and comparison

The lower molars have goat folds along the mesial side (Fig. 2E). The ectostylid is weak and the enamel is rugose. A longitudinal tubercle is present

along the mesio-labial side. The entostylid is sharp and prominent. The protoconid and metaconid are pointed (Fig. 2E). The prae-entocristid and the post-entocristid are sloped downwardly. The fossettes are narrow and crescent shape. The metaconid rib is weaker than the entoconid rib. The hypoconulid in the third molar is prominent, oval shaped with a furrow on the lingual side and it is inclined towards the labial side. The studied dental material is characterized by goat fold and small ectostylid. The prominent goat folds on the lower molars are the characters that support their inclusion into the genus *Gazella* (Fig. 2E). The studied dental sample fit well with that of the already described sample of *Gazella* sp., reported from the Lower Siwaliks (Pilgrim, 1937). The sample is insufficient and can be assigned to cf. *Gazella* sp.

TABLE I.- COMPARATIVE MEASUREMENTS (MM) OF THE CHEEK TEETH OF THE DHOK BUN AMIR KHATOON RUMINANTS.

Taxa	Number	Nature/Position	Length	Width	W/L
<i>M. cf. gluten</i>	PUPC 69/731*	rM2	14.5	14.5	1.00
	PUPC 13/54*	IM2	13.6	14.3	1.05
	PUPC 68/353*	lm2	11.9	7.90	0.66
	PUPC 13/55*	lm2	13.8	8.00	0.57
<i>Gazella</i> sp.	PUPC 67/83*	rm2	14.7	9.4	0.63
		rm3	21.0	8.8	0.41
	PC-GCUF 11/05	rm2	16.0	10.0	0.63
	PC-GCUF 11/04	rm2	17.0	11.0	0.65
		rm3	22.5	10.0	0.47
	PC-GCUF 11/10	lm3	22.0	10.5	0.48
	PC-GCUF 11/68	lm3	23.0	11.0	0.47
	PUPC 84/131	rm1	13.3	9.0	0.67
	PUPC 84/127	lm1	12.0	8.0	0.66
		lm2	14.0	8.0	0.57
	PUPC 84/133	rm2	15.4	9.7	0.62
		rm3	20.0	9.0	0.45
	PUPC 94/21	lm3	22.0	9.0	0.40
	PUPC 02/15	lm3	22.0	10.2	0.46
	PUPC 97/20	lm3	20.0	8.6	0.43
PUPC 86/30	lm3	21.0	9.0	0.42	
PUPC 02/22	rm3	21.5	9.0	0.41	
<i>C. sindiensis</i>	PUPC 13/47*	IM2	16.4	16.4	1.00
<i>G. browni</i>	PUPC 13/56*	i2	15.9	9.4	0.59

* The studied specimens. referred data are taken from Akhtar (1992), Khan *et al.* (2009a) and unpublished specimens.

Family Suidae Gray, 1821
Subfamily Hyotheriinae
Genus *HYOTHERIUM* von Meyer, 1834
cf. *Hyotherium*

New material

PUPC 13/48, partial probably lower molar.

Description and comparison

The material is very poor in nature (Fig. 2F). The part of the molar is preserved. The molar is bunodont with moderately deep furchen. The cingular remnant is present. The enamel is moderately thick. The preserved cusp is inflated to small extent. The material is scanty and it is too difficult to assign it precisely. However, the morphological features and open lingu-labial notches of the molar share some characteristics with *Hyotherium* (Pickford, 1988).

Subfamily Tetraconodontinae Lydekker, 1876
Genus *CONOHYUS* Pilgrim, 1926
cf. *Conohyus sindiensis*

New material

PUPC 13/47, 1M2.

Description and comparison

The second upper molar has broader the anterior pair of cusps than the posterior pair ones (Fig. 2G). The molar is almost unworn. The metacone is slightly smaller than the paracone. The protocone lie more distally than the paracone. The furchen is shallow. The anterior cingulum is prominent. The labial cingulum is developed at the median valley end. The feature of the molar: relatively broadly spaced, tall cusps, poorly developed labial cingulum and great buccal and lingual swelling of the base associates the smaller tetraconodont lineage *Conohyus*. *Conohyus sindiensis* is a moderate sized member of the genus and *C. indicus* is a larger species of the genus (Pickford, 1988; Vander, 1999). Morphometrically, the sample agrees to *Conohyus sindiensis*.

Order Perissodactyla Owen, 1848
Family Rhinocerotidae Gray, 1825
Subfamily Rhinocerotinae Gray, 1821
Tribe Rhinocerotini Gray, 1821
Subtribe Rhinocerotina Owen, 1845
Genus *GAINDATHERIUM* Colbert, 1934
cf. *Gaindatherium browni*

New material

PUPC 13/56, i2.

Description and comparison

The incisor is small and semicircular in outline (Fig. 2H). The incisor is delicate with thin enamel and the enamel layer is damaged in most of the tooth surface. The incisor is comparatively slim and weak representing a female animal. Its worn surface is short. It is straight and its worn surface faces posterolaterally. Morpho-metrically, the studied sample resembles with the Middle Miocene Rhinoceros species *Gaindatherium browni* (Colbert, 1935; Heissig, 1972) and can be assigned to cf. *Gaindatherium browni*.

DISCUSSION AND CONCLUSION

The Middle Miocene deposits of Dhok Bun Amir Khatoon, Pakistan have produced a good record of ruminants (Cheema, 2003; Khan *et al.*, 2008, 2011; Ghaffar, 2010; Samiulla *et al.*, 2012). The remains of ruminants are the most utilizing source in the determination of ecological changes. In ruminants, as habitat indicator, mostly Bovidae remains have been used which reflects their importance (Kappelman, 1988). The rhinocerotids, tragulids, bovids and giraffids are common elements of the fauna (unpublished data).

The Dhok Bun Amir Khatoon's faunal composition suggests a fluvial depositional environment with two successive fossiliferous layers. The lower layer resembles to the late Oligocene and early Miocene localities of Bugti hills (Khan *et al.*, 2008; Welcomme *et al.*, 2001). Nevertheless the presence of *Chilotherium blanfordi*, ruminants (*Dorcatherium* sp., *Eotragus* sp. *Eotragus* large size sp., *Palaeohypsodontus* sp.), and a listriodont suid in the lower layer suggests an Early Miocene age (Welcomme *et al.*, 1997, 2001; Antoine and Welcomme, 2000). The upper level represents the fauna of the upper Chinji

Formation. The selenodonty of the Dhok Bun Amir Khatoon ruminants may be interpreted for rubbery foods which may have been the marsh vegetation due to the depositional environment.

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ETHANOL FERMENTATION BY LOCALLY ISOLATED MUTANT STRAIN OF *SACCHAROMYCES CEREVISIAE* FSAT-89

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Abstract.- The present study describes the ethanol fermentation by mutant strain of locally isolated *Saccharomyces cerevisiae fari-15*. Thirty cultures of yeast were isolated from different local habitats (air, water, soil etc.). The culture *Saccharomyces cerevisiae fari-15* with maximum ethanol yield (8.5%) was selected and exposed to UV radiations for different time periods (10, 20 and 30 seconds). The strain irradiated for 30 seconds gave maximum ethanol yield. This strain was selected and designated as *S. cerevisiae* FSAT-89. Batch and Fed-batch fermentation processes were carried out in shake flasks. Fermentation conditions sugar concentration, temperature, pH, inoculum size, inoculum age, aeration, time course were optimized for maximum ethanol production. Maximum (9.3 %) ethanol production was obtained at temperature 35 °C, pH 4.5, inoculum size 6.0 % (v/v) and inoculum age 24 h and sugar concentration 15 %. Fed-batch fermentation was carried out to enhance ethanol yield in short time period. Ethanol fermentation under fed batch fermentation was maximum at 5th stage of fermentation. The results revealed that *S. cerevisiae* FSAT-89 showed high efficiency for general strain improvement than wild-type.

Keywords: Sugarcane molasses, ethanol fermentation, *Saccharomyces cerevisiae* FSAT-89, UV mutagenesis, optimization.

INTRODUCTION

In the present state of energy crisis, efforts are being made to reduce the dependence upon fossil fuels and search for renewable and alternate sources. Ethanol is the most important alternative liquid fuel that declines negative environmental impacts of fossil fuel burning (Cardona and Sanchez, 2007). It can be used in unmodified petrol engines with current fueling infrastructure and it is easily applicable in present day combustion engine, as mixing with gasoline (Hansen *et al.*, 2005). Ethanol is a neutral liquid with pleasant smell. The diluted solution of ethanol has a sweet taste while more intense solutions have very strong taste (Patil, 1991). The melting and boiling points of ethanol are -114.1°C and 78.5°C, respectively, and also has density of 0.789 g/ml at 20°C. Ethanol is

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also used as an antifreeze agent in radiators of motor vehicles (Kaur and Kocher, 2002). Ethanol is mainly produced by fermentation of sugary materials. Various raw materials like sugarcane juice and molasses, sugar beet, beet molasses, Sweet sorghum and starchy materials like sweet potato, corn cobs and hulls, cellulosic materials like cocoa, pineapples and sugarcane waste and milk/cheese/whey using lactose hydrolyzing fermenting strains are reported in the literature (Sree *et al.*, 2000; Agrawal *et al.*, 1998; Morimura *et al.*, 1997; Bulawayo *et al.*, 1996; Silva-CA-da *et al.*, 1995; Beall *et al.*, 1992; El-Diwany *et al.*, 1992; Othaman *et al.*, 1992). *S. cerevisiae* is the widely used industrial microorganism for ethanol production and is commonly recognized as "bakers yeast" or "brewers yeast". The yeast converts reducing sugars (glucose, fructose and sucrose) into CO₂ and ethanol present in the raw material used (Moreira *et al.*, 2005; Converti *et al.*, 2003; Walker *et al.*, 1990). The present work describes ethanol fermentation from sugarcane molasses by locally isolated mutant strain of *S. cerevisiae*.

MATERIALS AND METHODS

Molasses

Sugarcane molasses collected from Shakarganj Mills Limited Jhang, Punjab, Pakistan was used for ethanol fermentation by *S. cerevisiae*. Molasses with initial sugar content of 49 % was diluted to 30% sugar content. Molasses was pretreated by adding concentrated H₂SO₄, heated at 80°C for 30 min and left overnight. The upper shiny black layer of molasses was collected to be used as fermentation medium while precipitates of trace metals (yellowish brown) left at the bottom.

Organism

Yeast cultures were isolated from different local habitats (water, soil, air etc.) by pour plate method. Independent young colonies were transferred to yeast extract peptone glucose (YPG) agar slants and incubated at 30°C for 24 h. The isolated cultures were screened to check their ethanol producing efficiencies through fermentation technique. The best culture of *S. cerevisiae* was selected and identified under the microscope (Nikon Eclipse E 200).

UV mutagenesis of S. cerevisiae

Twenty four hours old culture of *S. cerevisiae* was used for UV mutagenesis following the method described by Petrea (Mukhtar *et al.*, 2010). The 24 h old culture grown on YPG agar plates was placed under UV lamp

(CAMAG, USA) at a distance of 55 cm and were irradiated at 254 nm for 10, 20 and 30 seconds. The agar plates were kept in the dark for 1 h. The irradiated yeast colonies were then spread onto YPG agar plates and kept at 30°C for incubation (EHRET BK4444, Germany) for three days and then mutant strain was transferred onto agar slants.

Fermentation techniques

Batch fermentation

Batch fermentation was carried out in 500 ml flasks containing clarified sugarcane molasses (15 % sugar concentration) with initial pH of 4.5. The fermentation medium was supplemented with salt solutions. The fermentation medium was inoculated with 24 h old mutant strain of *S. cerevisiae*.

Fed-batch fermentation

Fed-batch fermentation was carried out in 1000 ml Erlenmeyer flask containing 1000 ml medium with initial pH of 4.5, 15% (w/v) sugar concentration, 6% (v/v) inoculum size at 35°C. After 24 h, 600 ml fermented broth was taken out from the flask under aseptic conditions and in return 600 ml fresh sterilized medium was added to the flask and the fermentation was continued. The process was repeated similarly for another four stages up to 120h.

Analytical method

Qualitative analysis of ethanol

About 5 to 10 ml fermented sample was taken and a pinch of potassium dichromate and a few drop of H₂SO₄ were added. The color of the sample turned from pink to green which indicated the presence of ethanol.

Quantitative analysis of ethanol

The ethanol content in the fermented broth was measured by ebulliometer (Mukhtar *et al.*, 2010).

Analysis of reducing sugars

Reducing sugars in fermented mash were estimated by rapid method (Zaidi, 1998).

RESULTS AND DISCUSSION

Isolation and screening of yeast cultures

A total of 30 cultures of yeast were isolated from different local habitats (air, water soil etc.) and screened for ethanol production efficiencies (Table I). The locally isolated *S. cerevisiae* YS-15 gave better results (8.5%) after 48h of incubation.

Extensive studies have been carried out on the fermentation process of ethanol by fungus, bacteria and especially through yeast cells (Bajaj *et al.*, 2001). However, *S. cerevisiae* remained the organism of choice (Moreira *et al.*, 2005; Converti *et al.*, 2003; Walker *et al.*, 1990). This is due to the fact that these facultative anaerobes utilize Embden-Meyerhof pathway by having invertase genes and invertase enzymes for the conversion of sugars to ethanol and carbon dioxide or other metabolites (Fregonesi *et al.*, 2007; Kreutzfeldt and Witt, 1991).

Improvement of culture through UV mutagenesis

The culture of *S. cerevisiae* YS-15 was improved through UV mutagenesis to enhance ethanol production. *S. cerevisiae* YS-15 was exposed to UV radiation for different time periods (10, 20 and 30 Sec.) (Table II). The best culture was selected for the optimization of fermentation conditions. *S. cerevisiae* FSAT-89 gave better results (9.3%) than wild-type as compared with the work reported by other workers (Petrea, 2008).

Effect of sugar concentration

Sugar concentration in molasses was varied (15, 20, 25 and 30 %) to study their effect on ethanol production by *S. cerevisiae* FSAT-89 (Fig.1). Maximum ethanol production (9.3%) was obtained in the medium containing 15% sugar content. Further increase in sugar concentration decreased ethanol production. It is due to the fact that medium viscosity was increased because of higher sugar concentration, which resulted in the decreased metabolism, hence reduction in the ethanol production (Monte *et al.*, 2003; Amutha and Paramasamy, 2001).

Fermentation conditions

Temperature 35°C, pH 4.5, inoculum Size 6.0 % (v/v), inoculum age 24 h, time of fermentation 48 h.

TABLE I.- CULTURES OF YEAST ISOLATED FROM DIFFERENT LOCAL HABITATS.

Sr. No.	Isolates	Ethanol production (%)
1	fari-1	8.3
2	fari-2	7.5
3	fari-3	6.8
4	fari-4	5.2
5	fari-5	8.2
6	fari-6	8.0
7	fari-7	7.3
8	fari-8	6.5
9	fari-9	7.0
10	fari-10	7.8
11	fari-11	5.8
12	fari-12	7.2
13	fari-13	7.9
14	fari-14	6.4
15	fari-15	8.5
16	fari-16	4.5
17	fari-17	6.6
18	fari-18	4.9
19	fari-19	7.4
20	fari-20	3.9
21	fari-21	5.0
22	fari-22	8.4
23	fari-23	7.6
24	fari-24	3.5
25	fari-25	4.8
26	fari-26	6.3
27	fari-27	8.1
28	fari-28	5.1
29	fari-29	6.2
30	fari-30	7.7

TABLE II.- UV MUTAGENESIS OF *SACCHAROMYCES CEREVISIAE* FARI-15.

Sr.No.	UV exposure time (Sec.)	Ethanol production (%)
1	10	8.7
2	20	8.9
3	30	9.3

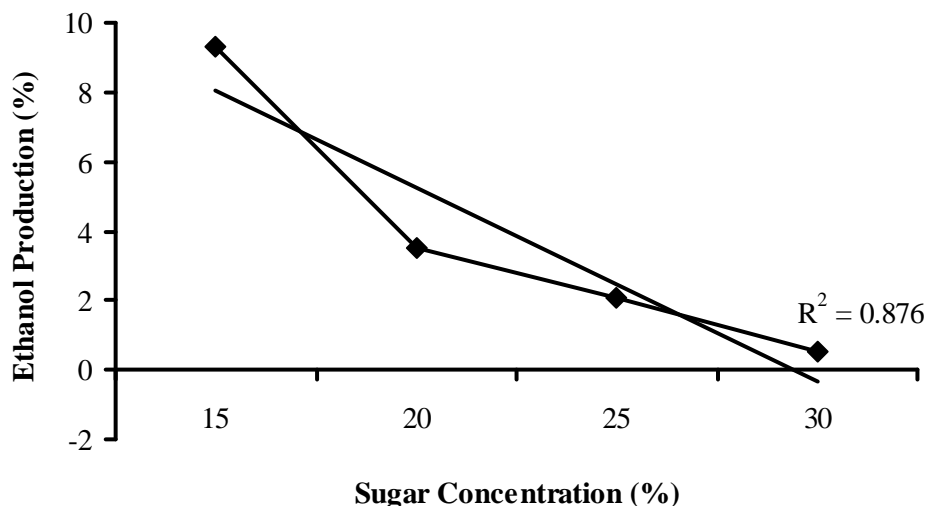


Fig. 1. Effect of sugar concentration on ethanol production.

Effect of temperature

Temperature has significant effect on ethanol production. The effect of temperature on ethanol production by *S. cerevisiae* FSAT-89 was investigated at different temperatures (25–40°C). Ethanol production was increased with increase in temperature up to 35°C (Fig.2). Maximum ethanol yield (9.3%) was obtained at 35°C. Further increase in temperature greatly reduced ethanol yield. This finding is in agreement with other workers (Sridhar *et al.*, 2002; Unaldi *et al.*, 2002).

Effect of pH

Effect of initial pH of fermentation media was studied in the pH range of 4.0-5.5 (Fig.3). The maximum conversion of sugar to ethanol by *Saccharomyces cerevisiae* FSAT-89 was achieved at pH 4.5. Ethanol yield was decreased by increase in pH due to the lower metabolic rate of the yeast cells. With increase in pH yeast produces acid rather than alcohol (Amutha and Paramasamy, 2001; Kourkoutas *et al.*, 2004; Mathewson, 1980).

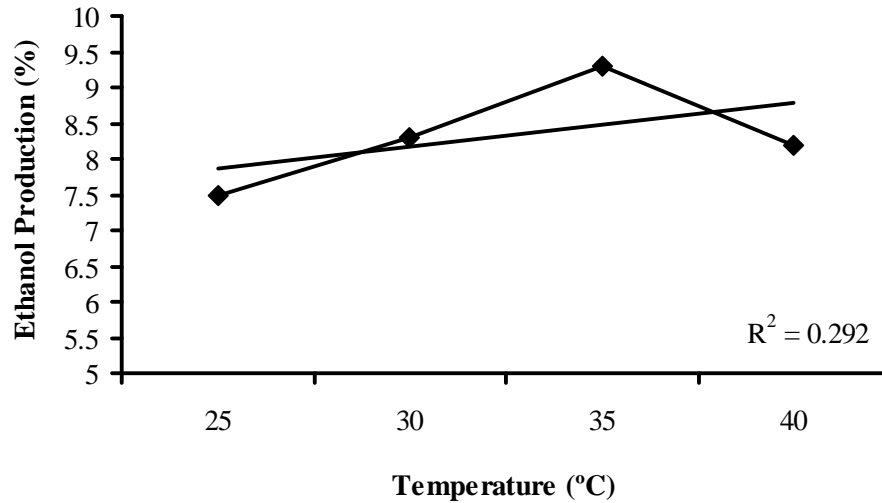


Fig. 2. Effect of temperature on ethanol production.

Fermentation conditions

Sugar concentration 15 %, pH 4.5, inoculum size 6.0% (v/v), inoculum age 24 h, time of fermentation 48 h.

Fermentation conditions

Sugar concentration 15%, temperature 35°C, inoculum size 6.0 % (v/v), inoculum age 24 h, Time of fermentation 48 h.

Effect of inoculum size

The size of inoculum in ethanol fermentation is of great importance in completing the fermentation process. Different sizes of inoculum ranging from 4.0-10.0 % (v/v) were used to inoculate the fermentation media (Fig. 4). Maximum ethanol production was achieved when inoculum size was 6.0 %. Further increase in inoculum size did not result in the considerable enhancement of ethanol production. This finding is not in agreement with other workers who reported 3% inoculum size for maximum ethanol production (Fregonesi *et al.*, 2007; Tahir *et al.*, 2010; Alegre *et al.*, 2003).

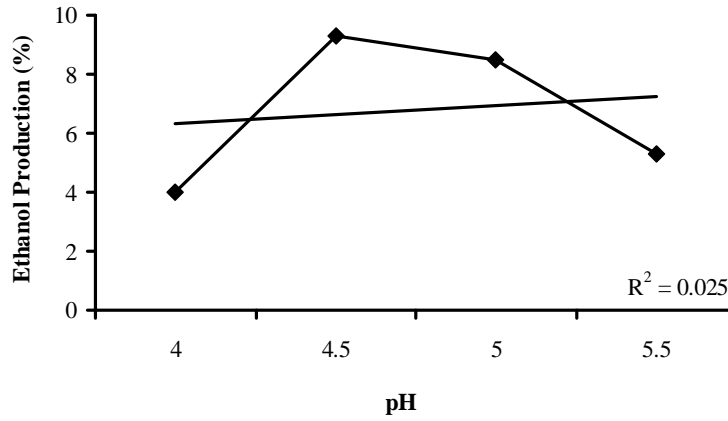


Fig. 3. Effect of pH on ethanol production.

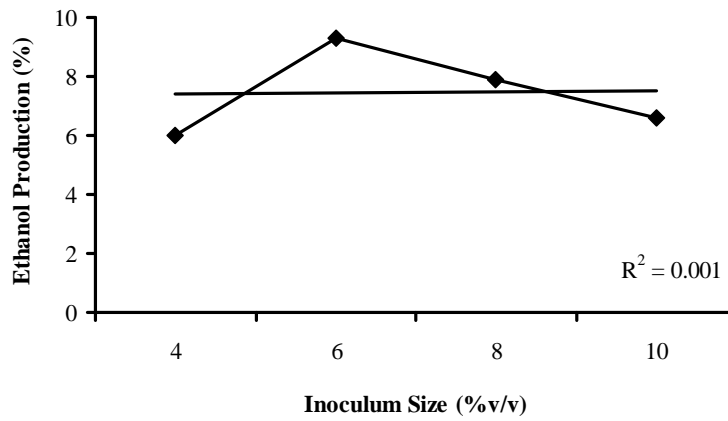


Fig. 4. Effect of inoculum size on ethanol production.

Fermentation conditions

Sugar concentration 15%, temperature 35°C, pH 4.5, inoculum age 24 h, time of fermentation 48 h.

Effect of inoculum age

Inoculum age is very important in obtaining maximum ethanol production

with minimum time. The inoculum of different age (12-48 h) was used to inoculate fermentation media (Fig. 5). Maximum ethanol production (9.5%) was obtained when 24 h old inoculum of *S. cerevisiae* FSAT-89 was used. This work is in agreement with the work done by other worker (Manikandan and Viruthagiri, 2010).

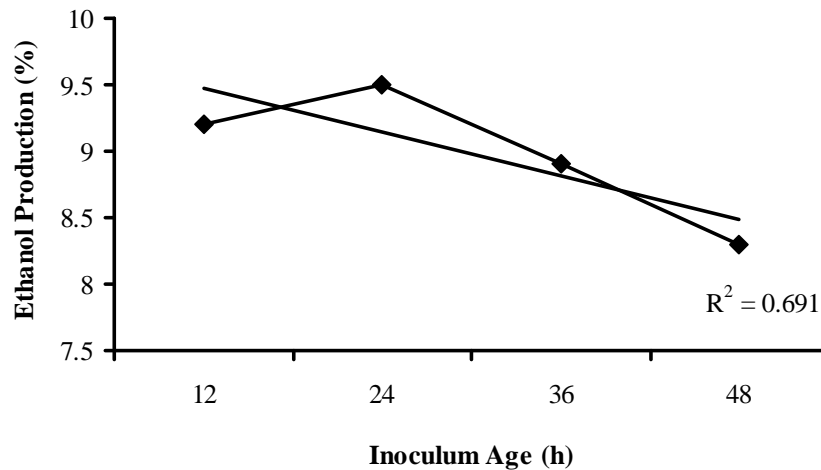


Fig. 5. Effect of Inoculum Age on Ethanol Production

Fermentation conditions

Sugar concentration 15%, temperature 35 °C, pH 4.5, inoculum size 6.0% (v/v), time of fermentation 48 h

Effect of aeration

The effect of aeration on ethanol production by *S. cerevisiae* FSAT-89 was studied by varying volumes of fermentation medium from 200-400 ml (in 500 ml Erlenmeyer flask). Maximum ethanol production was obtained, when 400ml working volume was used in 500 ml Erlenmeyer flask while low production was obtained at other volumes (Fig. 6). It is important to avoid a high degree of aerobic metabolism which utilizes sugar substrate but produces no ethanol. It has been found that trace amounts of oxygen may greatly stimulate yeast fermentation (Tahir *et al.*, 2010).

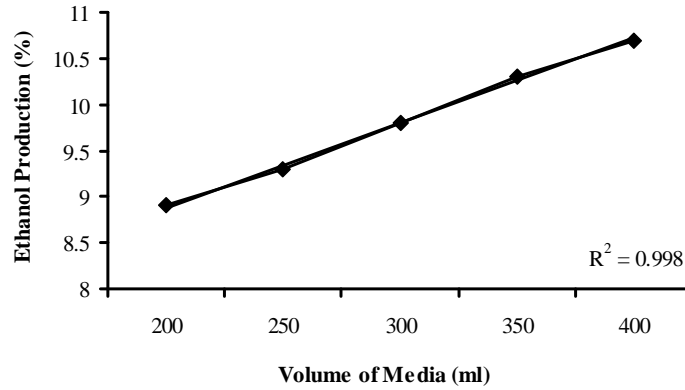


Fig. 6. Effect of aeration on ethanol production.

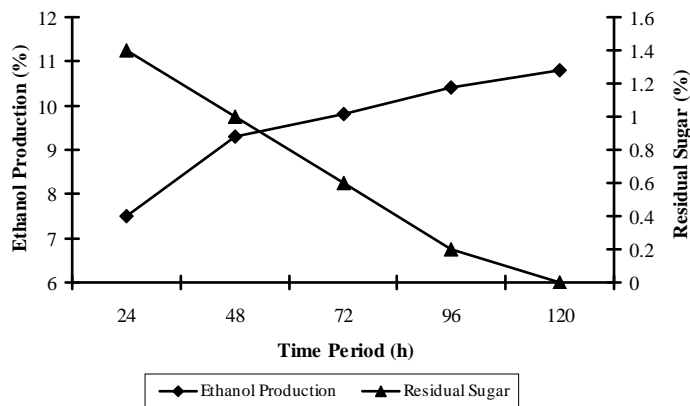


Fig.7. Time course of ethanol production.

Time course of ethanol fermentation

Fermentation profile of ethanol production was studied by carrying out fermentation for different time periods ranging from 24-120 h (Fig. 7). It was found that maximum ethanol (10.8%) was produced after 120 h with complete utilization of reducing sugars by *S. cerevisiae* FSAT-89 (0.00% residual sugars). This work is in agreement with the work done by other workers (Asli, 2010; Mariam *et al.*, 2009; Pramanik, 2003).

Fed-batch fermentation

The ethanol production by fed-batch fermentation was investigated under same optimum conditions as were used in batch process up to six stages. Maximum ethanol production (10.2 %) was observed at 5th stage and then gradually decreased (Table III). Fed- batch process gave higher ethanol yield (9.5 %) than batch fermentation. This is in agreement with the work done by other workers (Vu and Le, 2010; Laluce *et al.*, 2009).

TABLE III.- FED-BATCH ETHANOL FERMENTATION BY *SACCHAROMYCES CEREVISIAE* FSAT-89

Sr.No.	Fermentation stage	Ethanol production (%)
1	1 st	8.9
2	2 nd	9.0
3	3 rd	9.1
4	4 th	9.3
5	5 th	9.5
6	6 th	7.9

CONCLUSION

It was concluded from the present research work that *S. cerevisiae* FSAT-89 showed high efficiency (9.3%) in batch fermentation under optimized conditions (temperature 30°C, pH 4.5, inoculum size 6%(v/v), inoculum age 24 h). Fed-batch fermentation was carried out to enhance ethanol yield in short time period.

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**RELIABILITY OF URINE PUS CELL COUNTS ON MICROSCOPY
CORRELATED WITH URINE CULTURE COLONY COUNTS ON
CULTURE MEDIA FOR UTI DURING PREGNANCY**

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Abstract.- The urine pus cell counts reliability upto a level of significance as a significant pyuria was correlated with urine culture colony counts as a significant bacteriuria for the diagnosis of UTI during pregnancy. The correlation between significant pyuria and bacteriuria will be ensured the interpretation of urine pus cell counts under un-centrifuged microscopic examination as a rapid screening test which either investigate the UTI during pregnancy or reinvestigate the limit of urine pus cell counts in pregnant women. Present study of significant pyuria was recognized by following semi quantitative method for urine sample wet mount film preparation and 5-8 consecutive fields examination microscopically by using 40x objective lens as a high power field 2-4 pus cell/HPF. The significant bacteriuria was also recognized on the basis of urine sample measured quantity characterizing calibrated loop method for culture by following the semi quantitative culture method for urine sample culture colony count estimation visually as a colony forming unit per milliliter quantity $>10^5$ CFU/ml. In our study 56% urine samples out of 100 were positive by characterizing 2-4 pus cell/HPF under un-centrifuged microscopy called significant pyuria then on CLED agar culture was also positive by evaluating $>10^5$ CFU/ml called significant bacteriuria and 35% urine samples out of 100 was negative by characterizing 1-2 pus cell/HPF called insignificant pyuria. Gynecological clinicians can easily interpret significant pyuria for the management of UTI during pregnancy.

Keywords: Significant pyuria, Bacteriuria, Urinary Tract Infection (UTI), High Power Field (HPF), Colony Forming Unit (CFU).

INTRODUCTION

The reliability of urine pus cell counts upto a level of significant number as a significant pyuria and urine culture colony counts upto a level of significant

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growth as a significant bacteriuria has correlated for the diagnosis of UTI during pregnancy (Lin and Fajardo, 2008). Gynecological clinicians deal pregnant women suffering from UTI because they are enormously aware from bacterial UTI and their causative complications during pregnancy (Colgan *et al.*, 2006).

The ingestion and digestion capability as a phagocytic activity containing WBC or neutrophils detect bacteria as a foreign particle then either engulf or secrete leucocidin toxin which lyse and dead the neutrophils in the form of dead host cell endogenous material as a pus cell then excretes in urine by the process of blood filtration through kidneys as a part of human excretory system (Madiagan, *et al.*, 2006; Ahmad, *et al.*, 2015).

Significant pyuria is recognizing by following the semi quantitative method as a high power field under microscopic examination (Monica, 2006) then culture by following the semi quantitative culture method as a colony forming unit per milliliter under visual examination (John and Michel, 2006).

MATERIALS AND METHODS

Total 100 urine specimens were collected where 70 from GNSSH, Multan road, Lahore and 30 from SMCH, Raiwind road, Lahore with the coordination of Gynecological outpatient department. Urine specimens after collection were labeled and transported to the Microbiology laboratory without any delay (Monica, 2006).

Significant pyuria is recognizing by following the semi quantitative method for urine sample wet mount film preparation manually and 5-8 consecutive fields examination microscopically by using 40x Objective lens as a high power field characterizing 2-4 pus cell/HPF (Thomas, *et al.*, 1996).

CLED agar media was prepared in disposable petri plate under sterilization by autoclaving (Mackey and Sandys, 1966). Urine sample measured quantity characterizing calibrated loop method was used for culture on CLED agar then incubate at 37°C for overnight period (Monica, 2006; Shahzad, *et al.*, 2013).

Significant bacteriuria is also recognizing by following the semi quantitative culture method for urine sample culture colony count estimation visually as a colony forming unit per milliliter quantity of urine sample characterizing $>10^5$ CFU/ml (Thomas, *et al.*, 1996).

RESULTS AND DISCUSSION

Out of 100 urine samples 35 (35%) were showed insignificant bacteriuria ($<10^4$ CFU/ml) by characterizing 1-2 pus cell/HPF and 56 (56%) were showed significant bacteriuria ($>10^5$ CFU/ml) by characterizing 2-4 pus cell/HPF and 9 (9%) were also showed significant bacteriuria ($>10^5$ CFU/ml) by characterizing 10-12 pus cell/HPF as shown in Table I.

TABLE I.- CHARACTERIZATION OF BACTERIURIA WITH DIFFERENT NUMBERS OF PUS CELL/HPF COUNT

Pus cell/HPF		CFU/ml		Total
		< 10000 CFU/ ml	<100000 CFU/ ml	
1-2 Pus Cell/HPF	Count	35	0	35
	% of Total	35.0%	.0%	35.0%
2-4 Pus Cell /HPF	Count	0	56	56
	% of Total	.0%	56.0%	56.0%
10-12 Pus Cell / HPF	Count	0	9	9
	% of Total	.0%	9.0%	9.0%
Total	Count	35	65	100

65 urine samples out of 100 were positive for significant bacteriuria ($>10^5$ CFU/ml) by characterizing 2-4 pus cell/HPF and 10-12 pus cell/HPF respectively as shown in Table II.

Correlation between pus cell/HPF and bacterial CFU/ml were significantly positive by characterizing p-value = 0.0001 and $r = 0.935$ as shown in Table IIIa,b.

Relationship between 2-4 pus cell/HPF and $>10^5$ CFU/ml in 56 (56%) urine samples and 10-12 pus cell/HPF in 9 (9%) urine samples has showed 100% sensitivity (Table I). Lin and Fajardo (2008) had also evaluate durine esterase and nitrite test for the evaluation of urine culture test with 100% sensitivity then correlate with our study results.

Out of 100 urine samples 56 (56%) characterize 2-4 pus cell/HPF and 9 (9%) urine samples characterize 10-12 pus cell/HPF on un-centrifuged urine microscopy method (Table I). The same study method was adopted by Pryles and Elicot (1965) and assessed 90% sterile urine specimens, 39% $>100,000$

organisms/ml urine specimens containing 10 pus cell/C. mm then correlated with $>10^5$ CFU/ml. The results of both studies were same as our work.

TABLE II.- CHARACTERIZATION OF 2-4 PUS CELL/HPF AND 10-12 PUS CELL/HPF

Pus cell/HPF	CFU/ml		Total
	< 10000 CFU/ ml	<100000 CFU/ ml	
1-2 Pus Cell/HPF	35	0	35
2-4 Pus Cell /HPF	0	56	56
10-12 Pus Cell / HPF	0	9	9
Total	35	65	100

TABLE III.a- CORRELATION BETWEEN SIGNIFICANT PYURIA AND BACTERIURIA

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.000E2 ^a	2	.000
Likelihood ratio	129.489	2	.000
Linear-by-Linear association	78.387	1	.000
N of valid cases	100		
No. of valid cases	100		

TABLE IIIb.- CORRELATION BETWEEN PUS CELL/HPF AND BACTERIAL CFU/ml

	Value	Asymp. Std. Error ^a	Approx. T ^b	Approx. Sig.	
Interval by interval	Pearson's R	.890	.021	19.305	.000 ^c
Ordinal by ordinal	Spearman Correlation	.935	.023	26.087	.000 ^c
No. of Valid Cases		100			

Bacterial CFU/ml and pus cell/HPF shows relatedness in which 65 urine samples show 2-4 and 10-12 pus cell/HPF on urine direct microscopy and $>10^5$ CFU/ml on urine culture (Table II). The same practice was performed by Lam *et al.* (1969) where Gram stain procedure was performed which showed 80-95% CFU evaluation for similar results.

On statistical analysis, it was proved that significant pyuria (2-4 and 10-12 pus cell/HPF) and significant bacteriuria ($>10^5$ CFU/ml) were showed a

significant positive correlation by obtaining p -value = 0.0001 and $r = 0.935$. In another study James and Arthur (1963) showed the same kind of comparison between wet film examination for cell counting (pus cell/HPF) and urine culture evaluation for colony counting (CFU/ml) where 65% correlation indicates UTI.

Gynecological clinicians can easily interpret significant pyuria on direct urine microscopy for the early management of UTI during pregnancy.

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BIODIVERSITY AND DISTRIBUTION OF THE ORTHOPTERN INSECTS OF PAKISTAN

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Abstract.- The present paper deals with the grasshoppers belonging to families Acrididae, Dericorythidae, Pamphagidae, Pyrgomorphidae, Tetrigidae and Eumastacidae collected from the different ecological zones of Pakistan for the last forty years and lodged at Sind Entomological Museum, Sindh University (SEM). Most of the species of Pamphagidae are endemic with restrictive habitat at higher altitude ranging 1500 to 2500 meters from sea level. However, during the present study the species of family Pamphagidae has been recorded from a new locality *i.e.* Ranni Kot and at low sea level. More detailed study on the Orthoptera of Ranni Kot (Sindh), Mekran (Balochistan), mountainous areas of Khyber Pakhtunkhaw, Zhob, Quetta and Naushki of Balochistan will bring some more interesting facts. Beside this, the species not previously reported from this area including rare species are *Aularches miliars pseudopunctatus* Kevan, *Pterorthacris subcallosa* Uvarov, *Pyrgomorpha inaequaulipennis* Bolivar, *P. hemiptera* Uvarov, *P. cognota miniata* Uvarov (Pyrgomorphidae); flightless genera *Paraconophyma kashmirca* Mishchenko, *Paraconophyma minuta* Mishchenko, *Kabulia balucha* Uvarov, *Kabulia* sp. montanae species of subfamily Gomphocerinae (Acrididae), *Dericorys* spp, *Conophoma* spp. (Dericorythidae); *Bolivaritettix nilgricus* (Hebard), *Eucrotettix monatanus* (Hancock), *Thoradonota nodulosa spiculoba* Hancock (Tetrigidae), *Gomphomastax calavata*, *G. bulbosus*, *G. monsoonia* and *G. moderata* (Gomphomascidinae). Furthermore, occurrence of many of the previously recorded species has been confirmed and their distribution has been extended to new localities.

Key words: Acrididae, Dericorythidae, Pamphagidae, Pyrgomorphidae, Tetrigidae, Eumastacidae, ecological zones, rare species.

INTRODUCTION

The grasshoppers are one of the very familiar group of insects to mankind. The fact is that the locusts, the notorious members of the group, are of common sight during swarms. Among the desert fauna the desert locust (*Schistocerca gregaria*) is recognized as a threat to agriculture production in Africa and south west Asia for thousands of years. A published account of locust invasion in North Africa date back to about AD811. Locust scourges are referred to in the Bible as

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well as in Quran Shareef. “So we sent (plagues) on them wholesale death Locusts Lice Frogs and Blood: signs openly Self-explained; but they were steeped in arrogance a people given to sin.” (Surah 7. Al-Araaf, verse 133, Makkah). “They will come forth their eyes humbled from (their) graves (torpid) like locusts scattered abroad.” (Surah 54. Qamar, verse 7, Makkah).

The systematic of Orthoptera of Pakistan has been studied after and before the creation by a number of authors over a considerable period of time. These include Walker (1870), Stal (1873, 1876), Brunner Von Wattenwyl (1893), Bolivar (1902, 1912, 1914, 1918), Hancock (1911), Kirby (1914), Uvarov (1921 a,b, 1923, 1925, 1931, 1932, 1941a,b, 1942a,b, 1943, 1954, 1966, 1977), Mishchenko (1936, 1952) Ramme (1952), Dirsh (1950, 1954, 1958, 1961, 1975), Willemse (1951), Bei-Bienko and Mishchenko (1951 a,b), Dirsh and Uvarov (1953), Mohyuddin (1955), Ahmed (1958), Kevan (1959), Akbar (1959), Kevan and Akbar (1964), Kevan and Wagan (1987), Kevan and Hsuing (1988, 1989), Kevan *et al.* (1969, 1972, 1973, 1974), Akbar (1963, 1966 a,b, 1968, 1971, 1973), Hollis (1963, 1965, 1968, 1971, 1975), Jago (1963, 1967, 1969, 1971, 1977, 1984, 1996 a,b), Baloch (1966), Moeed (1966, 1971), Noushaba (1967), Sajida (1967), Shams-ul-haque (1968), Mason (1973), Irshad *et al.* (1977), Hsiung, and Kevan, (1975), Popov and Kevan (1979), Ahmed (1980), Ritchie (1981, 1982), Ritchie and Pedgley (1989), Solangi (1987), Wagan (1988, 1990), Wagan and Mughal (1990, 1992), Wagan and Solangi (1990), Grunshaw (1991), Blackith (1992), Wagan and Kevan (1992), Wagan *et al.* (1992), Suhail, (1994), Sergeev (1995), Tokhai (1996), Wagan and Naheed (1996, 1997), Yousuf (1996), Ingrisich (1989), Tokhai *et al.* (1999), Eades (2000, 2012), Baloch (2000), Baloch and Wagan (2000), Garai (2002), Wagan and Naheed (2000, 2001a,b), Wagan and Soomro (2001), Riffat *et al.*, (2002), Sultana and Wagan (2003), Khahid *et al.*, (2004), Soomro and Wagan (2005), Wagan and Riffat (2006), Soomro (2007), Riffat (2008), Wagan and Riffat (2006), Riffat and Wagan, (2007, 2008), Riffat *et al.* (2007, 2013), Bughio (2012) and Bughio *et al.* (2011, 2012, 2013).

The zoogeographical position of the Pakistan is very peculiar because this country lies at the transitional zone among three of the world's six major zoogeographical regions *i.e.* the Palaearctic, the Oriental and the Ethiopian. The Oriental fauna zone is bounded on the west by the river Indus and on the north by Himalayas and extending eastward to embrace south west China the Philippines and most of Indonesia. Most of the dry mountainous country to the west of the Indus River falls outside of the Oriental region and forms the part of the

Palaeartic region. Extreme south-west of the country represents Ethiopian region Serjeev (1995). Orthoptera of Pakistan is also poorly studied by Song (2010). Recently it has become particularly challenging to work on Pakistan fauna due to logistically and politically. Further more there are, however, considerable gaps in our knowledge of the grasshoppers from this region and it was considered that it might prove useful, in the light of this to study accumulation of Orthoptera collected from various provinces and those remote areas of Pakistan not easily to visit. Now a days during 1972 to 2012 and now housed at Department of Zoology, University of Sindh, Jamshoro. The present study does not pretend to rectify situation to any major degree but it will fill in certain gaps. The system of classification followed is that of Orthoptera species on line version 5.0/5.0

MATERIALS AND METHODS

This paper is based on the samples collected by author and his students from Sindh (1972-75, 1982-1988, 2002-2012), Balochistan (1981, 1993-195, 2002-2012), Punjab (1992, 1995-1997, 2002-2012), Khyber Pukhtun Khwa (KP) (1985, 1987, 1992, 1995, 1997, 2002-2012) and Gilgit Baltistan (1992 only).

Pakistan is situated between 24° N and 37° N Latitude and between 61° E and 75° E Longitude. On the southern part is the Arabian Sea, on the west is Iran, on the northwest part lies Afghanistan, China is towards the northeast. On the east it has a long border with India and with China we have common frontier with the Gilgit Agency and Baltistan (now Gilgit Baltistan Province).

The important geographical features of the country are the high lands and inland basin of Balochistan and the low lands of the Punjab and the lower Indus. To the West of Sindh are the Kirthar Mountains and northwestern areas adjoining the Kalat division. These are dry and arid, with Palaeartic topography. Several inland basins are formed by the separate ranges of these mountains south of Quetta. In the north, Chaghai hills separate Balochistan from Afghanistan. The Mekran ranges composing of parallel ridges aligned west to east are found along the Balochistan coast. The wide valley of Dasht river separates the coastal ranges from the inner ranges of Kech and Kolwa and in its lower reaches broadens out into Deashtiari plain on both sides of the Iran border. The sand stone ranges of Sulaiman and the Kirthar Mountains connect the high plateau of Kohat and Waziristan in the north western Pakistan and south and south-west region. The KP province is mostly mountainous and rocky, and is crossed by several mountainous ranges, including the Hindu-kush in the north-west, the Himalayas

in the northeast, and the Sulaiman and Safed Koh ranges in the west. Climate is dry-sub humid and cold. The eastern region consists of the sand belt stretching from the borders of Bahawalpur (Punjab) to the Runn of Kutuch and is part of Rajistan Desert called Thar. The northern Punjab is a vast semi arid plain and are extensively irrigated by various rivers. Southern Punjab-Cholistan is dry and hot and north is sub humid and cold. Much of the lower Indus Plain is occupied by the Thar Desert of Sindh with mostly parallel and ridged aligned north east to south. West of Thar lies Deltaic area with rich alluvial soil crossed by numerous river channels. The present work was undertaken from 1972 to 2013. Many expeditions were undertaken during the same period throughout the Pakistan. During such expeditions, the areas of wide geographical nature were visited ranging from agricultural lands, semi desert/desert, semi mountainous and mountainous areas having the vegetation of grasses, crops, herbs and shrubs for the purpose to collect specimen. The various districts of the different provinces of Pakistan visited (Fig. 1) for the collection of grasshoppers, both of minor and major economic importance. The material was preserved by conventional methods and stored in standard entomological cabinets with labels showing locality, date of collection and collector name. Nephthalene balls were placed in boxes to prevent the specimens from the attack of ants and other insects.

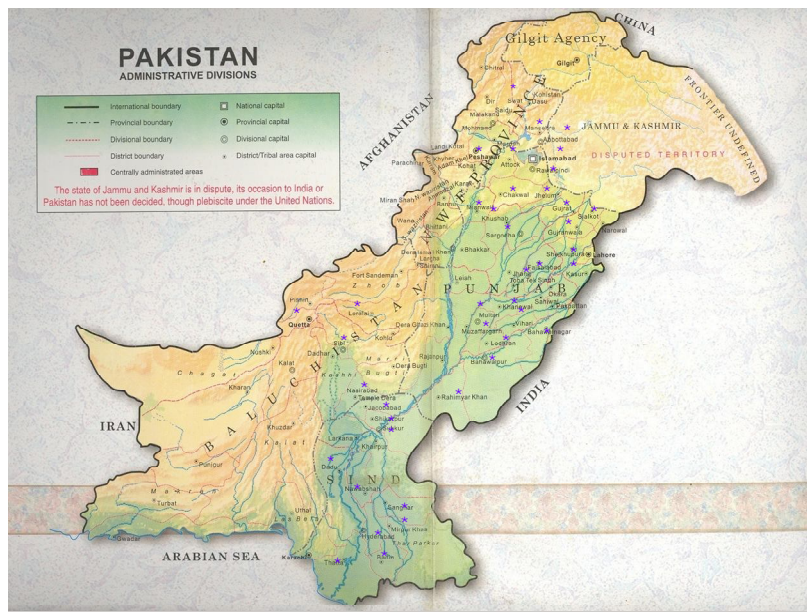


Fig. 1. Map of Pakistan showing the surveyed Districts.

RESULTS AND DISCUSSION

As a result of surveys in various representative localities of ecological zones of Pakistan, a total of 31,200 specimens in six families collected have been shown in Table I. The list of species and their distribution in various provinces of Pakistan is shown in Tables II-VII. The largest family is Acrididae with 120 species followed by Pyrgomorphidae 15 species, Tetrigidae 14 species, Pamphagidae 06 species, Dericorythidae 06 species and Ghomphomasticidinae 02 species. Table VIII shows the number of species reported by various workers.

TABLE I.- NUMBER OF SPECIMENS COLLECTED FROM DIFFERENT PROVINCES OF PAKISTAN.

Family	Number of specimens
Acrididae	25,000
Dericorythidae	100
Pamphagidae	100
Pyrgomorphidae	5000
Tetrigidae	1000
Eumastacidae	50
Total	31250

Superfamily: Acridoidea

Family Acrididae

This is the largest family of Orthoptera, and indeed, of all Orthopteroids. It includes all the true locust and grasshoppers. Twelve subfamilies of this family were studied and are given below.

Acrididae: Acridinae (Table II A, Fig. 2)

A few tribes of Acridinae (Acridini, Truxalini, and Phlaeobini) are composed mainly of phytophilous and are widely distributed throughout the country along road sides, agriculture crops, and water channels. The Acridini and Truxalini are characterized by large size, head conically ascending with flattened antennae, eyes situated towards the apex of head, hind femora strongly elongated and narrow. In all species the female is very much larger than the male. The hind wing is hyaline or slightly greenish in *Acrida* L. (Fig. 2A) while in *Truxalis* Fabricius brightly colored with tessellated pattern formed by darkened transverse veinlets. The most pronounced colour is found in *Truxalis fitzgarldi* (Fig. 2B, C)

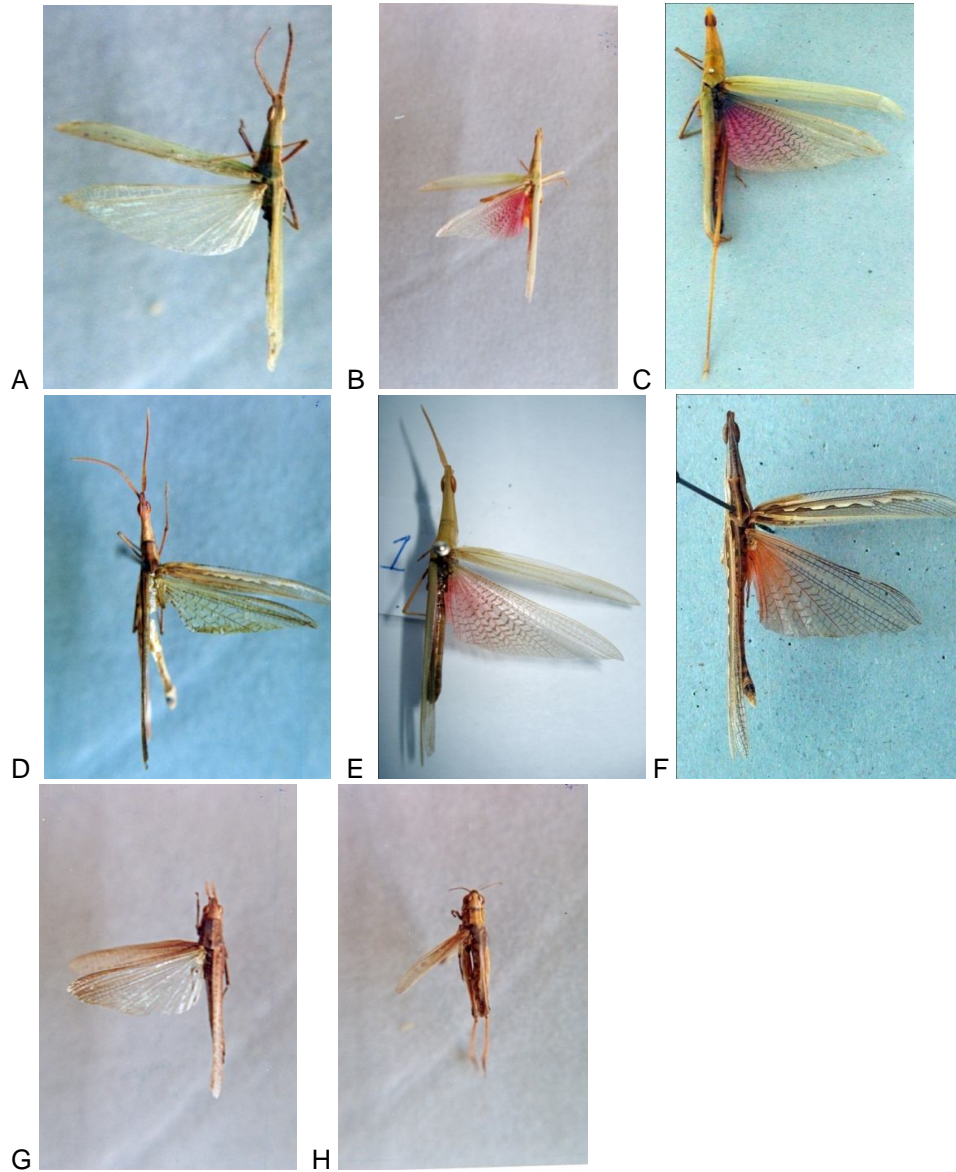


Fig. 2. A, *Acrida exaltata* (Walker), male; B & C, *Truxalis fitzgeraldi* (Dirsh) B, male C, female; D & E, *Truxalis eximia eximia* (Eichwald) D, male; E, female; F, *Truxalis procera* Klug, male; G, *Phlaeoba tenebrosa* (Walker), male; H, *Duroniella laticornis* (Krauss), male.

TABLE. II. - SHOWING THE DISTRIBUTION OF FAMILY ACRIDIDAE IN VARIOUS PROVINCES OF PAKISTAN.

	Sindh	Balochistan	Punjab	Khyber Pakhtunkhwa	Gilgit Baltistan
(A) Subfamily Acridinae					
Tribe Acridini					
<i>Acrida exaltata</i> (Walker)	+	+	+	+	-
Tribe Truxalini					
<i>Truxalis fitzgeraldi</i> (Dirsh)	+	+	+	-	-
<i>T. eximia eximia</i> (Eichwald)	+	+	+	+	-
<i>T. procera</i> Klug	-	+	-	-	-
Tribe Phlaeobini					
<i>Phlaeoba tenebrosa</i> (Walker)	+	+	+	+	-
<i>Phlaeoba panteli</i> Bolivar	+	+	+	+	-
<i>Duroniella laticornis</i> (Krauss)	+	+	+	+	-
<i>Duroniella laeviceps</i> Uvarov	+	+	+	+	-
(B) Subfamily Calliptaminae					
Tribe Calliptamini					
<i>Calliptamus italicus italicus</i> Ramme	-	+	-	+	-
<i>C. balucha balucha</i> Uvarov	-	+	-	-	-
<i>C. barbarus barbarus</i> Costa	-	+	-	+	-
<i>C. tenuicercis</i> Tarb.	-	+	-	+	-
Genus <i>Sphodromerus</i> Stal					
<i>Sphodromerus undulatus undulatus</i> (Kirby)	+	+	+	+	-
<i>S. undulatus afghanus</i> B. Bienko	-	+	-	-	-
<i>S. undulatus pedestris</i> Uvarov	-	-	+	-	-
<i>S. undulatus salinus</i> Uvarov	-	-	+	-	-
<i>S. luteipes rubripes</i> Uvarov	-	+	-	-	-
<i>S. Indus</i> Soomro and Wagan	-	-	-	+	-
Genus <i>Acorypha</i> Krauss					
<i>Acorypha glaucopsis</i> (Walker)	+	+	+	+	-
(C) Subfamily Catantopinae					
Tribe Catantopini					
<i>Diabolocatantops innotabilis</i> (Walker)	+	+	+	+	-

Continued

	Sindh	Balochistan	Punjab	Khyber Pakhtunkhwa	Gilgit Baltistan
Tribe Paraconophymatini					
<i>Paraconophyma kashmirica</i> Mishchenko	-	-	+	+	-
<i>Paraconophyma minuta</i> Mishchenko	-	-	-	+	-
Subfamily Cyrtacanthacridinae					
Cyrtacanthacridini					
<i>Anacridium aegyptium</i> (L)	+	+	+	+	-
<i>Anacridium rubrispinium</i> B. Bienko	+	+	+	+	-
<i>Cyrtacanthacris tatarica</i> (L)	+	-	+	+	-
<i>Schistocerca gregaria</i> (Forsk.)	+	+	+	+	-
<i>Patanga succinata</i>	-	-	-	+	-
<i>Patanga japonica</i> (Bolivar)	-	-	-	+	-
Subfamily Eyprepocnemidinae					
Tribe Eyprepocnemidini					
<i>Eyprepocnemis alacris alacris</i> (Audinet-Serville)	+	+	+	+	-
<i>E. alacris impicta</i> (Uvarov)	+	+	-	-	-
<i>E. rosea</i> Uvarov	-	-	-	+	-
<i>Heteracris littoralis</i> (Rambur)	+	+	+	+	-
<i>H. adpersa</i> (Redtenbacher)	+	+	+	+	-
<i>H. notabilis</i> Uvarov	+	+	+	+	-
<i>H. persa</i> Uvarov	-	+	-	-	-
<i>Shirakacris shirakii</i> Bolivar	-	-	-	+	-
Genus <i>Cataloipus</i> I. Bolíva					
<i>Cataloipus cognatus</i> (Walker)	+	-	+	-	-
Genus <i>Choroedocus</i> I. Bolívar					
<i>Choroedocus illustris</i> (Walker)	+	+	+	+	-
<i>C. robustus</i> Serville	+	-	-	-	-
Genus <i>Tylotropidius</i> Stal					
<i>Tylotropidius varicornis</i> (Walker)	-	-	+	+	-

Continued

	Sindh	Balochistan	Punjab	Khyber Pakhtunkhwa	Gilgit Baltistan
(F) Subfamily Gomphocerinae					
Tribe Arcypterini					
<i>Aulacobothrus punjabensis</i> Naheed and Wagan	-	-	+	-	-
<i>A. luteipes</i> (Walker)	+	+	+	+	-
<i>Crucinotacris decisa</i>	-	+	+	-	-
<i>Leionotacris bolivari</i> (Uvarov)	+	+	+	+	-
<i>L. beeshamsensis</i> Riffat and Wagan	-	-	-	+	-
Docioptaurini					
<i>Docioptarus tartarus</i> (Stchelkanovtzev)	-	+	-	-	-
<i>D. nigrogeniculatus</i> Tarbinsky	-	+	-	+	-
Gomphocerini					
<i>Chorthippus indus</i> Uvarov	-	+	+	+	-
<i>Ch. angulatus</i> Tarbinsky	-	-	-	+	-
<i>Ch. dorsatus</i> (Zett)	+	+	-	+	-
Ochrilidini					
<i>Gonista sagitta</i> Uvarov	+	+	+	+	-
<i>Gonista rotundata</i> Uvarov	+	+	+	+	-
<i>Ochrilidia ahmadi</i> Wagan and Naheed	-	-	+	-	-
<i>O. beybienko</i> Cejan	-	+	-	-	-
<i>O. gracilis gracilis</i> (Krauss)	+	+	+	+	-
<i>O. hertata hertata</i> Bolivar	-	+	-	-	-
<i>Oxypterna afghana</i> Ramme	+	-	+	-	-
<i>O. akbari</i> Moeed	+	-	-	-	-
<i>O. isoformis</i> Moeed	+	-	-	-	-
<i>O. scapularis</i> Moeed	+	-	-	-	-
Genus <i>Brachycrotaphus</i> Krauss					
<i>Brachycrotaphus longiceps</i> (I. Bolivar)	-	+	+	-	-
Genus <i>Gelastrohinus</i> Brunner					
<i>Gelastrohinus semipictus</i> (Walker)	+	+	+	+	-

Continued

	Sindh	Balochistan	Punjab	Khyber Pakhtunkhwa	Gilgit Baltistan
<i>Genus Mesopsis</i>					
<i>Mesopsis cylindricus</i> (Kirby)	+	+	-	-	-
<i>M. iranicus</i> (Uvarov)	-	+	-	-	-
<i>Genus Stenohippus</i>					
<i>Stenohippus mundus</i> (Walker)	+	+	+	+	-
<i>S. trapezoidalis</i> (I.Bolivar)	-	-	+	+	-
<i>S. xanthus</i> (Karny)	-	-	+	+	-
<i>Genus Leva</i>					
<i>Leva indica</i> (I. Bolivar)	+	-	+	-	-
(G) Subfamily Hemiacridinae					
Tribe Hieroglyphini					
<i>Hieroglyphus akbari</i> Riffat and Wagan	-	+	-	-	-
<i>H. banian</i> (Fabricius)	-	-	+	+	-
<i>H. nigrorepletus</i> I. Bolivar	+	+	+	+	-
<i>H. oryzivorus</i> Carl	+	-	-	-	-
<i>H. perpolita</i> (Uvarov)	+	+	+	+	-
Tribe Leptacriini					
<i>Leptacris tokhai</i> Tokhai <i>et al</i>	-	+	-	-	-
(H) Subfamily Oedipodinae					
Acrotylini					
<i>Acrotylus humberians</i> , Saussure.	+	+	+	+	+
<i>A.insubricus insubricus</i> , Scopoli	-	+	-	-	-
<i>A. patruelis</i> , Herrich-Schaffer.	-	+	-	-	-
<i>A longipes longipes</i> , Charpentier	+	-	-	-	-
<i>A.longipes subfasciatus</i> , Bei- Bienko.	+	+	+	+	-
Epacromini					
<i>Aiolopus thalassinus</i> <i>thalassinus</i> , Fabricius	+	+	+	+	-
<i>A.thalassinus tamulus</i> , Fabricius.	+	+	+	+	-
<i>A.simulatrix simulatrix</i> , walker.	+	+	+	+	-

Continued

	Sindh	Balochistan	Punjab	Khyber Pakhtunkhwa	Gilgit Baltistan
<i>Hilethera aelopoides</i> , Uvarov.	+	+	+	+	-
<i>H. turnica</i> Uvarov	-	+	-	-	-
<i>H. balucha</i> Bughio et al.	-	+	-	-	-
Locustini					
<i>Locusta migratoria</i> Linnaeus	+	+	+	+	-
<i>Gastrimargus africanus sulphureus</i> , Bei-Bienko	+	+	+	+	+
<i>Oedaleus abruptus</i> , Thunberg.	-	-	+	-	-
<i>O. roscens</i> , Uvarov.	+	-	+	-	-
<i>O. senegalensis</i> Krauss.	+	+	+	+	-
<i>S. notabilis cincitipes</i> Uvarov	+	-	-	-	-
<i>Scintharista notabilis pallipes</i> Uvarov.	+	+	+	+	+
<i>Scintharista notabilis brunneri</i> Saussure	-	-	-	+	+
Oedipodini					
<i>Oedipoda coerulea</i> , Linnaeus.	-	+	-	-	-
<i>O. fadtshenkoi pamicica</i> Saussure.	-	+	+	-	-
<i>O. himalayana</i> Uvarov	-	-	-	+	+
<i>O. miniata atripes</i> , Bei-Bienko.	-	+	-	+	+
<i>Mioscirtus wagneri rogenhoferi</i> , Saussure.	+	+	+	+	-
Sphingonotini					
<i>Sphingonotus akbari</i> Wagan and Naheed.	+	-	+	-	-
<i>S. balteatus balucha</i> Uvarov.	-	+	-	-	-
<i>S. balteatus himalayanus</i> Uvarov	-	+	-	-	+
<i>S. hussaini</i> Wagan & Naheed	-	-	+	-	-
<i>S. longi pennis</i> Saussure.	-	+	+	-	-
<i>S. maculatus petraeus</i> , Bei-Bienko.	-	+	-	-	-
<i>S. nebulosus discolor</i> Uvarov	-	+	-	-	-
<i>S. nebulosus tokhai</i> Bughio et al	-	+	-	-	-
<i>S. predtechenskyi</i> Mistshenko.	-	-	+	-	-
<i>S. rubescens rubescens</i> , walker.	+	+	+	+	+

Continued

	Sindh	Balochistan	Punjab	Khyber Pakhtunkhwa	Gilgit Baltistan
<i>S. rubescens afghanicus</i> , Mistchenko	-	+	-	-	-
<i>S. savignyi</i> Saussure	+	+	+	+	+
<i>S. sindhensis</i> Bughio et al	+	-	-	-	-
<i>Sphingoderus carinatus</i> (Saussure)	+	-	-	-	-
Genus <i>Chloebora</i>					
<i>Chloebora crassa</i> (Walker)	+	+	-	-	-
<i>C. grossa</i> saussure	-	-	+	+	+
(I) Subfamily Oxyinae					
<i>Oxy hyla hyla</i> Audinet- Serville,	+	+	+	+	-
<i>O. fuscovittata</i> (Marschall)	+	+	+	+	-
<i>O. bidentata</i> Willemse	+	+	+	+	-
<i>O. velox</i> (Fabricius)	+	+	+	+	-
(J) Subfamily Spathosterinae					
<i>Spathosternum prasiniferum</i> (Walker)	+	+	+	+	-
(K) Subfamily Teratodinae					
<i>Kabulia balucha</i> Uvarov	-	+	-	-	-
<i>Kabulia sp.</i>	-	+	-	-	-
(L) Subfamily Tropidopolinae					
<i>Tropidopola Longicornis</i> <i>longicornis</i> (Fieber)	+	+	+	+	-

in which the basal area of the hind wings is purple black in the female and bright red in male.

The genera *Phlaeoba* stal and *Duroniella* I. Bolivar of Phlaeobini tribe were studied. The species of genus *Duroniella* (Fig. 2H.) shows a lot of color dimorphism. In some specimens head and pronotum are marked with the double bands and some are with single band and some variation was recorded in the lateral carinae of pronotum. However this genus *Duroniella* is in need of revision. The species of this genus occurs in cultivated fields among thick grasses in dry meadows, damp places and near the roadsides.

Acrididae: Calliptaminae (Table II B, Fig. 3)

The Calliptaminae are characteristic form for the steppe, semi-desert, desert and rocky landscapes of the studied areas. *Sphodromerous undulatus undulatus* and *Acorphya glaucopsis* are robust species found on rocky slopes and occur in all the provinces whereas *S. undulatus salinus* Uvarov *S. undulatus pedestris* Uvarov occur in the Punjab while other species are restricted to Baluchistan and KP provinces. The species of tribe Calliptamini of this subfamily Calliptaminae shows very different dispersal behavior. *Calliptamus italicus italicus* and *C. barburus barburus* are characterized by their divergent adaptations (Termir, 1991). *C. barburus barburus* shows very marked color morphism, especially in South and south east of its geographical distribution Jago (1963).

Acrididae: Catantopinae (Table II C, Fig. 3)

Two tribes Catantopini and Paraconophymatini were recorded. A single species *Diabolocatanops innotabilis* (Walker) (Fig. 3F) of tribe Catantopini was collected and studied. The male cercus of this species is variable and this species is widely distributed throughout the country. Two species of the flightless genus *Paraconophyma* Uvarov of tribe Paraconophymatini were collected and studied *P. kashmirica* Mishchenko (Fig. 3F) and *P. minuta* Mishchenko (Fig. 3D). These species occur among the bushes of tall pine trees in Bala Kot (KP) and Murree hills (Punjab). The organs of flight are very short in *P. minuta* and this species is reorded for the first time from this area.

Acrididae: Cyrtacanthacridinae (Table IID, Fig. 3)

Members of this family have been called “bird-locusts” because of their generally large size and strong flight. It includes some of the most destructive species e.g. *Schistocerca gregaria gregaria* (Forsk.) (Fig. 3H) and a number of important pest species such as the so called Bombay locust *Patanga succinata* (Johannson). Other species are *Anacridium aegyptium* (L.), *Anacridium rubrispinum* B.Bienko (Fig. 3G) and *Cyrtacanthacris tatarica* (L.) (Fig. 3I)

A few species of Cyrtacanthacridinae settles the arid and semi-arid lands of Pakistan.

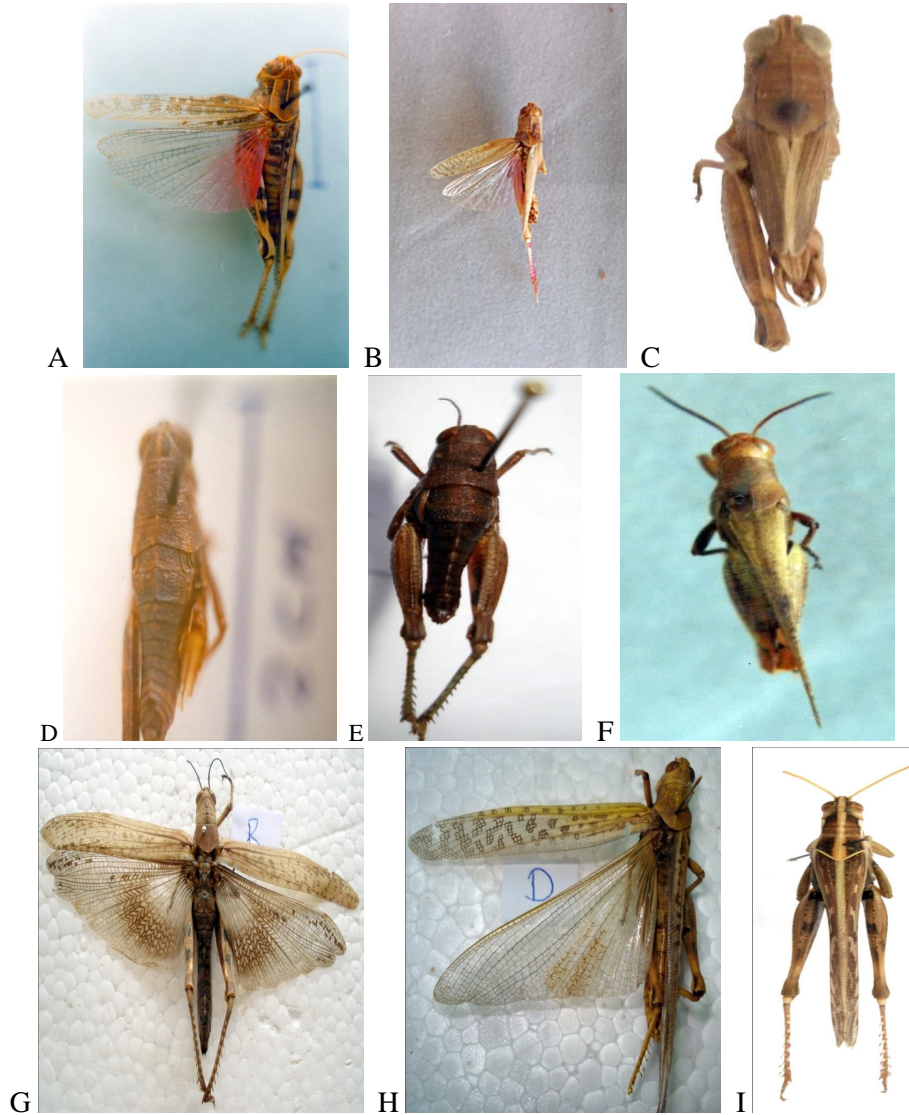


Fig. 3. A, *Calliptamus italicus italicus* Ramme, female; B, *Sphodromerus undulatus undulatus* (Kirby); C, *Acrophya* sp; D, *Paraconophyma minuta* Mishchenko, female; E, *Paraconophyma kashmirica* Mishchenko, male; F, *Diabolocatantops innotabilis* (Walker), male; G, *Anacridium rubrispinium* B. Bienko, female; H, *Schistocerca gregaria* (Forsk.) male; I, *Cyrtacanthacris tatarica* (L.), male.

Acrididae: Eyprepocnemidinae (Table II E, Fig. 4)

The species of this subfamily are widely distributed and dominant often in the local Orthopteran communities. The genera *Eyprepocnemis*, Fieber, *Heteracris*, Walker and *Shirakacris*, Dirsh of tribe Eyprepocnemidini can easily be separated on the basis of male cercus which is narrow and pointed apically in *Eyprepocnemis*; flattened, broadly rounded and down curved distally in *Heteracris* and widened at both ends and narrow in the middle in *Shirakacris shirakii* Bolivar. This last species is restricted to Swat valley (KP). *Eyprepocnemis alacris alacris* (Audinet-Serville) was collected from all the provinces whereas *E. alacris impicta* (Uvarov) occurs in Sindh and Balochistan provinces while *Eyprepocnemis rosea* Uvarov (Fig. 4B) which is easily recognizable by small size and rose coloured hind wings seems confined to hill country restricted to the mountainous areas of Manshera (KP). *Heteracris adspersa* (Redtenbacher); *H. littoralis* (Rambur) (Fig. 4E) and *H. persa* Uvarov whose tegmina have brown spots the first two species are widely distributed throughout the country while *H. persa* Uvarov is restricted to Balochistan. *H. notabilis* (Uvarov) (Fig. 4A) tegmina without spots occurs in Sindh and Punjab provinces. *Choroedocus illustris* (Walker) (Fig. 4C) characterized by very large male cercus and is widely distributed. *Tylotropidius varicornis* (Walker) (Fig. 4F) whose hind femur long, inflated at base, strongly narrowed distally occurs in the mountainous areas of Punjab and Manshera (KP). Two females of *Cataloipus cognatus* (Walker) (Fig. 4D) were recorded from Sindh.

Acrididae: Ghomphocerinae (Table II F, Fig. 5)

The species of subfamily Ghomphocerinae include both plain and widely distributed forms and montane highly localized species. The great majorities are of comparatively small size and are very largely graminivorous. The species of Dociostaurini were collected from Baluchistan and Khyber Pakhtunkhwa provinces whereas the species of other tribes Arcypterini, Gomphocerini and Ochrlidini occur in all the provinces of Pakistan. The tribe Ochrlidini include an economically and ecological important genus *Ochrlidia* Stal (Jago, 1977) which is widely distributed throughout country this tribe also include genera *Gonista* I. Bolivar, *Oxypterna* Ramme and *Kirmania* Uvarov. This latter genus has not been recorded authentically from Pakistan. Two species of genus *Gonista* namely, *Gonista rotundata* Uvarov (Fig. 5A) and *G. sagitta* an Iranian origin species have been recorded from the Oriental part of country.

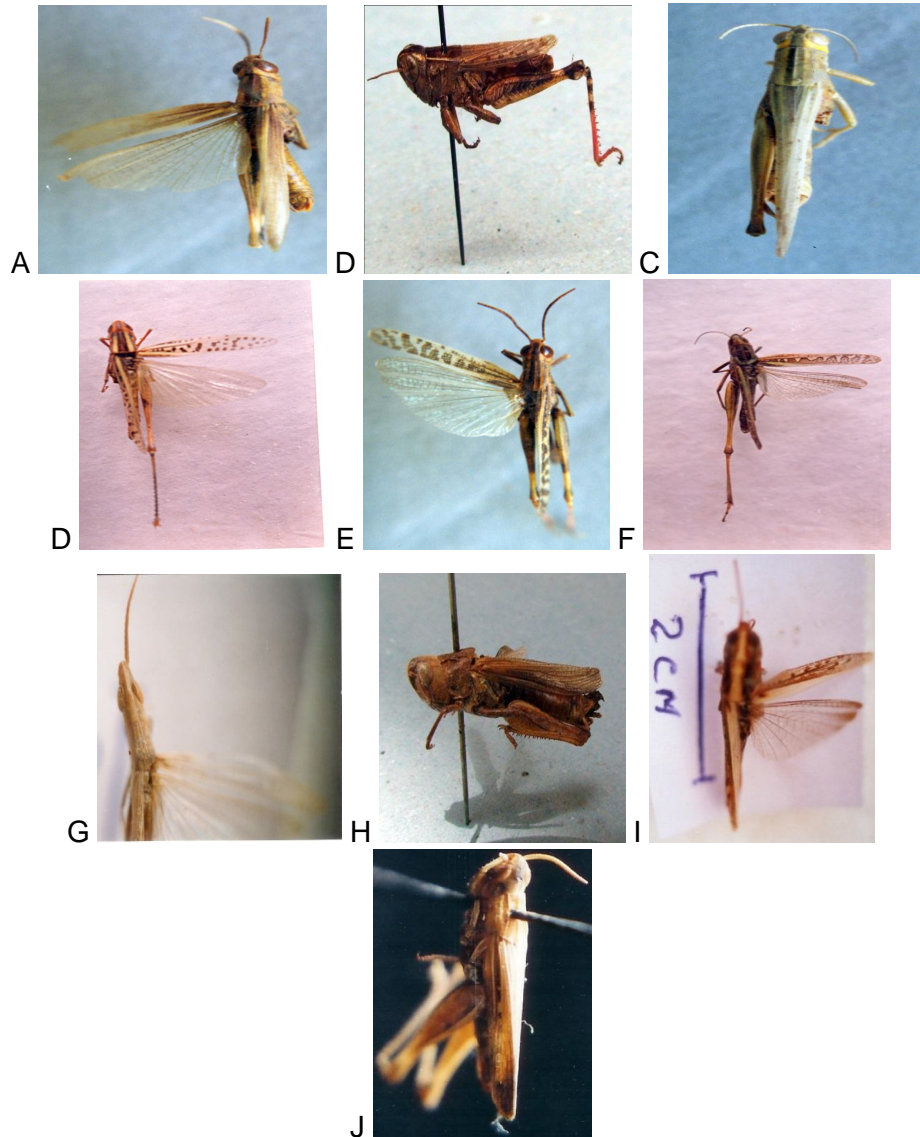


Fig. 4. A, *Heteracris notabilis* (Uvarov), male; B, *Eyprepocnemis rosea* Uvarov, male; C, *Choroedocus illustris* (Walker), male; D, *Cataloipus cognatus* (Walker), female; E, *Heteracris littoralis* (Rambur), male; F, *Tylotropidius varicornis* (Walker), female

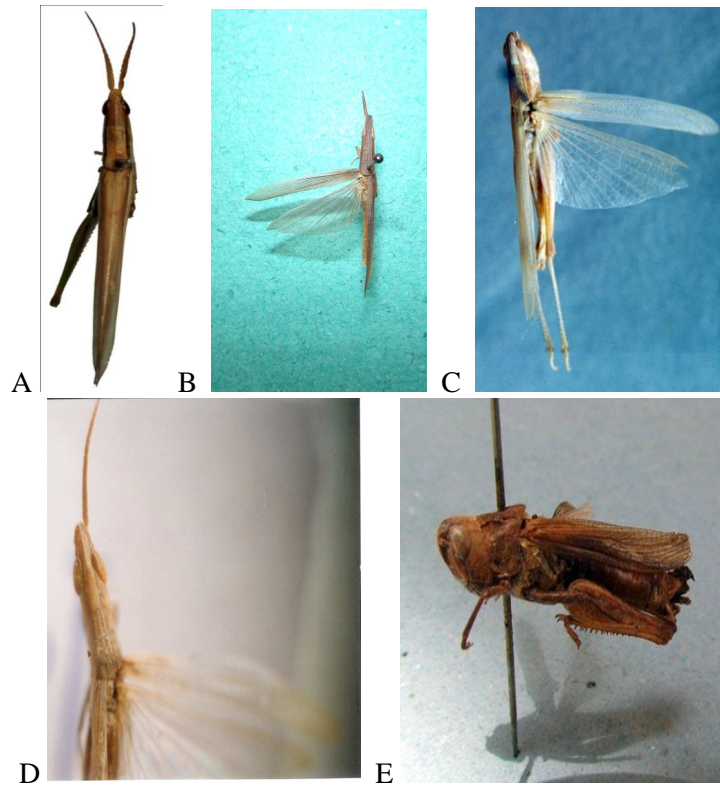


Fig. 5. A. *Gonista rotundatta* Uvarov , male; B, *Oxypterna afghana* Ramme, male; C, *Ochrilidia geniculata* (Bolivar), male; D & E, *Brachycrotaphus longiceps* (I. Bolivar), male; F, *Chorthippus indus* Uvarov, female.

The genus *Oxypterna* was described by Ramme (1952) from a single female from Aghanistan as *Oxypterna afghana* (Fig. 5B), latter on it was revised by Uvarov (1954) from a single male from Iran. The genus *Oxypterna* is characterized by medium size, head longer than pronotum, latter with longitudinal ridges, flattened antennae, tegmina with pointed apices. Moed (1966, 1971) erected three new species namely, *O. akbari*, *O. isoformis* and *O. scapularis* on a small number of specimens collected from Miani forest Hyderabad mainly on the basis of number of hind tibial spines, antennal segments and in the degree of truncateness of tegminal apices. Our study based on the external morphology as well as the male and female subgenital plates and on the large number of specimens collected different districts of Sindh shows that in *Oxypterna afghan* the male subgenital plate is much broader as compared to *O.*

akbari and the female subgenital plate have the middle process as long as or slightly shorter than the lateral lobes in *Oxypterna afghana* while in *O. akbari* it is for from reaching lateral lobes. We arrive at the conclusion that *O. isoformis* is synonym of *Oxypterna afghana* and *O. scapularis* is synonym of *O. akbai*. These species are widely distributed throughout Sindh in agriculture fields, among thick grasses along water channels, in dry meadows, damp places and near the roadsides. Ahmed (1980) collected only 2 specimens of *Oxypterna afghana* from Sindh while Wagan and Naheed (1997) reported single specimens from Punjab whereas Yousuf (1996) did not collect a single specimen of this genus from Pakistan. Species of Dociostaurini and Gomphocerini tribes are of small size, occur in the Mountainous areas of KP and Balochistan e.g. *Chorthippus indus* Uvarov (Fig. 5F).

A single male and female of *Brachycrotaphus longiceps* (I. Bolivar) (Fig. 5E) was collected from the (Zhob area) Balochistan and Chakwal (Punjab), respectively. A single species of genus *Gelastrohinus* Brunner, namely *Gelastrohinus semipictus* (Walker) was recorded, detailed study will result in more species. This species is widely distributed and occurs in cultivated fields and among thick grasses along water channels. *Mesopsis iranicus* (Uvarov) was recorded from the Quetta and Zhob areas of Balochistan while *M. cylindricus* (Kirby) was collected from Sindh. *Stenohippus* species are widely distributed throughout the country.

Acrididae: Hemiacridinae: (Table II G, Fig. 6)

Two tribes namely Hieroglyphini and Leptacriini were studied. The species of tribe Hieroglyphini are important pest of agriculture. A single genus *Hieroglyphus* Krauss with five species were studied. Tribe Leptacriini is represented by a single species *Leptacris tokhai* Takhoi *et al.* (1999) (Fig. 5C) recently described as a new species from Zhob area of Balochistan.

Acrididae: Oedipodinae (Table II H, Figs. 7, 8)

The subfamily Oedipodinae are usually called band-winged grasshoppers, also known as subfamily Locustinae are characteristic feature of arid and semi arid regions of Pakistan. Mostly are geophiles living in open grounds and others are phytophyles (found at vegetations, grasses, herbs and shrubs). A high portion of the species exhibit flash coloration in flight drawing attention to themselves by their brightly colored hind wings (Red, Yellow or Blue) and suddenly disappearing when settled. This assist in defeating predators but it may have

been developed primarily as a recognition signal. The subfamily Oedipodinae is a large one, occurring throughout the world. There are about 185 genera and a very large number of species. The most infamous included member of the subfamily is *Locusta migratoria* Linnaeus, various subspecies of which occur from time to time in devastating swarm over much of the old world (Vickery and Kevan, 1983).



Fig. 6. A, *Hieroglyphus perpolita* (Uvarov), male; B, *H. oryzivorus* Carl, male; C, *Leptacris tokhai* Tokhai *et al.* male.

Most species of mainly tropical and subtropical Epacromini, Locustini, Oedipodini, and Trilophidini are limited by the southern part of the palaeartic including its arid and semi arid lands (Serjeev, 1995). The species of tribe Epacromini are widely distributed throughout the country. The *Aiolopus* species (Fig. 7A) occur in both green and brown form and are dominant and widely distributed throughout the country. *Hilethera balucha* (Fig. 7B) has been recently described as a new species by Bughio *et al.* (2013) from Balochistan. Tribe Acrotylini is represented by five species and subspecies. *Acrotylus humberians* Saussure (Fig. 7C) and *A. longipes subfasciatus*, Bei-Bienko have yellow hind wing with short lunar black band occur in agriculture field, desert, and mountain and are widely distributed throughout the country. *A. insubricus insubricus*, Scopoli (Fig. 7D) and *A. patruelis* Herrich-Schaffer have red hind wing with an incomplete black band in the centre were collected from Balochistan whereas *A. longipes longipes*, Charpentier has the yellow coloured hind wings without any black band recorded from Sindh only. This later species and *A. insubricus insubricus*, Scopoli and *A. patruelis*, Herrich-Schaffer are less common than *Acrotylus humberians* and *A. longipes subfasciatus*.

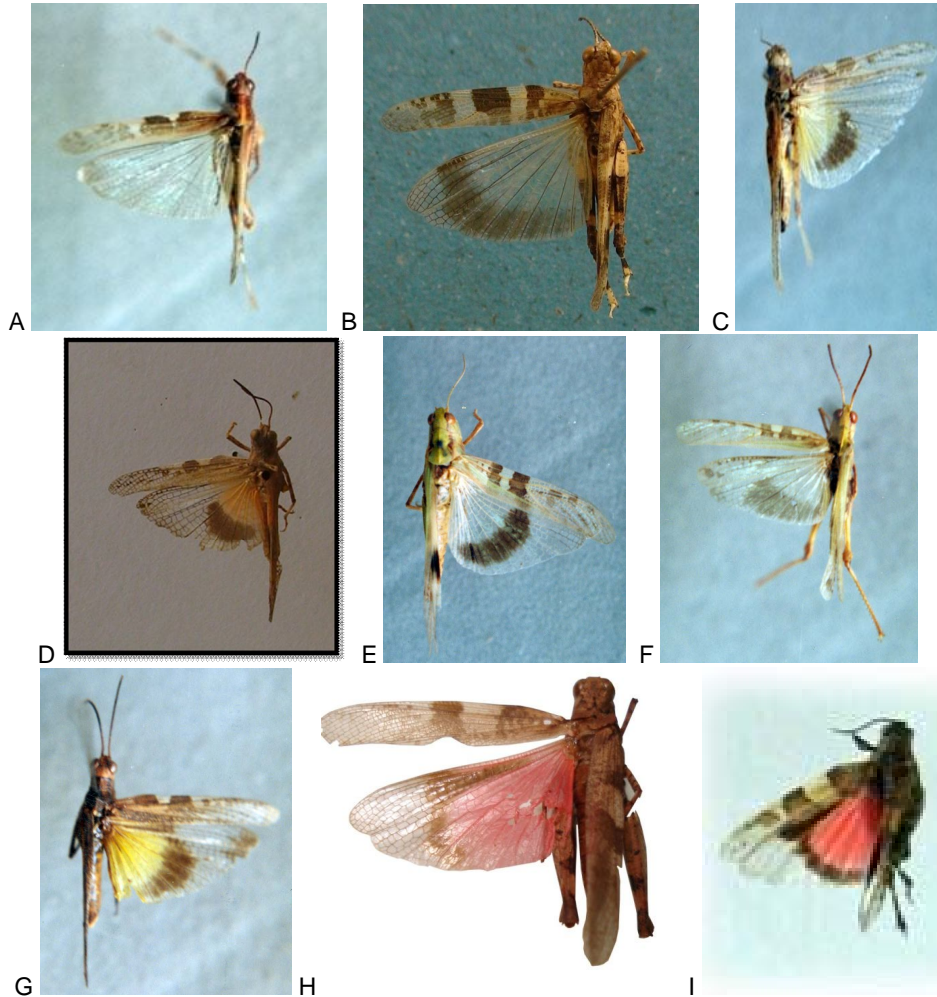


Fig. 7. A, *Aiolopus thalassinus thalassinus*, Fabricius, female; B, *Hilethera turnica* Uvarov, male; C, *Acrotylus humberians*, Saussure, female; D, *A. insubricus insubricus*, Scopoli, male; E, *Oedaleus senegalensis* Krauss, female; F, *O. abruptus*, Thunberg, male; G, *Mioscirtus wagneri rogenhoferi*, Saussure, male; H, *Oedipoda miniata atripes*, Bei-Bienko, male; I, *O. himalayana* Uvarov, male.

The species of Locustini tribes includes some of the important pest of agriculture, *Locusta migratoria* Linnaeus. The species *Gastrimargus africanus sulphureus* Bei-Bienko, *O. rosecens* Uvarov, *O. senegalensis* Krauss (Fig. 7E) has been recorded from the various provinces of the country while *Oedaleus*

abruptus, Thunberg (Fig. 8F) is restricted to Punjab province. The genera *Scintharista* and *Chloebora* Uvarov are color full insects. *Scintharista notabilis brunneri* Saussure (Fig. 8F) this subspecies has yellow hind wings and occur in Gilgit Baltistan while the two other subspecies have red hind wings, *S. notabilis cincitipes* Uvarov (Fig. 8D) this subspecies have dirty white hind tibia was collected from Thar desert (Sindh) while *Scintharista notabilis pallipes* Uvarov (Fig. 8E) with red coloured hind tibia has been recorded from all the provinces of Pakistan. The two species of genus *Chloebora* studied i.e. *Chloebora grossa* Saussure (Fig. 8A) have pink hind wings, collected from the river bed in Bisham(KP) and Simly Dam Islamabad (Punjab) while the other species *Ch. crassa* Walker (Fig. 8B,C) having yellow or red wings have been collected from Jung shahi (Sindh).

The *Oedipoda coerulescens* Linnaeus *O. fadtshenkoi pamirica* Saussure, *O. miniata atripes*, Bei-Bienko (Fig. 7G) and *O. himalaya* Uvarov (Fig. 7H) of tribe Oedipodini are characterized by rough pronotum and the dark band of hind wing is usually with short or long radial arm were collected from steppe, semi-desert, desert and rocky landscapes While *Mioscirtus wagneri rogenhoferi* Saussure (Fig. 7I) pronotum smooth dark band of wings is without radial arm was collected from the road sides and near cultivated fields. The tribe Sphingonotini consists from terricolous grasshoppers and is widely distributed through the arid lands of the world. All species of this tribe large genus *Sphingonotus* are geophilous and are found on bare ground with scattered vegetation. *Sphingonotus rubescens* (Walker) is a very variable species and commonest representative of the genus in Pakistan. The hind wings and hind tibia are very faintly blue. *Sphingonotus savignyi* Saussure (Fig. 8G) is also very common species has clear hyaline wings with a transverse black band. This species has been collected from the cultivated fields and from the rocky, semi desert and desert areas having the scattered mixed vegetation of herbs and grasses. This species is widely distributed and most common. *Sphingonotus balteatus himalayanus* (Fig. 8H) and *Sphingonotus balteatus balucha* Uvarov (Fig. 8I) have a very wide dark band wing which almost touching the inner margin in former subspecies and in later subspecies not touching the inner margin.

Acrididae: Oxyinae (Table II, I, Fig. 9)

A single genus *Oxya* Audinet Serville with four species of Oxyini tribe were studied and all are important pest of rice and other crops and are widely distributed and abundant in rice, maize, sugarcane and other agriculture field.

These species are characterized by having lower external lobe of hind knee spine like and hind tibia usually expanded towards apex.

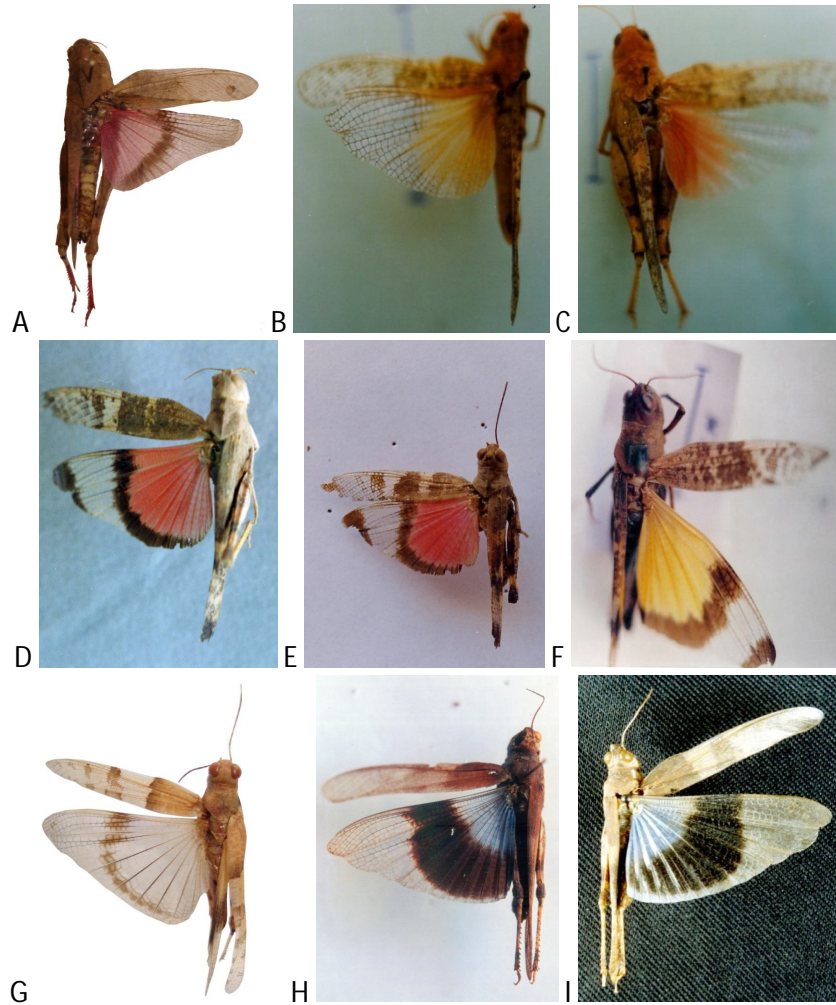


Fig. 8. A, *Chloebora grossa* Saussure, female; B & C, *Chloebora crassa* (Walker), male, female; D, *Scintharista notabilis cincitipes* Uvarov, female; E, *Scintharista notabilis pallipes* Uvarov, male; F, *Scintharista notabilis brunneri* Saussure, female; G, *Sphingonotus savignyi*. Saussure, male; H, *S. balteatus himalayanus*, Uvarov, male; I, *S. balteatus balucha* Uvarov, male.

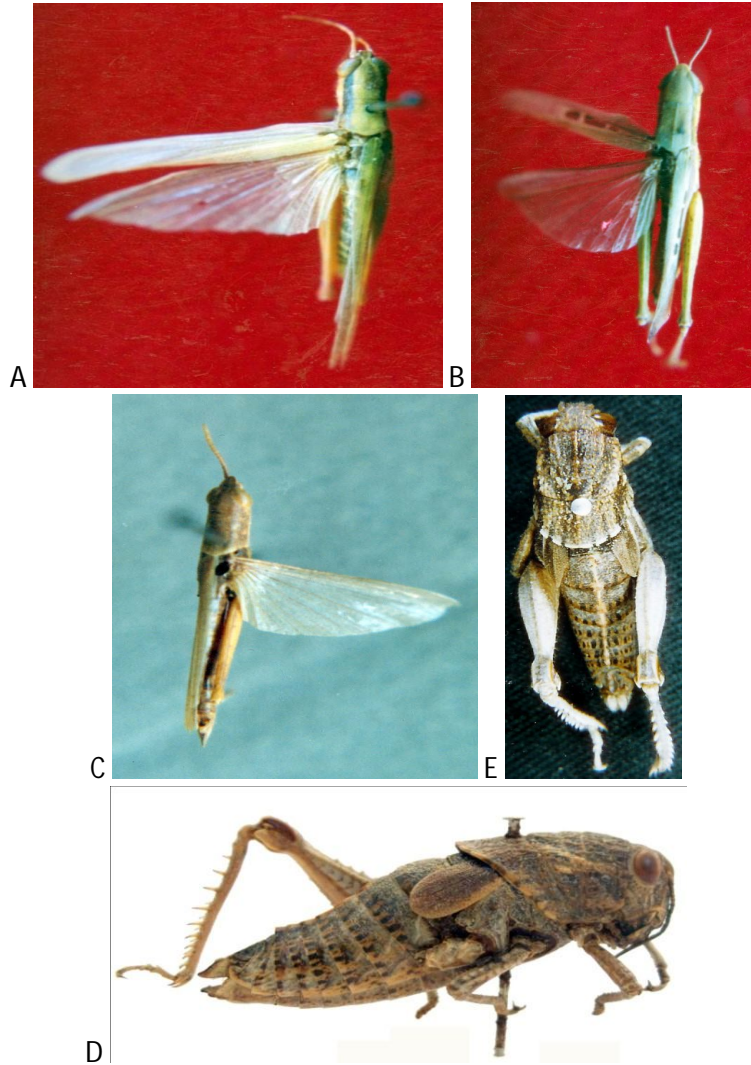


Fig. 9. A, *Oxya fuscovittata* (Marschall), female; B, *Spathosternum prasiniferum* (Walker), male; C, *Tropidopola Longicornis longicornis* (Fieber), male; D, *Kabulia balucha* Uvarov, female; E, *Kabulia* sp.

Acrididae: Spathosterinae (Table II, J, Fig. 9)

A single Genus *Spathosternum* Krauss with single species *Spathosternum prasiniferum* (Walker) (Fig. 9B) of Spathosterini tribe was studied. This species

is very common, widely distributed and occurs in cultivated fields among thick grasses in dry meadows, damp places and near the roadsides.

Acrididae: Teratodinae (Table II, K, Fig. 9)

Two female specimens of flightless genus *Kabulia* were collected from Zhob area of Balochistan. One specimen is *Kabulia balucha* Uvarov (Fig. 9D.) while another specimen (Fig. 9E) which is near to *Kabulia balucha* but differs in the structure of pronotum, more material is needed to confirm the species.

Acrididae: Tropidopolinae Table II, L, Fig. 9)

A single species *Tropidopola longicornis longicornis* (Fieber) (Fig. 9C) of Genus *Tropidopola* stal of tribe Tropidopolini studied, it occurs near the river banks.

Dericorythidae (Table III, Fig. 10)

Eades (2000) raised the status of subfamily Dericorythinae to family level and includes three subfamilies namely Dericorythinae, Conophomatinae and Iranillinae. The latter subfamily has been not recorded from Pakistan while the species of first two subfamilies are the endemic of Saharan-Gobian subregion including the Balochistan and Khyber Pukhtunkhawa but one species enter into the eastern side of the Indus Plain.

Dericorythidae: Dericorythinae (Table III, Fig. 10)

The species of Dericorythinae are characterized by the presence of hump or crest in the region of prozona formed by median carina of pronotum and the lateral carinae absent. Five species (Table III) collected from the desert of Nauskhi and the mountainous of Quetta (Balochistan). *Dericoyis tibialis* (Pallas) (Fig. 10A), *D. xenosterna* Uvarov (Fig. 10B) *Dericoyis* sp. (Fig.10C). One species *Dericoyis tibialis* (Pallas) penetrate into eastern side of the Indus Plain and has been collected from Punjab and Sindh.

Dericorythidae: Conophomatinae (Table III, Fig. 10)

The conophymatini grasshoppers settle the mountains of west Himalayas. It includes many wingless enedemics, which are chiefly associated with the outer ridges of such mountains previously four species namely *Conophyma*

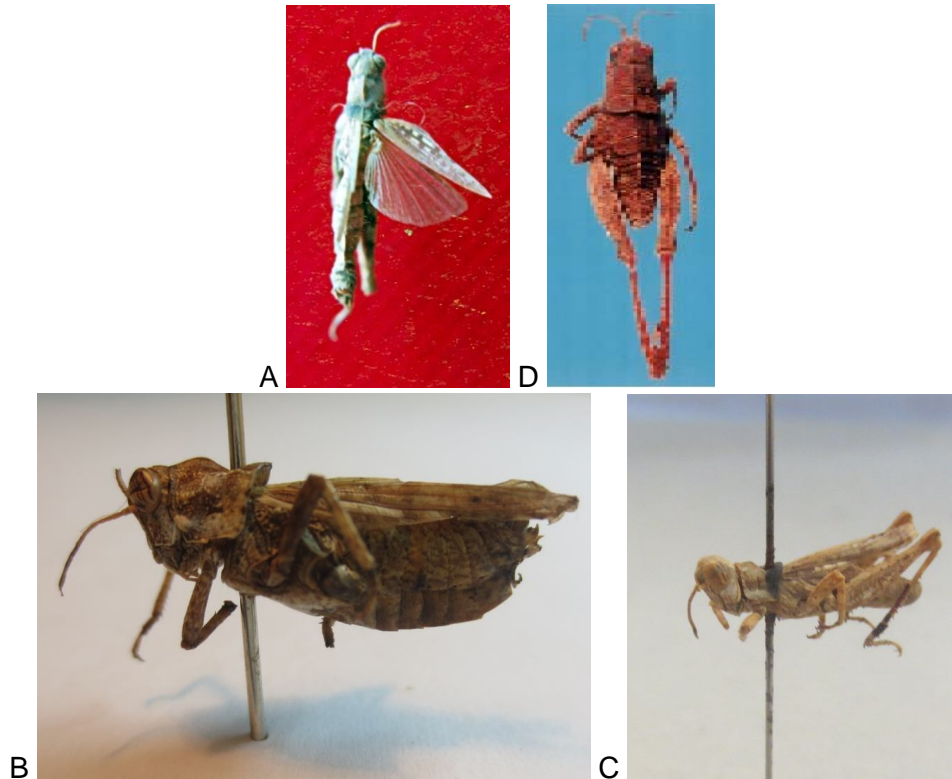


Fig. 10. A, *Dericorys tibialis* (Pallas), female; B, *D. cryosterna* Uvarov, female; C, *Dericorys* sp.; D, *Conophoma* sp.

kashmiricum Mistsh, *C. mitchelli* Uvarov, *C. indicum* Mistsh from Kashmir and *C. baludzhianum* Mishtsh from Pakistan have been recorded. Presently a single specimen of *Conophoma* sp. (Fig. 10D) has been recorded from the Osho valley i.e 10 km from Kalam (Swat) towards Mahomandh Lake (KP). The collected specimen differs from previously reported 4 species in having small size and is closely related to *Conophyma cercatum* (Ramme) but 2mm larger and lateral carinae well developed in pro and metazoana Whereas in *Tharnmatophyma cercatum* lateral carinae is not well developed in pro –and- metazoan. General coloration: Brown, hind femur with ventral inner aspect and most of the inner side red in color. Hind tibia reddish in color. Measurements given in millimeter (mm). Antennal length 7.5, pronotal length 3.75, Hind femurlength 7.8, Hind tibia length 7.0 total length 16.5 This specimen might prove to be a new but we hesitate to describe it as a new on the basis of single specimen and in the absence of male.

TABLE III.- DISTRIBUTION OF FAMILY DERICORYTHIDAE IN VARIOUS PROVINCES OF PAKISTAN.

Species	Sindh	Balochistan	Punjab	Khyber Pakhtunkhwa	Gilgit Baltistan
<i>Dericorys albidula</i>	-	+	-	--	-
<i>D.ramchandrai</i> Uvarov	-	+	-	--	-
<i>Dericorys tibialis</i> (Pallas)	+	+	+	+	-
<i>D.cryptosterna</i> Uvarov	-	+	-	-	-
<i>D.xenosterna</i> Uvarov	-	+	-	-	-
Subfamily					
<i>Conophomatinae</i>					
<i>Conophoma</i> sp.	-	-	-	+	-

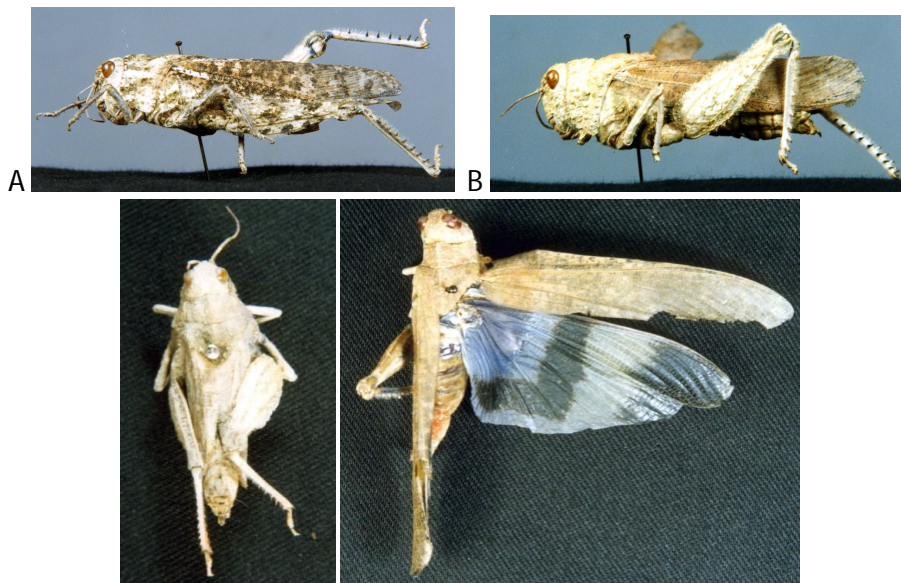


Fig. 11. A and B, *Eremocharis* spp.; C, *Eremocharis granulosa brachycera* (Kirby), female; D, *Eremopeza gigas* (Kirby), male.

Family: Pamphagidae (Table IV, Fig. 11)

Pamphagidae include usual forms for the arid plains and mountains of Middle Asia, Balochistan and Khyber Pakhtunkhawa. There are many endemic genera and species, which may be connected with the different types of the

deserts and semi-deserts (Stone ,Clay sandy) and this family has been recorded from the Balochistan , Khyber Pakhtunkhwa(formerly North western Province of Pakistan) and presently we have collected from low sea level *i.e.* Ranni Kot Sindh.The species identified are *Eremopeza gigas* (Kirby) (Fig. 11D.) and *Eremocharis granulosa brachycera* (Kirby) (Fig. 11C.) and other material *Eremocharis* spp (Fig. 11A,B) is under the process of identification.

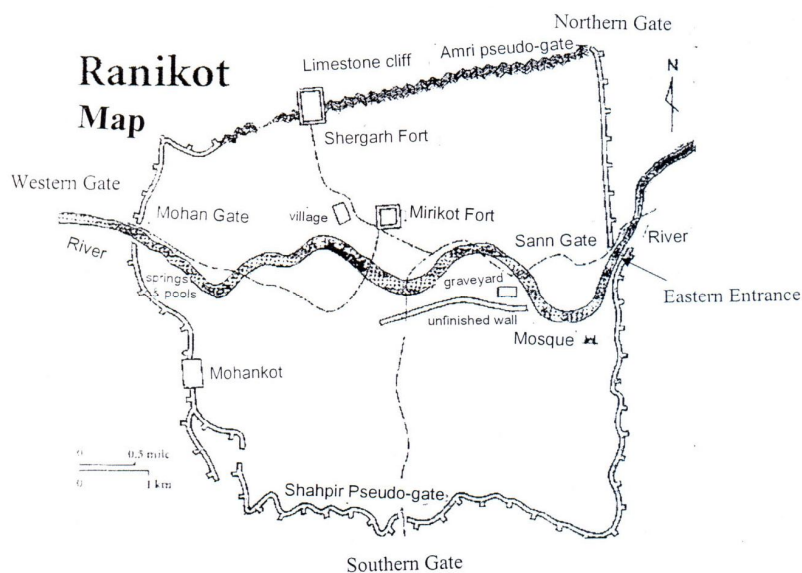


Fig. 12. Map showing the internal view of Ranni Kot.

Superfamily: Pyrgomorphoidea

Note: Ranikot (Fig. 12) is the most famous historical place of Sindh. This enchanted fort is located in Lakhi Mountains of the Lakhi range on the right side of the mighty River Indus at a distance of about 35 km from the present day town of Sann. A mountainous ridge, Karo Takkar, running north to south, forms its western boundary and the Lundi Hills forms its Eastern boundary. Mohan Nai, a rain- stream enters the fort from its rarely used Mohan gate, where it is guarded by a small fortification, changes its name to Reni or Rani Nai or rain-stream-Rani. It runs through it tumbles in a series of turquoise pools to irrigate fields and leaves the fort from its most used “Sann Gate” on the eastern side. It then travels about 33 km. The most of the 35-40 km long wall is made up of natural cliffs and barricades of mountainous rocks at places rising as high as 2000 feet sea level.

As one enters the fort one can find hills, valleys, streams, ditches, ponds, pool, fossils, scattered vegetation of shrubs, herbs and grasses, building structures, bastions watch towers ravelins ammunition deposits, fortress-all inside ranikot, adding to more its beauty and mystery, a spring emerging from an under ground water source near the Mohan gate is named as Parryan jo taru (the spring of fairies).

TABLE: IV.- DISTRIBUTION OF FAMILY PAMPHAGIDAE IN VARIOUS PROVINCES OF PAKISTAN.

Species	Sindh	Balochistan	Punjab	Khyber Pakhtunkhwa	Gilgit Baltistan
<i>Eremopeza gigas</i> (Kirby)	+	+	-	-	-
<i>E. cinerascens</i>	+	+	-	-	-
<i>aurantipes</i> Uvarov					
<i>Eremocharis granulosa</i>	-	+	-	-	-
<i>granulosa</i> (Walker)					
<i>Eremocharis granulosa</i>	-	+	-	-	-
<i>brachycera</i> (Kirby)					
<i>Eremocharis sp 1</i>	+	-	-	-	-
<i>Eremocharis sp 2</i>	+	-	-	-	-

Family: Pyrgomorphidae (Table V, Fig. 13)

The pyrgomorphidae contains some of the most colourful grasshopper species mainly inhabit the arid and semiarid areas but they are associated with local river valleys. A single subfamily Pyrgomorphinae with six tribes studied are given below.

Tribe Atractomorphini

Atractomorpha acutipennis blanchardi Bolivar (Fig. 9A) of this tribe was studied. This species is widely distributed and occurs in cultivated fields among thick grasses in dry meadows, damp places and near the roadsides.

Tribe Chlorizeinini

Pterorthacris subcallosa Uvarov (Fig. 13G) is a rare species in world collection. This species was described by Uvarov (1921a) from a single male from Pusa Bihar, India, at the same time two more males were reported from the type locality. Its female was described by Kevan and Wagan (1987) from Hyderabad, Sindh Pakistan, but no exact locality was mentioned.

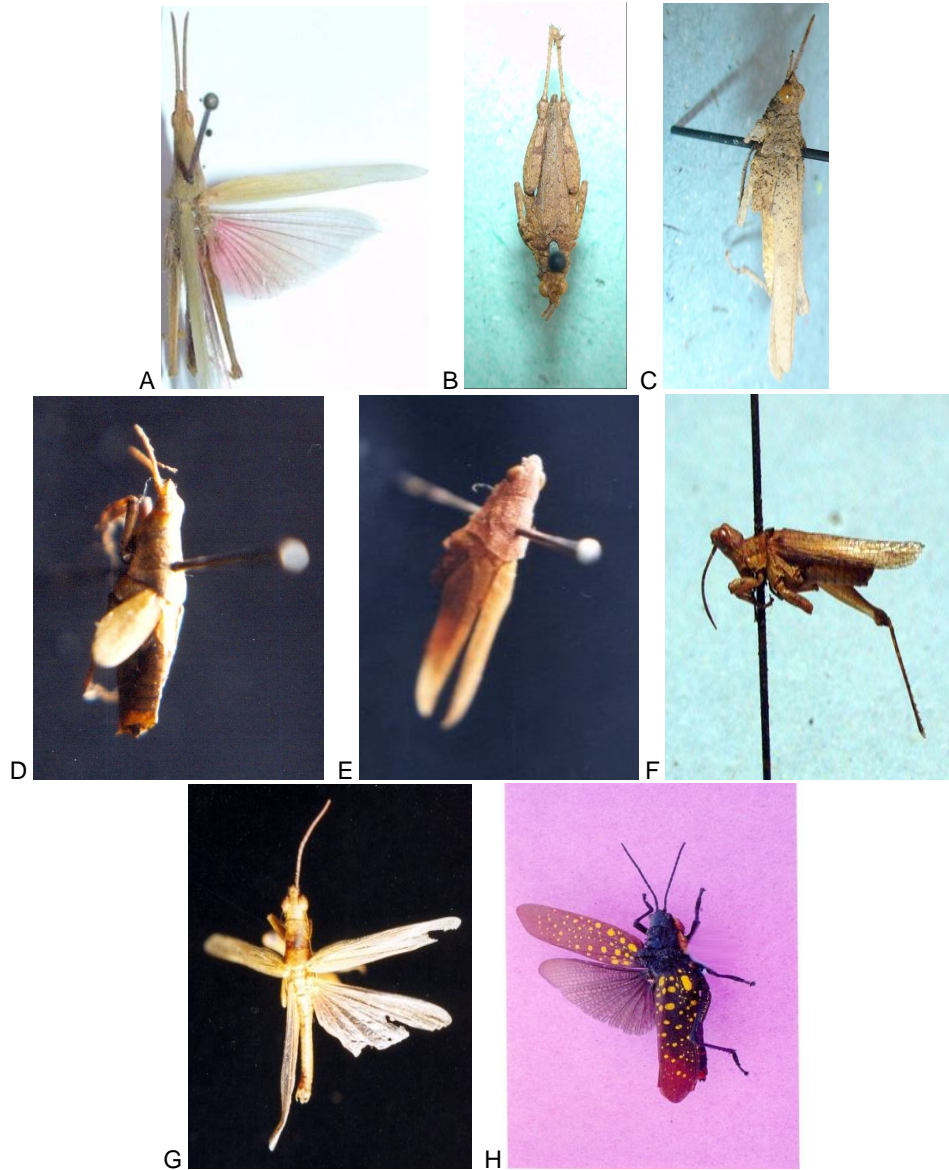


Fig. 13. A, *Atractomorpha acutipennis blanchardi* Bolivar, female; B, *Chrotogonus tracypterus tracypterus* (Blanchard), female; C, *Tenuitarsus orientalis* Kevan, female; D, *P. inaequalipennis* Bolivar, female; E, *P. hemiptera* Uvarov, female; F, *Pyrgomorpha bispinosa deserti* Bei-Bienko, male; G, *Pterorthacris subcallosa* Uvarov, male; H, *Aularches miliars pseudopunctatus* Kevan, female.

TABLE: V.- SHOWING THE DISTRIBUTION OF FAMILY PYRGOMORPHIDAE IN VARIOUS PROVINCES OF PAKISTAN.

Subfamily Pyrgomorphae Tribe and Species Atractomorphi	Sindh	Balochistan	Punjab	Khyber Pakhtunkhwa	Gilgit Baltistan
<i>Atractomorpha acutipennis</i> <i>Blanchardi</i> Bolivar	+	+	+	+	-
Chlorizeinini <i>Pterorthacris subcallosa</i> Uvarov	+	-	-	-	-
Chrotogonini <i>Chrotogonus tracypterus</i> <i>tracypterus</i> (Blanchard) <i>C. tracypterus robertsi</i> Kirby <i>C. homadolemus</i> <i>homadolemus</i> (Blanchard) <i>Tenuitarsus angustus</i> Blanchard <i>T. orientalis</i> Kevan	+	+	+	+	+
	-	+	+	+	-
	-	+	-	-	-
	-	+	-	-	-
	+	-	-	-	-
Pyrgomorphi <i>Pyrgomorpha bispinosa</i> <i>bispinosa</i> (Walker) <i>P. bispinosa deserti</i> Bei- Bienko <i>P. cognatus miniata</i> Uvarov <i>P. conica teretecornis</i> (Brulle) <i>P. inaequaulipennis</i> Bolivar <i>P. hemiptera</i> Uvarov	+	+	+	+	-
	+	+	+	+	-
	-	+	-	-	-
	+	+	+	+	-
	+	-	-	+	-
	+	-	+	-	-
Taphronotini <i>Aularches miliars</i> <i>pseudopunctatus</i> kevan	-	-	+	-	-
Poekilocerini <i>Poekilocerus pictus</i> (F.)	+	+	+	+	-

Tribe *Chrotogonini*

Two genera namely, *Chrotogonus* Serville and *Tenuitarsus* I. Bolivar were studied and are given below.

The genus *Chrotogonus* having body rough, with tubercles and granules distinctly flattened and it occurs in wide range of habitat including garden. According to Kevan (1959) the Indo Iranian species *Chrotogonus trachypterus* occurs in two subspecies i.e. *C. trachypterus trachypterus* and *C. trachypterus robertsi* occurs on the eastern and western side of Indus plain, respectively. The Iranian subspecies *C. trachypterus robertsi* Kirby is south eastern Palaearctic Eremian and is found on the arid plateau from Baluchistan and Afghanistan to eastern Persia and is limited on the north by the mountains of Elburz to the Hindu-Kush; the other north Indian subspecies *C. trachypterus trachypterus* (Blanchard) (Fig. 13B) seems to favor less arid conditions and is found south of Himalayas from the foot hills of Baluchistan to Assam and South in Peninsular India to about 16 N, thus having more oriental than Palaearctic range. A third species namely *C. homalodemus homalodemus* similar to above species but smaller and more slender occurs in the sandy area of Mekran Balochistan. *Tenuitarsus* characterized by slender body with brown and rust coloured speckles is Palaearctic-Eremian but extend into peninsular India and to Africa south of Sahara. *Tenuitarsus angustus* Blanchard is truly Eremian, extending from the deserts of North West Africa to Egypt, Arabia, southern Israel, Iraq, south east Persia and Balochistan but is replaced in other parts of Pakistan and in north west India by *T. occidentalis*. It is replaced in India and eastern part of Pakistan by the somewhat less specialized *T. orientalis* (Fig. 13C) (Kevan, 1959).

Tribe *Poecilocerini*

A single species *Poecilocerus pictus* (F.) of this tribe was studied which is widely distributed throughout country.

Tribe *Pyrgomorphini*

The species of the genus *Pyrgomorpha* of tribe Pyrgomorphini are small variable color, green or brown. The species *Pyrgomorpha bispinosa bispinosa* and *P.b.deserti* (Fig. 13F) are widely distributed and occurs in wide range of habitat including garden. Two specimens of *Pyrgomorpha hemiptera* Uvarov (Fig. 13E) were collected from (Khewra salt range) Punjab and Thar desert (Sindh) and a single specimen each of *P. inaequaulipennis* Bolivar (Fig. 13D)

P.c. tereticornis and *P. c. minima* was collected from Sindh, Punjab and (Mekran) Balochistan, respectively. It is possible that third species *P. bispinosa* nr. *incognita* might occur in Pakistan.

According to Hsiung and Kevan (1975) the *conica-bispinosa-cognata* group of species of the genus *Pyrgomorpha* superficially are very similar and difficult to separate even for a specialist.

Tribe Taphronotini

Aularches miliars pseudopunctatus Kevan (Fig. 13H) a single female and four 1st instar nymph of this subspecies have been collected from Simly Dam Islamabad. All nymphs are dark black coloured whereas in adult female the frons and lower part of pronota are yellow, sides of abdomen have red spots and ovipositor is red. This sub species is being reported for the first time from Pakistan previously reported from N .UttarPradesh, Himayal Pardesh, Kashmir, Nepal, Sikkim, S. Tibet.

Superfamily: Tetrigoidea

Family: Tetrigidae (Table VI, Fig. 14)

The species of Tetrigidae occur throughout the country and is connected with forest or forest steppe zones. Some species are widely distributed through rivers, valleys of an arid area. The species of *Tetrix* Latreille, *Hedotettix* Bolivar, *Paratettix* Bolivar and *Euparatettix* Hancock occurs in a rice field apart from this they also found on grassy field. *Critettix* sp. (Fig. 14B) occur on the margin of river banks, rice field, on wet and dry rocks and even in the desert area (Riffat *et al.*, 2013). *Bolivaritettix nilgricus* (Hebard), *Eucrietettix montanus* (Hancoc) and *Thoradonota nodulosa spiculoba* Hancock. (Fig. 14C) these species previously not reported from this area. The *Eucrietettix montanus* (Fig. 14A) have been collected from the fallen leaves in hilly areas of Murree (Punjab).

Superfamily: Eumastacoidea

Family: Eumastacidae

Subfamily: Gomphomastacinae (Table VII, Fig. 14)

Subfamily Gomphomastacinae is the endemic group for the mountains of Tien Shan, Pamiro-Allay, Nanshan, Karakoram the Hindu-kush and Himalayas.

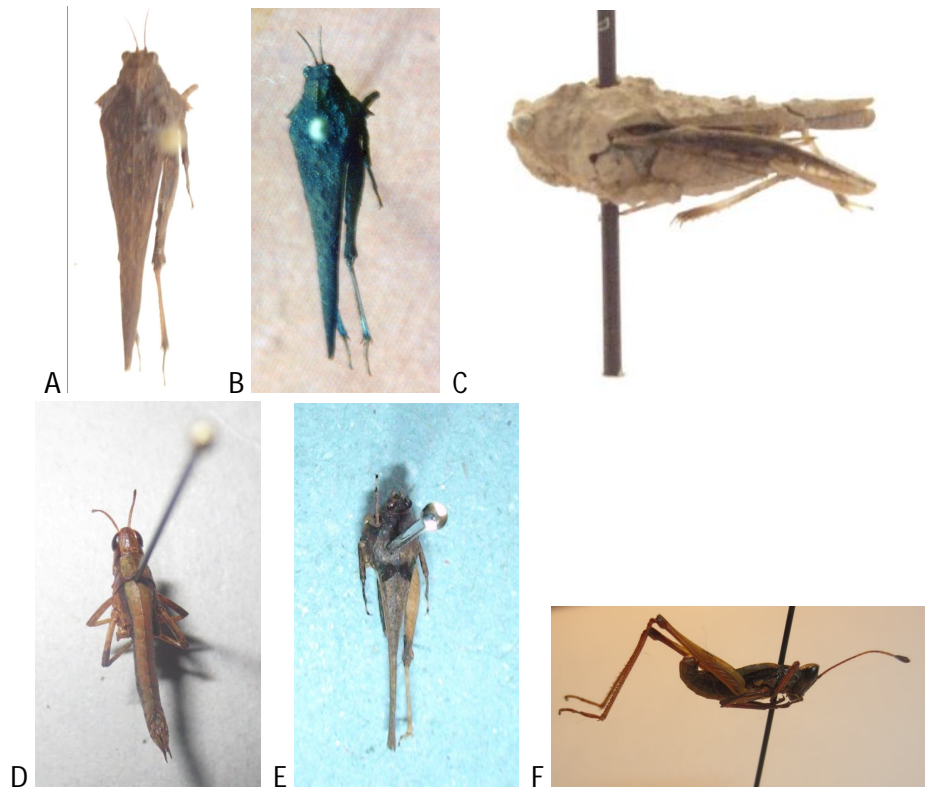


Fig. 14. A. *Eucriotettix montanus* (Hancock), female; B, *Critettix* sp. female; C, *Thoradonota nodulosa spiculoba* Hancock, female; D, *Paratettix cingalensis* (Walker), male; E, *Gomphomastax calavata* (Ostr.), female; F, *Gomphomastax bulbosus* Garai, male.

Most species have very localized settlements, which are associated definite part of each ridge (Serjeev, 1995). Two species of genus *Gomphomastax* namely, *Gomphomastax bulbosus* (Fig. 14F) and *G. calavata* (Fig. 14E) were collected and studied. *Gomphomastax bulbosus* was described a new species by Garai in 2002 from Babusar top mountain, presently we have collected one male and one female from Thandyani Abbotabad. Our specimes generally agrees with the description of Garai 2002 and we confirm the presence of this species and its distribution has been extended to new locality *i.e.* (Thandyani) Abbotabad. A single specimen of *G. calavata* (Ostr.) is collected from a new locality Osho valley *i.e* 10 km from Kalam (Swat) towards Mahomandh Lake (KP).

TABLE: VI.- DISTRIBUTION OF FAMILY TETRIGIDAE IN VARIOUS PROVINCES OF PAKISTAN.

Tetrigidae	Sindh	Balochistan	Punjab	Khyber Pakhtunkhwa	Gilgit Baltistan
Meterdorinae					
<i>Bolivarietettix nilgricus</i> (Hebard)	-	-	+	-	-
<i>B. ghumtitanus</i> (Hancock)	-	-	+	+	-
Scelimeninae					
Criotettigini					
<i>Criotettix sp</i>	+	-	-	-	-
Thoradontini					
<i>Thoradonota nodulosa</i> <i>spiculoba</i> Hancock	-	-	+	+	-
<i>Eucriotettix</i>					
<i>Eucriotettix</i> <i>maculatus</i> (Kirby)	-	-	+	-	-
<i>Eucriotettix tricarinatus</i> (Bolivar)	-	-	+	-	-
<i>Eucriotettix</i> <i>montanus</i> (Hancock)	-	-	+	-	-
Tetriginae					
Tetrigini					
<i>Tetrix mundus</i> (Walker)	+	+	+	+	-
<i>Copotettix annandalei</i> Hancock	-	-	+	+	-
<i>Coptotettix fossulatus</i> (Hancock)	+	+	+	+	-
<i>Coptotettix rugosus</i> (Hancock)	+	-	-	+	-
<i>Hedotettix gracilis</i> (Haan)	+	+	+	+	-
<i>Paratettix cingalensis</i> (Walker)	+	+	+	+	-
<i>Ergatettix dorsiferus</i> (Walker)	+	-	+	-	--

Grasshoppers constitute one of the most important group of insects occurring in Pakistan. Although, the group contains a number of species that have to be considered as a serious pest of agriculture and horticulture including the notorious desert locust. Most of the species were collected from grasses, herbs and shrubs. However, some species, e.g. *Acrida exaltata* (Walker), *Aiolopus*

TABLE VII. -DISTRIBUTION OF FAMILY EUMASTACIDAE IN VARIOUS PROVINCES OF PAKISTAN.

Subfamily	Sindh	Balochistan	Punjab	Khyber Pakhtunkhwa	Gilgit Baltistan
Gomphomastacinae					
<i>Gomphomastax bulbosus</i> Garai	-	-	-	+	-
<i>Gomphomastax calavata</i> Garai	-	-	-	+	-
<i>Gomphomastax monsoonia</i> Garai	-	-	-	+	-
<i>Gomphomastax moderata</i> Garai	-	-	-	+	-

TABLE VIII.- COMPARATIVE NUMBERS OF PREVIOUS RECORDED SPECIES AND PRESENT FINDINGS.

Family	Ahmed (1980)	Yousuf (1996)	Present finding
Acrididae	86 species	95 species	121 species
Dericorythidae	02	03	05
Pamphagidae	03	02	05
Pyrgomorphidae	08 species	06 species	15 species
Tetrigidae	03 species	Nil	13 species
Eumastacidae	Nil	02	02
Total	102	108	161

thalassinus thalassinus (F.), *A. thalassinus tamulus* (F.), *Aiolopus simulatrix simulatrix* (Walker), *Oxy hyla hyla* Serville, *O. velox* (F.), *O. fuscovittata* (Marsch.), *Obidentata* Willemse, *Acrotylus humberianus* Saussure, *Trilophidia annulata* (Thunberg), *Mioscirtus wagneri rogenhoferi* (Saussure), *Oedaleus abruptus* (Thunberg), *Heteracris littoralis* (Rambur), and *H. adspersa* (Redtenbacher) *Eyprepocnemis rosea* Uvarov, *Hieroglyphus nigrorepletus*, I. Bolivar, *H. oryzivorus* Carl, *H. perpolita* (Uvarov) were collected from cultivated field of rice, sugar cane, maize, jowar and vegetable fields, where they are regarded as a minor to considerable pests. Many of the species are inoffensive and restricted to habitats where they have little impact on human activities. Indeed, amongst this latter group are to be found some of the most beautiful insects of desert and mountains. The bright colors of species that depends flash coloration as part of their defenses rivals the butterflies in beauty included amongst this group are *Sphodromerus undulatus undulatus* (Kirby), *Oedipoda himalayana* Uvarov, *Chloebora grossa* Saussure *Scintharista notabilis pallipes*

Uvarov *Sphingonotus balteatus himalayanus* Uvarov, *Acrophya glaucopsis*. Many species are well known for their association with dry or even arid conditions and these generally live on the surface of the ground between rather than on vegetations.

The relatively large size of grasshoppers and their abundance, at least for some species make them an important food resource for vertebrates including reptiles, birds and mammals. Grasshoppers are also key organisms for sustaining a large variety of other invertebrate species in Pakistan. These include insects that are obligatory parasites. It is interesting to point out that previously all species of Pamphagidae have been collected from the higher altitudes ranging from 1500-2500 meters from above sea level. It is also evident from the available literature *i.e.* Uvarov (1943), Bei-Bienko and Mishchenko (1952) and Mishchenko (1952), Janjua (1957) and Ahmed (1980) that these species are restricted to higher altitudes provinces and considered to be endemics. However, during the present study the species has been recorded from a new locality *i.e.* Ranni Kot and at low sea level. More detailed study on the Orthoptera of Ranni Kot will bring some more interesting facts. The growth of our knowledge on the grasshoppers on Pakistan since this country becomes an independent in 1947 is shown in (Table VIII). It is very likely that this number will increase further as and when extensive surveys are carried out.

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BIO-ECONOMIC IMPACTS OF VERTEBRATE PESTS ON CROPS WITH SPECIAL REFERENCE TO RODENT PESTS IN PAKISTAN AND OTHER COUNTRIES

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Abstract.- Vertebrate pests of economic importance are distributed worldwide in all kinds of agro- forestry systems.. Throughout the world, wheat, rice, maize, sugarcane, groundnut, coconuts and oil palm are severely damaged by various rodent species. The loss of human health and lives cannot be assigned any price tag, as rodents are carriers of at least twenty severely debilitating human diseases. Estimates of food losses to rodents vary wide in Pakistan and in many other countries, which in majority cases are not statistically defensible. Such estimates are aimed to quantify the problem horizontally as well as vertically with the main objective to develop projects and framing national rodent control policies. A more reliable and critically analysed data is available on rice and of various cereal crops being affected by outbreaks of rats and mice in Africa, Australia and Latin America. In 11 Southeast Asia rice growing countries, a loss of 5% of rice production amounts to approximately 30 million tons - enough rice to feed 180 million people for 12 months. One of the best estimates of post- harvest rodent impact is from Central Punjab, Pakistan where 0.33 billion tons of rice, wheat and maize worth US \$30 million is consumed by house rats in villages of Pakistan. On the average, crop losses in Pakistan range from 10 -50%. In Pakistan, estimates of pre- post-harvest losses caused by vertebrate pests worth Rs. 5.7 billion are reported.

Key words: Economic impacts, Pakistan, pre- post-harvest losses, rodent damage, vertebrate pests.

INTRODUCTION

Vertebrate pests/deteriogens are of great concern world wide and in Pakistan too. Among these, rodents are the most important pests at the global

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level. Rodents have three major impacts. The first is the substantial damage they can cause to field and horticultural crops, forest plantations, and vegetation of range lands. The second is the losses they cause post-harvest to stored food and feed products. The third and often overlooked impact is on the health of small farmers and urban communities, and hence, influences productivity out-put of humans and health-care costs. The loss of human health and lives cannot be assigned any price tag. Serious attempts have not been made to evaluate health risks in monetary terms. In poorer farming communities, if a rodent zoonotic causes disability to a poor farmer for a month at a key time then it may lead to no crop, a late crop, or reduced crop yield. Each can lead to a debt treadmill (Singleton, 2001). Rodents are carriers of at least twenty severely debilitating human diseases (Mills, 1999; Meerburg *et al.*, 2009). The role rodent's play in contributing to health and safety risks is often overlooked or ignored while quantifying human conflicts with wildlife. The impact of rodent – borne diseases on human health, safety and natural resources is considerable (Gratz, 1996).

Evaluation and quantification of impacts of rodents on growing crops and destruction to stored food products is at best meager except in few cases where damages have been evaluated in terms of reduction in yield and resultant economic loss. Too little is known world wide about the quantum and value of damage that rodents inflict annually on crops and their products in stores. Rodent damage estimates, in most cases, are used as indices of rat activity or for comparing management strategies. In majority of cases, damage and loss assessment surveys/studies have been conducted to quantify the problem horizontally as well as vertically with the main objective to develop projects and framing national rodent control strategies. Measuring crop damage and yield loss is labour intensive and complicated in majority cases because uniform sampling techniques are difficult to develop. Accurate assessing damage is critical to document the extent of losses, justifying when to employ management strategies, and evaluating the effectiveness of management practices. Adequate sampling techniques have only been developed for a few crops and in few situations (FAO/CAB, 1971).

Estimates of food losses to rodents vary wide in Pakistan and Southeast Asian countries. In most cases, such estimates are not statistically defensible. However, in Pakistan, majority of the studies conducted by Vertebrate Pest Management Programmes of Pakistan Agricultural Research Council (PARC) were well designed (quadrant, multiple or stratified sampling techniques) and results, in most cases, are statistically reliable.

From the assessment of impacts of rodents on pre-and post-harvest systems, it is clear that rodents play a significant role in influencing food security and poverty alleviation programmes for the rural poor communities. Consequently, the purpose of this technical paper is to critically examine the relationship of vertebrate pests to the production, storage, and processing of agricultural crops and utilization by humans. In no way, this literature analysis attempt is complete or inclusive except of Pakistan. Of other countries selected data have been gathered to high light various concepts, problems, and seeking sustainable solutions to these most important identified rodent pest problems (Geddes, 1992; Singleton, 2001) or one of the major constraints to agricultural production (Grist and Lever, 1969; Hopf *et al.*, 1976; Hoque *et al.*, 1988; Singleton, 2001).

Various attempts have been made in the past and during the last three decades to compile and evaluate the depredation impacts of rodent pests to crops and stored food products. Hopf *et al.* (1976) made the first attempt to collect, compile, and document rodent damage to growing crops and to farm and village storage in tropical and sub-tropical regions of the world. The estimates were based on postal questionnaire response. A year later, Jackson (1977) evaluated the damage estimates of rodent pests of some selected countries. In these two works less detail data were provided for Europe, North and Latin Americas. Meehan (1984) documented estimates of losses due to commensal rats and mice to urban properties and stored products. Geddes (1992) and Geddes and Iles (1991) provided some more comprehensive information on rodent damage, including other vertebrate pests. Prakash (1988) and Hoque *et al.* (1988) tabulated rodent damage estimates in Asia and Southeast Asia including Pacific islands. A more reliable and critically analyzed data on rice damage estimates have been provided by Singleton (2003) and of various cereals being effected by out-breaks of different rodent species in Africa and Latin America by Stenseth *et al.* (2003). The works of these authors, combined together, give a global overview of the bio-economic impacts of rodent pests on crop production.

In Pakistan, Khan (1990) comprehensively reviewed damage and losses caused by vertebrate pests by enumerating results of studies up to 1988. Although little has been published, a number of burrowing rodent species cause water loss, and the attendant risks of flooding, by excavating earthen dams, irrigation canals, or flood control structures, and plastic tubing of drip irrigation systems (Khan *et al.*, 2007).

CROP DAMAGES AND LOSSES TO RODENT PESTS

The pre-harvest losses caused by rodent damage is a problem dating back hundreds, and probably thousands of years, The chronic and frequent acute nature of rodent depredation has led to a level of acceptance by the growers in Asia and Africa. Many farmers have taken the view that for every 10 rows of grain sown, two are planted for the rats. To some extent, in some regions, this perception, also, exists in some areas of Pakistan.

It has been estimated that between a fifth and a third of the world's food supply never reaches the table because of losses to rodents. In India their depredations will deprive a hungry people enough grain to fill a freight train stretching more than 3,000 miles (Canby, 1977). This kind of situation, also, exists in some regions of Pakistan. Tracking these conflicts becomes increasingly important in developing and justifying policies on rodent damage management by government agencies.

Out-breaks of rodent populations is a unique ecological phenomenon which has been less studied or understood. The losses thus caused result into total destruction of crops. Such cases have been reported from East African countries and wheat growing regions of Australia. In 1989, out-break of the hairy-footed gerbil (*Gerbillus gleadowi*) in Tharparkar desert of Pakistan was reported by Vertebrate Pest Control Institute (VPCI). The persistent drought years followed by heavy rainfall were identified as the possible climatic factors of this out-break. The estimated damage caused to millets and gowar in four talukas was 13,748 and 3,995 tons, respectively (Khokhar and Rizvi, 1991). An identical situation was reported in 1956 in the district of Sialkot that revealed that the quantity of grains (rice and wheat) lost due to rodents was enough to feed the population of the district (0.3 million) for ten years. In Southeast Asia (11 countries), a loss of 5% of rice production amounts to approximately 30 million tons; enough rice to feed 180 million people for 12 months (Singleton, 2001). Throughout the world, wheat, rice, sugarcane, coconuts, and oil palm are severely damaged by various species of rodents. Post-harvest damage to stored food products is, also, very serious. Poultry farms are heavily infested with commensal rats and cause economic damage to birds, eggs, structures, and contaminate feed with droppings, hairs and urine.

In Pakistan, field rats, wild boar, and porcupine are important vertebrate pests and are distributed in majority of agro-ecological zones of Pakistan.

Parakeets have been identified as serious pests of maize, sunflower, citrus and some other fruits. Porcupine (*Hystrix indica*) is a singular pest of economic importance which is distributed throughout the country; including Azad Jammu and Kashmir (AJ &K). Rice-based wheat, groundnut-fallow-wheat cropping systems, and crops of dry lands are highly vulnerable to rodent damage. Rodent damage and yield loss assessment are yet to be made from these areas. Details of pre-and post-harvest estimates of losses caused by vertebrate pests are shown in Table I. Results of research based studies/surveys have been detailed in Table II.

TABLE I.- ESTIMATES OF PRE AND POST HARVEST LOSSES CAUSED BY VERTEBRATE PESTS IN PAKISTAN.

Pest	Crop	Mean damage*	US \$ (M) value of loss**	Rs. (Million)
Rats and mice	Wheat	3.5%	52.910	841.269
Rats and mice	Rice	8.0%	64.634	1027.681
Wild boar	Sugarcane	7.5%	37.900	602.610
Wild boar	Maize	6.7%	10.170	161.70
Wild boar	Wheat	5.2%	78.461	1247.529
Rats and mice	Sugarcane	9.2% stalk	8.843	140.604
Parakeets	Citrus	8.6%	24.113	383.396
Parakeets	Maize	7.5%	11.391	181.117
Parakeets	Sunflower	15.7%	1.586	25.217
Pika	Apple orchards (total loss)	2.0%	1.905	30.289
Porcupine	Forestry (partial)	20.0%	1.258	20.000
Porcupine	Maize	0.4%	0.607	9.651
Porcupine	Potatoes (other vegetables not included)	2.0	1.966	31.26
House sparrow	Wheat	2.26%	34.100	524.190
Voles	Apple	2.0% (yield loss)	1.905	30.289
Rats	Groundnuts	3.0% (total loss)	0.919	14.612
Wild boar	Forestry	5.0%	1.886	30.000
Rodents	Food storage	3.0%	26.038	414.000
TOTAL			360.592	5733.413

* From VPCL Manual and current research

** Exchange rate Rs. 15.9 = US \$ 1.00

ESTIMATION OF DAMAGE AND LOSSES DUE TO RODENT PESTS

Estimates of damage and losses are, to some extent, available in the world's technical literature, including government documents and technical project reports of the FAO, GTZ, DFID, US-AID, Aus-AID, E.U., Belgium and

TABLE II.- PRE- AND POST-HARVEST CROP LOSSES DUE TO VERTEBRATE PESTS IN PAKISTAN.

Pest	Crop/commodity	Location/region	Nature of damage	% Damage/loss estimates	% Yield reduction/ economic value	Comments	Reference
Field rats	Rice	Lower Sindh	Pre-harvest	10-50	15-75	up to total crop destruction	Wagle, 1927
"	Rice+Wheat	Sialkot Distt.	"	-	Equal to feed 0.3 million population for 10 years	Extrapolated estimates	Anon, 1953 (Survey report)
"	Rice	Punjab	"	6-10	6.0	Estimated	Greaves & Khan, 1975
"	"	Lower Sindh	"	10.4	-	Estimates of two districts	Greaves <i>et al.</i> 1977
"	"	Lower Sindh	"	-	10	Due to sub-surface hoarding	Fulk, 1977
"	"	Lower Sindh	"	2-43	19	Results of simulated study	Fulk & Akhtar, 1981
"	"	Punjab (03 districts)	"	16.1	13.7	Estimated	Khan, 1987
"	Wheat	Faisalabad Distt.	"	7.5	-	Estimated	Beg <i>et al.</i> 1977/a,b
"	"	Central Punjab (04 districts)	"	-	5.0	Estimated	Beg <i>et al.</i> 1978
"	"	Central Punjab, NWFP	"	0.1-6.1	2.3	Estimated	Fulk <i>et al.</i> 1980
"	"	Gujrat district	"	3.3-8.4	-	Estimated	Brooks <i>et al.</i> 1991
"	"	Faisalabad	"	2.04	-	Estimated	Ahmad <i>et al.</i> 1986
"	"	Muzaffarabad, AJ & K	"	-	141.5kg/ha or Rs. 3361/ha	Estimated	Maqbool <i>et al.</i> 2011
"	Sugarcane	Central Punjab (03 districts)	"	11.0	4-15	Estimated	Beg <i>et al.</i> 1979
"	"	Punjab and Sindh	"	7.86	7.7-10.7	Estimated	Fulk <i>et al.</i> 1980
"	"	Thatta Distt.	"	24	-	Based on food habits studies	Smiet <i>et al.</i> 1980
"	"	Thatta Distt.	"	-	PKR. 140.6 m annually	Based on 1986-87 production and prices	Khan, 1990

Continued

Pest	Crop/commodity	Location/region	Nature of damage	% Damage/loss estimates	% Yield reduction/economic value	Comments	Reference
"	"	Sindh & Punjab	"	8.33	-	Estimated	Geddes & Iles, 1991.
"	"	Thatta & Sanghar Karachi district	"	7.5-9	-	Estimated	Ali <i>et al.</i> , 2003
Fields Rats	Coconut	Karachi district	"	30.2 nuts/per palm/year	27 thousands annually	Estimated	Khan, 1978
"	"	Karachi & Malir districts	"	15-20	-	"	Ali <i>et al.</i> , 2003
"	Date-palm	Nok kundi, Turbat, Panjgour	"	22.1 20.1 13.4	PKR. 5.33 m annual loss	"	Ahmad & Pervez, 2008
"	Groundnut	Rawalpindi division	"	21.5	-	"	Brooks <i>et al.</i> , 1986
"	Groundnut	Punjab	"	-	17% reduction in yield	Estimated	Islam, 1987
"	Forest trees	Pasni, Balochistan	"	8.53	-	"	Khokhar <i>et al.</i> , 1999
Porcupine	i) Forest trees ii) Nurseries iii) Fruit trees iv) Crops and vegetables	Country wide & AJK	"	22.8 29.56 10.94 8.17	- - - -	up to 50%	Pervez, 2006 Khan <i>et al.</i> , 2007 Nawaz & Ahmad, 1974 Greaves & Khan, 1978
"	Maize	AJK	"	10.7	-	Survey Results	Khan <i>et al.</i> , 1997
"	Wheat	"	"	3.5	-	"	Khan <i>et al.</i> , 1997
"	Rice	"	"	1.67	-	"	Khan <i>et al.</i> , 1997
"	Potato	Attock	"	17.56	-	Designed Study	Khan <i>et al.</i> , 2000
"	Plastic tubing of drip system	Bakkar	"	-	PKR.	Repair twice	Khan <i>et al.</i> , 2000
"	Rice canal	Sindh	Canal Breach	-	70,000/replacement cost PKR. 3.2 billion loss	done 12 flooded villages survey data	Khan <i>et al.</i> , 2007
Bird pests	Wheat	Central Punjab	Pre-harvest	1.14-3.22	Av. 2.18 of total production of Punjab	Estimated	Rashid, 1972
house sparrow	"	Rahim Yar Khan	"	1-10	-	-	Bashir 1978
"	"	Central Punjab	"	Up to 50	-	-	Mirza, 1979
"	"	Punjab & Sindh	"	Up to 44	3.0 actual loss	Questionare survey results	Fulk and Lathiya, 1979

Continued

Pest	Crop/commodity	Location/region	Nature of damage	% Damage/loss estimates	% Yield reduction/economic value	Comments	Reference
"	"	Punjab & Balochistan	"	10-15	172,000 mt PKR: 209 m	Estimated	Roberts, 1976
Crows	"	Khanawal district	"	Serious	-	Removal of seed and seedlings	Yousuf, 1982
Parakeets	Maize	Rahim Yar Khan	"	3-50	-	Estimated, small sample	Roberts, 1974
Parakeets	Maize	Punjab Sindh NWFP Faisalabad	"	10 5% 15% 0.41%	-	Estimated	Bashir, 1980
"	"	NWFP Punjab Rahim Yar Khan	"	10.61% 27.62% upto 100%	50846 mt PKR: 114.41 m	Studied small sample Estimated	Ahmad <i>et al.</i> 1987 Khan & Hussain, 1990 Roberts, 1974
"	Sunflower	"	"	30% 45% 22.4% 11.72% 16.61% 2.6-12.7%	-	Estimates of experimental fields Estimated	Bashir <i>et al.</i> 1981 Besser, 1982 Khan & Ahmad, 1983 Shafi <i>et al.</i> 1986
"	"	Punjab Sindh Multan Punjab (07 districts) Sindh (09 districts) Punjab (11 districts)	"	17.24	74,503 mt PKR: 204.88 m	Estimated, survey data	Hussain <i>et al.</i> 1991
House sparrow	Lentils	Karachi district Faisalabad	"	24-55%	-	Estimated	Ahmad & Ali, 1978
"	Millet Sorghum Rice	Barani lands Lower Sindh	"	3-10% 3-12% 20%	-	Estimated	Roberts, 1978
Weavers	"	"	"	-	-	Estimated	Grist & Lever, 1969
Wild boar	All crops	Punjab	"	16 mt annually	-	Estimated	Inayatullah, 1973
"	-	Punjab Punjab	"	6.8-35.5%	PKR: 50 m annually	Interview data Survey based data	Mirza, 1978 Shafi & Khokhar, 1986
"	Sugarcan	"	"	-	-	-	-

Continued

Pest	Crop/commodity	Location/region	Nature of damage	% Damage/loss estimates	% Yield reduction/economic value	Comments	Reference
"	Wheat	Central Punjab	"	3.0%	PKR: 250 m annually	Estimated	Ahmad <i>et al.</i> 1986
"	Maize	Faisalabad district	"	6.7-10.0%	-	Data of 33 villages	Ahmad <i>et al.</i> 1987
"	Wheat	Fateh Jhang	"	7.3%	-	Various villages data	Brooks <i>et al.</i> 1989
"	Groundnut	Chakwal	"	0.09%	PKR: 6.4 m annually	Estimated	Brooks <i>et al.</i> 1986
"	Sugarcane	Punjab	"	11.3%	PKR: 130.9 m annually	Extrapolated estimates	Brooks <i>et al.</i> 1989
"	Wheat		"	3.0%	-	Extrapolated data	Brooks <i>et al.</i> 1989
"	Maize	All Pakistan	"	6.7%	16 mt annually	Extrapolated data	Brooks <i>et al.</i> 1989
"	Sugarcane, Wheat, Maize		"	-	PKR: 1,525 m	Estimated	Khokhar & Rizvi, 1998
"	Rice	Punjab (04 districts)	"	-	22.75%	Estimated	Shafi <i>et al.</i> 1989
Lagomorphs	Apple trees	Ziarat, Balochistan	Debarking of trees	21.61	PKR: 21.21m/year/season	Estimated	Shafi <i>et al.</i> 1989
"	"	"	"	20	-	Estimated	Khokhar & Fulk, 1976
Rabbits	Chick pea	Islamabad, NARC	"	21.7	-	Estimated	DWRC, 1986
"	Ground nut	Chakwal	Pre-harvest	12.6	-	Estimated	Brooks <i>et al.</i> 1986
Small mammals (Rats, Pikas, Porcupine)	Fruit orchards	Balochistan	"	-	PKR 31.28 m annually	Estimated	Mian <i>et al.</i> 1988
Commensal rats & mice	Stored grains (Rice, wheat, etc.)	Countrywide	Post-harvest	4-6	-	-	Anon, 1952
"	Various grains	Countywide	"	5.0	-	Estimated	Nasir <i>et al.</i> 1957
"	Wheat	"	"	1.0	-	Random sampling estimates	Nasir <i>et al.</i> 1957
"	"	"	"	4-10	-	-	Hafiz & Hussain, 1961
"	Rice	Karachi Rice Storage Complex	"	-	US \$ 800-1000/year/storage	Estimated	Greaves and Khan, 1974

Continued

Pest	Crop/commodity	Location/region	Nature of damage	% Damage/loss estimates	% Yield reduction/economic value	Comments	Reference
"	Wheat	Countrywide (farm level)	"	23	-	Estimated	MICAS Associates, 1976
"	Food commodities	Ration shops, Karachi	"	77.7 shops infested	-	Survey data	Greaves, 1979
"	Wheat & Rice	Countrywide	"	5.0	-	Estimates at village and market level	Shafi, 1985
"	Rice	Punjab (whole sale markets of 05 cities)	"	740 kg/shop	4,000 t/year	Estimates of 5,500 shops	Ahmad <i>et al.</i> 1995
"	Wheat	Punjab	"	39% infested	-	56 godowns of 25 PASSCO Centres	Ahmad <i>et al.</i> 1988
"	Rice, maize, wheat	Countrywide	"	-	0.33 billion mt US \$ 30 m/year	Estimates based on rodent population	Mustaq-Ul-Hassan, 1992
"	Various food commodities	Rawalpindi city	"	100% shops infested	10.8 Kg filth/ shop	Estimates of 8 shops	Hussain & Iqbal, 2002

Denmark, ORSTOM (French Scientific Research Institute for Development) and published research work. Pre-70's, in many cases, these estimates were fabrications, guess work or blowing up of some preliminary data. By that time and little later, reliable damage data were just not available. At that time the most common base for calculation was the "one rat/person" myth derived from Boelter (1909), without any factual basis.

A citation indicates \$ 2 by feeding, \$ 4 by damaging and \$ 6 by contamination- a total of \$ 12/rat/person (Anon, 1975). This formula, if used, compounds losses. However, during 80's and 90's reliable data were generated. Later on these figures were critically examined and analyzed, in terms of economics, by different researchers (Singleton and Petch, 1994, 2001; Stenseth *et al.*, 2003)

The methods of sampling to measure damage and yield loss have been described and discussed in detail by Rennison and Buckle (1987), and Buckle (1994) These methods are statistically sound and the resultant figures are defensible. During the last two decades, these methods were being widely used in rice crop. The economic benefits of management practices cannot be evaluated unless the crop losses caused by rodents are not measured and given monetary value. Measuring crop damage on anything but a very small scale can only be practically achieved by taking samples from the crop. While estimating the damage and yields various factors need to be considered such as varietal susceptibility, early and late sowing varieties, and time of sampling. The best data collected will be 1-2 days before harvest. This will represent actual loss, near to accuracy. Assessing or predicting yield loss principally depends on the rodent species and their populations at the time of sampling. Therefore, there is a strong need to develop such relationship and be considered when calculating yield and economic losses.

Estimates of crop damage from various countries are summarized in Table III. The great variability in damage and loss cannot be explained by geography and crop variety. This documentation is not intended to be exhaustive nor representative of all geographic areas, nor should most of the estimates be considered reliable. In real situations, the specific statistics are of relatively little consequence. What is important is that the food removed from human consumption by rodents cannot be regained and is unacceptable when food deprivation exists as a major world problem (FAO, 1973).

TABLE III.- CROP LOSSES DUE TO RODENT PESTS IN DIFFERENT COUNTRIES.

Country	Crop/commodity	Nature of damage	% damage/loss	% Yield Reduction/ Economic value	Comments	Reference
Asian countries	Rice	Pre-harvest	5-10 per annum	30 million t	Enough to feed 180 m people for a year	Singleton, 2001
Bangladesh	"	"	>50	32-60%	Estimates of districts	Islam <i>et al.</i> 1993
China PDR	"	"	5-10	-	Few data	Zhao, 1996
India	"	"	5-15	-	Country wide data	Sridhara, 1992; Chopra <i>et al.</i> 1996; Parshad, 1999; Rao, 2003
India, Andhra Pradesh	"	"	Out-break of rodent population	3,302 mt	Out-break of rodent population	Rao, 2003
Indonesia	"	"	5-30 per annum	25-30 % US \$ 1.7 billion/year	Enough to feed 65 m people/year	Buckle, 1988, Singleton <i>et al.</i> 2003
Laos PDR	"	"	15	-	Out-breaks in upland	Bounneuang <i>et al.</i> 2003
Malaysia	"	"	5	87332 t, US\$ 7.3 m	Estimated	Lam, 1982
Malaysia	"	"	4-5	-	Data on economic impact not known	Singleton and Petch, 1994
Myanmar	"	"	5-40	-	Estimated, no national data	Singleton, 2001
Philippines	"	"	5 (2-18)	-	National survey data	Schaefer, 1975
Philippines	"	"	5	US\$ 67.3 m	Based on estimates of 1975	Hoque <i>et al.</i> 1988
Philippines	"	"	5	8%, US\$ 55.3 m	Estimates of 1953-54	Sumangil, 1990

Continued

Country	Crop/commodity	Nature of damage	% damage/loss	% Yield Reduction/ Economic value	Comments	Reference
Philippines	"	"	3-5	-	30-50% in some years	Singleton, 2001
Thailand	"	"	-	US\$ 2.3.m	Low land rice	Tongtavee <i>et al.</i> 1990
Thailand	"	"	6-7	-	Variable data	Singleton and Petch, 1994
Vietnam	"	"	-	10-15%	Average yield losses	Singleton, 2001
Cambodia	"	"	-	0.3% of national production	No national data	Singleton, 2001
SE Asia	"	"	6	36 million t	Enough to feed 212 m people/ year	Singleton, 2003
Indonesia	"	"	40	-	800,000 ha data	Soekarna, 1968
Indonesia	"	"	5-15	17%, 160,000 t	Estimated	Indrarto, 1984
Indonesia	"	"	15 annual	-	-	Geddes, 1992
Indonesia	"	"	17	8.21 million t, US \$ 1.0 billion	Estimated	Geddes, 1992
Argentina	"	"	20%	-	Out break data	Rodriguez, 1993
Guyana	Sugarcane	"	10%	172/Kg, 3.1% loss of sugar	Estimated	Bates, 1960
Guyana	"	"	-	12500 mt	When outbreaks occurred	Bates, 1962
Jamaica	"	"	10%	249Kg/ha; 4.2% loss of sugar	"	Metcalf & Thomas, 1966
India	"	"	10%	76Kg/ha; 3.9% loss of sugar	"	Gupta <i>et al.</i> 1971
India Punjab	"	"	2.1-31%	161-240 Kg/ha	Estimate of standing & lodged crop	Bindra and Sagar, 1971

Continued

Country	Crop/commodity	Nature of damage	% damage/loss	% Yield/Reduction/ Economic value	Comments	Reference
Philippines	"	"	10%	183Kg/ha; 3.7% loss of sugar	"	Estioko, 1978
Caribbean Islands (Barbados)	"	"	-	US \$ 2.25 million/year	1974, one crop season estimates	Williams, 1984
Australia	"	"	-	40,000 mt	Estimated	Anon, 1975
Australia	"	"	2-5%	US \$ 1-2 m annual	Moderate damage	Caughley <i>et al.</i> 1994
Australia	"	"	-	825, 000 t	Estimates of 1999-2000	Smith, <i>et al.</i> , 2003
Egypt	"	"	5-20	US \$ 50 m	Up to 80%	Ibrahim, 1972; Shuyler, 1970
Florida	"	"	5-11	-	Stalk damage survey data	Samol, 1972
Indonesia (Java)	"	"	30-100	-	Estimates of 14,000 ha	Soekarna, 1968
Mauritius	"	"	-	37 mt/ha	Estimated	Williams, 1953
Philippines	"	"	2-10	11-25%	Survey of 6farms	Porquez & Ledesma, 1970
World	"	"	3%	US \$ 40/ha	Estimated	Economist, 1981
Bangladesh	"	"	7.5%	14.3% weight loss	-	Haque <i>et al.</i> 1985
Hawaii	"	"	-	Loss of 0.45 t of sugar	-	Teshima, 1968
Argentina	"	"	12-20	-	Estimated	Rodriguez, 1993
India	Wheat	"	2.7-21.3%	25-72 Kg/ha	Average loss in 3 villages	Bindra & Sagar, 1971
Bangladesh	"	"	-	5 times destruction of the quantity eaten	Simulated enclosure study	Haque <i>et al.</i> 1980
Bangladesh	"	"	12.1	77000 t/annum	Country-wide data	Poche <i>et al.</i> 1982

Continued

Country	Crop/commodity	Nature of damage	% damage/loss	% Yield Reduction/ Economic value	Comments	Reference
Australia	"	"	5-30%	Aus \$ 100 million nationally	Out breaks data	Caughley <i>et al.</i> 1994
"	"	"	-	3-4% reduction in production	Out breaks data	Singleton, 1997
"	"	"	3.4	\$ 40 million	Simulated study	Brown, 2005
"	All cereals	"	5-30	US \$ 18/ha	Out break data	Stenseth <i>et al.</i> 2003
Nepal	"	"	25	-	As high as 60%	Fall, 1975
Iran	"	"	-	30,000 t	Estimated	Kaukenin & Rampaud, 1986.
Morocco	"	"	40-70	-	Out break data	Fiedler, 1988
Tunisia	"	"	10-15	-	"	Fiedler, 1988
China	"	"	-	15 million t	"	Zhang <i>et al.</i> 1999
Tanzania	Maize	"	5-15/annum	400,000 t annual, US \$ 45 m	Sufficient to feed 2.3 m people/year	FAO, 1973
Kenya	"	"	34-100	-	Out-breaks data	Leris, 2003
Congo DR	"	"	70% farmers suffer	-	Interview data	Taylor, 1968
Iraq	"	"	68% ears high	33% loss/year	-	Brooks, 1975
Egypt	"	"	42	-	Fields replanted	Shuyler, 1970
Nigeria	"	"	10-90	-	Out break data	Fiedler, 1988
Bolivia	"	"	-	-	Estimated	Rodriguez, 1993
Tanzania	Maize, Sorghum, pulses	"	-	48% yield loss	Out-breaks data	Mwanjabe <i>et al.</i> 2002
Malaysia	Oil palm	"	5%, 240 kg oil/ha/annum	M \$ 115 m	Based on 1981 prices	Khoo, 1984
India	"	"	11.2-52.3	-	Estimated	Rao, 2003
India	Coconut	"	4.5-55.0	-	Estimated	Rao, 2003
Pacific Islands	Cocunut	"	0.035-0.11 nuts/tree/week	-	Minor Damage	Smith, 1968

Continued

Country	Crop/commodity	Nature of damage	% damage/ loss	% Yield Reduction/ Economic value	Comments	Reference
China	Pastures	"	50.0	-	Very serious	Wang, 1984
Mongolia	"	"	70-100	-	Very serious	Davaa, 1978
Philippines	Various grains	Post-harvest losses	40-210 kg/warehouse	US \$ 80/unit	-	Benigno & Sanchez, 1984
Bangladesh	"	"	-	US \$ 29.50/unit/six months	Extra polated interview data	Bruggers, 1983
Bangladesh	"	"	10.5 mt	US \$ 620 m	Estimates of entire country	Mian <i>et al.</i> 1984
USA	"	"	House hold damage	US \$ 900 m/ annul	Estimated	Clinton, 1969
India	Rice	"	25-30	US \$ 5.0 billion annually	-	Hart, 2001
India	Various grain	"	140 kg/godown	-	Detailed study	Spillet, 1968
India	"	"	5.9	-	Estimated	Deoras, 1975
India	"	"	25	-	Estimated	Girish <i>et al.</i> 1974
Thailand	Rice	"	-	US \$ 2.0 m	Estimated	Tangtavee <i>et al.</i> 1990

Various attempts have been made to develop methods and techniques for rodent damage assessment in crops and of the post-harvest systems. An early attempt by FAO was made to develop damage assessment method for sugarcane (FAO/CAB, 1971). Metcalfe and Thomas (1966) followed this technique for assessing rodent damage to sugarcane in Jamaica and Hawaii. The Rodent Research Centre (Philippines) developed a sampling technique to determine damage to growing rice about 3 weeks before harvest. It is easy to apply on a large area and where rice has been planted in hills and rows. After 1,000 hills are counted, the % damaged tillers is easily calculated (Sanchez *et al.*, 1971; Benigno, 1979). An assumption is made that the % tiller cut approximate the quantity of rice yield reduction at the time of harvest. In practical sense, this relationship is conservative one and under estimates yield loss. Simulated studies have shown that % yield reduction is much higher than the proportion of tillers cut at harvest (Sanchez, 1974; Fulk and Akhtar, 1981). This method is still widely used in Asian countries. Random quadrat sampling in wheat, groundnut, and others crops is still considered reliable. Large area sampling is being done by using stratified sampling technique; the resultant data is statistically defensible. In Pakistan, using this technique damage assessment has been made in sunflower, maize and wheat. Still, there are many researchable issues while calculating damage by vertebrate pests. Varietal preference, and early sown varieties, cropping systems, rodent species and population abundance are some of the important factors which need to be quantified and considered in loss assessment.

Further to it, we need a better understanding of the trade-off between the costs and benefits of the tools and technologies for control. The outcome of a cost-benefit analysis must depend on the value of a crop, the timing, the costs, and effectiveness of control techniques. Unfortunately, ecologists tend to ignore economics, economists typically have simplistic understanding of ecology, and pest control managers commonly underrate both (Stenseth *et al.*, 2003). Rodent management requires a scientific approach, and the science of rodent management requires managers to conduct experiments (Sinclair, 1991). Efforts of individuals from a variety of disciplines will become the template for developing future management research and methods, and formulating a policy. A synthesis of these facts is, therefore, necessary, and since ecology and economics are so closely linked in pest systems, an integrated bio-economic approach is required in rodent pest management. Therefore, a bio-economic model incorporating basic ecological principles will provide a valuable framework for pest management systems - one which properly takes into account both ecology and economics. This will help the farmers to make decisions every year

about whether or not they will invest in rodent control and, if yes, to what extent. In this way the concept of “Farmer’s Lead” rodent control will be implemented.

TABLE IV.- COST/BENEFIT RATIOS OBTAINED THROUGH RODENT CONTROL TRIALS IN VARIOUS CROPS, PAKISTAN

Rodenticides return	Bait	Cost	Return*	Ratio
Rice				
i) Zinc phosphide (2%)	4.00	6.00	360.00	1:00
ii) Coumatetralyl (0.0375%)	8.00	120.00	1361.00	1:11
iii) Brodifacoum (0.005%)	4.00	160.00	1417.00	1:9
iv) Aluminium phosphide	0.95	210.00	443.00	1:2
Wheat				
i) Zinc phosphide (2%)	5.00	75.00	653.00	1:9
ii) Coumatetralyl (0.005%)	7.000	105.00	323.00	1:3
iii) Brodifacoum (0.005%)	2.50	145.00	812.00	1:6
Sugarcane				
i) Zinc phosphide (2%)	7.50	112.50	864.00	1:8
ii) Coumatetralyl (0.0375%)	1.88	30.0	1239.0	1:23
	4.0	93.00	4865.00	1:52
iii) Brodifacoum (0.005%)	1.57	203.00	5614.00	1:27
Groundnut**				
i) Coumatetralyl (0.0375%)	2.03	250.00	28634.00	1:23
ii) Flocoumafen (0.005%)	0.90	450.00	27305.00	1:19

* Based on GOP Support Prices of 1985-86

** Based on market prices of 2006-07

Cost-effective surveillance, research and development, and operational implementation are increasingly becoming standard practices in rodent damage management. Thus, rodent control managers are required to adopt standard industrial practices of product development, and approach which necessitates teams of investigators having diverse disciplinary knowledge and experience.

SOME MAJOR CASES OF RODENT DAMAGE

Pakistan

The first ever case of rodent damage to crops in Pakistan was reported in 1956 from Sialkot District. A survey carried out revealed that the quantity of grains (rice and wheat) lost to rodents was enough to feed the population of the

TABLE V.- ESTIMATES ON DAMAGE TO FOREST PLANTATIONS BY INDIAN CRESTED PORCUPINE IN DIFFERENT AREAS OF PAKISTAN.

Species	Locality	Damage (%)		Reference	
		CG	PG		
<i>Dalbergia sissoo</i>	Changa Manga	-	-	1.02	Nawaz & Ahmad, 1974
	Chichawati	-	-	2.04	Greaves & Khan, 1978
	Chichawati	11	-	-	Ahmad & Chaudhry, 1977
	Mianwali	3	-	-	
	Shorkot	8	-	-	
	Jhang	5	5	5	
	Kundian	7.5	-	-	
	Karluwala	1.4	0.7	2.1	
	Bhakhar	-	-	2.8	
	Changa Manga	-	-	24.29	Nawaz & Ahmad, 1974
	Changa Manga	9	-	-	Mian and Hussain, 2006
	Chichawati	-	-	51.2	Greaves & Khan, 1978
	Mianwali	34	-	-	Ahmad & Chaudhry, 1977
	Shorkot	9	-	-	
Fateh Jhang	65	-	-	Mian and Hussain, 2006	
Daphar	-	-	5-16		
Changa Manga	-	-	52.54	Nawaz & Ahmad, 1974	
Chichawati	-	-	70.8	Greaves & Khan, 1978	
Mianwali	12	-	-	Ahmad & Chaudhry, 1977	
<i>Pinus roxburghii</i>	Tarbela Watershed	-	-	60.0	Khan <i>et al.</i> , 2000
"	Tarbela Watershed	-	-	0.5-24.0	Hussain, 2004
<i>Robinia pseudoacacia</i>	Tarbela Watershed	-	-	42.0	Khan <i>et al.</i> , 2000
<i>Eucalyptus sp.</i>	Muzafargath	-	-	21.54	Mian and Hussain, 2006
	Lal Sohanra	-	-	0.5	
	Chichawati	-	-	19.33	

CG = Complete girdling, PG = Partial girdling

district (0.3 million at that time) for 10 years. As long ago as 1927, Wagle drew attention to the prevalence of rice-field rats in lower Sindh as one of the three probable chief causes (the other being malaria and seasonal flooding) of the lack of development in this region. At that time losses in many areas were said to range from 10% to over 50% of the rice crop. This approximate 15.75% reduction in yield. This situation has been confirmed by simulated field studies which have indicated that average reduction in rice yield attributable to rodents was 19% (Fulk and Akhtar, 1981). The author of this report also observed many rice fields in Thatta district during 80's which were completely harvested by rats. In 1974, VPCI/FAO began field studies which indicated that general conditions in the remoter rural areas of Thatta district remained much as described by Wagle. Such reports are also available from Bagh district of Balochistan where rats completely devastated wheat fields. Similar situations have been recorded in Pashin, Uthal and Mastung. In Pakistan, estimates of pre- post harvest losses caused by vertebrate pests worth Rs. 5.7 billion have been reported (Table I.).

Complete and partial destruction of millet and gowar crops has been reported from Tharparkar. In 1989 out-breaks of the hairy-footed gerbil was recorded by the staff of VPCI, Karachi. As a result of this 13,748 and 3,995 tons of millet and gowar were lost due to rodent damage in four talukas of the district (Khokhar and Rizvi, 1991).

Recent studies conducted under a coordinated ALP Project of PARC indicated that Indian crested porcupine has emerged as a major pest in all the agro-ecological zones of Pakistan (Khan *et al.*, 2000). The deterioration impacts of this pest on forestry and agricultural systems have been documented in Tables V-VIII. Beside this, the porcupines damage plastic tubing of drip irrigation system and cause breaches in barrage and link canals. One such breach in 2002 in the Rohri canal flooded 12 villages and caused 3.2 billion rupees loss (Khan *et al.*, 2007).

The most important aspect of porcupine damage, in Pakistan, occurs in forestry and reforestation areas, Nawaz and Ahmad (1974) calculated a loss of 1, 36,136 cft of wood in Changa Manga plantations, the value of which was estimated at PKR. 0.9 million annually. On the basis of annual incremental loss of timber in Changa Manga plantations, Greaves and Khan (1978) estimated economic loss around US \$25/ha in this plantation. Economic losses were probably double in the heavily infested forest at Chichawatni as compared with that calculated for the Changa Manga forest (Greaves and Khan, 1978). Hussain (2004) calculated bio-economic impact of porcupine damage in Tarbela

Watershed areas of Khyber Pakhtunkhwa (KPK). He estimated the losses based upon the material cost from nursery raised transplants of *Pinus roxburghii* at Rs. 8/plant, excluding cost of loss of time/ season, establishment, transportation and other related monetary resources. Assuming an average of 40% mortality of transplants based upon two studies (Khan *et al.*, 2000; Hussain, 2004), and plant density of 1,075/ha, the economic losses were Rs. 3,440/ha. If this estimate is amplified to 5 divisions of Tarbela Watershed, the total economic loss may run into millions of rupees. Khan *et al.* (2000) estimated economic losses suffered by irrigated forest plantation in the Punjab and natural forests in KPK and suggested annual economic loss of about US \$60- 70/ha. The accuracy of these economic losses could be much improved by more exact studies of the damage phenology and its relation to the actual and potential value of timber extracted at different stages in the forest management cycles.

Khan *et al.* (2000) estimated that 108 kg of potato seed/ha or 18% of the total seed cultivated was lost due to porcupine damage. Alkon and Saltz (1985) also reported damage to irrigated potatoes in the Negev desert of Southern Israel and was estimated at 1.3 ton/ha or 0.6% of the crop valuing US \$30/ha.

One of the best estimates of rodent impact post-harvest is from a detailed study of rodent pests in central Punjab in Pakistan, where for every person living in a village, there were 1.1 house rats. Extrapolating the results from this regional study to the national level, it was estimated that 0.33 billion mt (rice, maize, and wheat) worth US \$ 30 million were consumed by house rats in the villages of Pakistan every year (Mustaq-Ul-Hassan 1992). The study did not consider the impact of rats in and around major cities.

TABLE VI.- ESTIMATES ON DAMAGE TO FOREST NURSERIES STOCKS/ TRANSPLANTS BY INDIAN CRESTED PORCUPINE IN DIFFERENT PLANTATIONS IN PAKISTAN

Plant species	Locality	Damage (%)	Reference
<i>Melia azedarach</i>	Chichawatni	19.0	Greaves and Khan (1978)
<i>Bombax ceiba</i>	Chichawatni	16.0	
<i>Morus alba</i>	Changa Manga	14.97	Mian and Hussain (2006)
	Mianwali	1.01-25.57	
<i>Dalbergia sissoo</i>	Mianwali	61.4	
<i>Pinus roxburghii</i>	Tarbela Watershed	60.0	Khan <i>et al.</i> (2000)
"	Tarbela Watershed	38.1	Hussain (2004)

TABLE VII.- ESTIMATES ON DAMAGE TO FRUIT TREES BY INDIAN CRESTED PORCUPINE IN DIFFERENT ORCHARDS IN PAKISTAN

Species	Damage (%)	Reference
Citrus (<i>Citrus sinensis</i>)	20.0	Greaves and Khan (1978)
Apple (<i>Malus domestica</i>)	3.3-18	
Papaya (<i>Carica papaya</i>)	16.67	Pervez (2006)
Cherry (<i>Prunus cerasus</i>)	7.5	
Wild fig (<i>Ficus carica</i>)	7.7	
Wild Sheena (<i>Pistacia sp.</i>)	5.0-28.85	
Peach	1.2	
Grapes	1.20	

Recently (2005-2007), scientists of VPCI, Karachi implemented an ALP Project on the management of rodents infesting date-palm orchards at Nok-Kundi and Mashkale, Balochistan. The study revealed rodent damage of 22.1% to trees in Nok-Kundi, 20.1% in Turbat, and 13.4% in Panjgour. Alone in Nok-Kundi, the yield of infested trees reduced by 25-50% resulting in the harvest of low quality yields. Economic losses of PKR.5.33 million were calculated only in Nok-Kundi which indicated the seriousness of the problem (Ahmad and Pervez, 2008).

TABLE VIII.- ESTIMATES ON DAMAGE TO CROPS AND VEGETABLES BY INDIAN CRESTED PORCUPINE IN DIFFERENT AREAS OF PAKISTAN.

Crop	Location	Damage (%)	Reference
Maize	Central Punjab	0.38	Ahmad <i>et al.</i> (1987)
	AJ & K	10.7	Khan <i>et al.</i> (1997)
Wheat	AJ & K	3.53	Khan <i>et al.</i> (1997)
	Central Punjab	8.5	Mian and Hussain (2006)
Rice	AJ & K	1.67	Khan <i>et al.</i> (1997)
Groundnut	Rawalpindi Division	0.2	Brooks <i>et al.</i> (1986)
		20.2	Mian and Hussain (2006)
Potatoes	Attock	17.56	Khan <i>et al.</i> (2000)
	Balochistan	2-20	Pervez (2006)
Sweet potatoes	Balochistan	4-36	Pervez (2006)
Cabbage, pea, carrot	Balochistan	1-4	Pervez (2006)
Onion	Bhakhar	0.9-5.4	Mian and Hussain (2006)

International scenario

Rodents cause extensive damage to ripening sugarcane where ever it is grown, from Asia to Africa, Latin America, the Pacific region, and Australia ((Fiedler *et al.*, 1987; Fall, 1980; Tobin *et al.*, 1990). Rats gnaw on the internodes of growing stalks, thereby killing stalks, diminishing yields, or allowing infection by bacteria or fungus, which reduces cane quality and sugar yield. Losses are difficult to quantify but can be substantial (Redhead, 1980; Hampson, 1984; Haque *et al.*, 1985; Rampaud, 1993; Engeman *et al.*, 1998). Generally, at 10% stalk damage approximately 4% loss of sugar has been estimated. Details of losses have been described in Table III.

In 11 rice producing countries of Asia, under traditional rice farming systems, rodents cause chronic losses in production in the range of 5-15% per annum (Singleton, 2003). However, in many cases, this figure has risen dramatically over the last few decades. This has happened in areas/regions where cropping frequency has increased from one to two or three crops per year. Today, it is not unusual for the small rice farmers to report chronic yield losses of 20-30% per annum, rising to 50% or even total crop loss in certain seasons. Such kind of condition, even to day, exists in Thatta district, Sindh. Therefore, in such situations, farmers in Indonesia, Bangladesh and Philippines abstain from growing a second or third rice crop because of the expected severe rodent damage. This 'foregone' loss in productivity is not taken into account (Singleton, 2003).

More than 90% of the world's rice is produced and eaten in Asia (Khush, 1993). In Asia, a loss of 5% of rice production amounts to approximately 30 mt; enough rice to feed 180 million people for one year (Singleton, 2003). The highest reported impact by rodents in Southeast Asia is in Indonesia where around 17% (160,000 mt) of rice production is lost (Geddes, 1992, Indrarto, 1984). The total rice growing area damaged by rats is more than double that damaged by leaf folders and more than triple that damaged by stem borers in the rice growing countries (Van Elsen and Vande Fliert, 1991). In majority of rice producing countries of Asia moderate to high priority is given by governments for rodent control so as to prevent severe damage to crops. In most countries, except few; including Pakistan, field rats have been ranked as No. 1 pests of paddies.

At International Rice Research Institute (IRRI), the scientist's major concern is to high value of their experimental crops. In 1987 a survey of 51

scientists at the station suggested that rat damage occurred in 86% of 171 field experiments causing complete loss of data in 6.4% of experiments and partial loss of data in 59% (Ahmed *et al.*, 1987). These losses were quantified at US \$ 370,000 per annum. At National Agricultural Research Centre (NARC), Islamabad, similar situations existed in the past. However, from 2009 efforts are being made to make experimental and production areas free of rats. As a result of this more than 80% rodent populations have been reduced by now and will remain continued till the objective is achieved.

WHY RODENT CONTROL?

The quest to control the depredations of rodents, especially in agricultural systems, has been going on for thousands of years. Aristotle (384-322 BC) recounts: “the rate of propagation of field mice in country places, and the destruction that they cause, are beyond all telling”.

Although the last 50 years in particular have provided good progress with rodent pest management. Rural communities in many countries have a major concern that the rodents are literally competing with humans for food. Despite a clear increase in rodenticide use in the world, crop losses have not significantly decreased during the last 40 years. However, there are some cost-effective stories of rodent control in Philippines, Indonesia and Pakistan. In Philippines, from 5% losses of <0.52% were claimed to be achieved due to national rodent control programme between 1976-80 (Hoque *et al.*, 1988). According to Sumangil (1990) losses of rice in the Philippines were reduced from US \$ 34 million to 3.5 million as a result of organized rat control programmes. From Indonesia reports are available which indicate that cost-effective ratio of 1:25 can be achieved in rice through effective rodent control (Indrarto, 1984).

In Pakistan, Greaves *et al.* (1977) obtained 50-fold return on the cost of materials in paddies through large-scale trials in many areas of Thatta and Badin districts. Also, in groundnut crop an increased yield of 60.4% with cost-benefit ratio of 1:21 was obtained (Khan *et al.*, 2009). Again, earlier to it, Khan *et al.* (1984) evaluated the use of acute and anticoagulant baits in wheat and rice crops in central Punjab, and obtained 27 to 36% higher grain yields, respectively. For more details see Table IV.

In Bangladesh, two national strategic multi-media rodent control campaigns were organized and analyzed in detail. Net profits were calculated at US \$ 800, 000 for each campaign, based on a single crop and season (Adhikarya

and Posamentier, 1987). Calculated benefits would be a multiple of this figure, if all crops could have been surveyed and the reduction in structural damage and human sufferings could be quantified. Further field studies in the same country have shown clearly that losses can be reduced by 40-60% at farm level also (Posamentier, 1989).

Dubock (1984) and Richards (1988) described some examples of rodent control in urban and rural situations, including warehouses, in various Asian and Central American countries. The cost-benefit ratios ranged from 1:2 to 1:30. Hernandez and Drummond (1984) found that in Cuban warehouses the loss of 1% of the amount available to human consumption could be readily preventable by standard control techniques.

CONCLUSION AND RECOMMENDATIONS

- Like other Asian countries, vertebrate detriogens in Pakistan, specially rodent pests damaging field crops and stored grains, and their impacts are of economic importance. The resultant yield loss and economic value cannot be ignored as a policy. We are writing off more than PKR.10 billion annually because of the absence of a National Rodent Control Programme.
- Within the National Agricultural Research Systems (NARS), excluding PARC, vertebrate pest management subject activity is lacking in the four provinces and AJ&K. The capacity of PARC programmes do not allow them to reach and handle Vertebrate Pest Management (VPM) problems in each and every corner of the country. Therefore, it is high time to establish such programmes at the provincial level.
- Rice-based wheat cropping system of Punjab and Sindh is highly vulnerable to rodent attack that needs to be assessed in terms of yield loss and economic value.
- Dry land crops in all the provinces need to be investigated from various angles of vertebrate pest management so as to identify productivity constraints.
- Indian crested porcupine is widely distributed in the four provinces and AJ&K and cause serious damage to forest plantations, fruit orchards, maize, potato, and natural flora of medicinal value. Deterioration impacts need to be evaluated and quantified.
- Field rats have been identified by VPCI as serious pests of date-palm in Balochistan. Their impacts on suckers mortality, yield, fruit quality loss,

and economic value need to be thoroughly investigated.

- Introduction of new crops in different ecologies and associated emerging issues/problems be watched carefully, in respect of rodent damage susceptibility.
- Assessment of rodent filth (droppings, hair, urine etc) in pos-harvest systems is required to meet the international obligations under WTO/SPS and be integrated with grain quality testing protocols.
- The current damage assessment methodologies need revisit by VPM biologist and statistics experts, and possibility of using IT tools be explored.
- The capacity building and human capital development of existing VPM programmes of PARC need immediate attention so as to undertake VPM agenda effectively and for seeking solutions to the problems being faced by different stake-holders.

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TABLE V.- ESTIMATES ON DAMAGE TO FOREST PLANTATIONS BY INDIAN CRESTED PORCUPINE IN DIFFERENT AREAS OF PAKISTAN.

Species	Locality	Damage (%)			Reference
		CG	PG	CG+PG	
<i>Dalbergia sissoo</i>	Changa Manga	-	-	1.02	Nawaz & Ahmad, 1974
"	Chichawatni	-	-	2.04	Greaves & Khan, 1978
"	Chichawatni	11	-	-	Ahmad & Chaudhry, 1977
"	Mianwali	3	-	-	
"	Shorkot	8	-	-	
"	Jhang	5	5	5	
"	Kundian	7.5	-	-	
"	Karluwala	1.4	0.7	2.1	
"	Bhakhar	-	-	2.8	
<i>Morus alba</i>	Changa Manga	-	-	24.29	Nawaz & Ahmad, 1974
"	Changa Manga	9	-	-	Mian and Hussain, 2006
"	Chichawatni	-	-	51.2	Greaves & Khan, 1978
"	Mianwali	34	-	-	Ahmad & Chaudhry, 1977
"	Shorkot	9	-	-	
"	Fateh Jhang	65	-	-	Mian and Hussain, 2006
"	Daphar	-	-	5-16	
<i>Melia azedarach</i>	Changa Manga	-	-	52.54	Nawaz & Ahmad, 1974
"	Chichawatni	-	-	70.8	Greaves & Khan, 1978
"	Mianwali	12	-	-	Ahmad & Chaudhry, 1977
<i>Pinus roxburghii</i>	Tarbela Watershed	-	-	60.0	Khan <i>et al.</i> 2000
"	Tarbela Watershed	-	-	0.5-24.0	Hussain, 2004
<i>Robinia pseudoaccia</i>	Tarbela Watershed	-	-	42.0	Khan <i>et al.</i> , 2000
<i>Eucalptus sp.</i>	Muzafargarh	-	-	21.54	Mian and Hussain, 2006
	Lal Sohanra	-	-	0.5	
	Chichawatni	-	-	19.33	

CG = Complete girdling, PG = Partial girdling

TABLE II.- PRE- AND POST-HARVEST CROP LOSSES DUE TO VERTEBRATE PESTS IN PAKISTAN.

Pest	Crop/commodity	Location/region	Nature of damage	% Damage/loss estimates	% Yield reduction/economic value	Comments	Reference
Field rats	Rice	Lower Sindh	Pre-harvest	10-50	15-75	up to total crop destruction	Wagle, 1927
"	Rice+Wheat	Sialkot Distt.	"	-	Equal to feed 0.3 million population for 10 years	Extrapolated estimates	Anon, 1953 (Survey report)
"	Rice	Punjab	"	6-10	6.0	Estimated	Greaves & Khan, 1975
"	"	Lower Sindh	"	10.4	-	Estimates of two districts	Greaves <i>et al.</i> 1977
"	"	Lower Sindh	"		10	Due to sub-surface hoarding	Fulk, 1977
"	"	Lower Sindh	"	2-43	19	Results of simulated study	Fulk & Akhtar, 1981
"	"	Punjab (03 districts)	"	16.1	13.7	Estimated	Khan, 1987
"	Wheat	Faisalabad Distt.	"	7.5	-	Estimated	Beg <i>et al.</i> 1977a,b
"	"	Central Punjab (04 districts)	"	-	5.0	Estimated	Beg <i>et al.</i> 1978
"	"	Central Punjab, NWFP	"	0.1-6.1	2.3	Estimated	Fulk <i>et al.</i> 1980
"	"	Gujrat district	"	3.3-8.4	-	Estimated	Brooks <i>et al.</i> 1991
"	"	Faisalabad	"	2.04	-	Estimated	Ahmad <i>et al.</i> 1986
"	"	Muzaffarabad, AJ & K	"	-	141.5kg/ha or Rs. 3361/ha	Estimated	Maqbool <i>et al.</i> 2011
"	Sugarcane	Central Punjab (03 districts)	"	11.0	4-15	Estimated	Beg <i>et al.</i> 1979
"	"	Punjab and Sindh	"	7.86	7.7-10.7	Estimated	Fulk <i>et al.</i> 1980
"	"	Thatta Distt.	"	24	-	Based on food habits studies	Smiet <i>et al.</i> 1980
"	"	Thatta Distt.	"	-	PKR. 140.6 m annully	Based on 1986-87 production and prices	Khan, 1990

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Pest	Crop/commodity	Location/region	Nature of damage	% Damage/ loss estimates	% Yield reduction/ economic value	Comments	Reference
"	"	Sindh & Punjab	"	8.33	-	Estimated	Geddes & Iles, 1991.
"	"	Thatta & Sanghar	"	7.5-9	-	Estimated	Ali <i>et al.</i> 2003
Fields Rats	Coconut	Karachi district	"	30.2 nuts/per palm/year	27 thousands annually	Estimated	Khan, 1978
"	"	Karachi & Malir districts	"	15-20	-	"	Ali <i>et al.</i> 2003
"	Date-palm	Nok kundi, Turbat, Panjgour	"	22.1 20.1 13.4	PKR. 5.33 m annual loss	"	Ahmad & Pervez, 2008
"	Groundnut	Rawalpindi division	"	21.5	-	"	Brooks <i>et al.</i> 1986
"	Groundnut	Punjab	"	-	17% reduction in yield	Estimated	Islam, 1987
"	Forest trees	Pasni, Balochistan	"	8.53	-	"	Khokhar <i>et al.</i> 1999
Porcupine	i) Forest trees ii) Nurseries iii) Fruit trees iv) Crops and vegetables	Country wide & AJK	"	22.8 29.56 10.94 8.17	- - - -	up to 50%	Pervez, 2006 Khan <i>et al.</i> 2007 Nawaz & Ahmad, 1974 Greaves & Khan, 1978
"	Maize	AJK	"	10.7	-	Survey Results	Khan <i>et al.</i> 1997
"	Wheat	"	"	3.5	-	"	Khan <i>et al.</i> 1997
"	Rice	"	"	1.67	-	"	Khan <i>et al.</i> 1997
"	Potato	Attock	"	17.56	-	Designed Study	Khan <i>et al.</i> 2000
"	Plastic tubing of drip system	Bakkar	"	-	PKR. 70,000/replacement cost	Repair twicly done	Khan <i>et al.</i> 2000
"	Rice canal	Sindh	Canal Breach	-	PKR. 3.2 billion loss	12 flooded villages survey data	Khan <i>et al.</i> 2007
Bird pests	Wheat	Central Punjab	Pre-harvest	1.14-3.22	Av. 2.18 of total production of Punjab	Estimated	Rashid, 1972
house sparrow	"	Rahim Yar Khan	"	1-10	-	-	Bashir 1978
"	"	Central Punjab	"	Upto 50	-	-	Mirza, 1979
"	"	Punjab & Sindh	"	Up to 44	3.0 actual loss	Questionare survey results	Fulk and Lathiya, 1979

Continued

Pest	Crop/commodity	Location/region	Nature of damage	% Damage/loss estimates	% Yield reduction/economic value	Comments	Reference
"	"	Punjab & Balochistan	"	10-15	172.000 mt PKR: 209 m	Estimated	Roberts, 1976
Crows	"	Khanawal district	"	Serious	-	Removal of seed and seedlings	Yousuf, 1982
Parakeets	Maize	Rahim Yar Khan	"	3-50	-	Estimated, small sample	Roberts, 1974
Parakeets	Maize	Punjab Sindh NWFP	"	10 5% 15%	-	Estimated	Bashir, 1980
"	"	Faisalabad	"	0.41%	-	Studied small sample	Ahmad <i>et al.</i> 1987
"	"	NWFP	"	10.61%	50846 mt	Estimated	Khan & Hussain, 1990
"	Sunflower	Punjab Rahim Yar Khan	"	27.62% upto 100%	PKR: 114.41 m -	Estimates of experimental fields	Roberts, 1974
"	"	Punjab	"	30%	-	Estimated	Bashir <i>et al.</i> 1981
"	"	Sindh	"	45%	-	Estimated	Besser, 1982
"	"	Multan	"	22.4%	-	Estimated	Khan & Ahmad, 1983
"	"	Punjab (07 districts)	"	11.72%	US \$ 1.95 m	Serious damage in barani areas	Shafi <i>et al.</i> 1986
"	"	Sindh (09 districts)	"	16.61%	-	Estimated, survey data	
"	Citrus	Punjab (11 districts)	"	2.6-12.7%	74,503 mt PKR: 204.88 m	-	Hussain <i>et al.</i> 1991
"	Guava	Karachi district	"	17.24	-	-	Ahmad & Ali, 1978
House sparrow	Lentils	Faisalabad	"	24-55%	-	Estimated	Roberts, 1978
"	Millet	Barani lands	"	3-10%	-	Estimated	
"	Sorghum		"	3-12%	-	Estimated	
Weavers	Rice	Lower Sindh	"	20%	-	Estimated	Grist & Lever, 1969
Wild boar	All crops	Punjab	"	-	16 mt annually	Estimated	Inayatullah, 1973
"	-	Punjab	"	-	PKR: 50 m annually	Interview data	Mirza, 1978
"	Sugarcan	Punjab	"	6.8-35.5%	-	Survey based data	Shafi & Khokhar, 1986

Continued

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Pest	Crop/commodity	Location/region	Nature of damage	% Damage/loss estimates	% Yield reduction/economic value	Comments	Reference
"	Wheat	Central Punjab	"	3.0%	PKR: 250 m annually	Estimated	Ahmad <i>et al.</i> 1986
"	Maize	Faisalabad district	"	6.7-10.0%	-	Data of 33 villages	Ahmad <i>et al.</i> 1987
"	Wheat	Fateh Jhang	"	7.3%		Various villages data	Brooks <i>et al.</i> 1989
"	Groundnut	Chakwal	"	0.09%	PKR: 6.4 m annually	Estimated	Brooks <i>et al.</i> 1986
"	Sugarcane Wheat Maize	Punjab	"	11.3% 3.0% 6.7%	PKR: 130.9 m annually	Extrapolated estimates	Brooks <i>et al.</i> 1989
"	Sugarcane, Wheat, Maize	All Pakistan	"	-	16 mt annually	Extrapolated data	Brooks <i>et al.</i> 1989
"	Rice	Punjab (04 districts)	"	-	PKR: 1,525 m 22.75%	Estimated	Khokhar & Rizvi, 1998
Lagomorphs	Apple trees	Ziarat, Balochistan	Debarking of trees	21.61	PKR. 21.21m/year/ season	Estimated	Shafi <i>et al.</i> 1989
"	"	"	"	20	-	Estimated	Khokhar & Fulk, 1976
Rabbits	Chick pea	Islamabad, NARC	"	21.7	-	Estimated	DWRC, 1986
"	Ground nut	Chakwal	Pre-harvest	12.6	-	Estimated	Brooks <i>et al.</i> 1986
Small mammals (Rats, Pikas, Porcupine)	Fruit orchards	Balochistan	"	-	PKR 31.28 m annually	Estimated	Mian <i>et al.</i> 1988
Commensal rats & mice	Stored grains (Rice, wheat, etc.)	Countrywide	Post-harvest	4-6	-	-	Anon, 1952
"	Various grains	Countywide	"	5.0	-	Estimated	Nasir <i>et al.</i> 1957
"	Wheat	"	"	1.0	-	Random sampling estimates	Nasir <i>et al.</i> 1957
"	"	"	"	4-10	-	-	Hafiz & Hussain, 1961
"	Rice	Karachi Rice Storage Complex	"		US \$ 800-1000/ year/storage	Estimated	Greaves and Khan, 1974

Continued

Pest	Crop/commodity	Location/region	Nature of damage	% Damage/ loss estimates	% Yield reduction/ economic value	Comments	Reference
"	Wheat	Countrywide (farm level)	"	23	-	Estimated	MICAS Associates, 1976
"	Food commodities	Ration shops, Karachi	"	77.7 shops infested	-	Survey data	Greaves, 1979
"	Wheat & Rice	Countrywide	"	5.0	-	Estimates at village and market level	Shafi, 1985
"	Rice	Punjab (whole sale markets of 05 cities)	"	740 kg/shop	4,000 t/year	Estimates of 5,500 shops	Ahmad <i>et al.</i> 1995
"	Wheat	Punjab	"	39% infested	-	56 godowns of 25 PASSCO Centres	Ahmad <i>et al.</i> 1988
"	Rice, maize, wheat	Countrywide	"	-	0.33 billion mt US \$ 30 m/year	Estimates based on rodent population	Mustaq-Ul-Hassan, 1992
"	Various food commodities	Rawalpindi city	"	100% shops infested	10.8 Kg filth/ shop	Estimates of 8 shops	Hussain & Iqbal, 2002

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TABLE III.- CROP LOSSES DUE TO RODENT PESTS IN DIFFERENT COUNTRIES.

Country	Crop/commodity	Nature of damage	% damage/ loss	% Yield Reduction/ Economic value	Comments	Reference
Asian countries	Rice	Pre-harvest	5-10 per annum	30 million t	Enough to feed 180 m people for a year	Singleton, 2001
Bangladesh	"	"	>50	32-60%	Estimates of districts	Islam <i>et al.</i> 1993
China PDR	"	"	5-10	-	Few data	Zhao, 1996
India	"	"	5-15	-	Country wide data	Sridhara, 1992; Chopra <i>et al.</i> 1996; Parshad, 1999; Rao, 2003
India, Andhra Pradesh	"	"	Out-break of rodent population	3,302 mt	Out-break of rodent population	Rao, 2003
Indonesia	"	"	5-30 per annum	25-30 % US \$ 1.7 billion/year	Enough to feed 65 m people/year	Buckle, 1988, Singleton <i>et al.</i> 2003
Laos PDR	"	"	15	-	Out-breaks in upland	Bounneuang <i>et al.</i> 2003
Malaysia	"	"	5	87332 t, US\$ 7.3 m	Estimated	Lam, 1982
Malaysia	"	"	4-5	-	Data on economic impact not known	Singleton and Petch, 1994
Myanmar	"	"	5-40	-	Estimated, no national data	Singleton, 2001
Philippines	"	"	5 (2-18)	-	National survey data	Schaefer, 1975
Philippines	"	"	5	US\$ 67.3 m	Based on estimates of 1975	Hoque <i>et al.</i> 1988
Philippines	"	"	5	8%, US\$ 55.3 m	Estimates of 1953-54	Sumangil, 1990

Continued

Country	Crop/commodity	Nature of damage	% damage/ loss	% Yield Reduction/ Economic value	Comments	Reference
Philippines	"	"	3-5	-	30-50% in some years	Singleton, 2001
Thailand	"	"	-	US\$ 2.3.m	Low land rice	Tongtavee <i>et al.</i> 1990
Thailand	"	"	6-7	-	Variable data	Singleton and Petch, 1994
Vietnam	"	"	-	10-15%	Average yield losses	Singleton, 2001
Cambodia	"	"	-	0.3% of national production	No national data	Singleton, 2001
SE Asia	"	"	6	36 million t	Enough to feed 212 m people/ year	Singleton, 2003
Indonesia	"	"	40	-	800,000 ha data	Soekarna, 1968
Indonesia	"	"	5-15	17%, 160,000 t	Estimated	Indrarto, 1984
Indonesia	"	"	15 annual	-	-	Geddes, 1992
Indonesia	"	"	17	8.21 million t, US \$ 1.0 billion	Estimated	Geddes, 1992
Argentina	"	"	20%	-	Out break data	Rodriguez, 1993
Guyana	Sugarcane	"	10%	172/Kg, 3.1% loss of sugar	Estimated	Bates, 1960
Guyana	"	"	-	12500 mt	When outbreaks occurred	Bates, 1962
Jamica	"	"	10%	249Kg/ha; 4.2% loss of sugar	"	Metcalf & Thomas, 1966
India	"	"	10%	76Kg/ha; 3.9% loss of sugar	"	Gupta <i>et al.</i> 1971
India Punjab	"	"	2.1-31%	161-240 Kg/ha	Estimate of standing & lodged crop	Bindra and Sagar, 1971

Continued

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Country	Crop/commodity	Nature of damage	% damage/ loss	% Yield Reduction/ Economic value	Comments	Reference
Philippines	"	"	10%	183Kg/ha; 3.7% loss of sugar	"	Estioko, 1978
Caribbean Islands (Barbados)	"	"	-	US \$ 2.25 million/year	1974, one crop season estimates	Williams, 1984
Australia	"	"	-	40,000 mt	Estimated	Anon, 1975
Australia	"	"	2-5%	US \$ 1-2 m annual	Moderate damage	Caughley <i>et al.</i> 1994
Australia	"	"	-	825, 000 t US \$ 50 m	Estimates of 1999- 2000	Smith, <i>et al.</i> , 2003
Egypt	"	"	5-20	-	Up to 80%	Ibrahim, 1972; Shuyler, 1970
Florida	"	"	5-11	-	Stalk damage survey data	Samol, 1972
Indonesia (Java)	"	"	30-100	-	Estimates of 14,000 ha	Soekarna, 1968
Mauritius	"	"	-	37 mt/ha	Estimated	Williams, 1953
Philippines	"	"	2-10	11-25%	Survey of 6farms	Porquez & Ledesma, 1970
World	"	"	3%	US \$ 40/ha	Estimated	Economist, 1981
Bangladesh	"	"	7.5%	14.3% weight loss	-	Haque <i>et al.</i> 1985
Hawaii	"	"	-	Loss of 0.45 t of sugar	-	Teshima, 1968
Argentina	"	"	12-20	-	Estimated	Rodriguez, 1993
India	Wheat	"	2.7-21.3%	25-72 Kg/ha	Average loss in 3 villages	Bindra & Sagar, 1971
Bangladesh	"	"	-	5 times destruction of the quantity eaten	Simulated enclosure study	Haque <i>et al.</i> 1980
Bangladesh	"	"	12.1	77000 t/annum	Country-wide data	Poche <i>et al.</i> 1982

Continued

Country	Crop/commodity	Nature of damage	% damage/ loss	% Yield Reduction/ Economic value	Comments	Reference
Australia	"	"	5-30%	Aus \$ 100 million nationally	Out breaks data	Caughley <i>et al.</i> 1994
"	"	"	-	3-4% reduction in production \$ 40 million	Out breaks data	Singleton, 1997
"	"	"	3.4	US \$ 18/ha	Simulated study	Brown, 2005
"	All cereals	"	5-30	-	Out break data	Stenseth <i>et al.</i> 2003
Nepal	"	"	25	-	As high as 60%	Fall, 1975
Iran	"	"	-	30,000 t	Estimated	Kaukenin & Rampaud, 1986.
Morocco	"	"	40-70	-	Out break data	Fiedler, 1988
Tunisia	"	"	10-15	-	"	Fiedler, 1988
China	"	"	-	15 million t	"	Zhang <i>et al.</i> 1999
Tanzania	Maize	"	5-15/annum	400,000 t annual, US \$ 45 m	Sufficient to feed 2.3 m people/year	FAO, 1973 Leris, 2003
Kenya	"	"	34-100	-	Out-breaks data	Taylor, 1968
Congo DR	"	"	70% farmers suffer	-	Interview data	Drazo <i>et al.</i> 2008
Iraq	"	"	68% ears	33% loss/year	-	Brooks, 1975
Egypt	"	"	high	-	Fields replanted	Shuyler, 1970
Nigeria	"	"	42	-	Out break data	Fiedler, 1988
Bolivia	"	"	10-90	-	Estimated	Rodriguez, 1993
Tanzania	Maize, Sorghum, pulses	"	-	48% yield loss	Out-breaks data	Mwanjabe <i>et al.</i> 2002
Malaysia	Oil palm	"	5%, 240 kg oil/ ha/annum	M \$ 115 m	Based on 1981 prices	Khoo, 1984
India	"	"	11.2-52.3	-	Estimated	Rao, 2003
India	Coconut	"	4.5-55.0	-	Estimated	Rao, 2003
Pacific Islands	Cocunut	"	0.035-0.11 nuts/tree/week	-	Minor Damage	Smith, 1968

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Country	Crop/commodity	Nature of damage	% damage/ loss	% Yield Reduction/ Economic value	Comments	Reference
China	Pastures	"	50.0	-	Very serious	Wang, 1984
Mongolia	"	"	70-100	-	Very serious	Davaa, 1978
Philippines	Various grains	Post-harvest losses	40-210 kg/warehouse	US \$ 80/unit	-	Benigno & Sanchez, 1984
Bangladesh	"	"	-	US \$ 29.50/unit/six months	Extra polated interview data	Bruggers, 1983
Bangladesh	"	"	10.5 mt	US \$ 620 m	Estimates of entire country	Mian <i>et al.</i> 1984
USA	"	"	House hold damage	US \$ 900 m/ annul	Estimated	Clinton, 1969
India	Rice	"	25-30	US \$ 5.0 billion annually	-	Hart, 2001
India	Various grain	"	140 kg/godown	-	Detailed study	Spillet, 1968
India	"	"	5.9	-	Estimated	Deoras, 1975
India	"	"	25	-	Estimated	Girish <i>et al.</i> 1974
Thailand	Rice	"	-	US \$ 2.0 m	Estimated	Tangtavee <i>et al.</i> 1990

Some Abstracts

SECTION - I**CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY,
TOXICOLOGY****1. BIOCHEMISTRY****ELECTROPHORETICALLY RESOLVED SERUM PROTEIN FRACTIONS IN
WORKERS OF DYEING UNIT AT A TEXTILE INDUSTRY**

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Textile is the oldest industry in the world. It is as old as human civilization. The textile industry products are an elementary human requirement. In textile industry specially in dyeing unit workers are exposed to different hazardous chemicals. These chemicals cause adverse effects on the health of workers in textile industry. The study includes the comparison of serum protein fractions, resolved by SDS-PAGE of textile dyeing industry workers (n=30) with healthy control subjects (n=30). The quantification of separated protein fractions was carried out by Total Lab Quant software and for statistical analysis Student 't' test was used to detect the variation in particular protein fractions in textile industry workers when compared with healthy subjects. Thirteen protein fractions were detected ranging between 250-17 kDa. Protein fractions of 250, 224, 165, 71, 43, 23, 19 and 17kDa did not show considerable alterations, whereas, significant enhancement in 132 and 53kDa fractions was observed. In case of 87 and 66kDa fractions, significant decrement was seen, however, highly significant reduction was observed in 112kDa fraction. Altered Serum protein fractions in the textile dyeing industry workers are strong predictors of different ailments.

**A COMPARATIVE SPECTROPHOTOMETRIC DETERMINATION OF TOTAL
REACTIVE PHOSPHATE IN SOIL, COW MANURE, POULTRY MANURE, SUGAR
PRESS MUD, DIAMMONIUM PHOSPHATE, SINGLE SUPERPHOSPHATE AND ROCK
PHOSPHATE**

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The total reactive phosphate (TRP) is an important and valuable limiting nutrient which plays a vital role in primary productivity of an aquatic ecosystem. In the present study, seven matrixes /substractes of fertilizers & manures *i.e.*, soil, cow manure (CM), poultry manure (PM), sugar pressmud (SPM), diammonium phosphate (DAP), single superphosphate (SSP) & rock phosphate (RP) were studied for TRP releases in aquatic media contained in twenty-one glass tanks / aquaria. The experiment continued for six weeks and the TRP release was analysed weekly through the formation of heteropoly acid-phospho-molybdic acid being reduced to intensely blue

colour molybdenum complex by ascorbic acid using spectrophotometric / colourimetric technique. The method followed Lambert-Beer's Law at 880 nm with a minimum detection limit / concentration at 10 µg/L and light path of 2.5 cm using standard official methods for examination of water & wastewater. Physico-chemical analysis including electrical conductivity, temperature, pH, dissolved oxygen, carbon dioxide, hardness and alkalinity were also monitored / analyzed weekly during the experiment. The comparison of natural and artificial manures / fertilizers gave the results for high TRP releases in DAP following a descending order release pattern in SSP, SPM, CM, PM, RP and soil, respectively. However, DAP & SSP showed a rapid decline after twenty days while for SPM & CM, an equilibrium in TRP release and sink was established after twenty five days.

IN SILICO PREDICTION OF BIOSYNTHETIC PATHWAYS OF ARGININE AND PROLINE IN *THIOMICROSPIRA CRUNOGENA* XCL-2

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Arginine and Proline pathways were computationally annotated in *Thiomicrospira crunogena* XCL-2 genome, chemolithoautotrophic sulfur-oxidizing bacteria isolated from deep-sea hydrothermal vents. In *Escherichia coli* K12, 1-pyrroline-5-carboxylate dehydrogenase (EC Number: 1.5.1.12), ornithine carbamoyltransferase (EC Number: 2.1.3.3), argininosuccinate synthase (EC Number: 6.3.4.5) and argininosuccinate lyase (EC Number: 4.3.2.1) are involved in arginine biosynthesis. Whereas 1-pyrroline-5-carboxylate dehydrogenase (EC Number: 1.5.1.12) and pyrroline-5-carboxylate reductase (EC Number: 1.5.1.12) synthesize proline. Presence of all of these six proteins in *Thiomicrospira crunogena* proteome was confirmed by computational tools. Furthermore, genomes of other thermophiles and gammaproteobacteria were also blasted against *Thiomicrospira crunogena* but results were not found. These findings lead to the conclusion that the biosynthesis pathway of arginine and proline are not affected by any adaptive changes of *Thiomicrospira crunogena* genome to survive at elevated temperature (up to 200°C).

INTER-RELATIONSHIP OF MICRONUTRIENTS AND TOTAL ANTIOXIDANT CAPACITY AND THEIR ROLE IN PROGRESSION OF BREAST CANCER: PERSPECTIVE STUDY IN LOCAL POPULATION

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Human life is exposed to many risks of different diseases and cancer is one of those which cause death due to uncontrolled growth and spread of abnormal cells into the body. In present study, we analyzed different antioxidants and micronutrients in local population and data was collected from Jinnah and INMOL (Institute nuclear medicine and oncology) hospital of Lahore, for estimation of MDA, CAT, SOD, GSH, GPx, Vit A, C, E, Zn, Mn, Fe, Cu and Se in which we

investigate that there is a strong inter-relationship between oxidative stress and cancer. Different morphological, biochemical and molecular studies showed that oxidative stress plays a main role in the development of degenerative modification in the cells and tissues of the body. Since minor reduction or over production of antioxidants and essential micronutrients is responsible for the progression of cancers which produce free radicals and when free radicals are generated then free radicals attack on protecting cells and tissues of body. Naturally a balance is required between the oxidative attack of the free radicals and the antioxidative defense system of body but imbalance between them can be responsible for the progression of cancer.

EFFECT OF IRON OVERLOAD ON RENAL FUNCTIONS AND OXIDATIVE STRESS IN BETA THALASSEMIA PATIENTS

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Iron overload in beta-thalassemia leads to an enhanced generation of reactive oxygen species and oxidative stress. The cause of renal dysfunction is not known, but chronic anemia and oxidative stress due to tissue deposits of iron may be key factors, and antioxidative treatment can be useful to prevent this abnormality. Serum creatinine and Uric acid are significantly higher in patient groups (0.732 ± 0.23 mg/dl and 6.7 ± 0.94 mg/dl, $P < 0.05$) respectively as compared to control. Ferritin levels are significantly higher in patients as compared to control (3103.9 ± 1747.4 , $P < 0.05$). Hb levels are 14 ± 1.3 g/dl and 7.1 ± 1.03 g/dl in controls and patients respectively. No clear relationship exists between age and hematological parameters of thalassemic patients. Serum Ferritin level is positively related with Serum ALT, AST, ALP and MDA ($P < 0.05$). Serum MDA and serum ferritin of patients indicate that both erythrocytic free reactive iron and serum ferritin levels are important parameters to estimate the cellular damage.

EFFECT OF AGE RELATED MACULAR DEGENERATION ON LIPID PROFILE: A STUDY IN PAKISTANI PATIENTS

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The study was designed to evaluate the effect of AMD on lipid profile in normotensive patients. The study was prospective, randomized and case-control. A total of 3911 patients were screened, of which 90 patients had confirmed AMD. Control subjects (100 subjects) were selected for comparison. Patients were examined in the outpatient department of Al-Shifa Trust Eye Hospital Rawalpindi, Pakistan between February 2010 and February 2011. The study included: subjects of both genders aged 50 years or \geq , normotensive, non-diabetic with no family history of any such disease and no complication of posterior ocular chamber other than AMD. Age matched

healthy subjects with no symptoms of AMD were taken as control. Diagnosis of AMD was done through conventional diagnostic techniques by professional ophthalmologists. Blood was collected from patients and control subjects. Serum samples were analyzed for total cholesterol, triglycerides, LDL and HDL using commercially available kits. Data were compared with Student's t-test. Pearson correlation was calculated for relationship between different parameters. $P < 0.05$ was considered significant difference. Wet type AMD was found in 68.8% patients while 31.2% had dry type AMD. AMD patients had significantly greater total cholesterol concentration than the controls ($p < 0.034$). HDL/LDL ratio turned significantly lower in AMD patients ($P < 0.039$), while serum triglycerides, HDL and LDL were non-significantly different between the two groups. Total cholesterol in AMD patients was significantly correlated with TG, LDL and HDL ($P < 0.0001$). The study implicates that high cholesterol may be an important risk factor for retinal artery or vein occlusion leading ultimately to AMD.

EVALUATION OF BIOCHEMICAL MARKERS AND MALONDIALDEHYDE STATUS IN PATIENTS WITH THYROID GLAND DYSFUNCTION RECEIVING INTERFERON THERAPY

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It is evident that the incidence of thyroid dysfunction is the most frequent auto-immune reaction to interferon therapy. Despite the fact that a number of studies have been carried out about the varying manifestation of thyroid disease and its pathogenesis, association of thyroid disease with severity or response to interferon treatment are reported with different results. In the resultant thyroid dysfunction the serum levels of some liver enzymes like AST ($P = .002$), ALT ($P = .074$), ALP ($P = .017$) and total bilirubin ($P = .489$) were evaluated as a marker of liver injury. Moreover, another crucial biochemical parameter MDA ($P = .013$), one of the products of lipid peroxidation was also evaluated in interferon induced thyroid patients. The estimation of these various circulating biochemical parameters showed that in the interferon induced thyroid dysfunctions; some liver enzymes and oxidative status of the cells were imbalanced and related the hepatic injury.

EFFECTS OF FERROUS SULPHATE AND IRON NANOPARTICLES ON SOME HEMATOLOGICAL AND GROWTH INDICES OF FRESHWATER FISH *CIRRIHINUS MRIGALA*

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A 90 days feeding trial was conducted. to investigate the effect of iron in nanoparticles and bulkform on growth performance and some hematological indices of fingerlings of *Cirrihinus*

mrigala, Experiment was conducted in replicate in glass aquaria at a stocking density of 1.5g L⁻¹. The control group (C) of fish was offered 35% protein basal diet. while fingerlings of experimental groups T-I and T-2 were fed on 100 and 200 mg kg⁻¹ ferrous sulphate (bulkform of iron) respectively, whereas nT-I and nT-2 groups of fingerlings received 100 and 200 mg kg⁻¹ iron nanoparticles supplemented feed. respectively. No mortality was observed during the experiment. Groups of fish fed on iron supplemented diet showed significantly higher growth rate as compared to the control group of fingerlings while 100 mg Fe kg⁻¹ diet either in nanoparticles and bulkform showed significantly higher percentage weight gain compared to feed supplemented with 200 mg Fe kg⁻¹ diet. The comparison between nanoparticles and bulkform of iron supplemented diets revealed that growth performance was significantly higher in group of fish fed on nanoparticles enriched diet. Similar trend was shown by SGR and FCE. Iron supplemented diet also showed significant effect on hematological parameters. Statistically higher Hb level was observed in nT-I (10.50±0.29 g dl⁻¹) and T-I (9.5±0.50 g dl⁻¹) group of fish while MCH values also showed similar trend higher in nT-I (55.16±0.44 pg) and T-I (50.85±3.64 pg) group of fingerlings. Conversely, RBC showed higher and comparable values in nT-2 (2.79±0.05 x 10⁶ mm⁻³) and T-2 (2.53±0.04 x 10⁶ mm⁻³) group of fish. The results clearly indicate the need of iron supplementation in the feed of *Cirrihinlls mrigala* for obtaining better performance while also confirm the view that nanoform of matter have higher efficiency compared to bulkform.

HEMATOLOGICAL AND SERUM BIOCHEMICAL ALTERATION IN CATTLE AND BUFFALOES SUFFERING FROM NATURAL INFECTION OF BLACK QUARTER

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Hematological and serum biochemical changes in cattle suffering from natural outbreak of Black quarter (B.Q) in different areas of Punjab were studied. Blood samples from infected Cattle were subjected to Total Leukocyte Count (TLC), Total Erythrocyte Count (TEC), Differential leukocyte Count (DLC), hemoglobin (Hb) and Packed cell volume (PCV) while serum samples for estimation of Creatinine Kinase (CK), ALT and AST (n=50). It was found that mean erythrocyte count was decreased significantly (P < 0.05) while mean leukocyte count was increased significantly (P < 0.05) in diseased animals. On the other hand mean Hb, Platelets count and PCV were not significantly different (P > 0.05) in diseased animals as compared to normal healthy animals. Similarly mean DLC values were found varying to a great extent. It was noticed that mean neutrophils and lymphocytes (%) was increased significantly in diseased animals (P < 0.05) while those of monocytes and basophils (%) increased significantly (P > 0.05). On the other hand serum biochemical analysis of animals revealed that there was a significant increase (P > 0.05) in CK, ALT and AST levels as compared to normal healthy animals. Implication of these findings in the pathogenesis of B.Q is discussed.

EFFECT OF AQUATIC ENVIRONMENT ON MINERAL CONTENTS OF SOME FRESHWATER FISHES (CYPRINIDS)

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The objective of the present study is to investigate the effect of aquatic environment of different water bodies on mineral contents of some freshwater fish. For this purpose, three water bodies, Tarbela dam, River Soan and concrete water tanks were selected for the collection of five freshwater species, silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idella*), mrigal (*Cirrhinus mrigala*), rohu (*Labeo rohita*), and common carp (*Cyprinus carpio*). Mineral contents like Mn, Ni, Cd, Cu, Pb, Se, Zn and Co were examined in gills, skin, kidney, liver and muscle tissues of all five freshwater fishes with help of atomic absorption. There were considerable variations in the metals contents among different tissues of same species as well other species collected from different water bodies. The contents of metals founds in different tissues of different species varied for Mn: 0.43-6.0, Ni: 0.40 – 2.0, Cd: 0.04 – 0.49, Cu: 0.35 – 1.55, Pb: 0.32 – 1.55, Se: 0.97 – 17.05, Zn: 0.19 – 2.62 µg/g wet wt. Cobalt was not detected in any tissue of any species. All values were found under permissible range. The significant variation in the contents of metals accumulation in different tissues of fish collected from different water reservoirs clearly indicated the role of aquatic environment on the mineral composition of fish.

EFFECT OF AQUATIC ENVIRONMENT ON FATTY ACIDS COMPOSITION OF CYPRINUS CARPIO

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The major aim of the study was to determine the effect of aquatic environment on Fatty acids composition of *Cyprinus carpio*. For this purpose, two water bodies, Tarbela dam and River Soan were selected for the collection of samples. In *Cyprinus carpio* % age contents of SFA like hexanoic acid (C6:0) and Palmitic acids (C16:0) were significantly higher ($P < 0.01$) in fish from Tarbela dam while others SFA like Myristic Acid (C14:0) pentadecanoic acid (C15:0) and margaric acids (C17:0) were significantly higher ($P < 0.05$) in fish from River Soan. Among the MUFA myristolic acid (C14:1), palmitoleic acid (C16:1) and elaidic acid (C18:1n9t) contents were significantly higher ($P < 0.001$) in fish from River Soan compared to Tarbela dam fish samples, while other MUFA like heptadecanoic acid (C17:1), and eicosenoic acid (C20:1) contents were slightly but significantly higher ($P < 0.05$) in fish from River Soan. Among PUFAs all except Lenolic acid (C18:2c), Arachidonic acid (C20:4n6), eicosatrienoic acid omega 6 (C20:3n6:8, 11, 14) and eicosadienic acid (C20:2) appeared in significantly higher levels in fish samples from River Soan. The significant variation in the contents of fatty acids clearly indicated the role of aquatic environment of different water bodies on the chemical composition of fish.

**CORRELATION OF RENAL ISCHEMIA WITH OXIDATIVE STRESS AND
GENERATION OF REACTIVE NITROGEN SPECIES**

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Reactive oxygen species and nitric oxide are important mediators of tissue injury leading to renal ischemia. Reactive oxygen species and nitric oxide may form cytotoxic metabolites causing lipid peroxidation and DNA damage resulting in pathogenesis of ischemia-perfusion injury. The present study was therefore aimed to investigate the correlation of oxidative stress and generation of reactive nitrogen species with renal ischemia. Blood samples were collected from 25 renal ischemic patients as case group along with 25 normal individuals as control group. Plasma urea, uric acid and creatinine levels were monitored as markers of renal injury. Glutathione (GSH) oxidation, SOD, catalase and lipid peroxidation (MDA) were used as markers of oxidant stress. Nitric oxide (NO) was used as a biomarker of RNS formation. SOD, MDA, GSH, catalase and NO were assessed by spectrophotometric assay while renal parameters (urea, creatinine and uric acid) were estimated by enzymatic kit method. Significant increase in plasma creatinine, urea and uric acid were found that shows the renal injury. Significant decreases in catalase, SOD and GSH while significant increases in MDA and NO were observed after renal ischemia. The data thus obtained was evaluated statistically by using t-test according to which overall significant difference was observed in oxidative stress indices, reactive nitrogen species indices and renal indices in case group with renal ischemia as compared to control group as $p < 0.05$. Negative correlation was observed in oxidative stress parameters with NO and renal parameters except for MDA that shows positive correlation. The outcomes showed the increase in oxidative stress and generation of reactive nitrogen species. The data clearly demonstrates that oxidative stress and RNS generation occur in the kidney during ischemia.

**EFFECTS OF GRADED LEVEL OF VITAMIN C ON REPRODUCTIVE
PERFORMANCE OF BROODFISH OF SILVER CARP, *HYPOPHthalmichthys
MOLITRIX***

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The study was designed to examine the effect of dietary L- ascorbyl-2-polyphosphate on reproductive performance of broodfish of silver carp (*Hypophthalmichthys molitrix*). Both male and female broodfish of 3 years olds were stocked in a ratio of 1:1 and at a density of 400 kg /ha in nine earthen ponds. Experiment was conducted in replicate and three ponds were assigned as control receiving 35% protein basal diet while others two treated groups of fish were fed APP₂₅₀ and APP₅₀₀ supplemented diet respectively. After 70 days feeding trial on their respected diet, fish were bred through induced spawning and their reproductive performance was observed. In APP treated groups, % culled was 0 % as compared to control 6.7 %. Vitamin C supplementation significantly enhanced the fecundity and quality of egg compared to APP non supplemented diet,

while no significance difference was observed in reproductive performance of female fed APP₂₅₀ and APP₅₀₀ supplemented diet. Results of nine reciprocal crosses revealed that female broodfish fed with graded level of APP had significant effect on % hatching and fry kg⁻¹ female compared to male broodfish fed graded level of dietary APP. Hatching % and fry kg⁻¹ female body weight increased with increased in dietary vitamin C concentration.

RELIABILITY OF URINE PUS CELL COUNTS ON MICROSCOPY CORRELATED WITH URINE CULTURE COLONY COUNTS ON CULTURE MEDIA FOR UTI DURING PREGNANCY

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The urine pus cell counts reliability upto a level of significance as a significant pyuria was correlated with urine culture colony counts as a significant bacteriuria for the diagnosis of UTI during pregnancy. The correlation between significant pyuria and bacteriuria will be ensured the interpretation of urine pus cell counts under uncentrifuged microscopic examination as a rapid screening test which either investigate the UTI during pregnancy or reinvestigate the limit of urine pus cell counts in pregnant women. Present study of significant pyuria was recognized by following semi quantitative method for urine sample wet mount film preparation and 5-8 consecutive fields examination microscopically by using 40x objective lens as a high power field 2-4 pus cell/HPF. The significant bacteriuria was also recognized on the basis of urine sample measured quantity characterizing calibrated loop method for culture by following the semi quantitative culture method for urine sample culture colony count estimation visually as a colony forming unit per milliliter quantity > 10⁵ CFU/ml. In our study 56% urine samples out of 100 were positive by characterizing 2-4 pus cell/HPF under uncentrifuged microscopy called significant pyuria then on CLED agar culture was also positive by evaluating > 10⁵CFU/ml called significant bacteriuria and 35% urine samples out of 100 was negative by characterizing 1-2 pus cell/HPF called insignificant pyuria. Gynecological clinicians can easily interpret significant pyuria for the management of UTI during pregnancy.

BIOSORPTION OF MANGANESE BY GREEN ALGAE, *PITHOPHORA OEDOGONIA*

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Heavy metal contamination from industrial effluents is a serious issue of the day and needs immediate attention as it is adversely affecting the riverine ecosystems of Pakistan. These metals not only deteriorate the quality of water but also poses serious threat to life forms, both aquatic as well as terrestrial. The scenario is getting worsen day by day and demands to develop effective technology for the removal of such pollutants. In the present study, the process of biosorption for

the removal of Manganese [Mn (II)] ions from aqueous medium was employed by using dead biomass of green alga, *Pithophora oedogonia*. Biosorption capacity (q_{max}) of this ion was found 52 mg/g within 90 minutes after which no increase in biosorption was observed. However, biosorption of Mn decreased with increase in biomass concentration. Langmuir, Freundlich, and Temkin adsorption isotherm models were employed. Langmuir and Temkin isotherms which show mono layer sorption were found most suitable for biosorption of Mn by green alga. Pseudo-second order kinetic model was used to find out q_{eq} value (91.6 mg/g) and R² (Regression coefficient) which was 0.97. Fourier Transform Infra-red Spectroscopy (FTIR) revealed the presence of possible electronegative functional groups such as carbonyl, amino and alkyl on the surface of algal wall.

BIOCHEMICAL CHANGES IN SALIVA AND BLOOD OF DIABETIC PATIENTS

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Owing to the contribution of serum derived components into whole saliva it is hypothesized that the changes in serum composition caused by diabetes would be reflected in whole saliva. The present work was aimed to evaluate the biochemical changes in blood and saliva of diabetic patients. For this purpose total 50 blood samples were collected (25 from diabetic patients and 25 from normal individuals) from the Jinnah hospital Lahore. Total 50 saliva samples were also collected with the same ratio from Jinnah hospital Lahore. The serum from blood samples was separated by the centrifugation at 3000 rpm for 10 minutes. Serum and saliva was then analyzed for biochemical changes viz estimation of serum calcium, phosphate, total protein, alkaline phosphatase and salivary calcium, phosphate and amylase. Outcomes revealed that in diabetic patients, metabolism of minerals (calcium and phosphate) get altered significantly. In diabetic condition, phosphate decreased in blood and saliva with reduction in phosphate and amylase levels while other biochemical changes were found insignificant. It was suggested that saliva tests can be used to evaluate the diabetes and their oral health.

TO INVESTIGATE BIOCHEMICAL CHANGES IN SALIVA AND BLOOD OF CHRONIC KIDNEY DISEASE PATIENTS

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In present study serum and salivary biochemical changes in chronic kidney disease patients were analyzed. For this purpose serum calcium, phosphate, urea, creatinine and total proteins and salivary calcium, phosphate and alpha amylase were analyzed. Overall high serum calcium levels were found in chronic kidney disease patients. Serum phosphate levels were found high in chronic kidney disease patients as compare to normal individuals. Serum creatinine and urea levels were also elevated in chronic kidney disease patients. Salivary calcium levels increased insignificantly in chronic kidney disease patients as compare to normal individuals. While the salivary phosphate and

salivary alpha amylase levels decreased in chronic kidney disease patients. It was suggested that saliva tests can be used to evaluate the chronic kidney disease and oral health of such patients.

**EVALUATION OF ALTERATION IN THE LEVELS OF ELECTROLYTES IN
POLYARTHRITIS AND REACTIVE ARTHRITIS**

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The present study was conducted to investigate the evaluation of biochemical alteration in serum electrolytes (Na^+ , K^+ , Ca^{2+}) ALP, CRP and RA-factor in polyarthritis and reactive arthritis. Total 90 individuals were selected which were divided into three groups as control group, polyarthritis group and reactive arthritis group. Each group was consisted of 30 individuals. Blood samples were analysed for the estimation of serum electrolytes (Na^+ , K^+ , Ca^{2+}) ALP, CRP and RA-factor. The data was statically analysed by applying ANOVA. Non significant difference was recorded in (Na^+ , K^+), ALP, CRP level in polyarthritis and reactive arthritis group as compared to control group. Significant difference was recorded in the levels of calcium in polyarthritis and reactive arthritis as compared to control group. It was inferred that polyarthritis and reactive arthritis have profound effect on the metabolism of calcium but having no significant effect on potassium and sodium. Both types of disorders significantly alter and imbalance the quantity of alkaline phosphatase and CRP and R-A factor (an abnormal protein) is produced during polyarthritis.

**EVALUATION OF CORRELATION OF OXIDATIVE STRESS WITH CHRONIC
KIDNEY DISEASE IN PATIENTS WITH MODERATE TO SEVERE CHRONIC KIDNEY
DISEASE**

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The present study was aimed to investigate the correlation of oxidative stress with renal failure in CKD patients. For this purpose, oxidative stress was measured via SOD, GSH, MDA and catalase assessment. For renal dysfunction, basic renal parameters, urea, creatinine and uric acid were determined. 25 patients of moderate CKD and 25 patients of severe CKD were selected for the present study as case group along with 25 normal individuals as control group. Oxidative stress parameters (SOD, GSH, MDA and catalase) were assessed by spectrophotometric assay while renal parameters (urea, creatinine and uric acid) were estimated by enzymatic kit method. The data thus obtained was evaluated statistically by using ANOVA according to which overall significant difference was observed in oxidative stress indices and renal indices in case groups with moderate and severe CKD as compare to control group as $p < 0.05$. Positive correlation was observed in oxidative stress and the manifestation of CKD both moderate and severe CKD.

BIOCHEMICAL ANALYSIS OF ASCETIC FLUID OF CONGESTIVE HEART FAILURE AND HEPATITIS C PATIENTS

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The present study was aimed to investigate the correlation of ascetic fluid with congestive heart failure and hepatitis c patients. For this purpose, ascetic fluid was analyzed via TP, Amylase, glucose, HDL and LDL assessment. For biochemical analysis above parameters were estimated. 20 patients of ascetic fluid of congestive heart failure were taken as congestive heart failure with ascites group and 20 patients were selected as hepatitis C with ascites group as case group along with 20 patients of ascetic fluid only as control group. Ascitic fluid parameter glucose was tested by GOD/PAP test. Total protein was estimated by Lowery's method, Colorimetric method was used to estimate amylase and LDL was estimated by enzymatic colorimetric test .HDL was estimated by PEG/CHOD –PAP method. The data thus obtained was evaluated statistically by using ANOVA, according to which overall significant difference was observed except LDL values showed insignificant difference. Positive correlation was observed in the development of ascites and biochemical changes in both congestive heart failure and hepatitis C. It was inferred from outcomes that manifestation of both the disorders, congestive heart failure and hepatitis C is strongly related with significant biochemical changes in important constituents of ascetic fluid and hence disturb the normal homeostatic conditions of the body. Increased HDL, total protein, amylase and glucose levels while decreased level of LDL are the major observations under biochemical analysis of ascetic fluid produced in both the disorders.

SYNTHESIS OF ISATIN DERIVATIVES AND ESTIMATION OF THEIR BIOLOGICAL ACTIVITY

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In the present study several derivatives of isatin (A-L) were synthesized by condensation of Isatin with amines. All the synthesized derivatives (A-L) were Schiff bases. The structures were confirmed spectroscopically by IR and NMR. The biological activity of the synthesized compounds was evaluated for their radical scavenging antioxidant, antiacetylcholinesterase, antibutrylcholinesterase, antilipoxygenase and antityrosinase inhibitory potential. The outcome showed that amongs all these derivatives [(3Z)-3-(2-phenylhydrazinylidene)-1,3 dihydro-2H-indol-2-one] (K) expressed antioxidant activity, the derivative { 3-[(Z)-(2-oxo-1,2-dihydro-3H-indol-3-ylidene) amino]propanenitrile } (J) exhibited antiAChE, derivative [(3Z)-3-[(3-methylphenyl) imino]-1,3-dihydro-2H-indole-2-one] (F) revealed antiBChE activity, derivatives [(3Z)-3-[(2-sulfanylethyl)imino]-1,3-dihydro-2H-indol-2-one] (D) and the derivative [(3Z)-3-[(4-methylphenyl)-1,3dihydro-2H-indol-2-one] (E) showed antiLOX and derivative {N-[3Z)-2-oxo-1,2-dihydro-3H-indol-3-ylidene]hydrazinecarbothioamide} (I) expressed antityrosinase activity. some of these derivatives were novel with some novel group substitution with maximum biological activity. Further research is needed to explore their chemical and biological properties. These derivatives were observed biologically highly active compounds which can be used as antioxidant,

antiacetylcholinesterase, antibutyrylcholinesterase, antilipoxygenase and antityrosinase activity.

SYNTHESIS OF ISATIN DERIVATIVES AND ESTIMATION OF THEIR ANTIBACTERIAL ACTIVITY

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Isatin and its derivatives are potent antibacterial compounds. The present study was aimed to synthesize some novel isatin derivatives to estimate their antibacterial activity. 12 isatin derivatives were synthesized by condensation of Schiff bases to isatin. The derivatives were checked for their antibacterial activity against *Klebsiella pneumoniae*, *Escherchia coli*, *Salmonella aureus*, *Proteus vulgaris*, *Micrococcus*, *Pseudomonas aureoginosa* and *Aeromonas* bacterial strains. Disc diffusion method was used to evaluate the antibacterial activity of the derivatives against selected bacterial strains. Disc of isatin derivatives were prepared at concentration at 50 µg /disc, 200 µg /disc, 500 µg /disc, 2000 µg /disc and 5000 µg /disc. Whattmann filter paper was used for disc preparation. Antibacterial activity of isatin derivatives was evaluated by measuring the zone of inhibition (mm) at different concentration. Derivatives A (3Z)- (3-[(2-acetylphenyl)imino]-1,3-dihydro-2H-indol-2-one), derivative G (3Z)- (3-[(hydroxyimino)-1,3-dihydro-2H-indol-2-one] and derivative J (3-[(Z)-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)amino] propanenitrile) were found active against majority of bacterial strains. Derivative A was found active against *Salmonella aureus* and *Pseudomonas aureoginosa* at 50 µg /disc and against *Klebsiella pneumoniae*, *Salmonella aureus*, *Pseudomonas aureoginosa* and *Aeromonas* at 200 µg /disc and *Klebsiella pneumoniae*, *Salmonella aureus*, *Pseudomonas aureoginosa* and *Aeromonas* at 500 µg /disc and against *Klebsiella pneumoniae*, *Escherchia coli*, *Salmonella aureus*, *Proteus vulgaris* *Pseudomonas aureoginosa* and *Aeromonas* at 2000 µg /disc and 5000 µg /disc. Derivative G was found active against *Klebsiella pneumoniae*, *Escherchia coli*, *Salmonella aureus*, *Proteus vulgaris*, *Micrococcus*, *Pseudomonas aureoginosa* and *Aeromonas* at 50 µg /disc and 200 µg /disc while at 500 µg /disc, 2000 µg /disc and 5000 µg /disc showed significant activity against all tested bacterial strains except *Escherchia coli*. Derivative J was found active against *Salmonella aureus* at 50 µg /disc and 200 µg /disc and against *Salmonella aureus* and *Proteus vulgaris* at 500 µg /disc and against *Klebsiella pneumoniae*, *Escherchia coli*, *Salmonella aureus*, *Proteus vulgaris*, *Micrococcus* and *Aeromonas* except *Pseudomonas aureoginosa* at 2000 µg /disc and 5000 µg /disc. Among all these derivatives derivative G was found to be more active and potent than any other while all other derivatives showed insignificant antibacterial activities against all tested bacterial strains.

STATUS OF ANTIOXIDANT ENZYMES DURING LIVE HAULING OF ADVANCED FRY OF ROHU (*LABEO ROHITA*)

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Status of antioxidant enzymes at different time period during live hauling of advanced fry of *Labeo rohita* was investigated. Fry, average weight 2.45±0.25 g were packed in polyethylene

bags under pressurized pure oxygen, at a stocking density of 70 g L⁻¹ and transported for 12hrs. Transportation of fry started at 11.0 am and at that time water quality parameters like temperature, DO level and pH were 21°C, 5.6 mg eland 8.4 respectively while total ammonia was less than < 0.5 mg L⁻¹. Water quality parameters were changed with journey time. Total ammonia increased while pH were decline.d but remained in permissible limit. DO level decreased significantly and created an hypoxic condition. Antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione Reductase (OR) and peroxidases (POD) and LPO activity in brain, liver, gills and muscle tissues showed significantly enhanced activities at 2 hrs after loading and then decreasing trend was observed up to 8 hr. After 8 hr of journey time, LPO and antioxidant enzymes again showed higher activities and at 10 and 12 hr reached to levels that was significantly (P<0.05) higher than initial level.

**INHIBITION OF A-AMYLASE OF *TRIBOLIUM CASTENUM* AND HUMAN BY
AZADIRACHTA INDICA (*NEEM*) DERIVED COMPOUNDS**

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Starch is one of the major sources of glucose in our food. Starch is hydrolyzed by amylases present in animal, plants and microbes. Red flour beetle, *T castaneum* (Herbst) is one of the major starch dependent storage pests and is responsible for severe stored grain losses. Inhibition of insect amylase by natural compounds could provide safe, environment friendly and economically viable route. Efficacy of neem *Azadirachta indica* derived compounds including neem oil, Azadirachtin, saponin and leaf extract was checked on crude and purified amylase of human *T. castenum*. It was observed that neem derived compounds had higher potential to inhibit *Tcastenum* amylase as compared to human amylase.

**EFFECT OF FLUORIDE ON THE INTERNAL STRUCTURE OF LIVER, KIDNEYS AND
SPLEEN AND IMMUNITY OF *LABEO ROHITA***

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The present study deals with the immunomodulation due to the effect of fluoride in *Labeo rohita*. The toxic effect of fluoride on the humoral antibody production was studied. In addition, histopathological damage induced by fluoride in liver, kidneys and spleen was also noted. Results indicate that fluoride have negative impact on humoral antibody production. The humoral immunity of the fish was strongly suppressed in the group receiving high fluoride dose. High doses of fluoride also damaged the tissues of liver, kidneys and spleen. Necrosis, vacuolization and degeneration were observed in the vital organs under study. The fluoride intoxication also have prominent role in the deformation of the bones. Liver, Kidneys and Spleen also showed the proliferative responses by the production of pyroninophilic cells. The production of pyroninophilic cells was directly proportional to the amount of the toxicant to which the animals were exposed. The study shows that fluoride toxic dose not only damaged the skeletal bones but also decreased the immunity of the individuals.

DETECTION OF ENDOTHELIN (ET-1 AND ET-3) AS A NOVEL BIOMARKER OF CARDIO VASCULAR STRESS IN PATIENTS OF LIVER CIRRHOSIS WITH ASCITES

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Endothelin is an endothelial cell-derived peptide with greater vasoconstrictive potency than any known substance. ET system is particularly important in the control of systemic blood pressure (BP) and vascular tone. ET-1 and ET-3 are involved in cardiovascular dysfunctioning. The present study was aimed to investigate the detection of endothelin-1 and endothelin-3 in patients of liver cirrhosis with ascites and correlation of endothelin and oxidative stress with cardiovascular stress. Blood and ascetic fluid samples were collected from the patients of liver cirrhosis with ascites as case group and from normal individuals as control group. Endothelin-1 and endothelin-3 were detected by PCR in serum and ascetic fluid of patients and its quantification was done by Real-time PCR. ET-3 was quantified while ET-1 was not quantified which showed very low concentration of ET-1 to be calculated in both serum and ascitic fluid. Nitric oxide, CK, CK-MB, cholesterol, and CRP were estimated as cardio vascular stress parameters. Antioxidative enzymes glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MAD) a biomarker of oxidative stress were estimated in order to calculate oxidative stress. The obtained data was evaluated statistically by using t-test and Pearson correlation. Detection of endothelin was performed to evaluate the correlation of endothelin (ET-1 and ET-3) with oxidative stress and with the development of cardiovascular stress. Correlation was found between oxidative stress cardiovascular stress parameters with ET-3 (as ET-3 was quantified) and between individual parameters of oxidative stress and cardiovascular stress. Overall positive and highly significant ($P < 0.05$) correlation of CAT, SOD, MDA, GSH levels with ET-3 and with each other was observed and significant ($P < 0.05$) and positive correlation of ET-3 was found with NO, CK and CK-MB except cholesterol. It was concluded that increase production of endothelin (ET-3) under oxidative stress generates cardiovascular stress in patients of liver cirrhosis with ascites.

IDENTIFICATION OF CRYIC BINDING PROTEINS IN *SPODOPTERA FRUGIPEREDA* 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 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 *Spodoptera frugiperda* with *cry1C*. *cry1C* 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 (20-220KDa) 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.

EFFECT OF ZINC PRETREATMENT ON ESSENTIAL MINERAL ELEMENTS OF RAT BODY TISSUES CHALLENGED WITH LEAD

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The present study was conducted on female Sprague Dawley rats to evaluate the effect of zinc pretreatment on the essential mineral elements in rat tissues challenged with lead. Single, acute doses of lead acetate trihydrate (350 mg/kg b.w) and zinc sulphate hepta hydrate (50mg/kg b.w) were administered to the animals in single and also pretreated with each metal and assigned different groups. Animals were sacrificed after 18 hours. Concentration of injected metals and tissue minerals (K, Na, Ca, Mg, Fe, Mn, Cu and Ni,) were determined in the brain, lung, heart, liver kidney and blood through atomic absorption spectrophotometer. It is concluded from this study that lead causes a severe imbalance of tissues electrolytes and trace element concentration while a pretreatment with zinc significantly counters the toxicity of lead and plays an important role in the restoration of the normal concentration of electrolytes and some trace elements.

STRUCTURE MODELING OF BENZOATE TRANSPORTERS BENK AND BENE OF CORYNEBACTERIUM GLUTAMICUM

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In *C. glutamicum* genome, BenK is identified as benzoate transporter of aromatic acid/H⁺ symporter (AAHS) subfamily of major facilitator superfamily (MFS). Based on 2D structure, BenK is predicted to have 12 transmembrane α -helices that can be further divided into two halves, a characteristic of MFS transporters. It contains four small cytoplasmic- and six periplasmic-hydrophilic loops between transmembrane-spanning (TMS) segments and a large cytoplasmic loop between TMS6 and TMS7 while TMS1 is orientated outwards. BenE is predicted to have 11 transmembrane α -helices, a structure entirely different from MFS transporters. Five cytoplasmic and five periplasmic loops are located between TMS regions and TMS1 is orientated inwards. BenE does not have a well-conserved domain of 13 residues in cytoplasmic loop between TMS2 and TMS3; duplication of this domain between TMS8 and TMS9 and conserved charged residues in TMS1, TMS4 and TMS11, although these are the characteristics of AAHS family transporters. Some non polar amino acid residues and a motif are conserved in TMS10 of BenK and BenE. In 3D structure of BenK, all the 12 α -helices are arranged in a symmetrical pattern to form a central channel-like cavity for benzoate transport. The symmetrical structure suggests a reasonable explanation for bidirectional substrate transport by proteins in MFS. The 3D structure of BenE

seems bit symmetrical where benzoate is transported through a channel located in the center.

FATTY ACIDS COMPOSITION OF SILVER CARP (*HYPOPHthalmichthys MOLITRIX*) COLLECTED FROM TWO DIFFERENT WATER RESERVOIRS

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The study was design to determine the fatty acids composition of silver carp (*Hypophthalmichthys molitrix*) collected from Tarbela dam and concrete water tanks in the vicinity of Attock Refinery. Fatty acids analysis was performed on GC-MS and total thirty two FATs had been detected in silver carp. The percentage of SFA like palmitic acid (C16:0) and Stearic acid (C18:0), MUFA such as palmitoleic acid (C16:1) and elaidic acid (C18:1n9t) and PUFA like EP A (C20:5n3), DHA (C22:6n3) and Eicosatrienoic acid Omega 6 were significantly higher in fish from concrete water tanks compared to Tarbela dam while no significant differences were observed among others SFA, MUF A and PUFA.

TO EVALUATE THE ROLE OF ENDOTHELIN-1 (ET-1) PROTEIN IN THE DEVELOPMENT OF IRON LOAD AND HYPERGLYCEMIA IN THE PATIENTS SUFFERING FROM HEPATITIS C

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Previous evidences suggest that endothelin-1 alter the iron load and a leading cause of hyperglycemia in hepatitis C patients. The present study was aimed to investigate the role of Endothelin-1 (ET-1) on iron load and hyperglycemia. For this purpose blood samples were collected from 25 diabetic patients without HCV and 25 blood samples from hepatitis C patients without diabetes along with 25 samples of normal individuals. MDA and nitric oxide (NO) were assessed as markers of oxidative stress. MDA and NO were assessed by spectrophotometrically, while iron load and glycosylated Hb were estimated by enzymatic kit method. Endothelin-1 was detected by PCR. The data thus obtained was evaluated statistically by using ANOVA. Significant difference was observed in NO and MDA as $P < 0.05$ in non diabetic with hepatitis C while insignificant difference ($P > 0.05$) in NO and MDA levels was observed in diabetic patients without hepatitis C as compare to control group. Overall significant difference in serum iron level, glycosylated Hb, were assessed in both the groups diabetic without HCV and non-diabetic with HCV patients as compare to control group. It was concluded that the ET-1 is related to the generation of oxidative stress and the development of iron load and hyperglycemia in HCV patients.

2. BIOTECHNOLOGY

STUDY OF ISOZYME VARIATION IN DIFFERENT SPECIES OF *UCA* THROUGH ELECTROPHORESIS FOUND ALONG THE COAST OF KARACHI, NORTH ARABIAN SEA

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Isozyme survey of electrophoretically detectable genetic variation in known population of Fiddler crabs (*Uca* crabs) was done. Crabs belong to genus *Uca* was collected from Karachi coast of Sindh, Pakistan. Three species of *Uca* belong to family Ocypodidae *Uca iranica*, *Uca sindensis*, and *Uca urvillei* were determined by the use of native PAGE, vertical slab gel electrophoresis. The muscles were used to obtain the banding patterns of Carbonate Dehydratase (CD), Catalase (Cat) and Creatine Kinase (CK) isozyme from genus *Uca*. Sample was taken from chela muscles then homogenized, centrifuged, run on 10% vertical polyacrylamide gel in discontinuous buffer system and stained for specific enzyme. Two isozymes (Cd and CK) showed two bands in all three species of genus *Uca* and frequency of bands was (Cd1= 12.58, Cd2=68.25 and CK1=15.093, CK2= 66.39) where as isozyme catalase showed two bands and frequency of bands was (Cat1=9.026) in all three species, while the second band (Cat2) showed different frequency in *Uca iranica* (Cat2=71.36), *Uca sindensis* (Cat2=90.97) and *Uca urvillei* (Cat2=92.97) respectively.

ENHANCING THE DIGESTIBILITY OF OIL-SEED MEAL FOR ANIMAL FEED BY LIPASE PRODUCTION FROM *ASPERGILLUS NIGER* NRRL 330 UNDER SOLID STATE FERMENTATION.

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Fungal lipases have been used to improve the digestibility of animal feed by hydrolyzing the lipid content. In the present investigation, extracellular lipase producing potential of *Aspergillus niger* NRRL 330 using various oil seed meals was evaluated under solid state fermentation. The fermentation was carried out in 250 ml Erlenmeyer flasks. The lipase yield reached 27.9 ± 0.707 U/g after 72 h of incubation when 10 g of sunflower meal was employed as the basal substrate. When initial pH was kept at 6.0, lipase showed the maximum activity of 32.7 ± 0.707 U/g. Various diluents were evaluated for lipase production and the highest yield *i.e.*, 38.4 ± 0.989 U/g of lipase, during the course of study, was obtained when mineral salt solution to substrate ratio was 1:1 and inoculum level was 1.0 ml. It was concluded that oil seed meal has substantial potential to support fungal growth and lipase accumulation and, therefore, can be efficiently utilized in animal feed.

EFFECT OF ENZYMATIC PRETREATMENT OF WHEAT STRAW ON PULP AND PAPER QUALITYZOYA KHAN¹, ISHTIAQ-UR-REHMAN², SHAHNAZ CHOUDHRY^{1*} AND AMIR SAEED²¹*Department of Biotechnology, Kinnaird College for Women, Lahore, Pakistan*²*R&D Section, Packages Ltd. Lahore, Pakistan*

The present study was designed to investigate the effects of enzymatic pretreatment for delignification of wheat straw on pulping and bleaching processes at optimized conditions and to develop hand sheets of paper. High percentage yield of wheat straw was obtained during four hour pretreatment with 1% cellulase as compared to xylanase under sterilized and nonsterilized conditions. Cellulase was selected for wheat straw pulping to study physical, optical and chemical properties of unbleached and bleached pulp. Cellulase pretreatment resulted in high yield (91.5%), improved brightness (85.1%) and reduced kappa number of pulp at low chemical dosages of sulphite (14%) and hypochlorite (4.5%). Paper hand sheets formed from the treated pulp by TAPPI test method and checked for the physical properties exhibited acceptable quality. Hence, this study reveals the potential use of cellulase as a bio-pulping and bio-bleaching agent in pulp and paper industry in terms of reducing costs, improving quality and quantity of the product and reducing the environmental pollution impact of an industry.

STUDY OF EFFECT OF PH AND TEMPERATURE ON UPTAKE OF HEAVY (Cr⁺² AND Pb⁺²) METALS BY PSEUDOMONAS SP

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Biological method can be more effective alternate for the treatment of heavy metal bearing waste water resulting from humans and industrial activities. It has been reported that several gram positive and gram negative bacteria have the ability to remove the heavy metals and there by making water contaminant free. In present study this *Pseudomonas* sp. was purified and identified by standard morphological and biochemical tests. Effect of pH and temperature on selected isolate was investigated for uptake of two heavy metals (Cr⁺² and Pb⁺²). It was found that in acidic medium (pH: 5.0-6.0) *Pseudomonas* sp. showed increase in uptake of both metals (Cr⁺² and Pb⁺²) from growth medium while *Pseudomonas* sp. showed gradual decrease of Chromium removal at pH 7, however same bacterial strain showed gradual increase of Lead uptake from medium. Therefore, optimum pH for uptake of Chromium and Lead was found 6.0 and 7.0, respectively. In basic medium (pH: 8.0-9.0), Chromium uptake was continuously decreased throughout the incubation time (24 to 96 hours) however, Lead uptake was initially decreased till 48 hours then a sudden increase of Lead uptake was seen up to 96 hours. In case of temperature, *Pseudomonas* sp. showed Chromium removal only at 50°C while it remained suppressed at all other selected temperatures (20°C, 25°C, 37°C and 45°C). On the other hand *Pseudomonas* sp. removed Lead at all selected temperatures (25°C, 37°C, 45°C and 50°C) except 20°C. Optimum temperature found in this study for Chromium and Lead was 50°C and 37°C, respectively. Present investigation also revealed that *Pseudomonas* sp. preferred acidic environment (pH) for uptake of chromium (Cr⁺²) and it behaved as acidophilic, whereas, behaved neutrophilic as it preferred neutral environment for removal of Lead. It can be concluded that *Pseudomonas* sp. preferred high temperature for Chromium metal uptake and behaved as thermophilic and mesophilic in nature for Lead. At

optimum conditions for both metals, maximum uptake of metal by *pseudomonas* sp. was occurred with in three days (72 hours).

**FERMENTATION CONDITIONS FOR PROTEASES PRODUCTION BY *BACILLUS*
STRAIN PCSIR-NL37 IN CHEESE WHEY**

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Thirty eight bacterial strains were isolated from different samples *i.e.* chichen, feather, meat and soil collected from Lahore. On the basis of casein hydrolysis eleven strains were selected by well plate method. Among theses one isolate PCSIR-37 was selected and identified as *Bacillus* sp. on the basis of morphological and biochemical tests. Various cultural conditions such as incubation period (48 hrs.), incubation temperature (37°C), pH (11) and additional nutritional requirements were optimized for the enzyme biosynthesis using cheese whey as major carbon & nitrogen source by submerged fermentation. Protease production was increased 1.71 fold as compared to control.

**VERMICOMPOSTING OF *PARTHENIUM HYSTEROPHORUS* BY INDIGENOUS
EARTHWORM (*PHERITIMA POSTHUMA*)**

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Earthworms play vital role in soil fertility and decomposition of organic waste. About 700 million ton organic waste is generated in Pakistan annually. Generally it is burnt, so we lose 80% nitrogen and 25% phosphorus while degrading environment at the same time. Use of vermitechnology in agriculture is in conformity with the concept of sustainable environment. The present study encompasses the utilization of *Parthenium*, a noxious weed in addition to animal dung as earthworm (EW) substrate and composition of vermicompost. Locally found epigeic earthworms were collected from crop land and plant nursery of NARC. Four substrates were selected for the rearing of earthworm and data on EW biomass and composition of vermicompost was recorded. Highest biomass (64% increase) of EW and nitrogen content of vermicompost (0.4%) recorded in soil+*Parthenium*+animal dung (2:1:1) while highest P (7.2µg/g) recorded in soil+animal dung (1:1), were at Day 45 of incubation. Highest EW biomass (103% increases) in soil+*Parthenium*+animal dung (2:1:1), highest (5%) organic matter (OM), ambient pH (7.3), C: N (9:1) and population of diazotrophs and P-solubilizers in the range of 10⁷ cfu/g were recorded in the same substrate at 90 days. All these parameters indicate that the indigenous EW can improve the quality of vermicompost so produced by recycling parthenium effectively, thus bearing a promise to environment.

QUANTIFICATION OF CARBON DIOXIDE EMITTED FROM BRICK KILNS IN DISTRICT MIRPUR AND BHIMBER AZAD KASHMIR, PAKISTAN

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To calculate the annual emission of Carbon Dioxide (CO₂) gas in different areas of district Mirpur and bhimber azad Kashmir a study was conducted on 24th of February, 2012. A total of 106 brick kiln was monitored; the cumulative daily emission of CO₂ was 4.2 tons, the maximum emission of CO₂ was recorded on Sangot, Chamba and Khanpur sites which was 459.81 tons/annum while minimum at Islamghar area which was 57.48/annum. The annual emission of CO₂ was recorded on all sites was 1523.13 tons/annum. All the calculations were made by using carbon foot prints calculator and descriptive statistics.

BIODEGRADATION OF ENDOSULFAN BY FUNGAL ISOLATES

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The present study describes the isolation of fungal strains that can efficiently degrade the endosulfan. Four fungal strains (AE, BE, CE, DE) were isolated from six soil samples of the cotton field and were screened further to check their biodegradation ability. The fungal strain AE (*Aspergillus* sp.) showed the highest degradation rate of endosulfan (98.6%) in four days of incubation at 30°C. The conditions for the degradation of endosulfan by strain AE were optimized. Strain AE can completely degrade 0.1% endosulfan, using 2mL of spore inoculum at pH 4 within four days of incubation at 30°C.

PECTINASE PRODUCTION AND ITS CHARACTERIZATION FROM LOCAL BACTERIAL ISOLATES: ROLE OF ENZYME IN UPGRADATION OF FRUIT WASTES

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Microbial pectinases are the enzymes of industrial importance and they are being used commercially in various industrial sectors. In this study bacteria were screened for pectinase production from the soil, water, decaying fruits and vegetables samples. Initial screening of pectinase was carried out on LB medium containing pectin as sole source of carbon as well as the substrate of the enzyme. Thirteen isolates showed 0.9-2.6mm² zone of hydrolysis on pectin containing agar plates. On the basis of zone of hydrolysis four bacterial isolates (BCTL-SL-197, BCTL-FL-24, BCTL-VL-23 and BCTL-FL-27) were selected and they yielded the enzyme upto 59.0, 57.0, 58.0 and 58.0 µg/ml/min, respectively. These isolates were further studied for

optimization of pectinase production. Maximum Pectinase production appeared on LB medium containing 0.25% citrus pectin at pH 7 after within 48 hours of incubation. BCTL-FL-24 showed maximum enzyme production (119 μ g/ml/min) out of four bacterial strains. The enzyme assay conditions were optimized for characterization of enzyme. Maximum pectinase activity was obtained at pH 4.5, 1% citrus pectin and at 50°C. As regards the temperature of assay BCTL-VL-23 showed maximum activity at 40°C. Pectinase activity was tested against two lab prepared pectin samples from orange peel and apple pomace. BCTL-VL-23 showed maximum pectinase units upto 114 μ g/ml/min on orange peel pectin and BCTL-VL-24 could show an activity 9.71 μ g/ml/min against apple pomace pectin. In conclusion this study revealed that a number of bacteria in our local environment can be used to upgrade the solid wastes by using their extracellular enzymes and can also assist in reducing environmental pollution.

DIRECTED EVOLUTION OF *CLOSTRIDIUM PHYTOFERMENTANS* CELLULASE FOR ENHANCED SPECIFIC ACTIVITY ON SOLID CELLULOSIC SUBSTRATE

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Increasing specific activity of cellulase on solid cellulosic materials would be among top priorities for second generation biorefineries. However, the complicated relationship among heterogeneity of solid cellulosic materials and different action mode cellulase components results in great challenges in cellulase engineering. We applied directed evolution to the *C. phytofermentans* glycoside hydrolase family 9 processive endoglucanase (Cel9) for enhanced hydrolytic performance by using *B. subtilis* as a secretory protein host. Several CpCel9 mutants with both increased expression level and enhanced specific activity on the solid cellulosic material were obtained. The most active mutant had more than three-fold specific activity that of wild-type on regenerated amorphous cellulose and most mutation sites were located in the family 3 cellulose-binding module near to its catalytic module, which might guide glucan entrance to the catalytic module. This study suggested that directed evolution along with a secretory protein *B. subtilis* host and solid cellulosic substrates would be a powerful tool to evolve more active cellulase mutants for cost-effective biosaccharification process.

DESIGNING OF FUNGAL CONSORTIUM FOR THE REMOVAL OF ZN & CU FROM ELECTROPLATING EFFLUENT

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Fifty fungal strains were isolated from different local habitats. A consortium of fungal strains (*Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp.) was designed for the removal of Zn & Cu from electroplating effluent. After screening, best cultures were used for the removal of Zinc and Copper. The biosorption process was carried out in a batch process and the effects of contact time (30–120 min), initial pH (2.0–6.0), initial metal ion concentration (50–150 mg L⁻¹) and temperature (30–40°C) on the biosorption were investigated. Experimental results showed that the maximum biosorption capacity was achieved at pH 4.0. It was observed from results that the maximum

removal (69.5%, 30.3% of copper and zinc respectively) was obtained at 150 mg/l effluent concentration and further increase decrease the biosorption of Cu & Zn ions. The biosorption of Cu & Zn ions from was maximum after 20 min contact period at 40°C, pH 4.0 and 150 rpm with 7.0 g (wet weight) biosorbent. Zinc and Copper concentration was estimated by Atomic Absorption Spectrophotometer. FTIR spectrum of three fungal species showed that hydroxyl groups, primary and secondary amines, amides stretching, Imines, oximes, C-OH stretching and C-N-C bonding are present on its cell surface.

FUNCTIONAL CHARACTERIZATION OF A NOVEL ENDO-XYLANASE FROM *ACIDOTHERMUS CELLULOLYTICUS* 11B.

SAHER SHAHID

Highly active endo-xylanases have always been highly demanded in feed, paper and bio fuel industry for the degradation of xylan. This study describes the cloning, expression and characterization of a putative endo-xylanase from *Acidothermus cellulolyticus* 11B. This xylanase is active at a broad temperature (50-80°C) and pH (4-7) range. This enzyme of 33.5 kD was found to be highly active on birchwood as well as oat spelt xylan. However, it does not show any activity on CMC and Avicel. Its specific activity on birchwood and oat spelt xylan is 695.6 U mg⁻¹ of protein (20.8 U μmol⁻¹) and 592.5 U mg⁻¹ of the protein (17.7 U μmol⁻¹) respectively. Its Km value is 9.5 mg/ml and Vmax value is 2000 U/mg on birchwood xylan. This enzyme is an endo-xylanase that efficiently hydrolyses birchwood and oat spelt xylan forming xylobiose and xylose as major products. It forms a TIM barrel structure that is a ubiquitous characteristic of GH10 enzymes. Circular dichroism spectra showed disruption of α-helices on incubating the enzyme at high temperatures which correlated with loss of activity on incubation at these temperatures.

MOLECULAR CHARACTERIZATION OF METAL RESISTANT BACTERIA AND THEIR ROLE IN BIOREMEDIATION OF COPPER AND CADMIUM

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Copper is the most biologically used transition metal after iron. The biological functions of copper relates to its properties as a transition metal. However copper toxicity in living organisms causes a number of health hazards. Two metal resistant bacterial strains named as MBZ4 and MBZ6 isolated from the industrial waste water were provided by the Molecular laboratory of Zoology department, GC University, Lahore. The strains were grown as pure culture on LB agar. Microscopic observations show that MBZ4 are Gram positive rods while MBZ6 are Gram negative rods. To find out the optimum conditions for the growth, these metal resistant strains were incubated in range of pH and temperature. MBZ4 and MBZ6 showed maximum growth at pH7 and pH8 respectively whereas optimum temperature for both strains was 25°C. Minimum inhibitory concentrations of copper for MBZ4 and MBZ6 were 670 μg/ml and 650 μg/ml respectively while minimum inhibitory concentrations for cadmium were 240 μg/ml for MBZ4 and 270 μg/ml for MBZ6. Sequencing analysis identified MBZ4 as *Bacillus cereus* while MBZ6 as *Klebsiella*

pneumoniae. Both of the strains showed a remarkable capacity in bioremediation of copper. MNZ4 showed 80% removal of copper ions while MNZ6 showed 90% metal uptake after 24hrs.

SINGLE CELL PROTEIN OF PURPLE NON SULFUR BACTERIA (PNSB) FROM FRUIT WASTES

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Potential of photoheterotrophic purple non sulfur bacteria (PNSB) isolated from rice paddy field was evaluated with respect to single cell protein in different self-constructed low cost media *i.e.*, A-MII, M-MII, D-MII, A+M-MII, A+D-MII, A+M+D-MII with concomitant gas production. These 2% media were prepared by employing peels/wastes of *malus domestica* (apple), *mangifera indica* (mango) and *Phoenix dactylifera* (fruits of date palm). In order to explore the various aspects of usefulness of PNSB the present study focused on single cell protein production (SCP) from the low/zero cost substrates. Cultivation and optimization of facultative photosynthetic bacteria of *Rhodobacter* and *Rhodospseudomonas* sp. in these fruit wastes containing media as carbon source appeared promising. Bacteriochlorophyll and carotenoids showed peaks at 375, 590, 805 and 845nm and 405, 500 and 535nm, respectively. Maximum total cellular protein content were noticed upto 178.56 ± 0.04 $\mu\text{g/g}$ in *malus domestica* based medium. Thus biomass of PNSB may prove a good alternative of manure, fish feed and agricultural supplement because of its proteins richness.

STUDIES ON INOCULUM SIZE AND FERMENTATION MEDIA FOR THE PRODUCTION OF GLUCOSE OXIDASE BY UV MUTANT STRAIN OF *ASPERGILLUS NIGER* AN-14

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In this study, effect of inoculum size and fermentation medium was analysed for the enhance production of Glucose oxidase by UV mutant strain of *Aspergillusniger* AN-14. The wild strain of *Aspergillus niger* W-47 was subjected to UV mutagenesis to improve the strain for enhanced production of glucose oxidase. UV treated 11 isolates showing positive variations from parents were isolated and analysed for glucose oxidase production using shake flasks fermentation. Mutant strain AN-14 gave maximum and reproducible production of glucose oxidase (44.61 U/g of cell mass) as compared to wild strain (21.74 U/g of cell mass). Two folds increase in the production of glucose oxidase was observed by the mutant strain. Further experiments were carried out in a 7.5 liter capacity bioreactor with working volume of 5 liter. The optimization of the volume of fermentation medium and inoculum size was done and it was concluded that 65% volume of total bioreactor and 5% inoculum of the total volume of fermentation medium were found to be optimum for GOD production by the mutant strain. The optimized yield of Glucose oxidase for wild and mutant strains was recorded as 26.48U/g of cell mass and 50.39 U/ g of cell mass, respectively.

INDOLE ACETIC ACID PRODUCTION BY CHROMIUM REDUCING *PAENIBACILLUS BARCINONENSIS*

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The non symbiotic nitrogen fixing bacteria isolated from chromium contaminated soil was assessed for chromium reduction potential in nutrient broth. Production of plant growth promoting substances was analyzed in presence and absence of chromium. Molecular identification of the bacterial isolate and chromate reductase gene was performed. The results showed complete reduction of 100 ppm of chromium within 24 hrs of incubation at 37°C and 120rpm aeration. High level of indole acetic acid (IAA) (19mg/mL) was produced in absence of chromium while the isolate was capable of promoting plant growth in presence of chromium with production of 10 mg of IAA/mL with 50µg/mL of Cr(VI). The isolate was identified to be *Paenibacillus barcinonensis* while the gene was identified as chromate reductase. Sequences of 16S rRNA and chromate reductase gene were submitted in the NCBI DATA base under the accession numbers KC512769 and KC527053, respectively. The isolate could be utilized biotechnologically as an important tool for bioremediation and fertility enhancement of chromium contaminated soils.

ECONOMIC CULTIVATION OF *SACCHAROMYCES CEREVISIAE* AND POTENTIAL OF FRUIT WASTES FOR GENERATION OF SINGLE CELL PROTEIN

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New advances in biotechnological techniques have made possible potentially effective and economic use of fruit wastes into valuable products including single cell protein (SCP). Increasing concern about pollution caused by the agro industrial wastes has also stimulated the interest for conversion of these wastes into commercially valuable products. Provision of protein to serious protein energy malnutrition is a burning issue being faced by the fast growing populations of the world. The present study aimed at the utilization of fruit wastes to assess the best possible optimum conditions for the cultivation of *Saccharomyces (S) cerevisiae* and for the production of SCP. *S. cerevisiae* was cultivated on 2% media containing peels of apple, mango, watermelon and bagasse singly as well as in twelve different combinations. The media were inoculated with 1% 24 hours old culture of the yeast. The strain grew best at pH 7.0, 37 °C incubation temperature under non shaking conditions. The protein content of the substrates as well as the yeast cells was determined by Kjeldahl's method and fat by Soxhlet's method. A comparative study revealed that the watermelon peel showed the highest amount of protein followed by mango, bagasse and apple peels respective values of the parameter with 9.8%, 3.2%, 2.8% and 2.5%. The mango peels expressed the highest amount of fat followed by apple, bagasse and watermelon with having values of 3.95%, 1.82%, 1.085% and 0.995%, respectively. Highest growth *S. cerevisiae* was obtained in aqueous extracts of 2% watermelon peels and the yeast cell attained 32% total protein content on dry weight basis. These results indicate important biochemical attributes of the frequently produced from wastes and their efficiency for economic cultivation of *S. cerevisiae* which could be used as SCP source for supplementing animal feed formulations.

ISOLATION OF THERMOPHILIC-AMYLASE PRODUCING BACTERIA FROM HOT WATER HABITAT IN THE VICINITY OF AN OIL WELL

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Many bacteriological processes require familiar carbon source the glucose as feedstock. Non- food competing sources are now a days being searched for this purpose. In the present study thermophilic bacteria capable of cultivation in a medium comprising mainly of potato peels have been isolated. All the six bacterial isolates grew well at 55 °C were found gram positive bacilli. Five of the isolates expressed streptobacilli morphology while two of them also showed palisade cell arrangements too. One of the isolate expressed conspicuous, more or less spherical, gram negatively stained exocellular matrix owned by plumpy rods. Frequently the exocellular matrix was shared by more than one cells. Supernatants of the bacterial cultures raised in the select medium yielded starch hydrolyzing zones upto 20 mm diameter when loaded on filter paper discs. These bugs appear promising for obtaining industrially relevant (Thermostable) amylases while consuming food industrial wastes.

BIOACCUMULATION OF METALS IN DIFFERENT TISSUES OF THREE COMMONLY AVAILABLE FISH SPECIES FROM RIVER RAVI, PAKISTAN

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Bioaccumulations of cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), zinc (Zn), manganese (Mn), nickel (Ni) and mercury (Hg) were determined in eyes, gills, heart, intestine and kidney of three edible fish species (*Cirrhinus (C) mrigala*, *Labeo (L) rohita* and *Catla (C) catla*) from three polluted downstream sites (Shahdera = B, Sunder = C and Balloki = D) during high (post monsoon) and low (winter) flow seasons and compared with respective seasonal data of a less polluted upstream site (Siphon=A) of river Ravi by passing Lahore (the second largest city of Pakistan). The pattern of metal accumulation in studied organs was in the order of Zn > Fe > Mn > Cu > Cr > Pb > Ni > Hg > Cd. Mean concentrations of the metals were significantly higher in low flow compared to high flow season. Kidney showed greater metal bioaccumulation, in general, followed by intestine, heart, eyes and gills. Highest concentrations of Cr, Cu, Zn, Mn, Ni and Hg and lowest of Pb were detected in *C. mrigala*, whereas Fe and Pb showed higher and Zn, Mn, Ni showed lowest concentrations in *C. catla*. In contrast, lower concentration of Cd, Cr, Cu and Fe were recorded in *L. rohita*. Accumulation of metals was significantly different in organs among the sampling sites. Accumulation was greater in fish species dwelling downstream, indicating impairment of ambient water due to untreated discharge of industrial and municipal effluents into studied segment of the river. The measured concentrations of metals in fishes'organs indicate potential health risks for fish and food chain.

RANDOM MUTAGENESIS OF *ASPERGILLUS NIGER* FOR ENHANCED PRODUCTION OF GLUCOSE OXIDASE

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This study deals with the improvement of wild strain of *Aspergillusniger* IIB-31. The main aim of the work was to enhance the GOD yield of wild strain (24.57 ± 0.01 U/g of cell mass). The wild strain of *Aspergillusniger* IIB-31 was subjected to random mutagenesis by exposure to UV and X rays. It was also treated with chemical mutagens such as Ethidium bromide, Ethyl methane sulphonate (EMS) and nitrous acid. Mutagen treated 61 variants indicating the positive results were picked and screened for the glucose oxidase production using submerged fermentation. EMS treated E45, mutant strain gave the highest glucose oxidase production (69.00 ± 0.02 U/ mg of protein), which was approximately 3-folds greater than the wild strain IIB-31. The preliminary cultural conditions for the production of glucose oxidase using submerged fermentation from strain E45 were also optimized. The highest yield of GOD was obtained using 8% glucose as carbon and 0.3% peptone as nitrogen source at pH 7.

CULTIVATION OF *TRITICUM AESTIVUM* IN CELLULOLYTIC AND NITROGEN FIXING BACTERIAL CO-CULTURED BIOREMEDIATED CHROMIUM CONTAMINATED SOIL

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Chromium loaded effluents of leather tanning industries discharged in adjoining irrigation lands have raised soil as well as water pollution. A laboratory scale attempt was made for bioremediation of chromium contaminated soil artificially polluted with $1000 \mu\text{g/mL}$ of Cr (VI). Soil was incubated with co-culture of a cellulolytic isolate *B. amyloliquifaciens* and a nitrogen fixing isolate *B. megaterium* as potential bioremediation tools at predetermined optimized nutrient conditions for a 2 month period. During the experiment levels of Cr (VI) and CFU/mL were determined every 15 days in the soil leachate. Soil recovered so far was assessed further for its potential to promote plant productivity by growing *Triticum aestivum*. Seed germination and subsequent growth variables of seedlings were observed. Bioreduction of upto $850 \mu\text{g/mL}$ of Cr(VI) was successfully achieved at the termination of incubation period. Analyses of yield parameters showed significant improvement and enhancement of seed germination, metal tolerance index (MTI), seedling vigour index (SVI) and root/shoot ratios. Percent seed germination of 100%, 25% and 100%, MTI of 100, 7.2 and 134.14, SVI of 1121.25, 37.5 and 1156.25 while a root/shoot ratio of 0.61, 0.28 and 0.77 was observed for control, Cr(VI)contaminated and the bioremediated soils, respectively. Photosynthetic pigment (Chlorophyll a and b) were significantly higher in leaves of seedling grown in bioremediated soil as compared to control soil. This study contributes an important information for implementation of eco-friendly methods for the metal contaminated soils reclamation for safe and healthy agricultural practices.

PRODUCTION OF SINGLE CELL PROTEIN FROM FRUIT WASTE FOR CHICK FEED INDUSTRY

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In chick feed industry, the feasibility of providing cheaper and safer protein meal is quite less. The present study, therefore, focuses its attention on the production of single cell protein (SCP) from *Candida utilis* NRRL Y-900 while utilizing various fruit waste extracts. Amongst ten extracts evaluated, the one produced from the residues of mandarin orange supported maximum biomass production and was screened out for further studies. Various cultural characteristics including substrate concentration, type of production medium, incubation period, pH, inoculum size and supplementary nitrogen sources were optimized during the course of study. The highest biomass recorded after optimizing these parameters was 30.6 ± 0.24 mg/mL with the crude protein contents of 34% (w/w). An attempt to replace the expensive inorganic nitrogen source from the production medium with crude goat horn hydrolysate was carried out which demonstrated that it can potentially prove to be valuable nitrogen source supplement. The process is cost effective and environmentally benign as it helps to reduce the agro-industrial waste.

ISOLATION AND SCREENING OF THE ENTOPHYTIC FUNGI FOR THE PRODUCTION OF THE TAXOL

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Taxol is one of the most effective anti-cancer drugs ever developed. The natural source of taxol is the inner bark of several *Taxus* species, but it accumulates at a very low concentration and with a prohibitively high cost of extraction. The most promising approach for the sustainable production of taxol is by fungal cultures at an industrial level. Fermentation processes using taxol-producing fungi is an alternative way to produce taxol. In the present study, about 150 entophytic fungal strains were isolated from ten different plants. These strains were screened for the production of the taxol through static culture in 250ml Erlenmeyer flasks. Out of these 150 strains, about fifteen strains were found to produce the taxol. Among these, the strain IIB 10 was found to produce considerable amounts of taxol in the fermentation broth. This strain was isolated from the inner bark of *Taxus sp.* Strain IIB 10 was identified as *Cladosporium cladosporioides* according to morphology of the fungal culture and characteristics of the spores. The fungal taxol was analyzed by UV spectrophotometer, thin layer chromatography and was shown to be identical to the standard taxol and 10-deacetylbaconin III. Further analysis was performed with high-performance liquid chromatography for the quantitative estimation of the taxol.

**CHITINASE GENE FROM AN ISOLATE OF *STENOTROPHOMONAS MALTOPHILIA*
AND ITS INVOLVEMENT IN BIOLOGICAL CONTROL OF TERMITES**

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Biological control strategies remain important potential alternatives to the use of pesticides. Target molecular lytic activity of bacteria is one of a number of mechanisms that have implicated in biocontrol. Bacteria produce chitinases mainly to degrade chitin and utilize it as an energy source. Sixteen chitinolytic bacterial strains were selected on the basis of their chitinolytic activities and zones of clearance on solidified medium containing chitin. One of them was identified as *Stenotrophomonas (S) maltophilia* by 16S RNA sequencing. The *S. maltophilia* represents a rhizosphere bacterial species of potential insecticidal importance. In recent years, this gram-negative bacterium has become increasingly important in biotechnology. Its chitinase gene was targeted, amplified and sequenced. The *S. maltophilia* showed biological termite control potential when its broth culture, fluid cell free culture as well as the harvested cells were applied. Thus the chitinase enzyme of *S. maltophilia* appear promising for controlling the insect pests in an environment friendly way.

**CULTIVATION OF PLANT GROWTH PROMOTING BACTERIAL ISOLATES IN
SUGAR MILL SPENT WASH**

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Agro-industries have been continuously adding a lot of waste effluents to the environment. Mollasses, bagasse, spent wash and other chromium containing industrial effluents are very toxic and cause threats to animal and plant lives. Microbial biotechnology techniques are efficient for management of these problems where these effluents can be used either as supplements or as substitutes for chemical fertilizers. Six nitrogen fixing bacterial isolates were screened for their ability to grow in mollasses, bagasse, spent wash and yeast extract based media. Ability of these isolates to produce plant growth promoting chemical Indole Acetic Acid (IAA) was assessed in nutrient broth supplemented with 100µg tryptophan. All of these isolates produced IAA in an amount ranging from 28.58µg/ml to 44.82µg/ml. The maximum IAA was produced by NS13. These nitrogen fixing bacterial isolates were also assessed as bioremediation tools due to their ability to reduce toxic Cr(VI) into non toxic Cr(III) for the treatment of soil. All of these isolates reduced Cr(VI) in above mentioned media. 99% and 98.6% reductions were observed by the isolates NF1 and NF3, respectively in mollasses and yeast extract after 24 hours of incubation. Complete reductions by the isolates NF14, NF16, NS12 AND NS13 were observed after 48 hours of incubation. These information are relevant for the consumption of organic solid wastes and reduction of Cr(VI) in the contaminated soils.

STUDIES ON THE LIPASE PRODUCTION BY *ASPERGILLUS NIGER* THROUGH SOLID STATE FERMENTATION

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The present study includes the isolation and screening of different fungal cultures from soil samples for lipase production by solid state fermentation. Seven different lipolytic fungal strains were isolated and screened for the purpose. These isolates were identified on the basis of morphological and microscopic studies. Among these fungal strains, *Aspergillus niger* gave best reproducible results and hence selected for further studies. The optimization of the culture substrate was carried out and maximum lipase activity (5.12 ± 0.059 U/ml) was obtained using olive oil as substrate in a medium moistened with a diluent containing (g/ml): Dihydrogen potassium phosphate (KH_2PO_4), 0.1; Magnesium sulfate (MgSO_4), 0.05; Potassium chloride (KCl), 0.05; Ferric sulfate (FeSO_4), 0.001; glucose ($\text{C}_6\text{H}_{12}\text{O}_6$), 0.8; and peptone 2, (pH 6.5). Maximum lipase activity was observed when fermentation was carried out for 72 hrs with incubation temperature of 30°C.

ETHANOL FERMENTATION BY LOCALLY ISOLATED MUTANT STRAIN OF *SACCHAROMYCES CEREVISIAE* FSAT-89

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The present study describes the ethanol fermentation by mutant strain of locally isolated *Saccharomyces cerevisiae fari-15*. Thirty cultures of yeast were isolated from different local habitats (air, water, soil etc.). The culture *Saccharomyces cerevisiae fari-15* with maximum ethanol yield (8.5%) was selected and exposed to UV radiations for different time periods (10, 20 and 30 seconds). The strain irradiated for 30 seconds gave maximum ethanol yield. This strain was selected and designated as *Saccharomyces cerevisiae* FSAT-89. Batch and Fed-batch fermentation processes were carried out in shake flasks. Fermentation conditions sugar concentration, temperature, pH, inoculum size, inoculum age, aeration, time course were optimized for maximum ethanol production. Maximum (9.3%) ethanol production was obtained at temperature 35°C, pH 4.5, inoculum size 6.0% (v/v) and inoculum age 24 h and sugar concentration 15%. Fed-batch fermentation was carried out to enhance ethanol yield in short time period. Ethanol fermentation under fed batch fermentation was maximum at 5th stage of fermentation. The results revealed that *Saccharomyces cerevisiae* FSAT-89 showed high efficiency for general strain improvement than wild-type.

3. CELL BIOLOGY

ASSESSMENT OF ANTIOXIDATIVE STATUS AND POTENTIAL FREE RADICAL DAMAGE ASSOCIATED WITH PREGNANCY INDUCED HYPERTENSION IN LOCAL POPULATION

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Oxidative stress is a key factor in the pathogenesis of PIH. Inadequate trophoblast invasion may result in poor placental perfusion and placental oxidative stress. Maternal circulation will be affected which leads to activation of defense system against oxidative stress. Several exogenous antioxidants (e.g., vitamin E, vitamin C) and endogenous antioxidants (e.g. GSH, GPx, CAT, and SOD) play their role. But if the maternal antioxidant capacity is inadequate to deal with the increased load of oxidative compounds during pregnancy then PIH may occur. The available information on the role of antioxidants is contradictory. Therefore, the present study was done at Sharif Trust City Hospital to evaluate the role of antioxidants and free radicals and correlative factors in PIH. A total of 80 subjects were enrolled for the study 10 non-pregnant, 35 normal pregnancy and 30 patients with pregnancy induced hypertension. Antioxidants *i.e.*, glutathione, glutathione peroxidase, superoxide dismutase, catalase, vitamin E, vitamin C and an important nutrient calcium were measured in the above groups. MDA the marker of lipid peroxidation was also evaluated. All the antioxidants were decreased in PIH group as compared to normal pregnant and controls which was statistically significant. A negative correlation was observed between MDA and antioxidants which was statistically significant. The present study showed altered antioxidative status and increase in potential free radicals, suggesting the role of oxidative stress in PIH. Measurement of antioxidative status has important diagnostic and therapeutic implications in PIH.

EXPRESSION LEVEL STUDIES OF *TETRAHYMENA* COPPER METALLOTHIONEIN

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Metallothioneins are cysteine rich low molecular weight proteins which have ability to chelate metal ions through their sulfhydryl group. They have been reported in all groups of organisms including bacteria, protists, plants and animals. In present study expression of copper metallothionein of *tetrahymena* spp. was studied both at transcriptional and translational level. Real time analysis showed a relatively low basal expression of copper metallothionein, which goes to maximum after 15 minutes of copper exposure. This also confirmed the inducible nature of metallothionein genes. The translational level studies of copper metallothionein at translational level showed that they are labile and degrade quickly in bacterial expression system. Their

expression was increased by changing media composition and by introducing a His-Tag at N-terminal. Cloned gene of copper metallothionein showed an optimum expression after 6hrs at 0.1mM IPTG induction.

COMPARATIVE STUDIES OF SALIVARY AND BLOOD SIALIC ACID, LIPID PEROXIDATION AND ANTIOXIDATIVE STATUS IN ORAL SQUAMOUS CELL CARCINOMA (OSCC)

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Oral squamous cell carcinoma (OSCC) is considered to be a serious life threatening issue for almost two decades. Over production of lipid peroxidation (LPO) byproducts and disturbances in antioxidant defense system has been implicated in the pathogenesis of oral cancer. Lipid peroxidation and antioxidant status in OSCC patients were estimated and compared the sensitivity and specificity of circulating biomarkers (MDA, Sialic acid, Catalase, SOD, GSH and Neuraminidase) with β -2 microglobulin (β -2MG) at different thresholds in blood and saliva using receiver operating characteristics (ROC) curve design. Levels of MDA and Sialic acid were significantly increased ($P < 0.0001$) in plasma of OSCC patients as compared to healthy subjects whereas antioxidant level was significantly decreased ($P < 0.0001$). ROC analysis indicated that MDA in saliva is a better diagnostic tool as compared to MDA in blood and β -2MG in blood is better diagnostic marker as compared to β -2MG level in saliva.

ROLE OF PROGESTERONE RECEPTORS POLYMORPHISM IN THE MAMMARY TUMOR DEVELOPMENT: A STUDY OF SOUTHERN PUNJAB (PAKISTAN) POPULATION

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Progesterone, having same function like all other steroid hormones, synthesized from cholesterol and secreted from adrenal glands, gonads, brain and also during pregnancy in the placenta. Progesterone receptor (PR) mediates the physiological actions of progesterone and many research studies on gene and translated proteins have described their possible roles in carcinogenesis. The present research study was performed to assess the impact of *PROGINS* and +331G/A polymorphisms of progesterone receptor on cancer development in Pakistani population. 100 breast cancer patients and 115 controls individuals of different age groups were enrolled from Southern Punjab, Pakistan for the study. Genotyping was performed by using PCR restriction fragment length (RFLP) method. Different demographic parameters such as age, menopause age, breast feeding, pesticide exposure and breast cancer history were also analyzed for their association with mammary tumor development. Result demonstrated that +331G/A ($P: 0.01$) polymorphism

was significantly related to mammary tumor risk. Frequency of allelic forms of +331G/A homozygous wild type, heterozygous and homozygous mutant was 114(99.1%), 0 and 1 (0.9%) respectively in controls, while in cases it was 93(93%), 7(7%) and 0 respectively. However, there was no significant correlation of *PROGINS* (P: 0.4) polymorphism with mammary tumor risk. *PROGINS* allelic frequency was (72.2%), (23.5%) and (4.3%) in controls and 69(0.69%), 29(0.29%) and 2(2.0%) in cases respectively. A statistically significant association was observed between breast cancer risk and menopause status (P: 0.00). It is concluded from the results that *PROGINS* polymorphism don't play any significant role in breast tumor growth in our population, while wild +331G/A polymorphism is strongly linked to mammary tumor development. Physiological factor like menopause also increases the risk of breast tumor development in patients with +331G/A gene polymorphism, while other parameters have no role in tumor development in our study.

RING FINGER PROTEIN 13 MEDIATES ENDOPLASMIC RETICULUM STRESS INDUCED APOPTOSIS BY ENHANCING IRE1A STABILITY

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Endoplasmic reticulum (ER) is critical for maintaining cellular homeostasis by properly folding the newly synthesized proteins required for vital cellular processes. Disturbance in the folding capacity of ER inside its lumen could hamper cellular homeostasis leading to a condition known as ER stress. ER stress initially triggers an adaptive signaling pathway known as Unfolded Protein Response (UPR) in order to restore homeostasis by initiating three mechanisms; a) halting protein synthesis, b) upregulating chaperone proteins to assist folding of accumulated protein and c) elimination of misfolded proteins from the ER lumen. However, if UPR fails to restore homeostasis, signaling pathway is initiated leading to death of the cell. This loss of cells results in chronic conditions such as Type II diabetes, Parkinson's disease and Alzheimer's disease. The molecular mechanisms of ER stress induced cell survival and apoptosis are not fully understood, however, three important ER resident proteins are known to initiate signaling events that are believed to play role in ER stress and subsequent apoptosis. Finding new mediators of these proteins could prove valuable in understanding the whole mechanisms of ER stress mediated apoptosis. A genetic screen method was devised in our lab in which retroviral insertion mutation system was introduced in SHSY-5Y cells in orders to search for genes whose inactivation would confer resistance to apoptosis. Using this approach, Ring finger protein 13 (RNF13) was identified whose inactivation conferred survival against staurosporine and Tunicamycin-induced apoptosis. RNF13 gene was cloned into mammalian expression vector pXJ40 with 3'-Myc and 5'-Flag tag. Interestingly, RT-PCR and real time PCR results showed that overexpression of RNF13 induced splicing of X-box binding Protein 1 (XBP1) confirming role of RNF13 in ER stress. Furthermore, western blot analysis showed that overexpression of both, N-terminal as well as C-Terminal tagged RNF13 resulted in activation of c-Jun N-terminal kinase (JNK) in neuroblastoma cells. Moreover, SHSY-5Y cells were stably knockdown for RNF13 using lentiviral gene knockdown system. RT-

PCR and western blot analysis showed that RNF13 stable knockdown SHSY-5Y cells were resistant to Tunicamycin-mediated XBP1 splicing and JNK activation. Furthermore, RNF13 showed a strong interaction with IRE1 α in our Co-immunoprecipitation (CO-IP) assays. Finally, Cycloheximide chase experiment showed that RNF13-IRE1 α interaction increased the stability of IRE1 α . Altogether, our data suggest that RNF13 may act as a mediator protein affecting IRE1 α in inducing ER stress-mediated apoptosis.

HUMAN UMBILICAL CORD BLOOD SERUM; A BETTER ALTERNATIVE OF FETAL BOVINE SERUM

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Serum is essential component of cellular growth medium and consists of complex mixture of low and high molecular weight biomolecules required for cellular growth and maintenance. Fetal bovine serum (FBS) is extensively used in cell culture medium and its requirement is on the increase. Several alternatives of FBS in the form of goat, horse and human serum were tested but failed. Chemically defined medium are also in the market but they are very expensive and don't support growth as efficiently. There are also many scientific and ethical problems with the use of FBS so there is a need for an alternative which should have no ethical and scientific problems. Present study was conducted to test cord blood serum (CBS) as an alternative of FBS. HeLa, rMSC, hMSC, HBC-1 and HBC-3 were grown in the presence of 5, 10 and 15% FBS and CBS for 48 h. All cell lines were grown in the presence of 10% FBS and CBS for 0 to 120 hr to calculate doubling time. HeLa, rMSC, and hMSC showed better growth and proliferation in the presence of CBS as against HBC-1 and HBC-3 in the presence of FBS. CBS also resulted in better attachment of HeLa and rMSC in short time. Doubling time of HeLa, rMSC and hMSC reduced in the presence of CBS while increased in case of HBC-1 and HBC-3. This study proves that CBS can be a better alternative to FBS as it accelerates the proliferation of mesenchymal stem cells (MSCs) as well as HeLa cells and also helps the cells to better adhere. So in future it has a great potential to be used for cell culture, to humanize the products used for cellular therapy and regenerative medicine.

MOLECULAR CHARACTERIZATION OF *PARMECIUM* SPECIES BASED ON SMALL SUBUNIT RIBOSOMAL RNA

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The genus *Paramecium* is known for 250 years ago and has worldwide distribution. Some of its species are cosmopolitan and some are endemic. Representatives of genus *Paramecium* play an important role in water quality assessment and saprobic level determination. For these applications the correct and accurate identification of these species is very essential. However, the accurate taxonomy of *Paramecium* species is still a matter of debate. One of the most widely used markers are genes coding for various rRNAs. The present study was undertaken to isolate and

identify the *Paramecium* species on the basis of SSrRNA. Five waste water samples from three different localities (Lahore, Kasur and Sheikhpura) were collected. *Paramecium* species were isolated from these samples and designated as M1, M2, M3, M4 and M5. The small subunit rRNA gene of all five strains was amplified and subjected to sequencing. Phylogenetic comparison of the sequences obtained from these strains with 29 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 (99%) of M1 and M2 with *P. multimicronucleatum*, second with maximum affinities of M4 (99.6%) with *P. jenningsi* and third one supported the close association of M3 and M5 with *P. primaurelia*.

4. GENETICS

DEVELOPMENT OF MICROSATELLITE LOCI IN THE NORTHERN SNAKEHEAD (*CHANNA ARGUS*)

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Ten microsatellite markers were isolated and characterized from enriched genomic libraries of *Channa argus*. The loci were scored in 40 specimens from one natural population. We explored 6 to 17 alleles per locus with observed and expected heterozygosities ranging from 0.8000 to 1.0000 and 0.7269 to 0.9551 respectively, containing 0.830 average polymorphic information content (PIC). Significant deviations from Hardy–Weinberg equilibrium were detected only at one loci and linkage disequilibrium between three loci was found to be significant after applying Bonferroni correction ($p < 0.005$). These newly developed markers should be significant in population genetic assessment studies or construction of genetic linkage map for this important snakehead fish species in future.

PREVALENCE OF ABO BLOOD GROUP IN ISCHEMIC HEART DISEASE SUBJECTS VISITING PIC, LAHORE

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Ischemic heart disease (IHD) is caused due to damage to the myocardium that results from continued temporary oxygen deprivation. It is one of the most inconvenient and crucial medical problems of the civilized world. The association between Blood groups and IHD has been documented. This study was planned to determine association between blood groups and ischemic heart disease. The study was performed at Punjab Institute of Cardiology (PIC), Lahore. 200 subjects suffering from ischemic heart disease were sampled from PIC and 230 healthy control subjects were sampled from blood bank. Self design questionnaire was used to collect information regarding risk factors. Blood grouping was determined by using standard agglutination test. The prevalence of blood groups in Ischemic subjects was 34% in blood group A, 29% in blood group B, 14% in blood group AB and 23% in blood group O. In control group the distribution of A, B, AB and O blood groups were 20.9%, 34.4%, 12.6%, 32.2% respectively. Rh+ve factor was prevalent in 90.5% among IHD group and 92.6% in control subjects. The prevalence of IHD is more in males (63.5%) as compared to females (36.5%). Mean age was 56.4 ± 0.86 (yrs) and BMI was 26.4 ± 0.33 (kg/m^2). 58.5% were suffering from hypertension, 53% had diabetes, 45% had family history of cardiac disease, 35.5% do regular exercise, 58.5% use ghee and 58% were smokers. The study revealed that IHD risk is significantly associated with blood group A,

OCULOCUTANEOUS ALBINISM: MOLECULAR GENETIC STUDIES OF NONSYNDROMIC PHENOTYPE IN THE PAKISTANI POPULATION

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Oculocutaneous albinism (OCA) is genetically inherited as autosomal recessive group of heterogeneous disorders. Complete lack or a reduction of melanin biosynthesis in the melanocytes resulting in the loss of pigmentation in the eyes, skin and hair is the main cause of OCA. Other common vision problems in people with albinism include iris transillumination, farsightedness, nearsightedness, astigmatism, nystagmus, strabismus and photophobia. Mutations in the four known non syndromic OCA genes *TYR*, *OCA2*, *TYRP1* and *SLC45A2* have been known to cause isolated OCA sub types. No comprehensive analysis has been conducted to study the spectrum of OCA alleles prevailing in Pakistani populations, essential for identifying the genetic causes of OCA, clinical testing and genetic counseling. Fifty large Pakistani families with three or more affected individuals were enrolled from different areas of Pakistan and screened for known OCA [*TYR* (OCA1), *P* gene (OCA2), *TYRP1* (OCA3) and *SLC45A2* (OCA4)] genes and a candidate gene, *SLC24A5*. Protein function effects were evaluated using *in silico* prediction algorithms and *ex vivo* studies in human melanocytes. An exon-trapping assay was performed to determine the effects of splice-site mutations on wild type and mutant alleles. *TYR* gene screening revealed three novel [c.62C>T (p.Pro21Leu); c.104T>C (p.Cys35Arg); c.1231T>C (p.Tyr411His)] and four reported mutations [c.896A>G (p.Arg299His); c.1217C>T (p.Pro406Leu); c.1255G>A (p.Gly419Arg); c.832C>T (p.Arg278X)] found in ten families. *Ex vivo* studies demonstrated the retention of an EGFP-tagged mutant (p.Pro21Leu, p.Cys35Arg or p.Tyr411His) tyrosinase in the endoplasmic reticulum (ER) at 37°C. The co-localization assay also indicated the effect of temperature on the enzymatic activity of p.Cys35Arg and p.Tyr411His. A significant fraction of p.Cys35Arg and p.Tyr411His left the ER in cells grown at a permissive temperature (31°C), while no change in p.Pro21Leu enzymatic activity was observed. Sequence analysis of the *OCA2* gene revealed three novel (p.Asp486Tyr, p.Leu527Arg, c.1045-15T>G) and two known variations (p.Pro743Leu, p.Ala787Thr) in fourteen families. Exon-trapping assays with a construct containing a novel c.1045-15T>G mutation revealed an error in splicing, which might results in mRNA decay, thus causing OCA2 phenotype in affected individuals. Screening of *TYRP1* and *SLC45A2* genes did not revealed any pathogenic mutant variant. Based on the mutational analysis results of fifty families, it was hypothesized that OCA1 and OCA2 are more prevalent in Pakistani population by affecting 24 families out of 50 as compared to OCA3 and OCA4. It is also obvious that OCA2 mutations are widespread than OCA1 mutations in the Pakistani population. Study indicated a significant proportion of the cohort did not have mutations in known OCA genes.

**ASSOCIATION OF RHEUMATOID ARTHRITIS WITH ABO AND Rh BLOOD GROUPS
IN TEHSIL MURREE DISTRICT RAWALPINDI, PAKISTAN**

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Present study was designed to investigate the relationship of Rheumatoid Arthritis (RA) with ABO and Rh blood group systems in Murree, Pakistan. RA is an autoimmune disease characterized by swelling and tenderness of joints along with restricted movement of that part of the body. Two hundred patients of RA (which were diagnosed by physicians in Tehsil Headquarter Hospital, Murree) were selected and their ABO and Rh blood groups were determined by agglutination method in the laboratory of same hospital. History and other related data was collected and the final data of blood groups was analyzed statistically by using Chi Square test. Among the ABO blood groups, O blood group was most abundant and occurrence of RA was O > B > A > AB. There was a significant (p -value <0.05) difference between the groups. There was also a highly significant (p -value <0.001) difference between Rh+ve and Rh-ve blood groups. When both ABO and Rh blood groups were combined then O+ve was most abundant and AB-ve was least common. There was a highly significant (p -value < 0.001) difference between all the Rh+ve with their respective Rh-ve blood groups. The overall order of abundance was as O+ve > B+ve > A+ve > AB+ve > B-ve > O-ve > A-ve > AB-ve. A strong association of RA was found with Rh+ve blood groups and with O blood group.

**GENETICALLY MODIFIED RABIES VACCINE: A POTENTIAL CANDIDATE FOR
WILD LIFE RABIES VACCINATION IN PAKISTAN**

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South Asia is the flash point of rabies which contributes more than 50% of all deaths due to rabies in the world. The presence of same Arctic strain of rabies virus in cases of sylvatic and urban rabies revealed that same strain of rabies virus is moving freely across India, Pakistan and Afghanistan. Furthermore, virus spill over from wild life to domesticated carnivore and vice versa is common. The only effective way to prevent transfers of rabies virus among various reservoir animals and hence to save precious human lives is oral vaccination and immunization of wildlife. We developed a genetically modified attenuated rabies vaccine which might be a potential candidate for oral vaccination of carnivores. Rabies virus glycoprotein gene (RVG) was amplified, purified, sequenced and cloned. The cloned RVG was mutated to reduce its pathogenicity, enhanced immunogenicity and to create restriction sites. The mutated RVG was substituted with glycoprotein gene of ERA strain of rabies based vector. The modified rabies virus (PKG) was

recovered by reverse genetics and used for oral vaccination of mice. Rapid fluorescent focus inhibition test (RFFIT) was used to measure the rabies virus neutralizing antibodies (RVNA). The mean antibody titer of 20 vaccinated groups was ≥ 0.5 IU/ml ranges from <0.04 -7.2 IU/ml. Seventeen out of twenty mice (85%) survived when they were injected intracerebrally with challenge rabies virus. Three mice exhibiting RVNA less than 0.5 IU/ml died. All the control group mice did not show any RVNA and 80% of mice died when they were exposed to challenge virus. PKG induced rabies virus neutralizing antibody in mice model and protect them against rabies virus challenge. The interesting aspect of the study was that vaccine was administer through per oral route , hence PKG may be used as oral vaccine through baits to wildlife after further investigation and safety trial in future.

MAPPING OF A NOVEL NON SYNDROMIC *OCA5* LOCUS TO CHROMOSOME 4Q24

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Complete lack or a reduction of melanin pigment cause a hypopigmentation of the eyes, skin and hair resulting into a phenotype called OCA. *TYR*, *OCA2*, *TYRP1* and *SLC45A2* genes mutations have been known to cause isolated OCA sub types. For the identification of novel loci, linkage analysis has been proven to be a powerful means for rare, severe, and high-risk mutations. PKAB080 is one of the families excluded for all the known nonsyndromic genes causing OCA subtypes by the Sanger's sequencing method. Therefore a genome wide linkage analysis was performed on family PKAB080 by using the Illumina Infinium II Human Linkage-12 panel. However, the physical, clinical and hematological evaluation of affected individuals revealed no other obvious clinical phenotypes in family PKAB80. Therefore, the OCA segregating in the affected individuals was concluded as nonsyndromic. Family PKAB80 was enrolled in the Rajanpur district of Punjab with six affected individuals in four generations. In this family, the OCA phenotype is linked to a novel locus, *OCA5*, on chromosome 4q24. A maximum two-point LOD score of 4.34 was obtained for the marker D4S2961 in a linkage region of 1.06 cM interval. All the affected individuals of the family defined the proximal recombination at D4S421 and distal break point of the critical linked region at D4S2913. From these findings, the HUGO nomenclature committee designated the locus defined by family PKAB80 as *OCA5*. The linkage interval of *OCA5* is approximately 3.84 Mb and harbors 14. None of the candidate genes are known to be part of the melanin synthesis pathway. Screening of the coding exons and flanking intronic sequences of these genes in two affected individuals revealed no pathogenic variants. Identification of the *OCA5* gene will significantly further our knowledge of the molecular basis of OCA and improve genetic diagnosis and genetic counseling. There is still need to broaden the study by increasing the number of families and also there is a need of performing whole genome sequence for unlinked families to search for new genes causing albinism in Pakistani population.

CYTOGENETIC INVESTIGATION OF REPEAT BREEDER COWS

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Present study was carried out on some reported repeat breeder individuals (n=08) of nondescript cows at Para-veterinary hospital, University of veterinary and Animal Sciences, Ravi Campus, Pattoki. Nondescript cows has major share, which is approximately 70% in cattle farming in Pakistan. Chromosomal preparations were made using blood culture from animals under study. About 50 metaphase spreads were screened to detect the chromosomal aberrations and prepare the karyotype. The results showed that the percent of total numerical aberrations for repeat breeder group was 19.95%, while the percent of total structural aberrations were predominant and reached 62%. These results concluded that cytogenetic studies should be used as a diagnostic tool to determine the causes of low reproductive efficiency.

CLINICAL AND MOLECULAR GENETICS STUDY OF FAMILIES WITH SECKEL LIKE MALFORMATION: EVIDENCE OF A NOVEL SKELETAL DYSPLASIA?

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Seckel syndrome (OMIM 210600) is a rarely occurring hereditary disorder characterized by prenatal and postnatal growth retardation, short stature, abnormally small head and characteristic facial features including beak-like protrusion of the nose. We present two families with skeletal dysplasia in which the phenotypic presentation is similar to Seckel like syndrome. Two independent families with relatively similar symptoms of a skeletal dysplasia were studied from northern Punjab. We performed detail phenotypic assessment including physical examination, radiographic evaluation, and anthropometric measurements. For molecular genetics characterization highly polymorphic microsatellite markers were utilized from the six known candidate genomic intervals of Seckel syndrome. Genotyping was performed on the genomic DNA from the available affected and normal subjects from these families. The phenotype in the affected subjects in both families was characterized by prenatal growth retardation, very low birth-weight, postnatal developmental delay, short stature, small head, narrow face, bird-like facial appearance with sloping forehead, beak-like protrusion of the nose, malformed ears, and micrognathia. The associated limb defects included radial hypoplasia, oligodactyly, and general fragility of long bones. However, contrasting to the typical Seckel syndrome where dwarfism is proportionate, in both of our families there was disproportionate type of dwarfism which also involved both the axial and appendicular skeletal elements. The limbs were most severely affected in our cases. Other remarkable features in the present cases were relatively normal head circumference and normal IQ, which are not characteristics of Seckel syndrome. The molecular mapping studies of the known loci for Seckel syndrome did not show any region of shared homozygosity and haplotype in the affected

subjects. The excluded loci were 3q22-q24 (*ATR*), 18p11-q11, 14q, 13q12 (*CENPJ*), 2q14.2-q14.3, and 21q22.3 (*PCNT*), which are known for Seckel syndromes I, II, III, IV, and osteodysplastic primordial dwarfism types I and II, respectively. Clinical and genetics studies in both families lead us to conclude that the phenotypes in both families are not completely concordant with the Seckel syndrome. It is quite likely that we are dealing with hitherto unreported skeletal dysplasia which may phenotypically overlap with Seckel syndrome but with unknown genetic causation. The identification of the causative locus/loci cosegregating with the phenotype(s) in these families would help in the molecular characterization of these anomalies and would be valuable in providing genetic counseling and molecular testing to the other family members.

GENETIC STUDIES OF *TYRPI* AND *SLC45A2* IN PAKISTANI PATIENTS WITH NONSYNDROMIC OCULOCUTANEOUS ALBINISM

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Autosomal recessive Oculocutaneous albinism (OCA) is a genetic disorder characterized by the partial or complete loss of pigmentation in the skin, hair and iris which can severely impact an individual's quality of life. Genetic disease are common in Pakistani population due to inbreeding practices, however, genetic information regarding OCA in this population is limited. To the best of our knowledge, only one allele of *OCA3* (*TYRPI*) and no mutations in *OCA4* (*SLC45A2*) have been documented in the Pakistani population. We enrolled 75 large, multi-generation families segregating nonsyndromic OCA from various regions of Pakistan and conducted a comprehensive molecular analysis of *TYRPI* and *SLC45A2*. Molecular modeling of wild type and mutant proteins and exon trapping assay was performed to determine the effect of mutation. We have identified four Pakistani albinism mutations, including three *SLC45A2* alleles and one twenty-two nucleotides deletion in *TYRPI*, segregating in four large families respectively. A sequence analysis of *TYRPI* in our cohort revealed a twenty-two-base pair deletion (c.647_668del) in the coding region in family PKAB131. Deletion of these nucleotides is predicted to cause a frameshift in the reading frame that will result in a truncated (p.Glu216GlyfsX42) *TYRPI* protein. The affected individuals of family PKAB131 had golden hair with orange highlights, brown eyes with foveal hypoplasia and nystagmus. To date, this is the second mutation of *TYRPI* that causes an *OCA3* phenotype in a family of Pakistani origin. A sequence analysis of *SLC45A2* revealed three variants that are likely to be pathogenic in three different families. Two missense substitutions: p.Leu84Pro (c.251T>C) and p.Ala511Val (c.1532C>T) were identified in families PKAB053 and PKAB051, respectively. Both missense mutations affected amino acid residues that are conserved among the *SLC45A2* orthologs and these mutations are predicted to be deleterious. A putative splice site mutation in intron 3 (c.889-6T>G) segregating with nonsyndromic OCA is identified in family PKAB059. To determine whether c.889-6T>G alters the normal splicing of *SLC45A2* mRNA; we performed an exon-trapping assay. The results demonstrate that the c.889-6T>G mutation caused aberrant splicing. This will lead to a shift in the reading frame and a predicted stop codon which result in nonsense-mediated decay. In summary, *SLC45A2* mutations accounted for 4% [3 out of 75 families; 95% Confidence Interval (CI), 1.45 – 11.1%] of OCA in our Pakistani cohort study, while only one pathogenic mutation in *TYRPI*

(1.3%; 95% CI, 0.32 – 7.1%) was found. This knowledge, in combination with data from previous studies, will improve our understanding of the molecular epidemiology of OCA in Pakistan. The results of our study will be important for future OCA diagnoses, genetic counseling and functional studies of the TYRP1 and SLC45A2 proteins.

GENETIC EPIDEMIOLOGICAL STUDY OF HEREDITARY LIMB DEFECTS IN CHITRALI POPULATION, NORTHERN PAKISTAN

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Congenital limb defects are common in various populations and constitute the highest proportion among the total hereditary anomalies. Limb defects cause long term disabilities thus putting financial and psychological burden on the subjects, their families and the society. The incidence and spectrum of limb disorders varies greatly in different populations, the understanding of which is imperative for effective management and intervention. In order to get an insight into the spectrum of hereditary limb anomalies in northern Pakistan we have conducted a retrospective genetic epidemiological study in District Chitral, Khyber Pakhtunkhwa. A total of 120 independent subjects/families with hereditary limb anomalies were ascertained. Only one index subject in each family was included in this analysis. The affected index subjects had a mean age of 20.27 ± 17.54 (range 1-95 years). Detailed distributions of these cases were established with respect to phenotype, clinical variability and socio-demographic variables. There were 80 affected males and 40 females. Majority of the cases were sporadic in nature (n=90) while there were 30 cases with familial evidences of segregation. The most common limb anomaly observed in this cohort was polydactyly (n=95), followed by syndactyly (n=8), clubfoot (n=6), and oligodactyly (n=3), while there were 8 other limb defects with minor representations. In most of the cases upper limb was involved (n=81); lower limbs were affected in 31 subjects and in 8 subjects both upper and lower limbs were involved. In majority of the subjects the limb anomaly was unilateral (n=80) and there were 40 subjects with bilateral presentations (50% cases symmetrical). In 53 subjects there were postaxial defects, 41 had preaxial, while there were 11 cases with mesoaxial anomalies. Data on hereditary anomalies and particularly congenital limb defects is scarce for the Chitrali population. This study would help appreciate the spectrum of hereditary limb anomalies in this population and would be helpful in the comprehension of clinical and phenotypic aspects of limb defects.

GENETIC STRUCTURE OF HUMAN POPULATION OF CHICHAWATNI, CENTRAL PUNJAB PAKISTAN, AS REVEALED BY THE TEMPORAL VARIATIONS IN ALLELIC FREQUENCIES AND HETEROZYGOSITIES AT *ABO* AND *RH* LOCI.

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ABO and *Rh* loci at chromosomes 9q34 and 1p34-36 respectively, are classical genetic markers frequently used to get a preliminary insight into the population structure. However, owing

to their low resolution power and limited heterozygosity they need to be supplemented with more informative and modern genetic markers. The previous studies in the populations of Punjab, Pakistan, have suggested significant variations in the allelic frequencies and their heterozygosities at the *ABO* and *Rh* loci. It had been previously hypothesized that these variations could be the visible representations of ethnic, cultural and linguistic diversity in Punjabi population. However, there are a number of regions in Punjab for which no information is available regarding the polymorphisms at *ABO* and *Rh* loci. In order to partially fill this gap in knowledge, we have conducted a study in the population of tehsil Chichawatni, District Sahiwal, which is an old city situated at the crossroad of grand trunk road, river Ravi and the ancient city of Harappa. Data of ~20,000 individuals on *ABO* and *Rh* loci was gathered which spanned a period of 15 years (1995-2010). Our analyses revealed that allele r[O] at the *ABO* locus remained highly variable throughout the study period. Additionally other allelic systems and the locus heterozygosities were not demonstrating any specific ascending or descending trend. Compared with other populations of Punjab, locus heterozygosity was higher while absolute gene diversity and coefficient of differentiation were very low in the Chichawatni population. This preliminary study would help understand the patterns of allele frequencies and gene diversity at *ABO* and *Rh* loci which intern would be useful in drawing a gene diversity map of Punjab populations.

PCR BASED DIAGNOSIS OF FRAGILE X SYNDROME IN A COHORT OF MENTALLY RETARDED PAKISTANI SUBJECTS

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Fragile X syndrome (FXS) is the most common type of X-linked mental retardation which is caused by the mutations in *FMR1* gene leading to hyperexpansion of a trinucleotide repeat sequence. The frequency of FXS is estimated to be 1/4,000 in males and 1/8,000 in females. The present study was aimed at the development of a molecular diagnostic tool for the better detection of FXS subjects and to assess the implementation of this diagnostic protocol in a developing country like Pakistan. Study included 395 mentally retarded subjects with unknown etiologies. There were 76% males and 24% females. DNA extracted from blood was used for the initial screening of mentally retarded subjects by conventional PCR analysis of *FMR1* repeat polymorphisms. In conventional PCR, the *FMR1* expansion allele failed to amplify in 13 individuals (out of 395). These subjects were suspected to be the patient of FXS and were subsequently confirmed by methylation specific PCR (MS-PCR). MS-PCR detects the expansion alleles of *FMR1* region but cannot discriminate the inactive X-chromosome in female subjects. Hence, MS-PCR is suitable for the diagnosis of male FXS subjects only (Panagopoulos *et al.*, 1999). The expanded allele in FXS positive male and female subjects were then confirmed through southern blot analysis. Of the 395 mentally retarded subjects, 13 (10M and 3F) were detected to have expansion mutations in the *FMR1* (repeat size >300 bp), revealing the prevalence rate of 2.53% in males and 0.75% in females of FXS in mentally impaired population. This study suggested that conventional PCR complemented with MS-PCR and southern blotting could be the reliable and economical method for the detection of FXS in the Pakistani mentally retarded population. This is the first attempt towards the molecular diagnosis of mental retardation in the

Pakistani population. We anticipate that an accurate diagnosis with customized and focused rehabilitation and training programs would help in improving the status of individuals with mental ailments in Pakistan.

ASSOCIATION OF GENETIC MUTATION OF *CYP2D6* GENE WITH GENERALIZED TONIC CLONIC SEIZURES IN PAKISTANI PATIENTS

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Epilepsy is a chronic neurological disorder in which hyperexcitability of neurons cause seizures. It is a serious disorder as there is an association between the increased mortality and epilepsy. Epilepsy has more prevalence in developing countries than in developed countries. Pakistan is a developing country with the prevalence of epilepsy 0.999% of total population. A type of epilepsy is Generalized Tonic Clonic Seizures (GTCS). Cytochrome p450 enzyme has been reported to be associated with GTCS which is coded by *CYP2D6* gene. We investigated 25 patients with GTCS for identification of novel mutations in *CYP 2D6* gene and association of the mutations with the disease. PCR amplification and direct sequencing was performed for *CYP2D6* gene. Three genomic mutations were detected in the patients with GTCS; 214G>C and 232G>C in intron 1 and 2850 C> T in exon 6. None of these mutations were found in healthy controls which indicated the association of these mutations with the disease. This is the first report of *CYP2D6* gene mutations in Pakistani patients with GTCS.

AN INSIGHT INTO THE PHYLOGENY OF INDUS DOLPHIN (*PLATANISTA GANGETICA MINOR*) AND GANGIES DOLPHIN (*PLATANISTA GANGETICA GANGETICA*)

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Indus Dolphin (*Platanista gangetica minor*) and Gangies dolphin *Platanista gangetica gangetica* are two rarest fresh water and endangered riverine found in Pakistan and India has also not been studied in detail for their DNA make up and phylogenetic analysis. The traditional attempts to infer a phylogenetic relationship between the species of Riverine whales dolphin was based on the morphological information. The accepted classification had been changed multiple times from subspecies to separate species and back again. On the basis of morphological information Indus river dolphin (*Platanista gangetica minor*) is rated as sub species (*Platanista gangetica gangetica*). Geographically, there is no link between River Indus and river Gangies. In modern evolutionary biology, phylogeny is based on concatenated sequences obtained from multiple alignments of the core proteins and recognized core genes dissecting mitochondrial genome, employing latest computational tools. Mitochondrial genome for *P. gangetica minor* and (*P. gangetica gangetica*) are available. It is required to employ modern computational tools for

phylogenetic analysis, before a position is allocated for *P. minor* in an evolutionary tree. To address the quest, a set of genes coding for a mitochondrial core protein cytochrome b in *P. minor* and *P. Gangetica* was analysed. The phylogenetic inferences that (1) *P. minor* shares a common ancestor with *Platanista gangetica gangetica* (2) *P. minor*, shows a separate lineage from *Platanista gangetica gangetica*.

MOLECULAR ANALYSIS OF A PAKISTANI FAMILY WITH LCA IDENTIFIED A NOVEL SINGLE BASE DELETION MUTATION IN *GUCY2D* GENE

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Pakistan has the high rate of consanguineous marriages, which is the leading cause of genetic ophthalmic disorders including Leber Congenital Amaurosis (LCA). In this study we recruited a Pakistani family with inherited LCA and identified a novel homozygous deletion mutation in a pre-characterized LCA gene. Whole Genome scan was performed with SNP analysis using the GeneChip Mapping 250K NspI array (Affymetrix). For perfect segregation of disease locus in the entire family; STS markers were genotyped within the homozygous region. For statistical significance; linkage analysis was also done by using GeneHunter and Superlink packages of easy LINKAGE program. Sanger sequencing for mutation analysis was carried out by using BigDye Terminator version 3.1 cycle sequencing kit together with Genetic analyzer 3130, ABS. The gross clinical analysis showed common Leber Congenital Amaurosis symptoms among patients. Genome-wide autozygosity mapping revealed linkage to the *GUCY2D* gene on 17p13.1 locus, which has already been found mutated before in patients with LCA. Sequence analysis of this gene showed a novel one base pair deletion mutation (c.1573delC) in exon 7 which resulted in a frameshift and created a stop codon 40 codons after the frameshift. The encoded truncated protein has lost both protein kinase and guanylate cyclase domain. The results obtained by this study will be helpful for the genetic counseling and prenatal diagnostics of other Pakistani families at risk. This study also determined the genetic heterogeneity of *GUCY2D* in Pakistani population.

PREVALENCE OF CONGENITAL ANOMALIES AND NON-COMMUNICABLE DISEASES IN DISTRICT BHIMBER, AZAD JAMMU AND KASHMIR, PAKISTAN

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In Pakistan there is no systematic health surveillance registry monitoring congenital anomalies (CA) and non-communicable diseases (NCDs), particularly among the young and adult population strata. Better information on the distribution and prevalence of CA is essential for the intervention programs and for estimating any future increase in the genetic load from mutagens.

Similarly, basic information on NCDs is vital to quantify their impact on the masses and the risk factors associated with them. In order to get an insight into the morbidity profile of young and adult women of District Bhimber, a retrospective cross-sectional study was conducted. A total of 1,731 females were interviewed and physically examined to access any kind of morbidity. There was a total of 74 CA witnessed among recruited subjects. Hence, the prevalence of CA was 42.75/1,000. Limb anomalies had the highest presentation (n=51; 68.92%), followed by deaf-mute cases (n=7; 9.46%). Among the total CA as well as within the limb defects category, club thumb had the highest proportion (n=18), followed by brachydactyly (n=12), camptodactyly (n=8) and clinodactyly (n=7). Majority of CA had sporadic nature (n=63; 85%), while there were 11 cases with familial aggregation (15%). Regarding laterality, most of the limb anomalies were bilateral (n=31, 60.78%). Of the total bilateral cases, 15 (48.38%) had symmetrical phenotypes. The involvement of upper limb was more common compared to the lower limbs (n=36 vs 15). There were a total of 104 NCDs observed in the recruited females (prevalence 60.08/1,000), and majority of them were involving the skeleton. There was highest representation of subjects with certain type of limb amputation (n=19; 18.27%), followed by subjects with arthritis (n=15, 14.42%), and acroosteolysis (n=12, 11.54%). The prevalence of NCDs was significantly higher among the subjects belonging to rural areas and low literacy level ($p < 0.0001$). This is a pioneer study to ascertain the prevalence of CA and NCDs in District Bhimber. Additional epidemiological studies on extended sample sizes would be helpful in getting a comprehensive picture of morbidity and the risk factor associated with CA and NCDs.

5. HUMAN DISEASES

POTENTIAL RISK OF TRANSFUSED MALARIA IN PAKISTAN: COMPARISON OF NEW DIAGNOSTIC METHODS TO THE CONVENTIONAL ONE

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In Pakistan blood for transfusion is not screened for malaria, though this is an endemic disease of the country, and in certain times of the year the situation regarding transmission of this disease, becomes alarming particularly in Khyber Pakhtunkhwa. This issue has never been considered by health organizations of Pakistan; therefore effort is made to draw their attention for including malaria screening before blood transfusion. Blood samples were collected from donors visiting the major hospitals of Peshawar and Fatimid Foundation Pakistan. Besides taking the blood samples, relevant questions about the possibility of having blood infections were asked from the donors. Thick and thin blood smears were made on the slides. Rapid diagnosis test (RDT) and polymerase chain reaction (PCR) were carried out for the detection of *Plasmodium* parasites. DNA was extracted by rapid boiling method and processed for amplification of 100 bp species-specific sequences of the small subunit of the ribosomal RNA (18S SSU rRNA) of *Plasmodium* species. A total of five hundred (500) samples were collected from July 2011 to March 2012. Males were the most dominant group. Majority of the donors were in the age group of 15-35 years. Six positive cases for malaria parasite *Plasmodium vivax* were revealed using simple microscopy. RDT was somehow totally failed in the present study, while PCR method showed two positive results (also found positive by microscopy). PCR failed to detect the presence of *Plasmodium* in four samples found positive for the parasite by microscopy. The study proved the chances of malaria transmission via blood transfusion to the recipients, indicating the necessity of blood screening for malaria parasites. Simple microscopy though laborious but still found to be authentic, cheaper, reliable and precise for species or false positive results.

EPIDEMIOLOGY OF LYMPHOMA IN A HOSPITAL POPULATION IN DISTRICT FAISALABAD

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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 and Punjab Institute of Nuclear Medicine and District Head Quarter Hospital and Combined Military Hospital (CMH) Sargodha. 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. The study was aimed to investigate the occurrence and prevalence of lymphoma in a hospital population. Non Hodgkin lymphoma was most commonly occurring malignancy 57.21% followed by Hodgkin lymphoma 42.79%. There was a significant ($p < 0.05$) gender depicted difference in the prevalence

of lymphoma. The age limit was 4-80 years. The average age at diagnosis of patients was 30.9 ± 0.109 . The patients with blood group A⁺ (25.87%) were more affected with Lymphoma. Birth order 3rd (21.39%) showed the highest representation of all lymphoma. Rural (65.17%) population was significantly influenced than urban (34.83%) population. Patients with low level of education were more affected ($p < 0.01$) with lymphoma in contrast with highly educated patients. Highly significant consequences of consanguinity had been observed among patients and similar result was found for first cousin marriages in contrast to unrelated marriages. Highest percentage of patients among various surnames was observed in Arian (23.38%) and lowest in Sheikh (1.49%). Smoking was important risk factor for occurrence of lymphoma. 53.73% patients were smokers followed by non smokers (39.30%) and ex- smokers (6.97%). It was found that 50.25% patients belonged to the lower class followed by middle class (39.80%) and 9.95% in upper class. Commonly occurring lymphoid malignancy was non Hodgkin's lymphoma. The most prevalent subtype of non Hodgkin lymphoma was Diffused large B-cell lymphoma (31%) followed by B- cell lymphoma (20%), lymphoblastic lymphoma (8%), and small lymphoblastic lymphoma (7%). Cervical region (32%) was most commonly occurring site for tumors followed by axillary (24%) and inguinal (16%) regions. In case of Hodgkin lymphoma most commonly occurring subtype was mixed cellularity type (44%) followed by nodular sclerosis (23%). Autosomal recessive mode of the inheritance was observed in lymphoma patients.

GENITOURINARY INFECTIONS CAUSED BY *CANDIDA SPECIES* IN FEMALE POPULATION OF GILGIT, GILGIT- BALTISTAN PAKISTAN

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This 9 months study from July 2011 to March 2012 was undertaken to investigate the frequency and etiology of *Candida* urinary and genital tract infections in female population of Gilgit. A total of 629 specimens processed, 21.3% revealed heavy growth of *Candida species*. 575 specimens out of 629 specimens were urinary and 54 were genital. 112 out of 575 urinary specimens (19.4%) and 22 out of 54 genital specimens (40.7%) were positive for *Candida species*. *Candida albicans* was the predominant species both in urinary and genital tract infections having 59.8% and 40.7% respectively in urinary and genital tract isolates. In urinary tract isolates *Candida tropicalis* (20.5%) was second frequent species, while in genital tract isolates *Candida albicans* was followed by *Candida glabrata* (27.2%). In urinary isolates *Candida glabrata* was 12.5%, *Candida gluerimondii* 6.25% and *Candida krusei* 0.89% while in genital tract isolates *Candida tropicalis* and *Candida gluerimondii* were 4.5% each. Minimal inhibitory concentrations (MICs) of fluconazole against *Candida species* was determined by agar dilution method for a total of 134 *Candida* isolates. Out of 81 *Candida albicans* only one isolate was resistant ($\geq 64 \mu\text{g/ml}$), while one isolate was sensitive dose dependent (16-32 $\mu\text{g/ml}$) and the rest of 79 isolates were sensitive ($\leq 8 \mu\text{g/ml}$). 18 out of 24 *Candida tropicalis* isolates were sensitive ($\leq 8 \mu\text{g/ml}$), 4 isolates were dose dependent (16-32 $\mu\text{g/ml}$), and 2 isolates were resistant ($\geq 64 \mu\text{g/ml}$). Three isolates of *Candida glabrata* were sensitive dose dependent (32 $\mu\text{g/ml}$), and 17 resistant ($\geq 64 \mu\text{g/ml}$). Three isolates of *Candida gluerimondii* were dose dependent (32 $\mu\text{g/ml}$), and 5 were resistant ($\geq 64 \mu\text{g/ml}$). One isolate of *Candida krusei* was resistant ($\geq 64 \mu\text{g/ml}$).

STUDY OF SOME CYTOKINES IN CORONARY ARTERY DISEASE PATIENTS IN DISTRICT KHUSHAB AND SARGODHA

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Present study was conducted to study the inflammatory and anti-inflammatory cytokines among patients suffering from myocardial infarction (MI) in district Khushab and Sargodha of Punjab, Pakistan. Plasma concentration of tumor necrosis factor- α (TNF- α), interferon- γ (IFN- γ), interleukin-10 (IL-10) and tumor growth factor- β (TGF- β) were estimated and compared with healthy individuals. Concentrations of TNF- α and IFN- γ were significantly higher in MI patients as compared to control group. There was no difference for these two inflammatory mediators between MI males and MI females. Lower concentration of IL-10 and TGF- β was observed in MI patients as compared to healthy group, however no significant difference of these anti inflammatory cytokines was found between MI males and MI females. The results of current study revealed, severe inflammatory state in MI patients while the level of cytokines (TNF- α , IFN- γ , TGF- β and IL-10) in MI patients is not associated with gender. Protective effect of IL-10 and TGF- β in stability of atherosclerotic plaques was not observed which needs further investigation.

PATHOLOGICAL FACTORS INVOLVED IN THE CITRUS FRUIT BLEMISHES ON KINNOW MANDARINS AND ITS CHEMICAL MANAGEMENT

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Citrus is an important fruit of Pakistan. It is good source of mineral and vitamins in addition to carbohydrate, which are essential for human health. Citrus is attacked by different diseases which affect its quality and quantity. Among them citrus fruit blemishes is the most important. There are huge losses in yield and export of citrus in Pakistan due to the citrus blemishes. Survey of Kinnow Mandarins (*Citrus reticulata*) for fruit blemishes was carried out in Sargodha, Shahpur, Kot Momin, Bhalwal and Silanwali for disease incidence and severity. Results indicated that maximum disease incidence (86%) and severity (4) was found in Sargodha while minimum disease incidence (19%) and severity (1) was found in Silanwali. Isolated fungi were multiplied and purified on PDA. Most dominating isolated fungi (*Elsinoe fawcettii*) was evaluated for pathogenicity. Management of the *E. fawcettii* was done under *in vitro* and *in vivo* conditions. Fungicides; Antracol, Elite, Topsin-M, Expel, Ridomil Gold and Remover was used at different concentration (100 ppm, 200 ppm, 500 ppm). Inhibition zone technique was used. Antracol caused 100% growth inhibition of *E. fawcettii* at its 200 ppm concentration. As the most effective fungicide under *in vitro* conditions, Antracol was further tested in field trial. Data was recorded by using disease rating scale for citrus blemishes. The field trial results revealed that Antracol is the most promising fungicide and can be used for controlling the disease under field conditions.

EVALUATION OF FUNGICIDES FOR THE MANAGEMENT OF *COLLETOTRICHUM GLOESPORIOIDES*; THE CASUAL AGENT OF CITRUS WITHER TIP

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Citrus wither tip caused by *Colletotrichum gloeosporioides* is one of the most damaging disease of citrus causing reduction in flower set and yield losses. Present study was conducted to evaluate the efficacy of four fungicides; Elite, Ridomil Gold, Score and **Copper Oxychloride** at different concentrations (50ppm, 100ppm and 150ppm) under in vitro and in vivo conditions. The inhibition effect of fungicides was evaluated in *in vitro* condition against *C. gloeosporioides*. Elite and Score completely inhibited the mycelial growth of *C. gloeosporioides* at 100 and 150 ppm concentration followed by Copper oxychloride and Ridomil Gold at 150 ppm respectively. In field experiment different concentrations of fungicides were tested. The reduction in disease incidence and increased yield was recorded in all treatments at variable levels. Elite and Score were found to be more effective in the reduction of disease at their all concentrations followed by copper oxychloride at 100 and 150 ppm. Ridomil Gold was found to be least effective as it controls the disease only at its 150 ppm concentration. The results of our findings revealed the potential of these fungicides against *C. gloeosporioides* under in vitro and field conditions which could be used as a better option to manage the disease.

FREQUENCY DISTRIBUTION OF TUBERCULOSIS IN DISTRICT CHITRAL

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TB is a very serious health problem of present time. Millions of people world wide and in Pakistan are suffering from this disease. During present research, for the first time, frequency distribution of TB was studied in Chitral district, KPK, Pakistan. A total of 1055 individuals (586 female and 487 male) from all parts of Chitral were surveyed during present study. Eighty eight out of 1055 people had TB indicating 8.34% occurrence of TB in the study area. Gender wise 8.6% females and 8.0% males had TB in Chitral. Slightly higher frequency of TB patients (8.6%) was found in upper Chitral, as compared to relatively lower frequency of TB cases in lower Chitral (7.6%). Age wise, higher percentage of TB cases was found in older age groups. It was also found that in 25-36% cases, diagnosis of TB was based on symptoms only. Fortunately all of the patients diagnosed with TB, received or receiving treatment. Most of the patients receive treatment against TB from a private (NGO owned) health facility. 28% patients revealed that they also use spiritual methods of treatment in combination with medicines. In 37.5% cases, at least one or both of the parents of the patients had TB. This indicates strong possibility of genetic nature of the onset of the disease. It is suggested that this particular finding should be studied in more detail for better understanding of the prevalence of TB.

IN VITRO SENSITIVITY TESTING OF *LEISHMANIA TROPICA* CLINICAL FIELD ISOLATES KWH23 TO BUPARVAQUONE

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In the present study, activity of buparvaquone was evaluated against *Leishmania tropica* clinical isolate KWH₂₃ *in vitro*. Antileishmanial activity of the drug was interpreted in relation to the standard chemotherapy currently available for leishmaniasis. The drug was tested both against promastigotes and amastigotes in four different concentrations, with quadruplicate for each concentration. The mean % inhibition for each concentration was 78% (1.35 μ M), 44% (0.51 μ M), 20% (0.17 μ M) and 14% (0.05 μ M) in case of intramacrophagic (Intra THP-1) amastigotes. In case of promastigotes, mean % inhibition was 89%, 77%, 45% and 35% for the mentioned concentrations respectively. IC₅₀ was calculated to be 0.06543 μ M for amastigotes and 0.1523 μ M for Promastigotes. Buparvaquone showed comparable results, in its first concentration, to the 3rd concentration of amphotericin B 0.1388 μ M (77%) and 1st concentration of Miltefosine 90 μ M (79%), both of which were the most potent against *Leishmania tropica*.

PREVALENCE OF HEPATITIS IN VARIOUS PARTS OF DISTRICT CHITRAL

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Millions of people in Pakistan and all over the world are suffering from viral hepatitis. During present research, for the first time, frequency distribution of hepatitis was studied in district Chitral, KPK, Pakistan. A total of 1494 individuals (525 female and 969 male) from all parts of Chitral were surveyed. One hundred and fifty six people (74 female and 82 male) had Hepatitis. Overall% of Hepatitis patients in Chitral was found to be 10.4%. Percentage of hepatitis infected female was much higher (14.1%) as compared to hepatitis infected male (8.5%). Out of 156 patients, 77, 24 and 12 have Hepatitis A, B and C, respectively. Almost half of the patients suffering from Hepatitis have Hepatitis type A while Percentage of Hepatitis B patients among the infected people was 15.4% followed by Hepatitis C (7.7%). Most of the Hepatitis patients belonged to young - middle age group (11 – 40 years). In 119 cases (out of 156) diagnosis was done by pathological test while in 23.7% (37 out of 156) cases diagnosis was done on the basis of symptoms only. Out of 156 Hepatitis patients studied, 119 received some kind of treatment while 37 (23.7%) patients were not receiving any type of treatment. Majority of the patients (75) received treatment from government owned hospitals, 9 and 3 patients, respectively received treatment from Private and NGO operated facilities. Twelve patients were receiving spiritual (Dam Daruud, Taweez etc) in addition to medicines while 20 patients were receiving only spiritual treatment. Giving due consideration to the present findings it is recommended that public campaign should be launched on large scale to provide awareness about hepatitis and preventing the disease by adopting simple measures including regular hand washing, washing fruits and vegetables before eating them and getting vaccine against hepatitis etc.

**FIGHT AGAINST MYOCARDIAL INFARCTION IN NORTH PUNJAB, PAKISTAN;
COST-EFFECTIVE INTERVENTIONS**

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Coronary heart disease (CHD) is an important cause of mortality and morbidity in Pakistan. Temporal trends in the risk factors for Myocardial Infarction (MI) and impact of socio-economic status on these risk factors remain ambiguous. The objective of the present analysis was to investigate potential association between various risk factors and MI in North Punjab and to ascertain if this association varies by sex or age. Total of 515 patients were included in this study who admitted to coronary care units (CCU) or equivalent cardiology wards of participating hospitals in North Punjab, Pakistan. The analysis focused on identifying the socioeconomic status, life style, family history of MI and risk factors (hypertension, diabetes, smoking, and hyperlipidemia). Lipid profile was recorded from investigation chart of every patient. For statistical analysis we used 'Kruskal Wallis', Mann Whitney U' Wilcoxon and Chi-square tests. MI was appeared to be more common in males at the age of 41-60 years as compared to females with p value of 0.015. Patients with positive parental history of CHD experienced MI in younger age at BMI \leq 25 Kg/m² with p value of 0.001. Sedentary life style (63%) and smoking was (67%) with male predominance. Hypertension accounts for nearly 37.4%, hyperlipidemia 21.5% and diabetes 18% in rural and urban subjects. HDL (high density lipoprotein) cholesterol reduced, while LDL (low density lipoprotein) Cholesterol and hypertension rose with age. The mean cost of monthly medicine and physicians check-up charges per patient were Rs. 2381.132 (24.24 USD). The main burden of disease is faced by the person at the time of Surgery which reaches up to 600 thousand (6108.19 USD). Higher BMI, positive family history, smoking hypertension, hyperlipidemia, and diabetes are strong predictor of MI in North Punjab, Pakistan. Middle income people are facing the socio-economic burden to get their treatment. Better control of risk factors and the awareness of preventive strategies are needed.

FREQUENCY DISTRIBUTION OF DIABETES MELLITUS IN DISTRICT CHITRAL

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Diabetes mellitus is a serious disorder of human body that affects the body's ability to make or use insulin. Insulin is a hormone produced in the pancreas that helps transport glucose (blood sugar) from the bloodstream into the cells so they can break down it and use it for fuel. If remain ignored and untreated, diabetes can cause serious problems including blindness, heart diseases and kidney failure. There are two main kinds of diabetes viz; type I (insulin deficiency) and type II (insulin resistance) diabetes. Millions of people in Pakistan and all over the world are suffering from diabetes. To adopt better treatment / preventions strategies, information regarding prevalence of diabetes in a particular are is pre requisite. Unfortunately no reported work has been done on the frequency distribution of diabetes in northern areas (especially Chitral district) During present research, for the first time, frequency distribution of diabetes was studied in district Chitral,

Khyber PukhtoonKhawa, Pakistan. Out of 1356 individuals surveyed , 870 were male while 486 were female. Three hundred and one people had diabetes. Data in indicated that overall % of diabetes patients in Chitral was 22.2%. While 21.6 % males and 23.2 % females had diabetes in Chitral.

**MOLECULAR CHARACTERIZATION AND GENOTYPING OF DENGUE VIRUS
SEROTYPE 2 AND 3 IN *AEDE*S MOSQUITO POPULATION OF KHYBER
PUKHTUNKHWA AND THEIR OCCURRENCE OF NATURAL VERTICAL
TRANSMISSION**

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Dengue is currently one of the most important arthropod-borne diseases and may be caused by four different dengue virus serotypes from DENV-1 to DENV-4. With neither dengue vaccine nor treatment available, vector control strategies constitute a crucial mode to prevent or reduce disease transmission. By using reverse transcription polymerase chain reaction (RT-PCR) the DENV detection in natural *Ae. Aegypti* and *Ae albopictus* populations was conducted in the present study which might serve as a potential additional tool for early prediction systems of dengue outbreaks, leading to a vector control measures so that we may be able to reduce the disease transmission. The current investigation was also aimed to check the circulating serotypes and the occurrence of transovarial transmission in local mosquito populations of Khyber Pukhtunkhwa. According to the research, the dominant prevalence was of DENV-2 as it was 77% as compared to DENV-3 which was 22% in 6 positive pools out of 35 adult mosquitoes pools while that of the larvae obtained from adults showed 3 positive pools out of 17 pools which shows transovarial mode of transmission. The prevalence of DENV 2 and 3 infections was high in Peshawar as compared to other districts.

**STUDY OF SOME CYTOKINES IN DIABETIC PATIENTS IN DISTRICT KHUSHAB
AND SARGODHA**

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During present study concentration of inflammatory and anti-inflammatory cytokines of 300 patients with Diabetes Mellitus was studied in district Khushab and Sargodha of Punjab, Pakistan. Plasma concentration of tumor necrosis factor- α (TNF- α) interferon- γ (IFN- γ) interleukin-10 (IL-10) and tumor growth factor- β (TGF- β) were estimated and compared with healthy individuals. Concentrations of TNF- α and IFN- γ were significantly higher in Diabetes Mellitus patients as compared to control group. While no significant difference was observed for these two inflammatory markers in DM Male and DM female. Lower concentration of IL-10 and TGF- β was observed in DM patients as compared to healthy group but not much significant. When conc. of these anti inflammatory cytokines was compared among DM males and DM females again not much difference was observed. The results of study revealed inflammatory condition in DM

patients and inflammatory cytokines play crucial role in development of disease. Protective effect of IL-10 and TGF- β in stability of Diabetes Mellitus was not observed which needs further investigation.

CHARACTERISTICS OF INTRAOCULAR PRESSURE IN OPEN ANGLE GLAUCOMA PATIENTS FROM DIFFERENT AREAS OF PUNJAB VISITING LRBT HOSPITAL

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Present retrospective, analytical study that was performed between April 2012 and August 2012 on all cases of open angle glaucoma seen from Punjab during ophthalmological consultation at the Layton Rahmatullah Benevolent trust hospital from the duration of October 2011-May 2012. The aim of this research was to determine the characteristics of intraocular pressure associated with Open angle Glaucoma. The techniques used for the diagnosis of open angle glaucoma were an examination of ocular fundus using a biomicroscope, gonioscopy and far visual acuity. Indeed a drop in visual acuity and blindness were the main reasons for consultation in this study. 160 cases of open angle glaucoma were identified from Punjab, out of which 36 were women and 124 men. The OAG is predominant in the age of 51-61 years in both men and women. Blindness ratio is higher in men than women. Maximum patients have IOP range 22-31. People with OAG are often diagnosed later in life and have a difficulty in accepting the loss of vision. There is essential need for population based studies to provide up-to-date characterization of magnitude and nature of open angle glaucoma and blindness as well.

MALARIA: STILL A HEALTH PROBLEM IN THE GENERAL POPULATION OF BANNU DISTRICT, KHYBER PAKHTUNKHWA, PAKISTAN

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Malaria has been a global health problem threatening more than 40% of the world's population, and about 300- 500 million cases of malaria infection are reported every year. In the developing countries including Pakistan malaria infection is still mostly prevalent. Although significant measurements have been made for the control of malaria, but still it remains a frequent health problem in the highly epidemic region of Southern belt of Khyber Pakhtunkhwa. The current study was designed to re-evaluate the high incidence of malaria, species prevalence, and its demography in the human population of district Bannu, Khyber Pakhtunkhwa, Pakistan. A total of 823 blood samples from both sexes; 513 males and 310 females of varying age groups <1 up to 50< years were collected residing in different localities of both urban and rural areas. Out of 823 blood

samples, 223 (27.09%) subjects were found positive for plasmodiasis, while the distribution of species prevalence was observed as 186 (22.6%) and 25 (3.04%) for *Plasmodium vivax*, and *Plasmodium falciparum*, respectively along with a mixed infection of 12 (1.46%). Variation with high incidence (42.65%) was found in the age group of 21-30 years. Moreover, males were found to be more malaria infected (30.64%) than females (24.95%). In addition, the incidence of suspected malaria cases was found more frequent in rural population (33.42%) as compared to urban (21.00%) population. The present study showed that the burden of malaria was high in rural areas of district Bannu and more attention is needed to overcome and control the high epidemics of malaria in this region.

MOLECULAR CHARACTERIZATION AND CLINICAL EPIDEMIOLOGY OF HCV GENOTYPES FROM DISTRICT DIR (LOWER), PAKISTAN

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Hepatitis C virus (HCV) infection is very important as it accounts about 3% of the world's population. The relative prevalence of HCV genotypes varies among different geographic regions of Pakistan and thus has created a significant public health problem. This study was initiated to determine the prevalence of overall HCV genotypes and also to find association of HCV with clinical and epidemiological characteristics in Dir (Lower) district of Pakistan. A total of 100 blood samples were tested by multiplex PCR using type specific primers for HCV core region. Results indicated that the most prevalent genotype is 3. However 33 among 100 blood samples were found untypeable by the present genotyping method. Frequency of HCV was found higher in male individuals of age group (41-50 years) having up to 10,000 rupees monthly income. This study also revealed that a highly significant mode of HCV acquired by these patients was injection received at clinics (39%); ($P < 0.002$). There is great need of further investigation of genotypes by optimal methods and sensitive assays that will not only help to unravel other genotypes of HCV but will also help in the clinical management and prognosis of HCV infection.

WATER-BORNE DISEASES IN PAKISTAN

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Water is life; life originated from and depends on water; about 50-60% of human body consists of water. But it is not free of risk. Most common water-borne diseases (worldwide) include typhoid fever, paratyphoid fever, shigellosis (bacillary dysentery), cholera, leptospirosis, infectious hepatitis (hepatitis virus A, E), poliomyelitis and amoebic dysentery. Landmarks in the history of water-borne diseases include 1) John Snow's studies on epidemiology of cholera epidemic in

London in 1849 suggesting remedy before the causative agent was known, and 2) 1885 discovery of *Escherichia coli*, a cause of diarrhea, used as an indicator of the quality of drinking water. Developed countries have been able to control water-borne diseases to a large extent long ago, but underdeveloped countries are still facing the menace. Common water-borne diseases in Pakistan include amebiasis, giardiasis, diarrhea, cholera, hepatitis, hydatid disease, leptospirosis, typhoid fever, paratyphoid fever and shigellosis. Both water-borne and water-related diseases (e.g., malaria) comprise important infectious diseases. Due to variety of factors like overpopulation, unplanned urbanization, scarcity of water, poor sanitary conditions, prolonged load shedding, etc the challenge of water-borne diseases is turning from bad to worse. A number of water-borne or water-related diseases can be controlled or even eradicated by improvement in water supplies; case of dracunculiasis (dracunculiasis) is a classical example. Fresh water is a limited resource that has to be used judiciously. Poor quality drinking water is of great health threat throughout the developing world, and certainly so in Pakistan. We have to live with water-borne and water related diseases in the foreseeable future. Hence special focus on these diseases is warranted not only in our medical schools but also in all health and educational institutions.

**CLINICAL UTILITY OF POLYMERASE CHAIN REACTION (PCR) FOR THE
DIAGNOSIS OF CUTANEOUS LEISHMANIASIS IN PATIENTS ATTENDING MAJOR
TEACHING HOSPITALS IN PESHAWAR, PAKISTAN**

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The main objectives of this study were to identify *Leishmania* species by using semi-nested PCR in local Pakistani population and some Afghan refugees visiting Combined Military Hospital (CMH) and Kuwait teaching hospital in Peshawar. Exudates from cutaneous lesions for the *Leishmania* promastigotes culture were collected during September 2011 to March 2012. From total 123 exudates, 60 were found positive for promastigotes in M-199 medium at 26°C. Prevalence of leishmaniasis was higher among Pakistanis (91.7%) than in Afghan refugees (8.3%), which may be due to few Afghan patients' availability in Kuwait teaching hospital. Prevalence was higher among male (65%) as compared to female (35%), as male travel frequently out of home and normally sleep without shirt in open air during summers. Cutaneous leishmanial lesions were more common on exposed body parts (78.3%) and were with a diameter range of 0.1-6cm. Depending on the treatment and care, duration of the cutaneous lesions was from 1-12 months. Isolates of *Leishmania* species were identified after culturing in M199 medium. For the identification of *Leishmania* species present in cutaneous lesion semi-nested polymerase chain reaction (PCR) was performed. The PCR primers were used for the identification and differentiation of *Leishmania* species (*Leishmania tropica* and *Leishmania major*). PCR bands at 650 bp and 720 bp were reported on gel electrophoresis to separate mixed parasite-specific kinetoplast DNA sequences for the *L. major* and *L. tropica* respectively. DNA was extracted from positive cultures by using Phenol-Chloroform Isoamyl Alcohol technique. Out of the 60 positive cultures, 44(73.3%) were found positive for *L. tropica* infection, 10(16.7%) for *L. major* infection and only 6(10%) were positive for both *L. tropica* and *L. major*. It is concluded from our study that presently cutaneous lesions caused by extra- and intramacrophages amastigotes of *L. tropica* are the main source of infection in Peshawar and Jamrud (Khyber agency) and mixed infection with *L. tropica* and *L. major* also prevail in this region of Khyber Pukhtunkhwa.

***ENICOSTEMMA HYSSOPIFOLIUM* (WILLD) VERDOON, REPORTED AS REMEDY AGAINST MALARIA FEVER FROM NAGARPARKAR, SINDH**

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During ethno botanical survey of district Nagarparkar, Sindh, *Encicostemma hyssopifolium* (Willd) Verdoon was reported as remedy against malaria fever. Species was selected for chemical and clinical investigation in search of new herbal drug against malaria fever. Erythrocenturine was isolated from *Encicostemma hyssopifolium* from CHCl₃ fraction at the ratio of 1: 9 (CHCl₃: Hexane). UV, IR, EIMS, 1H-NMR and 13C-NMR (HMBC, HMQC, COSY & NOSY) were carried out to confirm the structure of compound. Clinical treatment of the compound showed its inhibition against Gram +Ve organisms, i.e *Micrococcus luteus* and *Micrococcus luteus* ATCC (American type cultural collection), 50% against serine protease and 70% as antioxidant agent.

ASSESSMENT AND CORRELATION OF TOTAL ANTIOXIDATIVE STATUS WITH VITAMIN C AND VITAMIN E IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) PATIENTS

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Present study demonstrates that there is increased oxidative stress in patients with COPD when compared to controls and oxidative stress is much higher in patients of low socioeconomic status when compared to patients of medium socioeconomic status. This study also emphasizes the decreased antioxidants namely serum glutathione, Gpx, catalase, vitamin C, vitamin E and SOD activity and increased levels of oxidants like MDA and NO in COPD patients when compared to controls. Antioxidant levels are particularly much decreased in patients of low socioeconomic status when compared to chronic medium class of patients. This study demonstrates the role of oxidative stress and antioxidant imbalance in pathogenesis of COPD. Hence by advising diet rich in antioxidants or supplementation of antioxidants may prevent the further oxidative damage in COPD patients. The present study strongly indicates that there is an association of low social class (reduced income, less education) with COPD that cannot be explained by confounding by smoking. The extent, to which the association is due to occupation, air pollution, or other factors, is, however, unclear.

FREQUENCY OF DENGUE FEVER IN DISTRICT LAHORE AND FUTURE GIS MAPPING

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Pakistan has experienced a number of dengue fever outbreaks since 1992 and becoming most rapidly spreading mosquito born viral disease now a day. In present study prevalence of dengue fever recorded from November, 2011 to May, 2012. Data was collected from nine towns of Lahore. Results showed that total 11,283 dengue cases were reported in different hospitals of nine towns. Highest prevalence was recorded in Data Ganj Baksh town (18.3%), Gulberg (13.3%), Cantt (12.6%). While equal prevalence (11.9%) recorded in Allama Iqbal and Samanabad towns. The highest prevalence of dengue fever (DF) with respect to sex was observed in males 11.9% (1347) in Data Ganj Baksh town. Moderately affected areas were Nishter, Ravi, Shalimar and Aziz Bhatti Towns containing about 9.8%, 8.7%, 7.2% and 4.5% cases of DF respectively. The least affected area was Wahga town, where 1.5% prevalence was recorded. The highest frequency per unit area was found to be at Data Ganj Baksh town. That is ranging from 54.01 to 67.57%. The prevalence of DF with respect to age in age group 21 to 30 years was found highest (23.14%) among all age groups. The possible reason for higher prevalence of dengue fever in males and in people falling between 21 to 30 years of age can be attributed to the fact that usually these people spend more time outdoors and thus have more chances of getting affected. It is recommended that keep surroundings of congested areas free from standing water. Potential breeding sites for mosquito needs to be inspected regularly.

PREVALENCE OF PULMONARY TUBERCULOSIS AND ITS PROMOTIVE FACTORS IN DISTRICT BAGH AZAD JAMMU AND KASHMIR.

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Tuberculosis (TB) is caused mainly by mammalian tubercle bacilli, *Mycobacterium tuberculosis* complex, having closely related species. *Mycobacterium tuberculosis* principally causes TB in humans and also animals that are in contact with infected humans. *M. tuberculosis* affect lungs but other parts of body are also affected by *M. tuberculosis*. TB in lungs is called pulmonary TB, 75% of the TB cases are pulmonary. Sneezing, cough, or spit of infected people is the main source of spreading. In the present study, descriptive survey approach was used, purposive sampling technique was used to obtain a sample of 111 persons affected by pulmonary TB from the population of District Bagh Azad Jammu and Kashmir comprises the children and adults (both male and female). Interview schedule was prepared to collect the data; the data was collected personally by visiting each patient at homes. To check the blood group antisera of Cenix Clone II and painless lancets were used. Females were found to be more affected by pulmonary TB than males. 58.5% of affected persons were uneducated. Within the different ethnic groups the Chaudhary and Raja Family have maximum persons affected by pulmonary TB (both have 18%

affected persons). Most have blood group B⁺ (27%), 28.8% belong to age group 21-40, 48.6% were use pipe water, 76.5% have toilet facility, 63.9% were positive sputum test. Pulmonary TB is caused by poor hygienic conditions and lack of awareness of pulmonary TB, and the production age is at high risk of pulmonary TB.

HEPATITIS C VIRUS INFECTION IN IDPS OF WAR AGAINST TERRORISM IN SOUTH WAZIRISTAN AGENCY, PAKISTAN

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Hepatitis C virus (HCV) is a major public health problem which causing chronic liver disease and affected an estimated 180 million people throughout the world. The main aim of the current study was to investigate the HCV infection and the various associated risk factors in IDPs of South Waziristan Agency. A total of 250 individuals of the age groups 01-60 years were screened for the detection of anti-HCV antibodies using 3rd generation assay. All the subjects were also analyzed for ALT and ALP level. Among the total individuals, 28.8% were found positive for the presence of anti-HCV anti bodies. These were further processed for Polymerase Chain Reaction (PCR), for the presence of HCV RNA confirmation and active infection. A total of 21.6% patients were found to have HCV RNA and were confirmed for active infection of HCV. Prevalence of HCV was found to be higher in males than females. Similarly higher active infection of HCV was found in the old aged peoples and illiterate peoples were more infected than educated peoples. The risk factors observed were dental surgery, skin tattooing, reuse of blades, general surgery, reuse of syringes, drugs addicts, blood transfusion and intrafamilial prevalence. Awareness programs and timed screening needs to prevent the transmission of this dreadful disease in the study area.

PREVALENCE OF ABO BLOOD GROUP IN PEPTIC ULCER PATIENTS VISITING DIFFERENT HOSPITALS OF LAHORE

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Peptic ulcer remains a significant public health problem. Prevalence of peptic ulcers is higher in third world countries where it is estimated about 70 per cent of the population, whereas developed countries show a maximum of 40 per cent ratio. Peptic ulcer is a disruption of mucosal integrity and may occur any where between lower oesophagus to jejunum. An association between peptic ulcers and blood groups has been documented. The present study was conducted to observe association of ABO blood groups with peptic ulcer disease. The study was carried from January 2012 to July 2012. Patients with peptic ulcer (n=200) were sampled from different hospitals. The healthy control subject (n=200) were collected from blood banks and transfusion clinics. Self structured Questionnaire was designed for this research work. Nutritional habits, smoking habits, use of caffeine, consumption of NSAIDs and their life style were noted. Blood grouping was

determined by using standard agglutination test. The prevalence of blood groups in peptic ulcer subjects was 30.5% in blood group A, 28.5% in blood group B, 6% in blood group AB and 35% in blood group O. In control group the distribution of A, B, AB and O blood groups were 36.33%, 20.67%, 8.33% and 34.67% respectively. Rh+ve factor was prevalent in 93.5% among peptic ulcer subjects and 89.67% in control subjects. Duodenal ulcer (53%) was more prevalent than Gastric ulcer (47%). Smoking, excessive caffeine intake and use of NSAIDs was higher in peptic ulcer subjects. The prevalence of peptic ulcer is more in males (57%) as compared to females (43%). It is concluded that among four different blood groups *i.e.* A, B, AB and O, subjects with blood group O were more prone to develop peptic ulcer.

MALARIA AMONG THE STUDENTS OF RELIGIOUS SCHOOLS OF BANNU DISTRICT, KHYBER PAKHTUNKHWA, PAKISTAN

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A parasitological survey was conducted from March to May 2002 to assess the prevalence of malaria, among the students of religious schools (Islamic Madaris) of Bannu District. A total of 581 male students of different age groups were examined. The prevalence of malaria was also examined in relation to urban and rural localities, nature of surrounding areas and presence of wire screen in windows of sleeping quarters. Of these 581 individuals, 3.61% were found positive only for *Plasmodium vivax*. No other species of *Plasmodium* was detected. The prevalence of malaria was highest (5.52%) in age group of 5-9 years, intermediate (3.37%) in age group of 10-14 years and lower (2.2%) in age group of 15-19 years, though the differences were not significant. The prevalence of malaria was significantly higher in Madaris located in marshy areas (6.94%) than in those having dry surroundings (2.51%). Similarly, prevalence of malaria was higher in rural settings (4.56%) than in urban settings (2.38%). Also prevalence rate of malaria was lower (1.80%) in Madaris where windows of sleeping quarters were screened than in those where windows were without screens (4.72%).

A STUDY ON DENGUE KNOWLEDGE, ATTITUDE, PRACTICE AND THEIR IMPACT ON Aedes Aegypti POPULATION IN LAHORE

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A study was conducted in 2010-2011 by questionnaire method to test knowledge, practice and its impact on control of *Aedes aegypti* population at Lahore. About 940 open ended questionnaires were received out of 1000, in which about 400 were from different houses of Lahore and 540 from different students (graduates, post graduates, intermediate, and secondary schools),

teachers, employees and workers. The results showed that the demographic characteristics of Dengue Fever/ Dengue Hemorrhage fever (DF/DHF) patients were significantly different ($P < 0.05$) for age and sex for the year 2010 and 2011. Chi-square analysis revealed significant ($\chi^2 = 366.29$) differences between alive DF/DHF susceptible patients in 2010-2011 and non-significant ($P = 0.13$) results between dead DF/DHF susceptible patients in 2010-2011. Area wise distribution of DF/DHF epidemic 2010-11 was also checked by Chi-square analysis and the results were significant showing variation in area wise distribution. Similarly, Overall patients of DF/DHF were more in the year 2011 than 2010. The female patients were higher than male patients and children and the age range in which the highest percentage of DF/DHF fall was between 16-25 years. From the present study, it can be concluded that such surveys should be carried out as the data generated through public knowledge not only helps in controlling *Aedes* population but also helps government in formulating policy against epidemics.

LIPID PROFILE OF NEONATES FROM DIABETIC AND NON-DIABETIC MOTHERS

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The metabolic components in neonates may be disturbed by gestational diabetes mellitus. In order to investigate, the lipid profile, body weight and sugar level were measured in 40 cord blood samples from gestational diabetes mellitus (GDM) and control mothers. The body weight and sugar level were increased in cord blood samples of neonates from GDM mothers as compared to control. Among the lipid profile, total cholesterol (TC), triglycerides (TG), low density lipoproteins (LDL), very low-density lipoproteins (VLDL) and ratio of cholesterol to HDL 'cholesterol (CHO/HDL: CRO) were higher in neonates of GDM mothers. High-density lipoproteins (HDL) were not significantly different between two groups. So it was concluded that the lipid profile in neonates might be disturbed by GDM.

ASSESSMENT OF DISEASE INCIDENCE AND SEVERITY OF CITRUS WITHER TIP (*COLLETOTRICHUM GLOEOSPORIOIDES*) IN DIFFERENT DISTRICTS OF PUNJAB

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Citrus fruits are one of the major export commodities of Pakistan, being an important fruit citrus is affected by a number of destructive diseases caused by fungal pathogens. Citrus wither tip disease (*Colletotrichum gloeosporioides*) causes twigs and branches as scorched by fire. A survey was conducted in different districts of Punjab; Sargodha, Khushab, Toba tek singh and Mianwali to assess the disease prevalence, incidence and severity of *C. gloeosporioides* on sweet orange (*Citrus sinensis*) and lime (*C. aurantiifolia*). Disease prevalence was maximum in Sargodha followed by Khushab and Mianwali. The survey's results indicated that disease incidence was maximum in Sargodha (93.5%) and minimum in Toba tek singh (41.5%). The severity (measured by disease rating scale) of citrus wither tip varied among the cultivars and districts. Disease severity was highest on *C. aurantiifolia* in Sargodha (3.87) followed by Khushab (3.13) and lowest in Toba

tek singh (1.27). The severity of citrus wither tip was maximum on *C. sinensis* in Sargodha (3.63) followed by Mianwali (2.97) and minimum in Toba tek singh (1.11). The results indicated that the severity of *C. gloeosporioides* varied not only with the districts but also with the cultivars. Our findings revealed the disease incidence and severity of citrus wither tip prevailing in different districts of Punjab, which would be helpful in better management options at proper location.

ASSOCIATION OF BREAST CANCER WITH OPESTY AND ESTROGEN IN HUMAN FEMALES

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Breast cancer is a leading cancer in women all over the world which is increasing day by day. Obesity is considered as one of the major causes of breast cancer. In both premenopausal and postmenopausal women obesity relates with breast cancer via estrogen activity in breast cells. Serum estradiol estimations were carried out in fifty five obese breast cancer women and in a control group of twenty three obese normal women. All the patients and controls were included premenopausal and postmenopausal women. The levels of estradiol have been investigated with the help of Enzyme Linked Immunosorbent Assay (ELISA). The levels of estradiol of breast cancer patients were compared with the control subjects. It was found that mean serum estradiol levels were significantly elevated, both in premenopausal ($P < 0.001$) and postmenopausal ($P < 0.01$) breast cancer patients, than in control subjects. Variance analysis of regression showed that estradiol levels increase significantly with increase in BMI in premenopausal, postmenopausal breast cancer patients and premenopausal control subjects but non significantly increase in postmenopausal control subjects. The results of the present studies clearly indicate that obesity is risk factor that contribute to breast cancer and have significant public health impact in women. These results also indicate that weight control may be an effective measure for breast cancer prevention in women.

MULTIPLE DRUG RESISTANCE PATTERN OF BACTERIAL PATHOGENS IN PATIENTS SUFFERING FROM URINARY TRACT INFECTION IN PESHAWAR, KHYBER PUKHTUN KHWA (KPK), PAKISTAN

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Urinary tract infection (UTI) is the most common problem in hospitalized and outdoor patients. It is mainly found in females because of the shortness of the urethra and its closeness to anus which facilitate entrance of fecal micro-flora to urinary tract. Aim of the study was to investigate the bacterial uropathogens and their antibiotic susceptibility in a tertiary care hospital, Peshawar, Pakistan. A total of 200 urine samples were analyzed and cultured on Cysteine Lactose Electrolyte Deficient (CLED) medium. All the bacterial isolates were identified by biochemical tests. Out of 200 samples, 113 were positive and 87 were negative. In positive samples, 36(31.9%) were male and 77(68.1%) were female. Out of 113 patients 80(70.8%) were hospitalized and 33(29.2%) were walk in patients. Out of those 113 positive samples, *E. coli* was the dominant

uropathogen 77(68.1%) followed by *Staphylococcus aureus* 13 (11.5%), *Proteus* spp. 9(8.0%), *Pseudomonas* spp. 6(5.3%), *Klebsiella* spp. 4(3.5%), and methicillin resistant *Staphylococcus aureus* 4(3.5%). Antibiotic susceptibility test was performed by disc diffusion method according to Clinical Laboratories Standard Institute (CLSI). Bacterial isolates showed resistance to ampicillin (72.0%), ciprofloxacin (53.1%), norfloxacin (51.3%) and trimethoprim-sulfamethaxazole (53.1%). Bacterial spp. resistant to other antibiotics was also prevalent. Meropenem was the most effective antibiotic against all the bacterial isolates. In conclusion, high incidence of single and multiple antibiotic resistant bacterial strains is matter of enormous concern. Meropenem was the drug of choice to control urinary tract infections.

CORRELATION OF BLOOD ASCROBIC ACID AND CHOLESTEROL LEVELS IN MYOCARDIAL INFARCTION

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Acute myocardial infarction commonly known as, heart attack is due to an interruption in blood supply to the heart. This is most commonly because of the occlusion of coronary artery following the rupture of an atherosclerotic plaque. Ascorbic acid has suggested preventive role in coronary artery disease (CAD) by inhibiting the atherosclerotic plaque formation, either by decreasing the oxidant-induced injury to the arterial wall or by decreasing the oxidation of low density lipoprotein (LDL). Ischemic heart disease had become the leading cause of death worldwide by 2004. It would become the second most important cause of disability by 2030, predicted by WHO in 2008 in the Global Burden of Disease, study is designed to investigate the significance and correlation of plasma ascorbic acid and cholesterol levels in patients with myocardial infarction. Blood samples collected from 100 patients of myocardial infarction, recruited from Faisalabad Institute of Cardiology (FIC) Faisalabad, were analyzed for ascorbic acid, cholesterol, triacylglycerol and other enzymic antioxidants like SOD, catalase and peroxidase levels. A significant high level of oxidative stress was found as indicated by the marked lower levels of vitamin and enzymic antioxidants. The cholesterol and triacylglycerol contents were found above the upper limit of their normal range. So it was concluded that oxidative stress has a prominent role in myocardial infarction. The inverse correlation of ascorbic acid with cholesterol and marked low levels of ascorbic acid indicate its considerable significance in myocardial infarction.

RELATIONSHIP OF MELATONIN WITH CHRONIC PERIODONTITIS

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Melatonin is a hormone secreted by the pineal gland and many other organs in body. Basically melatonin is an antioxidant. It has an enhancing effect on the immune system. Melatonin is also involved in bone remodeling. The purpose of this study was as follows: (1) To determine a possible link between serum melatonin levels and chronic periodontitis. (2) To determine the decline of serum melatonin level with age. (3) To determine the level of melatonin in diabetic

patients with increasing severity of periodontal disease. In this study 70 subjects were grouped into control comprising (5 young, 5 old) the diseased group comprised (30 young, 30 old) suffering from chronic periodontitis with, CPI 3 and CPI 4. Then among these diseased groups we further studied the melatonin level of chronic periodontitis in CPI3 and CPI4, diabetic and non-diabetic, the relation of gender with melatonin level. Serum melatonin levels were assessed using enzyme-linked immunosorbent assay (ELISA). The independent t-test was applied to find out the significance difference between melatonin levels of different groups. Serum melatonin was significantly decreased in diseased subjects as compared to control. Levels of melatonin were decreased in old control subjects as compare to young control. Melatonin was decreased in code3 ($P < 0.000$) when compared to code4. Decline level of melatonin in female was observed as compared to male. While serum melatonin was high in old patients when compared to young patients suffering from chronic periodontitis. Melatonin was also increased in diabetic patient with chronic periodontitis. The results indicated that the serum melatonin level varied in different disease conditions and showed the protective role against the infectious diseases as an antioxidant, anti-aging and free radical scavenger. Therefore, melatonin could be used therapeutically in chronic periodontitis. However, further research is required in this field of study.

COMPARATIVE EFFECTIVENESS OF SULFONYLUREA AND THIAZOLIDINEDIONES IN DIABETES MELLITUS TYPE 2

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The objective of the present study was to explore the comparative effectiveness of sulfonylurea and thiazolidinedione in Diabetes Mellitus 2. A total of 30 patients having Diabetes Mellitus 2 were recruited in the present study. They were further categorized into 2 groups of 15 each. One group was taking antidiabetic drug, Sulfonylurea (group B) while the other group was taking Thiazolidinedione (group C). 15 gender and age matched control subjects (group A) were included for comparison. HbA1c levels of all individuals were checked at zero and 4th week of administration of antidiabetic drugs. Mean age of group A, B and C was 41.2 ± 6.4 , 40.7 ± 6.3 and 43.7 ± 6.6 , respectively. A statistically significant difference ($P < 0.05$) was observed between group A and B, group A and C while an insignificant difference was observed between group B and C. Both of the antidiabetic drugs showed an almost similar response in controlling the HbA1c levels in patients with Diabetes Mellitus 2. The results of the present study however need further validation.

PREVALENCE OF BABESIA IN BUFFALO OWING TO MANAGEMENT PRACTICES AND ASSOCIATED RISK FACTOR, PUNJAB, PAKISTAN

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The epidemiology of babesia prevalence was investigated with respect to various associated risk factor and management practices in buffalo of district Toba Tek Singh (T. T. Singh), Punjab, Pakistan from 2009-2010. A total 60 (12.47%) out of 481 examined cases were found positive for

babesia infection. Month wise peak prevalence was observed highest in July (23.08%), while no positive case was registered in Dec-2009 and Jan-2010. among associated risk factor, animal age, the breed and sex showed strong relation ($P<0.05$) with babesia infection, while buffalo body condition were found non significant ($P<0.05$). Among management practices, the feeding system, housing, system, floor type, herd size were also found significant ($P<0.05$) determinant for babesia prevalence and watering system used for buffalo paid no effect ($P<0.05$) on babesia prevalence. The results indicate that associated risk factor, management practices and weather condition have significant role in babesia prevalence in buffalo.

AMPLIFICATION AND CLONING OF ENVELOPE GENE FROM PATIENT INFECTED WITH DENV-2

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Infection with dengue virus (DENV) is considered as serious public health issue internationally. According to estimation, throughout the world about 2.5 billion people are at infection risk. At the same time spread of dengue virus continuously enhanced the economic burden in sense of disease management and preventive measures. Dengue is now endemic in Pakistan. During 2010 and 2011 two major outbreaks of dengue have been appeared in Pakistan. Till now, no licensed vaccine is available. Thirty Blood samples were collected from dengue infected patients which were positive based on IgM and IgG. Dengue virus type 2 envelope protein encoding gene was amplified by Polymerase Chain Reaction using specific set of primers. Amplified envelope gene was cloned and confirmation was carried out through restriction digestion and sequencing. Genotyping of dengue patients were carried out through Polymerase Chain Reaction. Envelope gene was then amplified from dengue patients infected with DENV-2. The envelope gene of 1.5 kb was successfully amplified and cloned in pTZ57R1T. The cloned gene was then confirmed through restriction digestion and sequencing which show a required band size of 1.5 kb. Envelope gene sequence may helps to develop therapy for the treatment of dengue patients infected with genotype 2.

A PROSPECTIVE EPIDEMIOLOGICAL STUDY OF CONGENITAL ANOMALIES AND NON-COMMUNICABLE DISEASES IN FEMALE POPULATION OF DISTRICT RAHIM YAR KHAN, SOUTHERN PUNJAB, PAKISTAN

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Congenital anomalies (CA) have both milder and severe phenotypic manifestations. On their severe phenotypic extreme they may cause serious morbidity and long term disability which dramatically affect the quality of life. CA usually have genetic/hereditary nature and early onsets and have been most frequently reported for neonatal and hospital based cohorts. However, their distribution and impact on the adult population has not been much appreciated; particularly no data are available for the Southern Punjab population. Non-communicable diseases (NCD) on the other

hand, commonly have late incidences and they also adversely affect a significant proportion of adult population. The distribution and dynamics of NCD remain largely ignored in Pakistan. In order to get an understanding of the spectrum of CA and NCD in Southern Punjab we have employed a prospective epidemiological study design in district Rahim Yar Khan (RYK). A structured questionnaire was designed to collect information regarding CA, NCD and other socio-demographic correlates of female population of the district RYK. A total of 2,204 married females originating from RYK were recruited after formal consent approval. In this sample, 54 subjects were observed to have certain kind of anomaly. There were 30 cases with CA and a prevalence rate was estimated to be 13.6/1,000. Among these CA, limb defects were most prevalent (n=11; 20.37%), followed by skin anomalies (n=8; 14.81%), deaf and mute cases (n=4; 7.41%), and hemoglobinopathies (n=3; 5.55%). The affection status of subjects was significantly associated with the tehsil-wise distribution and ethnicity. Additionally, CA were significantly higher in subjects with parental consanguinity. On the other hand, there were 24 cases of NCD with all estimated prevalence of 10.88/1,000. The most frequently observed acquired anomalies were diabetes (n=12; 22.22%), asthma (n=4; 7.41%), and hyperthyroidism (n=4; 7.41%), and these were significantly higher in subjects belonging to tehsil Sadiqabad, rural origin and speaking Punjabi language. This study is the first attempt to understand the spectrum of CA and NCD in this population and would be helpful to estimate morbidity and its associated mortality in the Southern Punjab population.

SURVEY OF HEPATITIS C PATIENTS IN PARACHINAR, KHYBER PAKHTUNKHWA, PAKISTAN

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A survey of HCV Patients in Parachinar, KP, Pakistan was performed from March 2010 to March 2011. Hundred positive HCV cases were selected and interviewed. The highest prevalence of HCV was found in males 36% and females 8% with the group aged 16-30 years. Prevalence of HCV infection in married males 55% and females 10%. As for as demographic wise distribution of HCV infection was examined, it was found that the population in rural area was more affected as in males 72% and females 10%. When socioeconomic condition of the patients belong to high class people examined, it was found in males 74% and females 7% affected with HCV. Prevalence of HCV was higher in those people who had formal education in males 60% and in females 10%. Risk factors of HCV patients from higher to lower range as share syringe were found in males 31% and females 7%, patients with surgical operations were in males 17% and females 12%. It was found that health care workers, in males 14% and no female was affected. Sexually transmission of HCV, in males 3% and females 2%. Symptoms of HCV found in patients are given in descending order: abdominal pain in males 67% and females 12%; fatigue in males 65% and females 12%; fever in males 64% and females 6%; yellow skin color in males 53% and females 7%; dark color urine in males 35% and females 7%; rashes in males 35% and females 6%; vomiting in males 21% and females 4%; pale color stool in males 19% and females 5%. The HCV was diagnosed by strip, ELISA and PCR methods. Strip method was performed by males 9% and females 3%. The ELISA was performed by males 74% and females 9%. The PCR was performed by only males 2%.

RECENT TRENDS AND ECONOMIC BURDEN OF TYPE 2 DIABETES MELLITUS IN NORTH PUNJAB, PAKISTANANDLEEB BATOOL¹, NUSRAT JAHAN¹, ATIF HANIF² AND HONG XUE³¹*Department of Zoology, Government College University, Lahore, Pakistan.*²*Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore* ³*Applied Genomic Centre, Hong Kong University of Science and Technology, Hong Kong*
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Type 2 diabetes mellitus is increasing rapidly in Pakistan, evident from prevalence studies. A limited data is available on epidemiology of diabetes in North Punjab, Pakistan. There is a need to study epidemiology of diabetes for better planning of prevention and control. Therefore, the current study was designed to assess the characteristics, care and economic burden of diabetes in North Punjab. Demographic characteristics, clinical statistics and cost of management were studied in 591 subjects (diagnosed with type 2 diabetes according to WHO criteria). Statistical analysis was performed using 'Kruskal Wallis', 'Mann Whitney U' and chi-square test. Diabetes was highest in middle age group with 61.42% of total subjects. Urban Men and women were at higher risk of disease development 10.69% and 21.14% with diagnosis at early age ≤ 35 years, while rural population 2.94% and 4.11% respectively at the same age. Overweight (Body Mass Index > 25) and family history of diabetes were strong predictors at early age with $P = 0.01$ and $P = 0.003$ respectively. Retinopathy and nephropathy were three times higher in rural population than urban residents. Average annual cost per patient was 210.92US\$ (19,157 PKRs) and insulin treatment was of higher cost ($P < 0.001$) in comparison to oral medication. Educated and higher income groups spending more on diabetes management ($P < 0.05$) than other groups. Urbanization, BMI, positive family history and hypertension were determinants of diabetes onset in younger age. Families were spending 12-20% of their income to manage the disease. Further studies are needed to develop a comprehensive program for the control and prevention of diabetes.

GENOTYPING OF HEPATITIS C VIRUS INFECTION IN PREGNANT WOMEN IN THE PROVINCE PUNJAB, PAKISTANIMRAN RIAZ MALIK¹, FARHEEN REHMAN¹, GHUJAMMUJTABA² AND MEHMOOD UL HASSAN QAZI¹¹*Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Pakistan*²*Institute of Nuclear Medicine & Oncology (INMOL), PAEC, Lahore*

Hepatitis C virus is a plus stranded RNA virus and belongs to Flaviviridae family. About 200 million people are infected with HCV worldwide, which covers about 3.3% of the world's populations. There are at least 6 different genotypes of HCV and more than 90 subtypes Hepatitis C virus (HCV) and its genotypes vary widely between populations and geographical areas. The research was aimed to investigate the presence of Hepatitis C virus (HCV) and its genotypes in pregnant women in Punjab, Pakistan. Pregnant women with the history of Anti-HCV positive were included in this study. Blood samples were collected from 72 HCV positive pregnant women. PCR (Polymerase Chain Reaction) was done for the confirmation of active HCV RNA. Genotyping was done using genotype specific primers. Fifty eight patients were positive for HCV - RNA through polymerase chain reaction. HCV genotyping was done for further identification of different HCV genotypes. This study showed high percentage of HCV genotype 3a (79%), followed by genotype

untypable (10%), 3b (4%), 1a (4%), 1b (2%) and mixed genotype (2%). The common prevalence of HCV genotype in pregnant women is 3a in Punjab, Pakistan.

AMPLIFICATION AND SEQUENCE ANALYSIS OF CORE GENE ISOLATED FROM HCV INFECTED PATIENT

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Hepatitis C virus is a plus stranded RNA virus and belongs to Flaviviridae family. About 200 million people are infected with HCV worldwide, which covers about 3.3% of the world's populations. In Pakistan, prevalence of hepatitis C virus (HCV) is found to be more than 10 million people. The main objective of this study was to amplified full length core gene and determines the nucleotide sequence. Blood samples were collected from HCV infected patients which were positive based on biochemical and ELISA. Viral RNA was isolated from HCV patients and cDNA was synthesized using Reverse transcriptase enzyme. Hepatitis C core protein encoding gene was amplified using cDNA as template through Polymerase Chain Reaction. Amplified core gene was confirmed through sequencing. The polymerase chain reaction was carried out to amplify the core gene using two sets of primers. PCR product was run on 1.5% agarose containing ethidium bromide as staining solution and results showed that a target band of 573bp in size was achieved. The amplified product of core gene was then confirmed through sequencing. Full length core gene sequence of HCV may be helpful to developed genotype specific therapy.

EPIDEMIOLOGICAL, SEROLOGICAL AND MOLECULAR ANALYSIS OF HCV IN DIFFERENT RISK GROUPS

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Hepatitis C is an infectious disease affecting the liver caused by hepatitis C virus (HCV). A total 50 OPD subjects from INMOL were divided into four different risk groups. Group I (Intravenous drug use), Group II (Blood Transfusion), Group III (Sexual Activity) and Group IV (Haemodialysis). Blood samples were drawn and centrifuged at 2000g for serum separation. Epidemiological, serological and molecular analysis was studied of all risk groups. For determination of aminotransferases (ALT, AST) ready to use (Bio kit)-USA was used. Among 50 subjects; 7 (12%) and 5 (10%) had elevated level of ALT and AST, respectively; while 45 (90%) were ELISA positive, 32 (64%) were Anti-HCV positive and 26 (52%) were PCR positive. Taken together these findings we can conclude that PCR was found to be very specific and sensitive method to evaluate the presence of HCV infection.

HIGH DENSITY LIPOPROTEIN STATUS IN DIFFERING THYROID DISEASE STATUS IN PAKISTANI POPULATION

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Thyroid hormones regulate the metabolism of lipoproteins. Consequently altered thyroid gland activity affects whole body cholesterol homeostasis by manipulating plasma levels of low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C). Pakistani populations considered vulnerable in thyroid gland activity due to reduced iodine availability. There is increasing incidence of cardiovascular diseases in Pakistani population due to changes in life styles. The change in life style and vulnerable thyroid status may be setting a new adaptation in lipoproteins of local population. The aim of present study was to investigate the serum HDL cholesterol (whether reduced or increased) in differing states of thyroid gland activity. Serum HDL-C status after that treatment of thyroid dysfunction states was also one aspect of this study. Serum thyroid hormones were assayed by the radioimmunoassay. Serum HDL-C was measured by direct homogenous assay. In patients with subclinical hypothyroidism, serum HDL-C was slightly increased than controls (53.5 ± 3.6 vs 51.6 ± 3.42 ; P greater than 0.05). In hypothyroid state, the condition originates because of medication of hyperthyroidism, level of HDL-C was decreased (45.1 ± 3.32 vs 53.5 ± 3.62 ; P greater than 0.05). Thyroxine treatment decreased the HDL-C in hypothyroid subject in comparison to the disease state (45.5 ± 3.33 vs 53.5 ± 3.62 ; P greater than 0.05). Hyperthyroidism was associated with slight decrease in the concentration of HDL-C than the controls (49.4 ± 5.3 vs 51.6 ± 3.42 ; P greater than 0.05). Treatment of hyperthyroidism significantly decreased the level of HDL-C (37.5 ± 3.2 vs 49.4 ± 5.3 ; P less than 0.05). It was concluded from this study that hypothyroidism and hyperthyroidism have opposite effects on serum HDL-C. Hyperthyroidism results in a decreased HDL-C, while hypothyroidism resulted in increased HDL-C. Effect of both treatment of hypothyroidism and hyperthyroidism resulted in decreased HDL-C.

6. MICROBIOLOGY

MOLECULAR ANALYSIS OF *cueO*, A MULTI-COPPER OXIDASE IN LOCALLY ISOLATED COPPER RESISTANT *KLEBSIELLA PNEUMONIAE*

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CueO is a multicopper oxidase that takes part in copper homeostasis and resistance in members of Family Enterobacteriaceae. This protein protects periplasmic proteins from copper induced toxicity by converting copper from more toxic Cu⁺ to less toxic Cu⁺⁺ form. *cueO* (1.653 kb) has been amplified from *Klebsiella pneumoniae* KW and cloned in pTZ57R cloning vector followed by sequencing. Comparative analysis of the Sequence obtained through various bioinformatical tools viz. BLAST, multiple alignment and construction of neighbour joining tree revealed its scope of abundance and phylogenetic relationship with already known CueO sequences. Three dimensional structure was determined on the basis of homology modeling that was predominated by β strands with a few α helices. For expression analysis through IPTG induction, *cueO* gene was initially amplified with *Nde* I site at 5' end and cloned. Its expression profile will be studied in pET system in BLC C⁺.

TRANSFORMATION OF *STAPHYLOCOCCUS AUREUS* USING ELECTROPORATION TECHNIQUE THROUGH YEAST LYSATE

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In the present study, the research work was carried out on bacteria *Staphylococcus aureus* strain ATCC25923 and yeast *Saccharomyces cerevisiae*, the work was done to transform *S. aureus* from sensitive and non-virulent form to resistant and virulent form. The *S. aureus* pure culture was obtained from Veterinary University, Lhr and the colonies were obtained by spreading and streaking. The yeast colonies were also obtained by spreading and streaking. Different antibiotics were used and bacterial strain *S. aureus* showed maximum sensitivity against antibiotic AM10 and zone of inhibition was 17mm. while yeast showed resistance against AM10. The yeast plasmid was isolated. Electroporation of *S. aureus* was done in order to make the bacterial wall porous and to able bacterial DNA to accept foreign DNA or plasmid. The bacterial strain *S. aureus* after transformation became resistant against antibiotic AM10. *S. aureus* is Gram positive catalase +ve coccus which is the common bacteria in the environment. Electrocompetent cells of bacterial strain were prepared and yeast lysate was also prepared to isolate yeast genomic DNA. It was observed that after introducing yeast plasmid in bacterial strain ATCC25923, the bacterial strain became resistant to antibiotic and this genetic modification was carried out using the process of electroporation.

NATURAL THERAPY FOR MULTIDRUG RESISTANT ISOLATES

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Bacteriocins are well known for their antibacterial activity against Multidrug resistant strains (MDRs). *Lactobacilli* are known friendly bacteria for their antibacterial activities against pathogens. The antibacterial activity of different strains of *Lactobacilli* was analyzed against Multidrug resistant strains (MDRs). Objectives: (1) To screen the Multidrug resistant strains (MDRs). (2) To characterize the putative bacteriocins produced by selected bacteria (*Lactobacilli*). (3) To analyze the peptide component of the bacteriocins. (4) To check the antibacterial effect of putative bacteriocins. Well-Diffusion assay (Valgas *et al.*, 2007) was used for screening of putative bacteriocins produced by *Lactobacillus* strains against MDRs. Multidrug resistant strains were selected based on MAR (Multiple antibiotic resistance) index. Five bacteriocins obtained from *Lactobacillus* strains isolated from commercial products. These bacteriocins showed a strong antibacterial activity against selected MDRs. Decrease in zone sizes was observed when putative bacteriocins were treated with heat, SDS and Protinase k. It was observed that *Lactobacillus* showed a significant antibacterial activity *in-vitro* in the presence of putative bacteriocins against selected MDRs and further experiments are underprocess. Putative Bacteriocins produced by *Lactobacilli* exhibit significant antibacterial activity against MDRs. The peptidal component of these bacteriocins can be used as an alternative therapy. Hence, it is necessary to purify the antibacterial molecule out of putative bacteriocin for further analysis.

SCREENING OF *IN VITRO* BIOLOGICAL ACTIVITIES OF *CITRUS SINENSIS* PEEL AGAINST HUMAN BACTERIAL PATHOGENSBASHARAT MEHMOOD¹, UZMA AZEEM AWAN¹, TAHSEEN GHOU², SHAUKAT ALI¹
AND SAIQA ANDLEEB^{1*}¹*Biotechnology lab, Department of Zoology, The University of Azad Jammu and Kashmir, Muzaffarabad,* ²*Biochemistry lab, Department of Chemistry, The University of Azad Jammu and Kashmir, Muzaffarabad,**Corresponding author: drsaiqa@gmail.com

The antibacterial effect of peel extracts of *Citrus sinensis* was evaluated against several human bacterial pathogens including *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Staphylococcus epidermidis*, and *Serratia marcescens*. The solvents used for plant extractions were methanol, ethanol, chloroform and diethyl ether. Aqueous extract was also used. The *in vitro* antibacterial activity was analyzed by agar disc diffusion method and it was found that polar solvents had highly significant effect against *E. coli* and *K. pneumonia*. ABTS·+ free radical scavenging activity indicated that methanol and ethanol peel extracts had significant antioxidant activity. The potential antibacterial activity of active extracts was compared with the standard antibiotic through activity index. The results obtained in this study suggest that peels of *Citrus sinensis* could be used to treat diseases caused by the test microorganisms.

ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF EXTRACTS OF *EISENIA FOETIDA* AGAINST HUMAN BACTERIAL PATHOGENS

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Earthworms are macroinvertebrate and exist as the principal soil biomass. They have been widely used as therapeutic drugs for thousands of years. Therapeutic properties of earthworms including anti-inflammatory, anti-pyretic, anti-tumor, haemolytic and proteolytic activities are well known. In the present research work, experiments were conducted to investigate for the first time the antibacterial, antifungal and antioxidant activity of mucus and organic solvent extracts of *Eisenia foetida* against human bacterial and fungal pathogens. Antibacterial and antifungal activity of mucus and organic solvent extracts of *E. foetida* against human bacterial pathogens underwent investigation through an agar disc diffusion method while an ABTS+ free radical scavenging method assessed antioxidant activity. Results showed that the mucus extract of *E. foetida* produced a strong potent antibacterial activity against all tested bacterial pathogens as compared to the organic solvent extracts. The results clearly indicate that the mucus and solvent extracts contain effective antibacterial properties and bioactive compounds to inhibit the growth of bacteria and fungi. We conclude that mucus extracts of earthworm exist containing significant levels of antimicrobial activity and with future research could potentially be used against various infectious pathogens.

EVALUATION OF ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF *CITRUS SINENSIS* PEEL AGAINST FISH BACTERIAL PATHOGENS

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Antibacterial activity of different solvent extracts (fresh aqueous, methanol, ethanol, chloroform and diethyl ether) prepared through conventional solvent extraction method of *Citrus sinensis* peel were screened against fish bacterial pathogens. These pathogens were *Salmonella typhimurium* (KL1), *Shigella flexneri* (KL2), *Enterobacter amnigenus* (F1), and *Serratia odorifera* (F). Antibacterial activity was evaluated using agar disc diffusion method. The maximum antibacterial activity was exhibited by methanolic extract of *C. sinensis* against *Serratia odorifera* (10.0±2.16 mm) followed by other extracts against all tested bacterial pathogens. ABTS+ free radical scavenging activity indicated that methanol and ethanol peel extracts had significant antioxidant activity of 60.7% and 55.8%. *Citrus sinensis* extracts could be efficiently used against these tested pathogens as compared to standard antibiotics. This study may lead to the formulation of an antimicrobial drug and can be used as a potent natural antioxidant additive.

INHIBITORY EFFECT OF GARLIC (*ALLIUM SATIVUM*), GINGER (*ZINGIBER OFFICINALE*) EXTRACTS AND HONEY ON CLINICALLY IMPORTANT PATHOGENIC BACTERIA

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Herbs and spices have offered an excellent, important and useful source of antimicrobial agents against many pathological infections. In the present study the antimicrobial potency of fresh, naturally dried and commercial garlic (*Allium sativum*), ginger (*Zingiber officinale*) extracts and honey had been investigated against seven local clinical bacterial isolates. Five types of extracts of each garlic and ginger including aqueous, methanol, ethanol, chloroform and diethyl ether extracts had been assayed separately against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Staphylococcus epidermidis*, and *Serratia marcescens*. The antibacterial activity was determined by agar disc diffusion method. All tested pathogens were most susceptible to the garlic extracts of ethanol and methanol except *P. aeruginosa* and *E. coli*. Similarly, mostly chloroform and diethyl ether extracts of *Z. officinale* showed greater zone of inhibition against tested pathogens except *P. aeruginosa* and *E. coli*. 100% pure natural honey (H) showed strong zone of inhibition of diameter 41.6, 32, 43.3, 44.33, 32 and 43.33 mm against all tested human associated pathogens except *S. aureus* which showed moderate inhibition of 24.33 mm. In addition, synergistic effects when associated with MIC of honey were also studied. We found that all extracts of *A. sativum* and *Z. officinale* had strong antibacterial effect compared to recommended antibiotics through activity index analysis. All results were evaluated by ANOVA and LSD tests at $P < 0.05$. Antioxidant activity of extracts showed that many of these extracts had high scavenging potential. Thin layer chromatography profiling of all extracts of *A. sativum* and *Z. officinale* gave an idea about the presence of various phytochemicals such as tannins, phenols, alkaloids, steroids and saponins. Various R_f values of diverse phytochemicals provide valuable clue regarding their polarity and the selection of solvents for separation of phytochemicals. Significant inhibition of *S. aureus* was also observed through TLC-Bioautography. These results suggest that both *A. sativum* and *Z. officinale* are potential source of plants for controlling pathogenic bacteria when combined with honey and should be considered to decrease the cost in the management of diseases and threat of drug resistance.

IN VITRO SCREENING OF *ALLIUM SATIVUM* AND *ZINGIBER OFFICINALE* AGAINST SPOILED FISH PRODUCTS BACTERIAL PATHOGENS

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The antibacterial activity of *Zingiber officinale* and *Allium sativum* extracts against fish bacterial pathogens was studied. However, studies on antibacterial activity of garlic and ginger extracts against pathogenic bacteria in animals in Pakistan are still limited. Therefore, the aim of this study was to evaluate the antibacterial activity of garlic and ginger extracts along with

minimum inhibitory concentration (MIC) of honey against *Shigella flexneri*, *Enterobacter amnigenus*, *Salmonella typhimurium*, and *Serratia odorifera*. It was found that ethanol and methanol extracts of both natural and commercial dried *A. sativum* showed significant results against *Shigella flexneri* with maximum zone of inhibition (16 ± 6.0 mm, 17 ± 1.4 mm, 18 ± 3.0 mm and 15 ± 2 mm). Similarly, ethanol and methanol extracts of both natural and commercial dried *Z. officinale* showed significant results against *Enterobacter amnigenus* with maximum zone of inhibition (19 ± 0.81 mm and 12 ± 1.4 mm). Moderate zone of inhibition with MIC of honey was recorded against *Serratia odorifera* by NC, ND, and NE (10 ± 0.81 mm, 10.6 ± 1.24 , and 11.3 ± 1.24 mm), *Enterobacter amnigenus* by NC (8 ± 2.16 mm), and *Shigella flexneri* by NC and ND (10 ± 2.4 mm and 10 ± 0.81 mm). The synergistic effect of garlic and ginger along with MIC of natural honey was also measured against *S. flexneri*, *E. amnigenus*, *S. typhimurium* and *S. odorifera*.

STUDY OF ANTIOXIDANT, ANTIBACTERIAL AND PHYTOCHEMICAL ACTIVITY OF MINT (MENTHA SPICATA) AND BASIL (OCIMUM BASILICUM) AGAINST HUMAN PATHOGENS

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The antibacterial, antioxidant activities and phytochemicals of *Mentha spicata* and *Ocimum basilicum* extracts were evaluated with antibiotic susceptible and resistant bacterial pathogens. The antibacterial property of plant extracts were determined by agar disc diffusion method against microorganisms viz., *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Staphylococcus epidermidis*, and *Serratia marcescens*. The highest zone of inhibition of diethyl ether, chloroform and aqueous extracts of *M. spicata* was recorded against *E. coli* (43.33 ± 1.52 mm, 42.66 ± 2.51 mm) and *S. epidermidis* (32.33 ± 2.51 mm). Similarly, methanolic, chloroform and n-Hexane extract of *O. basilicum* showed significant zone of inhibition against *P. aeruginosa* (32.33 ± 2.51 mm, 39 ± 3.60 mm) and *S. pyogenes* (35 ± 0 mm). In addition, synergistic effects of *M. spicata* and *O. basilicum* when associated with MIC of honey were also studied. Phytochemical analysis indicated the presence of flavonoids in the crude extracts of *Mentha spicata*. Bioautography indicated significant effect on *S. aureus*. This study recommended these medicinal plants as alternative anti-infective agents in natural medicine for the treatment of infectious diseases.

MICROBIAL DIVERSITY IN INDUSTRIAL WASTEWATER AND THEIR POTENTIAL TO DEGRADE PHENOL

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Textile industrial sector is one of the most important and largest industrial sectors of Pakistan. Most of the textile industries contain phenol in their wastewater, Analysis show that phenol has serious health hazard when exposure take place to living beings. Most of the Bacteria present in industrial wastewater were critical for decomposing phenols and phenolic compounds;

they also are a valuable resource of novel metabolic abilities useful for industrial processes. The present study was conducted on textile industry wastewater, 8 strains were isolated from Chenab textile mill's wastewater. Tests were conducted to check either these strains were capable of degrading phenol or not, and what was the diversity of bacteria in wastewater. The isolates were subjected to phylogenetic analysis as well. Phenol estimation was done by Clesceri *et al.* (1998). It was found that only Chen9_27F belongs to pseudomonas specie and was able to utilize phenol as a sole source of carbon. To check microbial diversity DNA was isolated by phenol chloroform method and PCR was performed using universal primer B27F and U1492R whereas, sequencing was performed by MacroGen sequencing company (Korea). Diversity of microbes was evaluated by Phylogenetic Web Repeater (POWER) online available tool having POWER version 1.0 and phylip package version 3.5 and ClustalW version 1.83. Multiple sequence alignment was performed by ClustalW tool and multiple sequence alignment results showed that sequences shared 63.125% homology and dendrogram was constructed. None of the sequences have chimera region to check this Bellerophon (version 3.0) was used. Further work should be conducted in order to get microbes that help us in cleaning our environment on no wages.

MICROBIOLOGICAL INVESTIGATION OF AGRICULTURE SOIL AND EDIBLE VEGETABLES FOR HUMAN PATHOGENIC BACTERIA AND PARASITIC CONTAMINATION

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In rural areas of Pakistan, human excreta (fecal waste) from composting bio-toilets are applied to agriculture soil to increase soil fertility for various crops and vegetables. Given the variety of human pathogenic bacteria and parasites found in fecal waste and contaminated vegetables, this fertilization method, is of great concern to public health. The present study aimed to determine the bacteriological and parasitological load of human waste manure in soil and vegetables and to determine the sensitivity pattern of isolated bacteria for various antibiotics. The study was conducted in Oshikhandass valley of Gilgit-Baltistan. Samples were collected from seven sites of the study area and aseptically cultured by serial dilution on MacConkey agar, S.S. agar medium in the laboratory. Parasitological contamination was analyzed NaCl₂ saturated method and wet mount preparation technique. Seventy soil and vegetables samples were analyzed. The mean bacterial contamination in the soil samples was 9.23×10^5 cfu/g of sample, while the mean bacterial contamination in vegetables was 6.72×10^5 cfu/g of sample. The highest bacterial contamination was found for *Trifolium* spp, which was 6.33×10^3 cfu/g, followed by *Brassica* spp at 6.013×10^3 cfu/g, *Malva Sylvester's* at 5.99×10^3 cfu/g and *Lactuca* spp, and *Alium cepa* 5.68×10^3 cfu/g of sample. Out of 70 soil samples, 48 (68.57%) were positive for different human intestinal parasites such as *Ascaris Lumbricoid*, *Tricuris tricura*, *H. Nana*, *Gardia lumblia* Vegetative and *Entamoeba histolytica*; 32(45.71%) vegetables were positive for multiple parasites. The most common bacteria isolated from soil and vegetables samples was *E.coli*, *Salmonella*, *Proteus vulgaris*, *Staphylococcus aureus* and *Pseudomonas* sp. Most of the isolated bacteria were resistant to Ampicillin, Amoxicillin and Cephadrine while Ciprofloxacin, Cefixim and Azithromycin were the most effective antibiotics against isolated bacteria.

ASSESSMENT OF GASTRO-INTESTINAL PARASITES IN PIT LATRINE SAMPLES IN OSHKANDAS VALLEY, GILGIT-BALTISTAN, PAKISTAN

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Intestinal parasitic infections represent a global health problem. Specifically in developing countries, numerous environmental and socioeconomic factors have been identified to be main cause for the continued persistence of gastrointestinal infections. The present study was carried to assess the intestinal parasitic load (*Ascaris lumbricoid*, *Trichuris trichiura*, *Giardia lamblia* and *cryptosporidium*) in the rural village Oshkandas, Gilgit-Baltistan in ten pit latrine samples. The sample collection were analyzed in monthly intervals (October 2011 to Feb 2012) by NaCl₂ saturated solution flotation technique. The load of parasites in one gram sample ranged as; October *Ascaris lumbricoid* (16.67-33.34), *Trichuris trichiura* (2.66-4.00), *Giardia lamblia* (3.3-16.7) and *Cryptosporidium* (6.7-20.7); November (16.7-40), (8.7-7), (0-10) and (9.3-22.3); December (16-26.3), (0-4.7), (6.7-16.7) and (6.7-16.7); January (16.7-50.0), (5.00-6.7), (1.7-8.3) and (6.7-10.0) & February (25.0-56.7), (6.7-23.3), (3.3-16.7) & (6.7-20.0) respectively in one gram of sample. This study showed that *Ascaris lumbricoides* to have the highest load among the targeted parasites, followed by *Trichuris trichiura*, *Giardia lamblia* and *Cryptosporidium*.

CHARACTERIZATION AND ESTIMATION OF BIODEGRADATION POTENTIAL OF BACILLUS STRAINS ISOLATED FROM CRUDE OIL CONTAMINATED SOIL

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Screening of aerobic, spore-forming culturable hydrocarbon degrading bacteria from crude oil contaminated soil resulted in the collection of 15 strains. Careful phenotypic and biochemical characterization was done and 07 strains were selected for their biodegradation potential of crude oil fractions and mixtures (*n*-Hexane, benzene, petrol, kerosene, diesel, crude and engine oils). Two potential biodegraders (H8 & H9) from above 07 strains were subjected to PCR identification of the 16S rRNA gene using 27F and 1522R universal primers and showed high similarity with *Bacillus cereus* and *Bacillus pumilus* respectively. Biodegradation potentials of these isolates were evaluated by applying individual stain to 100 grams of sterilized soil contaminated with 10% crude oil at 37°C for 14 days in duplicates. Residual oil concentration was observed after every 48 hours by *n*-Hexane extraction method. Two controls, Positive with both microbial isolates and Negative with no microbial strain were also designed, Positive control showed the maximum 45.66% crude oil biodegradation till day 14 followed by H9 (41.71%), H8 (41.67%). The results show promising biodegradation of crude oil and its fractions by the indigenous microorganisms belonging to genus *Bacillus*, suggesting application of aerobic, spore-forming bacteria for effective biodegradation processes.

ROLE OF FIMBRIAE ADHESIONS OF ENTEROTOXIGENIC *ESCHERICHIA COLI* IN PATHOGENESIS AND BIOFILM FORMATION

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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.

PROCESS DEVELOPMENT TO OPTIMIZE THE ANTIBACTERIAL ACTIVITY OF *EUCALYPTUS TERETICORNIS* LEAF EXTRACTS AGAINST CLINICALLY IMPORTANT STRAIN *ESCHERICHIA COLI* USING RESPONSE SURFACE METHODOLOGY

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The present project was design to optimize the antibacterial activity of methanolic leaf extract of *Eucalyptus tereticornis* against clinically important strain *Escherichia coli* by using response surface methodology. The dry powdered extract of *Eucalyptus tereticornis* was dissolved with solution of dimethyl sulfoxide (DMSO) in order to prepare a stock solution. *Eucalyptus*

tereticornis have been used in order to obtain the methanolic extract for the in vitro study of antimicrobial activity. The strain was kept preserved at 4°C on nutrient agar. The strain *Escherichia coli* was used for the experiment. Experimental range and level of independent variables such as the extract of *Eucalyptus tereticornis* derivatives i.e., Limonene (X₁), 1, 8-Cineole (X₂), Terpinen-4-ol (X₃), pH (X₄) and Temperature (X₅) were studied at different levels 2µl-18µl, (6,7 and 8) for pH and temperature (37°C, 40°C and 42°C). Total 25 runs (a series of tests) in three blocks were performed to optimize the antibacterial activity of the experiment. Minitab statistical software release 15 is used for analysis of data and generation of response surface graphics. The results on the antimicrobial activity of medicinal methanolic extracts showed that all the concentrations were effective against tested microorganism with different zones of inhibition. The diameter of zones of inhibition exhibited by *E. coli* (26-32 mm). In all the cases, concentration of extract and effect of pH explained a significant variation in zones of inhibition. It is concluded that *in vitro* analysis of the extracts from fallen leaves of *Eucalyptus tereticornis* exhibited significant zones of inhibition against the *E. coli*. These encouraging results indicates that these ethno medicines have great antimicrobial potential and might be exploited as the natural antibiotics for the treatment of the several infectious diseases caused by the bacterial strain.

PROCESS DEVELOPMENT TO OPTIMIZE THE ANTIBACTERIAL ACTIVITY OF AZADIRACHTA INDICA LEAF EXTRACTS AGAINST CLINICALLY IMPORTANT STRAIN (*ESCHERICHIA COLI*) USING RESPONSE SURFACE METHODOLOGY

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The present study was design to determine the antibacterial activity of methanolic leaf extract of *Azadirachta Indica* against clinically significant strain *Escherichia coli* by using response surface methodology (RSM). The dry powdered extract of *Azadirachta Indica* was dissolved with solution of dimethyl sulfoxide (DMSO) in order to prepare a stock solution. DMSO was takes as 0.5g/ml. *Azadirachta Indica* have been used in order to obtain the methanolic extract for the *in vitro* study of antimicrobial activity. The strain *Escherichia coli* was used. Experimental range and level of independent variables such as the extract of *Azadirachta Indica* derivatives i.e., Azadirone (X₁), Gedunin (X₂), Azadiradine (X₃), pH (X₄) and Temperature (X₅) were studied at different levels 2µl-18µl, (6,7 and 8) for pH and temperature (37°C, 40°C and 42°C). Minitab statistical package (v. 15) was used for analysis of data and response surface graphs. The results on the antimicrobial activity of medicinal methanolic extracts showed that all the concentrations were effective against tested microorganism with different zones of inhibition. The diameter of zones of inhibition exhibited by *E. coli* (27-33mm). Concentration of extract of *Azadirachta Indica* and effect of pH showed a significant variation in zones of inhibition. The encouraging results indicates that these ethno medicines have great antimicrobial potential and may be used as the natural antibiotics for the treatment of the several infectious diseases caused by the bacterial strain and the synergistic effect from the association of different plant extract against pathogenic bacteria leads to the new choices for the treatment of infectious diseases. It would be advantageous to standardize methods

of the extraction and *in vitro* testing so that the search could be more systematic and interpretation of the results would be facilitated.

POLYHYDROXYBUTYRATE PRODUCTION BY *ENTEROBACTER AEROGENES*

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There has been considerable interest in the development and production of biodegradable polymer to solve the problem of pollution caused by the continuous use of synthetic polymer of petroleum origin. Polyhydroxyalkanoates (PHAs) are known to be accumulated as intracellular inclusion in some bacteria. They are accumulated intracellularly as carbon and energy reserves under certain conditions like, in the presence of different carbon sources and other nutrients. For PHB production bacterial strain *Enterobacter aerogenes* have been selected in this study. The maximum PHB yield was recorded under the cell dry weight basis respectively with molasses as the sole carbon source at P^H 7 and temperature 37°C for 48 hours. *E. aerogenes* has been screened for PHB production by Sudan Black B staining method. Polyhydroxybutyrate (PHB) has been extracted and purified by acetone-chloroform method. The maximum PHB production on CDW basis was 7.44 ± 0.20. The PHB biosynthesis from molasses (2.5%) was observed as 57.61±0.57% and 58.07±0.25% respectively at pH 7.0, 37°C and 150 rpm. Purification, quantification and analysis of functional group of PHB has been carried out by using HPLC. Thus present study has confirmed that *E.aerogenes* is a producer of PHB under optimal conditions and accumulate PHB granules within cell with low cost process.

CURRENT PATTERN IN ANTIMICROBIAL SUSCEPTIBILITY OF *SALMONELLA TYPHI* AND *SALMONELLA PARATYPHI A* AT A TERTIARY CARE HOSPITAL

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The prevalence of typhoid fever caused by *S. typhi* and *S. paratyphi A* in the community as well as hospital was determined in males and females belonging to different age groups. The bacterial isolates are collected from indoor and outdoor patients visiting or admitted at Military Hospital (MH) and Combined Military Hospital (CMH), Rawalpindi with typhoid or were suspected for typhoid. The antibiotic susceptibility test of selected *Salmonella typhi* and *Salmonella paratyphi A* was determined against 10 antibiotics commonly used for treatment of typhoid in hospitals and general practice. Overall, multi-drug resistance was observed in clinical isolates of *Salmonella typhi* and *Salmonella paratyphi A*, however the highest susceptibility was found against cefixime (100 per cent) and the highest resistance was observed against nalidixic acid (64 per cent). The susceptibility of these drugs varied greatly. The minimum inhibitory concentration (MIC) levels of *Salmonella typhi* and *Salmonella paratyphi A* for nalidixic acid was above the range of susceptibility > 128µg/ml. A total of 150 typhoidal salmonellae (isolated from patients blood) classified as fluoroquinolone susceptible according to the CLSI guidelines containing two distinct

subpopulations, with the majority of the salmonellae ~85 being ciprofloxacin sensitive (MIC $\leq 0.06\mu\text{g/ml}$) and a minority ~65 being low-level ciprofloxacin resistant (MIC $\geq 0.125\text{--}0.25\mu\text{g/ml}$) were evaluated. MIC levels of *S.typhi* showed a relatively high resistance rates against nalidixic acid, followed by ciprofloxacin. However, MIC level of *S.paratyphi A* also showed a resistance rates against nalidixic acid and in case of ciprofloxacin, all the isolates being inhibited within the susceptibility range. Thus screening with a $30\mu\text{g}$ nalidixic acid disk could be used to determine the low ciprofloxacin susceptibility

STUDIES ON THE EFFECT OF ACTIVATORS AND STABILIZERS ON ALKALINE PROTEASE FROM *BACILLUS SUBTILIS*

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The present study gives an insight into the effect of different enhancers and inhibitor on the activity and stability of alkaline proteases by *Bacillus subtilis* IH-72. The alkaline protease was strongly activated both by bivalent and monovalent cations such as Mg^{2+} , Mn^{2+} , Na^+ and K^+ . The enzyme activity was considerably enhanced in the presence of fructose, galactose, glucose and mannitol. The enzyme was stabilized up to 10 days by immobilization on activated charcoal and was efficiently stabilized up to 2 months by lyophilization. The enzyme remained stable up to 19 days both at 4°C and 30°C in the presence of Mn^{2+} . However, it exhibited significant stability up to 22 days at 4°C and 30°C in the presence of fructose, galactose and polyethylene glycol.

ISOALANTOLACTONE INDUCES OXIDATIVE STRESS IN PANCREATIC CARCINOMA PANC-1 CELLS BY DEPLETING INTRACELLULAR GSH

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In our previous report, we showed that isoalantolactone, a sesquiterpene lactone compound induced apoptosis in pancreatic carcinoma PANC-1 cells via reactive oxygen species (ROS) production and mitochondrial dysfunction. However, the pre-mitochondrial mechanism involved in ROS production and apoptotic cell death remained largely unexplored. The present study was therefore conducted to investigate the mechanism of ROS production by isoalantolactone in PANC-1 cells. We found that isoalantolactone induced oxidative stress in PANC-1 cells via glutathione (GSH) depletion. This isoalantolactone-induced GSH depletion, ROS production and apoptotic cell death were effectively inhibited or abrogated by N-acetyl-L-cysteine (NAC), a GSH precursor molecule. Pretreatment of cells with methionine, polyethylene glycol superoxide-dismutase (PEG-SOD) and PEG-Catalase failed to inhibit isoalantolactone-mediated GSH depletion indicating that GSH depletion is not associated with GSH extrusion and ROS production, respectively. The level

of GSSG was comparable in control and treated cells and the expression of glutathione reductase (GR) was not inhibited by isoalantolactone. The data demonstrated clearly that GSH depletion is not resulted from excessive ROS production. Next, we measured the mRNA expression of γ -glutamylcystein synthetase (γ -GCS). No change in mRNA expression of γ -GCS was observed. Incubation of isoalantolactone with GSH resulted in decreased concentration of isoalantolactone with increasing concentration of GSH. These sets of data demonstrate clearly that isoalantolactone depletes GSH in PANC-1 cells via direct conjugation with GSH which results in oxidative stress and mitochondrial mediated apoptosis.

CULTIVATION OF DIFFERENT HEAVY METALS RESISTANT *BACILLUS* SPECIES ON FRUIT PEELS

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Heavy metals pollution of water and soil habitat is consequent of industrial sector. Recalcitrant pollutants and their health and environmental deteriorative effects have necessitated remedial measures. Bioremediation is an environmental friendly way of detoxifying pollutants. In the present study, five bacteria *i.e.*, *Bacillus merisflavi*, *Bacillus amyloliquefacien*, *Bacillus safensis*, *Bacillus nelsonii* and *Bacillus megaterium* known to resist heavy metals Pb, Cu, Hg and Cr in the range of 1200 to 1250, 850 to 900, 45 to 50 and 1400 to 1600 $\mu\text{g/ml}$, respectively were grown on dried peels of watermelon, mango, apple, bagasse, potato, banana and their combinations to check their comparative growth potential. Growth conditions were characterized with 0.2% of a waste substrate, pH 7, incubation at 37 °C for 24 hrs. Growth assessment through OD_{600nm} indicated that all the bacterial species have potential of cultivation in these substrates. Maximum growth (OD_{600nm}) was recorded for *B. merisflavi* (0.241 \pm 0.001), *B. amyloliquefacien* (0.152 \pm 0.001), *B. safensis* (0.087 \pm 0.001247), *B. nelsonii* (0.061 \pm 0.002325) and *B. megaterium* (0.255 \pm 0.001656) in watermelon peels. These results show that the environmental burden of solid waste can be reduced by employing the agricultural byproduct/waste as a substrate for the heavy metals resistant bacterial growth.

IDENTIFICATION OF ANTIMICROBIAL ACTIVITY EXHIBITING BACTERIA FROM FRUITS AND VEGETABLES

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Emergence of resistance against antimicrobials has lead to an active research in the field of new antimicrobials. The antimicrobials may be natural or synthetic. Antimicrobial compounds are produced by certain bacteria as their own defense mechanism but could be used as either therapeutic, prophylactic measure in humans and animals or as food preservative in food industry. The objective of current study was to identify and characterize the antimicrobial activity possessing bacteria from fruits and vegetables. The antimicrobial substance producing bacteria were isolated from fruits and vegetables. Their antimicrobial potential was investigated by the disc diffusion and well diffusion method. The isolates possessing antimicrobial potential were identified by the

morphological and biochemical characterization. Antimicrobial activity of each isolate was determined under different conditions of temperature, pH and storage at 4°C. The antimicrobial potential was checked against Methicillin resistant strains of *Staphylococcus aureus* (MRSA), *Lactobacillus delbruecki*, *Lactobacillus fermenti* and *Lactobacillus casei* (vegetables) and *Staphylococcus* sp. (apple) were isolated. All of these strains except *Staphylococcus* inhibited the growth of MRSA isolated from contact lens. All isolates display optimal activity at 37°C and 5-6 pH. The antimicrobial compounds may be isolated and purified from broth cultures of the isolates identified and characterized in present study. Further studies are required to chemically identify the antimicrobial compound and investigate its potential either in health or preservation industry.

EVALUATION OF *CLADOPHORA* SP. OIL FOR THE PRODUCTION OF BIODIESEL AS RENEWABLE ENERGY SOURCE

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The expected depletion of fossil fuel within a century has compelled to search for renewable energy sources. The present study is concerned with the production of biodiesel from algal oil. In this context, *Cladophora* was cultivated and used for oil extraction. This algal oil obtained was analyzed for lipids contents using TLC. To optimize the lipase mediated transesterification of these oils for the production of Fatty Acid Methyl Esters (FAME), reaction conditions such as selection of alcohol, temperature, stirring time and oil to alcohol ratio were investigated. Among four different alcohols tested, methanol gave highest yield of biodiesel. Similarly, temperature 50°C and reaction time 6 hrs. were optimal for the transesterification of *Cladophora* oil with methanol. The maximum biodiesel conversion from *Cladophora* oil (75%) was obtained when oil to alcohol ratio was kept 1:8. ATR-FTIR for each biodiesel sample was also performed to analyze and compare them with diesel.

ANTIMICROBIAL POTENTIAL OF ESSENTIAL OIL EXTRACTED FROM CITRUS PEEL

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Essential oils are a potential source of bioactive compounds. In the present study essential oils were extracted from peels of *Citrus limon* (Lemon) and *Citrus aurantifolia* (Lime) by steam distillation. The chemical composition of these volatile compounds was determined by High Performance Liquid Chromatographic (HPLC) and Gas Chromatographic (GC) analyses. Citrus peel essential oils were screened for antimicrobial activity against two Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*), three Gram-negative bacteria (*Escherichia coli*,

Pseudomonas aurantiaca, *Serratia marcescens*) and one fungi (*Fusarium oxysporum*) using *in-vitro* bioassays. The zones of inhibition produced by pure oil extract of *C. limon* with the tested bacterial strains varied from 32 mm (*S. marcescens*) to 50 mm (*S. aureus*), whereas the inhibition zones obtained with *C. aurantifolia* ranged from 12 mm (*S. marcescens*) to 25 mm (*P. aurantiaca*, *S. aureus*). Among five bacterial strains, maximum inhibition was observed against *S. aureus* (50 mm) while minimum inhibition zone was displayed by *B. subtilis* (<10 mm). The extracted citrus oils were found to be least active against *B. subtilis*. The antifungal activity against *F. oxysporum* was more pronounced in *C. limon* (80 mm) than *C. aurantifolia* (30 mm). The essential oils at various concentrations in methanol (1:2, 1:3, 1:5 v/v) showed less antimicrobial activity with small zone of inhibition ranging from 10 mm (*S. marcescens*) to 20 mm (*S. aureus*). Thus pure oil of *C. limon* exhibited significant inhibitory effect and was more active against the tested strains than *C. aurantifolia*. Our results indicated that lemon peel essential oil possesses promising antimicrobial activity and can be a useful biological agent for the control of bacterial and fungal pathogens.

STUDY OF BIOACTIVITIES OF *PSEUDOMONAS AERUGINOSA* VIRULENCE FACTOR PYOCYANIN

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Pseudomonas aeruginosa (PA) is one of most clinically significant nosocomial infectious agent. Clinical significance of this bacterium is intensified due to phenomenon of its natural tendency for acquiring drug resistance mechanisms and as a result arising of resistant strains. PA produces pyocyanin (PCN), an important redox active virulence factor. PCN has been detected in higher quantities in sputum samples of PA infected Cystic Fibrotic patients, up to 100~M. These higher levels indicate the vital role it plays at the site of infection. PCN generates Reactive Oxygen Species which induce oxidative stress in biological systems. The study was aimed at isolation and characterization of PCN producing PA strains, optimization of physiochemical conditions and improvement of PCN yield, evaluation of its bioactivities and *in silico* analysis and structure prediction of proteins involved in PCN biosynthetic pathway and drug resistance mechanisms of PA. Different strains of PA were isolated and screened for PCN production. Isolate 55.1.a was selected for its higher degree of PCN production in comparison to other isolated and obtained strains. 16S rRNA gene segment was amplified and sequenced (GenBank accession # JX280426). Phylogenetic analysis revealed genetic similarities with all the reported sequences of PA. Antibiogram showed S-S.1.a susceptibility to fluoroquinolones and mostly the translation inhibitors antibiotics (72%). PCN batch yield was improved to three folds by optimizing physiochemical growth parameters. A correlation was established between PCN production and PA inoculum. Majority (62.5%) of bacterial and fungal clinical isolates were susceptible to bactericidal effects of PCN. Intra peritoneal PCN injection significantly altered oxidative stress levels in different organs of balb/C mice. Increased levels of oxidative stress were observed in lungs, heart and brain, lower in liver and spleen while muscle tissues showed no significant difference in comparison to control. PCN biosynthesis and drug resistance proteins of PA were modeled and evaluated for structural errors. This project was focused on evaluation of bioactivities PCN, its effects on murine model and *in silico* analysis of drug resistance mechanisms. Both of these cover our ultimate interest in PA research, the mechanisms by which PA damages human body and how does it manage to escape therapeutic agents. This study could be helpful in designing of novel therapeutic agents, virulence

inhibitors which target production of PCN during its biosynthesis and also the drug resistance mechanisms by affecting the efficiency of drug efflux pumps.

STUDY ON IMPROVEMENT IN AMINO ACID PRODUCTION BY MUTANT BACTERIAL STRAINS

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In the present study the project undertaken was aimed at the exploration of locally isolated bacteria for the production of amino acids and enhancement in amino acid production by using different mutant strains. For this purpose bacteria were isolated from soil samples collected from sugar factories area in pattoki and Lahore. A total 20 strains were isolated of which 9 produced significant amount of methionine, cysteine, valine, glycine, tryptophan, tyrosine, glutamic acid, and lysine in different fermentation media based on glucose, urea, molasses and vitamin. Amino acids are the fundamental building blocks for proteins and nutritionally important key element, are not only important biologically but they have copious industrial arduity. The major applications of amino acids include food industry (60%), feed additives (31%) and in the chemical, medicines and cosmetics industry as starting material (4%). The important advantage of these Fermentation media are that they are low cost or by employing this method it can upgrade variety of agriculture wastes like corn steep liquor or molasses. Another vantage of this fermentation is that it can engender 100% amino acid where as chemical process deplorably produce 50% amino acids which cannot be employed by the living organisms for protein synthesis. When newly isolated strains are used in biosynthesis, these strains are subjected to mutation to improve upon their ability to produce the desired product and increase the production. For this purpose different mutagen used according to their mode of action e.g Ethidium bromide, Ethyle methane sulfonate. sodium azide, UV irradiation and hydrogen peroxide.

PREVALENCE OF *ESCHERICHIA COLI* IN MILK AND DIFFERENT MILK PRODUCTS SOLD UNDER MARKET CONDITIONS AT LAHORE, PAKISTAN

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Milk and milk products are an important part of our diet. Quality of food counts much for human health, especially in developing countries where proper hygienic facilities are not practiced. In the present study 120 samples (60 from areas of better sanitation and 60 from those of poor sanitation) of milk and different milk products like Rasgulla, Gulabjamun, Barfi, Khoa and Dahi were screened for the comparative analysis of presence, absence and abundance of *Escherichia coli*. An overall 54% samples were contaminated with *Escherichia coli*. Number and ratio of contaminations was greater in samples from areas of poor sanitation than those of better sanitation

as expected. Whereas C.F.U counts of Dahi (yogurt) for both areas were amazingly higher counted up to $212.16 \pm 17.54 \times 10^3$ and $189.35 \pm 3.42 \times 10^3$ for the poor and better sanitation areas, respectively. Visualization of the milk products in terms of their cultivable *E. coli* contents declare fecal contamination and a possible route for travelling of enteric pathogens.

IMPORTED ORNAMENTAL FISHES ARE BRINGING PATHOGENS TO PAKISTAN

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A number of studies carried at Fish Disease and Health Management Laboratory Punjab University Lahore have revealed that the ornamental fishes imported from South East Asian countries into Pakistan bring loads of pathogens. Goldfish, *Carassius auratus* L. and many of its varieties like Shubunkin, Granda, Fain tail, Double tail, Comet, Black moor; Koi, *Cyprinus carpio*; Platy, *Xiphophorus maculatus* and guppy, *Poecilia reticulata* have been reported to be infected with some of these parasites (monogenean, *Dactylogyrus* sp. *Gyrodactylus* sp; protozoan, *Tricodina* sp, *Icthyobodo* sp, *Icthyophthirus* sp. crustacean, *Argulus* sp, *Lernaea* sp, and annelid, *Piscicola* sp.) and pathogenic fungi (*Aspergillus* sp. *Mucor* sp. Rhizopus sp. *Blastomyces* sp. *Alternaria* sp, *Penicillium* sp.). It is clear that importation of ornamental fish have significant elements of risk of spread of diseases in Pakistan. Such risks may have serious economic and biological impact on, 1) aquaculture industry, 2) ornamental fish industry, 3) aquatic environment and, 4) public health and animal health. These risk factors are discussed in the perspective of current importation of fish diseases with ornamental fishes.

INFLUENCE OF *ASPERGILLUS NIGER* STRESS ON ANTIMICROBIAL AND BIOCHEMICAL PROFILING OF *CICHORIUM INTYBUS*

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The present study was conducted to investigate the protein content, antimicrobial activity, phytochemicals, total phenolic compounds and enzymatic attributes of *C. intybus* under *Aspergillus niger*. The seedlings were inoculated from 1 to 7 dpi (day post inoculation), sample were harvested and extracted using 10 mM sodium acetate buffer (pH 5.2). Protein content, total phenolics, enzymatic activities and phytochemical constituents were measured by standard methods. The protein content was found higher at 3-4 dpi, while enzyme activities were best at 2-3. The phytochemical and total phenolic contents were measured at 3 dpi and phytochemical analysis revealed the presence of alkaloids, tannins, saponins, steroids, flavonoids, terpenoids and cardiac glycoside. The total phenolics like gallic acid (12.85 mg GAE/100 g), caffeic acid (8.51 mg GAE/100 g), vanillic acid (2.54 mg GAE/100 g), hydroxymethoxybenzoic acid (17.82 mg GAE/100 g), chlorogenic acid (1.55 mg GAE/100 g), m-coumaric acid (4.64 mg GAE/100 g) and ferulic acid (5.84 mg GAE/100 g) were recorded. From results, it is concluded that *C. intybus* has the ability to cope with *A. niger* at lower dpi.

7. MOLECULAR BIOLOGY

MOLECULAR CHARACTERIZATION OF *ars* GENES RESPONSIBLE FOR ARSENIC RESISTANCE IN *KLEBSIELLA PNEUMONIAE* CC

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Arsenic is well known potent toxic metalloid. Its pentavalent (As⁵⁺) and trivalent (As³⁺) ions cause obstruction of many cytoplasmic reactions. Arsenic resistance is due to the presence of multiple copies of arsenic resistance genes in arsenic (*ars*) operon. Arsenic operon can have variable combination of genes and may exist either in plasmid or chromosome. Current study is based on the study of genetic determinants in chromosome based *ars* operon in *Klebsiella pneumoniae* CC. This operon consists of three genes, i.e., *arsR*, *arsB* and *arsC*. *arsR* (330bp), *arsB* (1290bp) and *arsC* (426bp) were amplified from the genomic DNA of *Klebsiella pneumoniae* CC, cloned in pTZ57R and get sequenced. Expression primers were designed, nested *ars* genes were amplified, each having *Nde* I at 5' site and ligated in pET 21a for *E. coli* (BL 21) expression system

COPPER INDUCED TRANSCRIPTIONAL ACTIVATION OF *cus* EFFLUX DETERMINANTS IN COPPER RESISTANT ISOLATE OF *KLEBSIELLA PNEUMONIAE*

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Klebsiella pneumoniae, a facultative anaerobe, is a major component of microflora in stressed non clinical environments. It has great potential for resistance to high amounts of copper. Genetic determinants involved in copper resistance are categorized as *cue* (copper efflux) and *cus* (copper sensing) systems. In the present study, role of *cus* system has been focused. *cus* system comprises two operons, *cusRS* and *cusCFBA*, first regulatory and second structural in nature, both regulated in trans from a bi-directional promoter. Regulation of both operons at transcriptional level has been quantitatively measured through real-time PCR. Transcript levels of both *cusRS* and *cusCFBA* were monitored in the presence of non-lethal and sub-lethal copper concentrations at various time intervals in comparison to no metal, under aerobic and non-aerobic conditions simultaneously. Analysis of data obtained revealed that expression of *cus* determinants enhanced significantly in the presence of copper and these determinants played important role in copper resistance under both aerobic and anaerobic conditions.

MOLECULAR ANALYSIS OF *copA*, A COPPER TRANSLOCATING P-TYPE ATPASE OF *KLEBSIELLA PNEUMONIAE*

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Metals play an integral role in living systems. Copper ions present a dual challenge to life in that they are essential but can also be lethal when present in excess of cellular needs. *copA* gene,

a Cu(1) translocating p-type ATPase, exports excessive amounts of copper in *Klebsiella pneumoniae*. In present study, copper resistant strain of *Klebsiella pneumoniae* KW was used to study role of *copA* in Cu²⁺ resistance. Amplified *copA* gene (2.93 kb) was cloned and subjected to sequencing. Comparative analysis of *copA* sequence along with homologous gene sequences revealed that *copA* is a characteristic feature of copper resistance in all members of family Enterobacteriaceae. Topological analysis of the deduced protein sequence of CopA showed that it was a membranous protein as in hydropathy plot of the protein, six transmembrane helices were found. Modeling of three dimensional structure and Conserved Data Domain analysis revealed that CopA of *K. pneumoniae* KW comprised of four domains; two HMA, one E1-E2-ATPase and one HAD-like domain. These domains were found to harbor characteristic sequence features of P type ATPases. Cloned *copA* was expressed in *E. coli* BL21 C⁺ using pET21a⁺ as expression vector. CopA was found to be expressed in insoluble form as expression was found only in pellet of sonicated induced samples. 4 hours induction was found to be the best time duration for expression of CopA. CopA expresses equally within a broad range of IPTG *i.e.*, 0.02–0.1 mM. Expression of CopA at 17°C showed that protein became partially soluble at low temperature.

**CLONING AND EXPRESSION ANALYSIS OF CueR - A MERR LIKE
TRANSCRIPTIONAL ACTIVATOR - FROM *KLEBSIELLA PNEUMONIAE* ISOLATED
FROM INDUSTRIAL EFFLUENTS**

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Copper is an essential metal required by microbes and other organisms for its different structural and catalytic properties. However, differentiation and metabolism of cell is affected by higher concentrations of the metal. Cu toxicity is based upon generation of hydrogen peroxide radicals and its interaction with nucleic acids, proteins and lipid affecting cell membrane and other cell organelles. In *Klebsiella pneumoniae*, there are different resistance mechanisms working for copper homeostasis and resistance. Various genes have been reported that regulate these mechanisms. CueR, a MerR-like transcription activator protein, is responsible for maintenance of Cu metabolism. *cueR* (478 bp) from *K. pneumoniae* KW was cloned in pTZ57R cloning vector and subjected to sequencing. Analysis of deduced CueR sequence from *K. pneumoniae* KW provided its maximum sequence similarity within the same genus. All the homologous proteins already reported in the literature were found to be among Enterobacteriales only. No significantly homologous protein was found in other orders or at higher systematic level. Topological analysis of the protein sequence revealed the cytosolic and soluble nature of CueR with no membrane spanning regions. Three dimensional structure of CueR protein was designed on the basis of homology modeling. Its homodimeric functional form was also constructed that had a bisymmetrical form, a characteristic of transcription activators. According to Conserved Domain Database, three conserved sites in each monomer of CueR were found including dimer interface, DNA binding site and copper binding site. *cueR* along with *Nde* I restriction site at 5' end was amplified from pTZ57R-*cueR* and cloned in pET21a expression vector. Expression of CueR in BLC21 C⁺ induced with 0.05 mM IPTG for 6 hours was taken at 37°C which gave a 13 kDa protein band that was accurately the size of the respective protein. Fractionation of the induced sample revealed the presence of CueR band only in supernatant showing its soluble nature. Optimal conditions for the best expression were found to be 37 °C with 0.02 mM IPTG induction for 4 hours.

GENETIC DEGRADATION IN HATCHERY STOCKS OF *LABEO ROHITA* AS INFERRED FROM SSR MARKERSFAUZIA SULTANA¹, KHALID ABBAS^{1*}, SAJID ABDULLAH¹ AND ZHOU XIOYUN²¹*Department of Zoology and Fisheries, University of Agriculture, Faisalabad 38040 Pakistan*²*College of Fisheries, Huazhong Agricultural University Wuhan, 430070, P. R. China***Corresponding author: Abbas K., dr.abbas@uaf.edu.pk*

The genetic diversity in fish hatcheries is devastated due to anthropogenic intervention and poor management of broodstock. The present study was conducted to gauge the extent of genetic variability in hatchery stocks of *Labeo rohita*. The samples of *L. rohita* were collected from six selected hatcheries of the Punjab. A total five microsatellite markers were used to generate allele frequency data of the subject populations. The resulting genotypic data of each sample was analyzed for calculation of various indices of genetic diversity. The allelic polymorphism fluctuated from sample to sample over all the loci. The locus Lr 12 was found genetically more diverse while Lr 10 and Lr 21 were less diverse as compared to other loci. We examined low to moderate level of heterozygosity with the average values of H_o and H_e as 0.42-0.7 and 0.661-0.717 respectively. The inbreeding coefficient F_{IS} values showed the highest level of inbreeding in Mianchannu hatchery and lowest in Farooqabad hatchery. The analysis of F_{ST} , genetic distance AMOVA and UPGMA dendrogram revealed genetic closeness between Faisalabad, Farooqabad and Mianchannu hatcheries while PCA analysis showed genetic closeness between Sahiwal and Farooqabad hatcheries. The percent level of genetic diversity observed in each hatchery population was found as 25.24% in Kamalia, 31.23% in Sahiwal, 46.62% Sargodha, 48.4% in Faisalabad, 52.55% in Mianchannu and 62.39% Farooqabad respectively. Current knowledge on the genetic structure of *L. rohita* population will be useful for effective broodstock management and conservation of genetic integrity of the dwindling stocks of *L. rohita*.

GLUCOCORTICOIDS INDUCED INHIBITION OF PROTEIN KINASE C ATTENUATES KISSPEPTIN INDUCED ANTI-COAGULATION

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A small peptide named kisspeptin has been demonstrated to play an important role in down-regulating the matrix metalloproteinases which implicates its ability to dissolve the fibrin clot and therefore might act as an anticoagulant. Presently, the anticoagulatory role and mechanism of action of kisspeptin was investigated in vivo in laboratory rats. Adult Sprague-Dawley rats were exposed to 1 µg kisspeptin. Methylprednisolone acetate (100 µg) was used to block the action of kisspeptin. In one group, kisspeptin was administered 15 min before administering the glucocorticoid, while in the other group glucocorticoid was injected 30 min before kisspeptin administration. Kisspeptin and glucocorticoid were also administered alone. Coagulation assays of the blood samples were conducted at 30 min and 60 min to analyze the anticoagulatory effect of kisspeptin before and after glucocorticoid administration. Coagulation assays showed increased clotting time, bleeding time, prothrombin time, activated partial thromboplastin time and international normalized ratio. A noticeable decrease in plasma calcium concentration and increase in potassium concentration were the outcomes. A significant decrease occurred in the platelet

count, platelet distribution width, platelet crit, mean corpuscular volume, hemoglobin and hematocrit. Mean platelet volume showed negative correlation with platelet count. Glucocorticoid alone and glucocorticoid first and kisspeptin after administration revealed results opposite to that when kisspeptin was administered alone. No noticeable change was found in red blood cell count, mean corpuscular volume and mean corpuscular hemoglobin concentration of all groups. The present study showed that the anticoagulatory effects of kisspeptin are reversed upon glucocorticoid administration. The current study suggests that glucocorticoid application may prevent the anticoagulatory effect of kisspeptin via inhibiting the NF- κ B pathway.

BIASED EXPRESSION, UNDER THE CONTROL OF SINGLE PROMOTER, OF HUMAN INTERFERON α -2B AND *ESCHERICHIA COLI* METHIONINE AMINOPEPTIDASE GENES IN *E. COLI*, IRRESPECTIVE OF THEIR DISTANCE FROM THE PROMOTER

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Human interferon α -2b and *Escherichia coli* methionine aminopeptidase genes were cloned independently as well as bicistronically in expression plasmid pET-21a. Production of human interferon α -2b was comparable to that of *E. coli* methionine aminopeptidase when these genes were expressed independently in *E. coli* BL21-CodonPlus (DE3)-RIL. However, human interferon α -2b was produced in a much less amount whereas there was no difference in the production of methionine aminopeptidase when the encoding genes were expressed bicistronically. It is important to note that human interferon α -2b was the first gene in order, after the promoter, and *E. coli* methionine aminopeptidase was the next with a linker sequence of 27 nucleotides between them.

COMPARISON OF SNP VARIATION IN THREE NATIVE CATTLE (RED SINDHI, DHANNI AND CHOLISTANI) BREEDS

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Although a large number of single nucleotide polymorphisms (SNPs) have been identified from the bovine genome-sequencing project, few of these have been validated at large in Bosindicus breeds. We have genotyped 60 animals, representing 3 cattle populations of Pakistan, with the Illumina Bovine 700 K SNP BeadChip. These include 20 each samples of Red Sindhi, Dhanni and Cholistani cattle. Analysis of 500,383 SNP markers revealed that the mean minor allele frequency (MAF) was 0.23, 0.22 and 0.21 for Red Sindhi, Dhanni and Cholistani

respectively. There was no such significant difference of MAF observed among these three cattle breeds ($p < 0.001$). Across these cattle populations, a common variant MAF (≥ 0.10 and ≤ 0.5) accounted for an overall estimated 73.79% of the 500,383 SNPs. Investigation of these cattle populations showed that on average, 16.64% of the markers were monomorphic. The level of SNP variation identified in this particular study highlights that these markers can be potentially used for genetic studies in native cattle breeds of Pakistan.

**DEVELOPMENT OF A UNIVERSAL PRIMER SET FOR AMPLIFICATION OF
CYTOCHROME OXIDASE C SUBUNIT I (COI) GENE IN *URSUS THIBETANUS* AND
*URSUS ARCTOS***

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DNA Barcoding is one of the advance techniques widely accepted as tool for specie identification. Generating universal primers for amplification of COI gene of specific group of species is one of the targets of this molecular taxonomy approach. Keeping this in mind the present study was planned to design the primer pair for amplification of COI gene of bear species of Pakistan. Using mitochondrial genome sequence of eight Ursid species, available in GenBank database a degenerate primer set was designed manually and analyzed by IDT Analyzer. Under standard PCR conditions, 750 bp COI gene of *Ursus thibetanus* and *Ursus arctos* was amplified. The amplicons were sequenced and compared with each other as well as with all the available gene sequences of Ursid member species. Maximum homology (88%) was found between *Ursus thibetanus* and *Ursus americanus* and these two species have minimum genetic distance 0.080. On the other hand minimum homology was found between *Ursus thibetanus* and *Ailuropoda melanoleuca* and have greatest genetic distance of 0.206. The *Ursus arctos* was found to be closely related to *Ursus maritimus*. The study provided a primer pair which not only amplifies the COI gene in bears of Pakistan (*Ursus thibetanus* and *Ursus arctos*) but it is also suggested that this primer pair may also be used as universal primer for Ursids.

**STUDY OF THE ROLE OF RFRP-3 SIGNALING IN REGULATING HPG-AXIS IN THE
ADULT MALE RHESUS MONKEY (*MACACA MULATTA*)**

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Gonadotropin inhibitory hormone (GnIH) was first recognized as a bonafide regulator of reproductive axis in avian species. There is now credible evidence that it also plays an inhibitory role in the mammalian hypothalamic pituitary gonadal (HPG)-axis regulation. A putative inhibitory role of GnIH in the regulation of HPG-axis, under normal physiological conditions in adult male

higher primates was investigated in the present study. This was done by administering a GnIH antagonist, RF9 (adamantanecarbonyl-RF-2-NH₂), to adult male intact rhesus monkeys (*Macaca mulatta*) and assessing the testosterone response as an end parameter of the HPG-axis. The chair-restrained habituated animals were distributed into two groups having similar body weights. Control group (n=4) received normal saline (0.9% NaCl) as vehicle containing 0.0012% dimethyl sulfoxide (*DMSO*), while the treated group (n=4) received RF-9, dissolved in normal saline containing 0.0012% *DMSO*. On the first day of experiment, four bolus injections of normal saline (1ml/animal) were administered intravenously at 2-hr interval to the control monkeys. Similarly, on the second day of experiment, treated group animals were given four iv bolus injections of RF-9 (0.1mg/kg BW) at 2-hr interval. Serial blood samples were collected at the interval of 20 minutes during a 6-hr period which started just after first saline/RF-9 injection. Plasma testosterone concentrations were determined by using a specific enzyme immunoassay. Administration of RF-9 or saline did not affect mean plasma testosterone levels in the 6 hr sampling period. Overall means of plasma testosterone levels and plasma testosterone area under curve (AUC) after saline and RF-9 injections, remained comparable between the treatments. Comparison of overall mean testosterone and mean testosterone AUC in short time windows following each injection of RF-9 also revealed no effect of the treatment on plasma testosterone levels. In summary present study demonstrated no significant role of GnIH in HPG-axis regulation, in normal adult male higher primates. However, it remains to be seen if GnIH plays a role in regulation of the primate HPG-axis in physiological conditions like stress, season, lactation and prepuberty.

DETECTION OF *TOXOPLASMA GONDII* DNA IN ENVIRONMENTAL FRUITS AND VEGETABLES SAMPLES

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Toxoplasma gondii infections are prevalent in humans and animals all over the world. The aim of the study was to estimate the occurrence of *T. gondii* oocysts in fruits and vegetables and determine the genotype of the parasites. A total number of 200 fruits and vegetables samples were taken from shops and home gardens located in the area of Muzafarghar District. Oocysts were recovered with the flocculation method. Polymerase chain reaction (PCR) targeting the B1 gene was used for specific *T. gondii* detection. *Toxoplasma* DNA was found in 12 samples. Genotyping at the SAG2 locus showed SAG2 type I and SAG2 type II. This investigation is describing *T. gondii* DNA identification in a large number of fruits and vegetables samples with rapid molecular detection methods. The results showed that fruits and vegetables contaminated with *T. gondii* may play a role in the prevalence of toxoplasmosis in Pakistan.

GENE CLONING USING REAL-TIME PCR AMPLICONS OF *MYCOPLASMA GALLISEPTICUM* AND *MYCOPLASMA SYNOVIAE*

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Mycoplasma gallisepticum (MG) and *Mycoplasma synoviae* (MS) are important bacterial pathogens of many avian species including chickens. Studies were performed to clone the Real-time PCR amplicons of cytoadhesin encoding surface protein (*mgc-2* gene) of *M. gallisepticum* and variable haemagglutinin surface protein (*vlhA* gene) of *M. synoviae* (indigenous isolates). Gene cloning experiments were performed with TOPO TA Cloning[®] Kit (Invitrogen, USA) containing plasmid vector pCR[®]2.1-TOPO[®] and Top-10 chemically competent *E. coli*, using One Shot[®] chemical transformation procedure. Positive clones were selected as showing white colored bacterial colonies on surface of LB (Luria-Bertani) medium plate. Plasmid DNA purifications were performed using QIAprep Spin Miniprep Kit (QIAGEN Inc., USA). PCR amplifications were performed with specific primers on cloned purified plasmid DNA which have confirmed the presence of 69bp amplicon indicating *M. gallisepticum* insert and 65bp amplicon indicating *M. synoviae* DNA insert. In conclusion, these clones have exhibited a valuable addition into the genomic library. Furthermore, these may serve as ready to use positive control in various experiments pertaining to molecular detection and epidemiological investigations of the mycoplasma isolates.

MOLECULAR CLONING OF INTERFERON REGULATORY FACTOR 5 (IRF5) GENES IN RAINBOW TROUT, *ONCORHYNCHUS MYKISS*

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The IRF family of transcription factors plays diverse roles in coordinating innate and adaptive immune responses to pathogens mediated by the binding of pathogen-associated molecular patterns (PAMPs) to cell surface and cytosolic pattern recognition receptors. Most IRF studies to date have focused on the functional role of IRFs in antiviral responses, their functional and developmental roles in macrophages, dendritic cells, B and T cell lineages. RNA was extracted from cells and tissues of spleen and head kidney. RNA was converted to cDNA. Six primers four forward and two reverse primers were used during amplification of *IRF5* gene under different PCR conditions, PCR product was ligated, transformed and cloned. A portion of *IRF5* gene was amplified with the help of primers designed from Salmon fish STs (expressed sequence tags) data base. A putative protein less than 1000 base pair was obtained after running PCR products on gel. After sequencing and BLAST2 analysis the products were identified as IRF5 consisting of 678 nucleotides. When these nucleotides were translated, they resulted into 226 amino acids. These amino acid sequences aligned well with IRF5 gene of Salmon.

8. PHYSIOLOGY

EFFECT OF KISSPEPTIN-10 ON PLASMA TESTOSTERONE CONCENTRATION IN ADULT MALE GERMAN-SHEPHERD AND LABRADOR DOGS WITH POOR REPRODUCTIVE EFFICIENCY

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In the present study role of Kisspeptin-10 in regulation of reproductive efficiency in adult male dogs was investigated. Adult male German-shepherd dogs and Labrador dogs with poor libido (n=5/group) and reproductively healthy dogs (n=3/group) were used for this study. Kp-10 (50 µg/animal) was injected through a teflon cannula implanted in the saphenous vein. Blood samples were collected 90 minutes before and 180 minutes after Kp-10 administration. Plasma T levels were determined using Enzyme linked immuno assay (EIA) kits. Mean pre-treatment plasma T concentrations in healthy dogs were significantly ($P<0.01$) high as compared to mean pre-treatment plasma T concentrations in poor libido dogs. In reproductively healthy Labrador dogs, after Kp-10 administration, a significant increase in T levels was observed which reached its peak level ($P<0.001$) at 90 min post Kp-10 administration. Mean post-treatment plasma T concentrations in healthy Labrador dogs were significantly high ($p<0.001$) as compared to mean pre-treatment plasma T levels. In poor libido dogs a significant ($P<0.05$) increase in T levels was observed at 30 min. This increase attain a maximum levels ($P<0.001$) at 120 min post Kp-10 treatment at 0 min. Comparison between mean pre- and post KP treatment T concentrations also showed a significant ($P<0.001$) difference. In healthy German shepherd dogs a significant ($P<0.01$) increase in T levels was observed at 90 min and 120 min ($P<0.001$) as compared to 0 min sample. Mean pre- and mean post-treatment T concentration were also significantly ($P<0.01$) different. In poor Libido German-shepherd dogs, a significant ($P<0.01$) increase in T levels were observed at 60 min and 90 min ($P<0.001$) post Kp-10 administration at time 0. Comparison between pre- and post KP treatment also showed a significant ($P<0.01$) increase in T concentrations after Kp-10 treatment. These findings suggest that Kp-10 has similar effect in canines as in other animals. These findings also suggest that KP might have a role in maintaining reproductive efficiency in canines.

DEPRESSION ASSOCIATED OSTEOPOROSIS IN YOUNG ADULTS

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Metal disorders are illnesses that manifest in the brain. About 450 million people suffer from a mental or behavioral disorder. Mood disorders are a kind of mental problems characterized by a disturbance of mood or persistent emotional states that affect how a person acts, thinks and perceives his environment. Depression is a common mood disorder that presents with several comorbid illnesses. Hypercortisolism is associated with rather severe forms of depression and osteoporosis might be seen in those patients. Osteoporosis is a complex, multi-factorial condition characterized by reduced bone mass and impaired micro-architectural structure. In the present study, epidemiological survey was conducted in the general population for estimating prevalence of

depression, evaluating the biochemical markers in depressive subjects and also evaluating the possible pathogenic mechanism implicated in the loss of bone. A total of 2060 subjects were assessed with the Beck Depression Inventory-II. Serum calcium and alkaline phosphatase were measured in 367 subjects and cortisol in 89 subjects. Results of epidemiological survey demonstrated the prevalence of depression in the range of 42% ups and down, 25% mood disorders, 12.6% clinical depression, 14% moderate depression, 5% severe depression, 0.9% extreme depression. In the rural region, subjects of age group 14-18 years were more affected with percentage age frequency of 82.5% whereas in the urban age group of 19-24 years with percentage frequency of 90.3%. Analysis showed that females were leading the males $r = 0.79486$ and $r = 0.9967$ rural vs urban respectively. Depressed subjects had significantly increased level of serum cortisol ($P < 0.01$), alkaline phosphatase ($P < 0.01$) and but showed a decreased serum calcium ($P < 0.05$). Percent correlation showed the positive correlation among depression type and cortisol levels ($r = 0.381$, $P < 0.01$), alkaline phosphatase levels ($r = 0.183$, $P < 0.01$) and negative correlation with calcium levels ($r = -0.01$, $P < 0.05$). It also showed the positive correlation between cortisol and alkaline phosphatase ($r = 0.235$, $P < 0.05$), negative between cortisol and calcium ($r = -0.198$, $P < 0.05$), negative between calcium and alkaline phosphatase ($r = -0.23$, $P < 0.05$). The data suggested that increase in serum cortisol was related to depression and caused a decrease in the adsorption of calcium from intestine and renal tubule thus causing in turn elevation of alkaline phosphatase. Prevalence of depression was more common in females than males and the age group of 19-24 years showed more severity than other age groups *i.e.*, 14-18 years and 25-30 years in both rural and urban regions.

MELATONIN SUPPLEMENTATION OF EXTENDER IMPROVES THE POST THAW QUALITY OF BUFFALO BULL SPERMATOZOA

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Oxidative damage to sperm during cryopreservation is possibly one of the main causes of decline of their fertilizing ability. Present study aimed at evaluating the role of melatonin in extender for its effect on post thaw quality of buffalo bull semen. For this purpose, two consecutive ejaculates were collected from three Nili Ravi buffalo bulls using artificial vagina at weekly intervals for a period of three weeks (three replicates). Qualifying semen ejaculates were diluted (50×10^6 motile spermatozoa ml^{-1}) in Tris Citric Acid (TCA) extender with melatonin at 0 (control), 0.1, 0.5, 1, and 1.5 mM. Diluted semen was cooled to 4°C for 2 h and equilibrated for 4 h at 4°C. Straws were then kept in liquid nitrogen vapours for 10 min and stored in the liquid nitrogen. Thawing was performed after 24 h of storage, at 37°C for 30 seconds and sperm quality parameters were assessed at post thaw. Sperm progressive motility, plasma membrane integrity, acrosomal integrity, viability and chromatin integrity was improved ($P < 0.05$) in a dose dependent manner by supplementing melatonin at 0.1 mM and 0.5 mM in extender compared to control; while higher concentrations of 1 mM and 1.5 mM of melatonin resulted in a dose dependent decrease ($P \geq 0.05$) in aforementioned semen quality parameters. In conclusion, the addition of melatonin to buffalo bull semen extender at 0.5 mM improved the post thaw quality of buffalo bull semen.

MELATONIN IN EXTENDER IMPROVES THE FREEZABILITY OF SAHIWAL BULL SEMEN

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Antioxidants are used in semen extenders to reduce the effect of free radicals produced during freeze thaw cycle. It was hypothesized that melatonin supplementation of extender will improve the freezability of Sahiwal bull semen. The objective was to evaluate the effect of melatonin at 0.1, 0.5, 1.0 and 1.5 mM in extender on post thaw quality of Sahiwal bull semen. Semen was collected from three Sahiwal bulls with artificial vagina for a period of three weeks (two ejaculates/week). The ejaculates having $\geq 60\%$ motility were extended with Tris-citric acid extender containing melatonin at 0, 0.1, 0.5, 1.0 and 1.5 mM. Extended semen was cooled to 4°C in 2 hours and equilibrated for 4 hours at 4°C. Semen was filled in 0.5 ml straws at 4°C in the cold cabinet unit. The filled straws were placed in liquid nitrogen vapours (-196°C) for 10 minutes and cryopreserved. Sperm motility, plasma membrane integrity, viability and chromatin integrity of each semen sample was assessed after thawing. Melatonin at 0.1mM and 0.5 mM improved the post thaw quality of Sahiwal bull spermatozoa in a dose dependent manner, while higher concentrations of melatonin resulted in a dose dependent decrease in semen quality parameters. In conclusion, melatonin supplementation at 0.5 mM improved post thaw quality of Sahiwal bull spermatozoa.

METABOLIC AND STRESS HORMONES REGULATION DURING ENVIRONMENTAL STRESS

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Poultry industry is one of the largest growing industry of Pakistan. Rearing of birds is practiced throughout the year under different climates and may have impact on the alteration of plasma hormonal levels. Vitamin E / α -tocopherol is a lipid soluble vitamin and is known to be a major chain-breaking antioxidant. In this study, effect of Vitamin E on hormonal values is studied under cold stress, heat stress and control conditions. A total of 300 day old chickens, were procured from open market and maintained at the Poultry Research Institute, Rawalpindi. Birds were allocated into three groups (a) birds at 25 \pm 3°C (Group I; N = 100) (b) birds at 5 \pm 3°C (Group II; N = 100) (c) birds at 40 \pm 3°C (Group III; N=100) and were brooded to 21 days of age. In all groups, birds were further subdivided into two groups, one served as negative control and the other was given vitamin E (VE; 3g/Kg feed). The experiment was started from 21st day of age, and sampling of five randomly selected birds was carried out on 42nd day of age. Serum triiodothyronine (T₃), thyroxine (T₄) and cortisol hormone concentrations after the vitamin E treatment in heat and cold stress broiler was determined by commercially available ELISA kits. Heat stress induced

significant increase in serum cortisol level, but in VE treated group, its levels are quite suppressed. During normal temperature conditions, VE treatment showed no significant effect on serum cortisol level. Supplementation of VE decreased production of cortisol significantly in comparison of cold stress with heat stress. Heat stress caused significant depression in T3 levels as the age progressed, but normal temperature did not pose any significant change. VE supplementation results a significant increase in the serum T3 levels in both heat stress and normal temperature. During cold stress, VE supplementation resulted a non-significant change in T3 level. T4 concentrations were non-significantly different in heat stress and normal temperature. VE supplementation did not affect the concentration in both groups. However, during cold stress conditions, T4 concentrations were significantly lowered in controlled group, whereas VE fed group showed higher levels of T4. It was inferred from the study that supplementation of higher VE doses than the recommended one can be beneficial to alleviate the negative effects of cold stress and heat stress.

ASSESSMENT OF A CYTOKINE, IL-6, A VASCULAR GROWTH FACTOR, VEGF AND A HORMONE, LEPTIN IN PATIENTS WITH DIABETIC RETINOPATHY

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Type-II diabetes mellitus is a devastating metabolic disorder affecting millions of people around the world. Diabetic retinopathy is one of the three very significant microvascular complications of progressing diabetes that leads ultimately to blindness. Sustained hyperglycemia promote neovascularization leading to hemorrhagic retina. Factors like obesity, hypertension, and elevated random and fasting plasma sugar, raised cholesterol level, hyperlipidemia, and serum creatinine contributing to diabetes are very well known risk factors. The current study was designed to determine the specific parameters, viz. serum and vitreous vascular endothelial growth factor (VEGF), interleukin-6 (IL-6), and leptin in diabetic retinopathic patients. Serum levels were also compared with diabetic but non-retinopathic patients and normal healthy subjects. Over 2000 male and female patients of median age of 50 years ranging between 37-65 years were screened. The results demonstrated significantly higher ($P < 0.001$) concentrations of serum IL-6 (70%), leptin (64%) and VEGF (55%) in DNR, NPDR and PDR patients. Vitreous IL-6, leptin and VEGF concentrations were alarmingly increased (100%, 93% and 100% respectively $P < 0.001$) in NPDR and PDR patients. For conventional parameters significantly ($P < 0.001$) elevated BMI, RBS, FBS, HbA1c, TG, LDL, serum and urine creatinine and urinary protein concentrations were found in DR, NPDR and PDR patients. Values of these parameters were remarkably low ($P < 0.001$) in normal subjects. All parameters were affected linearly with the severity of the disease. Accordingly highest levels were found in PDR patients. Serum cholesterol concentrations were well in the range. HDL concentrations were significantly reduced ($P < 0.001$) in DNR patients, NPDR and PDR patients; but group comparisons showed slightly greater levels of HDL in NPDR and PDR patients than the DNR patients. TC/HDL ratio and LDL/HDL ratio were also increased in NPDR and PDR patients. Separate male and female comparisons did not show any significant differences with combined male and female analysis demonstrating that the disease prevalence is irrespective of gender; however a small female predisposition is evident from the data. Of 338 diabetic patients following were the frequencies of non-retinopathy and retinopathy: DNR (11%), NPDR (31.95%) and PDR (56.80%). Gender-wise, 38% (129) were males consisting of 12% DNR patients, 36% NPDR patients, and 52% PDR patients. Of 209 female patients, 11% were DNR patients, 29% were

NPDR patients, and 60% were PDR patients. The study points out that IL-6, leptin and VEGF can be significant diagnostic factors in clinical settings to predict the probability of retinopathy. They also demonstrated correlations, positive or negative, with some conventional parameters.

MELATONIN SUPPLEMENTATION OF *IN VITRO* MATURATION MEDIA IMPROVES THE *IN VITRO* FERTILIZATION RATE OF BUFFALO OOCYTES

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Oocyte maturation is the most critical step in the in vitro embryo production. Present study was conducted to study the effect of melatonin supplementation of in vitro maturation (IVM) media on maturation rate and in vitro fertilization (IVF) rate of buffalo oocytes. A total of 1179 ovaries were collected from abattoir at Sihala, Islamabad and transported to the laboratory in a thermos having saline solution at 37°C. Then cumulus oocyte complexes (COCs) were aspirated from the follicles having 2-8mm diameter. In experiment I, maturable grade oocytes were placed for maturation in IVM medium (MM; TCM-199 having bovine serum albumin (BSA) 6mg/ml, 10 IU/ml LH, 0.5 ug/ml FSH, 1 ug/ml estradiol-17 β and 50 ug/ml gentamicin) alone (control) or with melatonin at 250 μ M, 500 μ M and 1000 μ M in experimental groups covered with mineral oil. The maturation dishes containing oocytes were placed in an incubator in a CO₂ incubator at 39°C with 5% CO₂ and at 95% relative humidity for 24 hours. The maturation rate as assessed by cumulus expansion and nuclear maturation did not differ in media supplemented with melatonin at 250 μ M, 500 μ M, 1000 μ M and control. In experiment II, the oocytes were matured as described for experiment I. After maturation, oocytes were incubated in 50 μ l droplets prepared from the fertilization TALP medium having 10 μ g/mL heparin for sperm capacitation. The fertilization droplets were then kept for incubation at 5% CO₂, 95% humidity and 39 °C temperature for 18 hours. The fertilization rate as assessed by sperm penetration and pronuclear formation was improved when maturation medium was supplemented with 250 μ M melatonin compared to control. In conclusion, melatonin supplementation to serum free maturation media at 250 μ M improved the fertilization rate of buffalo oocytes.

PHYSIOGNOMIES OF OBESITY IN SPECIFIC FEMALE POPULATION OF KARACHI

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It is well implicit now that characteristics of obesity vary in different populations for subsequent health risks. The present study observes the features of obesity in the female populations from a few educational institutions. One hundred and sixty five obese females between 18 to 55 years of age volunteered for the study. The obesity specific data included body mass index (BMI), waist hip ratio (WHR), systolic blood pressure (SBP), diastolic blood pressure (DBP) and fasting blood sugar (FBG) levels. The mean age of the obese subjects was 29.280 \pm 0.7 years. The average BMI was 32.43 \pm 0.4; WHR: 0.84 \pm 0.005; SBP: 121.2 \pm 0.9 mmHg; DBP: 81.9 \pm 0.8 mmHg and FBG level at 99.25 \pm 1.32 mg/dl in the obese volunteers. The data predicted significant correlation between BMI and WHR. In comparison in control volunteers the average age was

22.54±0.752 year; BMI: 21.17 ± 0.488; SBP: 109.26±1.25; DBP: 79.41±1.7 and FBG: 95.71±1.25 mg/dl. In controls significant correlation was displayed between SBP and FBG (P>0.041) and DBP and SBP (P>0.001). The results were compared with other populations and are discussed.

WALNUT TRIM DOWN LIPID PROFILE AND BMI IN OBESE MALE IN DIFFERENT ETHNIC GROUPS OF QUETTA POPULATION, PAKISTAN.

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A total of 64 male obese subjects were randomized to observe the effect of 40 g of walnut in daily breakfast on lipid profile and Body Mass Index (BMI), Total Cholesterol (CHO), Triglyceride (TG) High Density Lipoprotein (HDL) cholesterol and Low Density Lipoprotein (LDL) cholesterol in obese male subjects of various ethnics, *i.e.* Baloch (B), Pathan (P), Hazara (H) and Punjabi (PU) residing in Quetta region of Balochistan, were designed. A batch of 32 obese male subjects, 8 from each ethnic group as a control and another batch of 32 obese males 8 from each ethnic group as treated were selected. Twelve hour fasting blood samples a day after stoppage of walnut were taken from obese control and obese walnut supplemented subjects. Daily walnut consumption in obese male subjects evidently demonstrated reduced BMI in all ethnic groups. Walnut supplementation in obese exhibited significant reduction in cholesterol level in Baloch (P < 0.001) and Punjabi (P < 0.01) males. There was profound and statistically significant [B (P < 0.05), P (P < 0.05), H (P < 0.01) and PU (P < 0.05)] elevation in HDL-C in all male ethnic groups. In male sub-population LDL-C was significantly [P (P < 0.01), H (P < 0.001) and PU (P < 0.05)] reduced in these groups. Walnut supplementation showed pronounced reduction [B (P < 0.001), P (P < 0.05), H (P < 0.001) and PU (P < 0.001)] in triglyceride levels. The positive influence of walnut on lipid profile suggests that walnut rich diet may have advantageous effects beyond changes in plasma lipid level.

OBESITY PHYSIOGNOMIES OF MALE VOLUNTEERS OF SOME EDUCATIONAL INSTITUTIONAL COMMUNITY AT KARACHI

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The characteristics of obesity in males are in contrast to the females. Thus obesity is assessed clearly in gender domains. The present work observes the features of obesity in the male populations at a few educational institutions in Karachi. A group of 142 males were sampled for the data of body mass index (BMI), waist hip ratio (WHR), systolic blood pressure (SBP), diastolic blood pressure (DBP) and fasting blood sugar (FBG) levels. The mean age of the volunteers was 28.22 ± 0.691 years; BMI: 32.3 ± 0.599; WHR ratio: 0.92 ± 0.01; SBP: 119.96 ± 1.28mm; DBP: 84.618 ± 0.932 mmHg and FBG 98.31 ± 1.59 mg/dl. The significant correlation was observed between DBP and SBP (P < 0.001) and FBG and BMI (P > 0.061). In comparison in control volunteers the average age was 24.54 ± 0.752 year; BMI: 22.27 ± 0.400; SBP: 105.26 ± 1.29 mmHg; DBP: 80.41± 1.7 mmHg and FBG 97.71 ± 1.25 mg/dl. Significant correlation was

displayed between SBP and FBG ($P > 0.041$) and DBP and SBP ($P > 0.001$). The results are compared and discussed.

TIME DEPENDENT CHANGES IN CIRCULATING BIOMARKERS DURING DIABETIC PREGNANCIES: PERSPECTIVE CASE STUDIES IN LOCAL POPULATION

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The aim of study was to estimate the changes of circulatory biomarkers in normal pregnancy and diabetic pregnancy in each trimester. The results showed imbalance between ROS and antioxidants. Increase oxidative stress throughout the pregnancy and compared that stress level with known diabetic pregnant group. In normal pregnancy the MDA ($1.82 \pm 0.16 \mu\text{mol/ml}$) levels were high but the level are much higher in diabetic pregnant ($4.48 \pm 0.32 \mu\text{mol/ml}$) group in 3rd Trimester. Similarly the levels of Sialic acid and neuraminidase are higher in diabetic pregnant group than normal pregnant group. As well as total cholesterol, HDL and TG is increase in diabetic pregnancy as compare to normal pregnancy. GSH, SOD and catalase (antioxidant stress markers) increased during pregnant group as compare it with diabetic pregnant group. So all the above results showed that ROS production increased in pregnancy and antioxidative stress enzymes balance was disturbed towards destruction more in diabetic pregnancy. The said biochemical parameters in trimester groups were statistically significant ($P < 0.05$).

COMPARATIVE EVALUATION OF SILAGE VERSUS HAY OF MILLET CONSERVED AT BLOOM STAGE AS BASAL DIET ALONG WITH CONCENTRATE SUPPLEMENT IN STALL-FED BUFFALO CALVES

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Twelve Nili-Ravi buffalo calves (age 10-12 months, and average body weights 157 ± 7.202 kg) were randomly divided into two groups A and B with 6 in each. Group A was fed millet silage while group B was offered millet hay *ad-libitum* as a basal diet. All the animals were supplemented with compound feed (Crude protein=15% and Total Digestible Nutrient=67%) @1% of body weight. The study lasted for 100 days (December to March). Millet green fodder was conserved as silage and hay (6-8 tons each) at bloom stage (about 30% DM) during October. Fodder was cut and chopped by chopper machine, conserved it into underground cemented pit, pressed by rolling of tractor and finally sealed with polyethylene sheet. Same fodder was used for millet hay making; whole stalks were sun dried and kept in roofed shed and chopped before feeding. The study lasted for 100 days (December to March). Daily feed intake and fortnightly weights were recorded. Growth trial was followed by a digestibility and nitrogen balance trial. Dry matter (DM) intake of

group A was higher than group B (5.45 vs 5.21 kg/head/day) but statistically the difference was non-significant ($P>0.05$). However, 10% higher ($P<0.05$) total body weight gain (59.58 vs. 54.04 kg) or average daily weight gain (596 vs 540 grams/head) were observed in group A compared to group B, respectively. The feed conversion ratio (FCR) *i.e.*, kilograms of feed consumed per kg weight gain was found to be 9.14 and 9.65 in groups A and B, respectively. The digestibility of DM, crude protein (CP), crude fibre (CF) and neutral detergent fibre (NDF) was significantly ($P<0.05$) higher in group A compared to group B. It may be concluded that millet silage is better than millet hay in terms of better weight gain, FCR and nutrient digestibility in buffalo calves.

IMMUNE RESPONSE OF MEAT TYPE CHICKEN DURING EXTREME WEATHER STRESS

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Bird's life is constantly challenged by stress whether either in wild or captivity. However, every avian species respond differently towards these stressors. Throughout the life of broilers these stressed conditions should be least to attain best performance. Among these, environmental stressors predominantly affect the bird's immune status (innate and humoral immune response). A number of 300 day old broiler chickens were procured and kept in environmental control house. At the age of 21 days, the birds were divided into three groups, (a) birds at $25\pm 3^{\circ}\text{C}$ (Group I; $N = 100$) (b) birds at $40\pm 3^{\circ}\text{C}$ (Group II; $n=100$) (c) birds at $5\pm 3^{\circ}\text{C}$. Each group was further divided into 2 subgroups. One subgroup from each temperature group was supplemented vitamin E (3g/Kg feed) while their counter subgroup served as control for their respective temperature categories. Five birds from each group were randomly selected and their blood was collected through direct heart puncture. Blood sampling was done at 21st, 28th, 35th and 42nd days of age. Before 7 days of blood collection, birds were given intravenous injection of 0.5% washed sheep red blood cells (SRBC). The serum antibody titer against SRBC was found to be significantly higher in all three temperature groups when supplemented with vitamin E as compared to control groups. Similarly, serum IgG and IgM titers were also significantly higher in vitamin E supplemented groups than in control groups. Also, serum antibody titer against ND was higher in vitamin E supplemented groups as compared to control group. From these results it can be concluded that vitamin E supplanted diet significantly improves the immune response of birds through increased antibody production and may also help them fight against different infectious diseases.

POTENTIAL OF HALOPHYTIC GRASSES AND SHRUBS AS CATTLE FODDER IN PAKISTAN

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Burgeoning world population, rapid urbanization and industrialization are exerting pressure on the already dwindling supplies of good quality water and arable lands; as a consequence food

production is not sufficient to meet the requirement of man and his animals. This study aims at exploring the potential of halophytic resources found in arid and/or saline areas of Sindh and Balochistan provinces of Pakistan as suitable alternative to conventional cattle feed. Perennial grass- *Panicum turgidum* Forssk. with a high potential yield of >50,000 kg ha⁻¹ year⁻¹ when raised with brackish water (EC 8-10 dS.m⁻¹) on saline land (EC 10-12 dS.m⁻¹), can be used both green and dry as a replacement of maize and wheat straw respectively. Secondary metabolite contents are not high enough to be harmful for animals and it is comparable to conventional fodder in terms of body weight gain of calves and quality of meat. Dried and chopped *Desmostachya bipinnata*- another perennial halophytic grass, could also replace wheat straw as a dry fodder when used in combination with green *P. turgidum* or maize. Energy ration in diet was vital for weight gain of animals; its absence sustained weight only for about 6-7 weeks after which the weight started to decrease and was restored only on supplementing the diet with the missing ingredients. Cotton seed cake (10% of energy ration) and molasses (5% of energy ration) could be replaced to a similar extent with *Prosopis juliflora* pods and *Manilkara zapota* (Sapodilla) fruit respectively, thus bringing down the cost of production. The findings of this study may be applicable under similar environmental conditions elsewhere like the region from Rajasthan in India extending all the way to Morocco including Pakistan, Middle East, North and Central Africa.

**SOUND PRODUCING APPARATUS OF *GRYLLUS TESTACEOUS* WALKER
(ORTHOPTERA:GRYLLIDAE) AND THEIR IMPORTANCE IN RECOGNITION OF
RELATED TAXA**

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Earlier the species of crickets Family Gryllidae were identified worldwide by their external morphological characters including those of their external male and female genitalia. Fulton (1932) was the first worker to use song to differentiate crickets species complex recognizing the bunch of sibling species, on the basis of songs and specific behavior of receptive females. Specimens of *G. testaceus* Walker were collected from Parachinar in Khyber Pakhtoon Khaw, appeared newly recorded from this locality of Pakistan the male specimen was subjected to Scanning technique of Electron Microscopy following the techniques of David *et al.* (2003). For SEM the specimen was boiled for a few minutes, and then the right tegmina was removed from the specimen and mounted on a stub, placed in a desiccator with Silica gel to dry. The sample was placed to coat with auto coater into JEOL model No. JFC-1500 Japan having gold target, which coated up to 300^oA, then scanned with Scanning Electron Microscopy, using JEOL Japan model No. JSM 6380A and studied as SEM pictures of the file from the ventral region at magnification 220X, from Centralized Science Laboratory, University of Karachi, Karachi. Stridulum is a complex sound producing apparatus in different species of Gryllidae. The morphology of teeth present therein appear similar among individuals of the same species and highly diversified among different related taxa. The stridulum pattern, the stridulatory file and the number of teeth there in appear important characters to distinguish males of *G. testaceus* Walker from other related taxa found in Pakistan.

SEX RATIO OF CALVES IN INDIGENOUS AND CROSSBRED DAIRY COWS; EFFECT OF BREEDING METHOD ON CALF SEX RATIO

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The number of male, female calves and their sex ratio in indigenous and crossbred dairy cows was investigated in present research work. The data used in this study was obtained from Livestock Development Research Centre, Muzaffarabad. A total of 259 (118 females, 141 males) births were studied, of which 27 (12 females, 15 males) were from indigenous, 123 (56 females, 67 males) from Indigenous × Jersey (F₁), 54 (26 females, 28 males) from F₁ × F₁ (F₂) and 55 (24 females, 31 males) from F₁ × Friesian cows. Sex ratio of calves from indigenous, F₁, F₂ and F₁ × Friesian cows was 100♀♀:125♂♂, 100♀♀:120♂♂, 100♀♀:108♂♂ and 100♀♀:129♂♂ respectively. Chi-square test showed that male and female births were not significantly (P > 0.050) different from each other in all the breeds groups. Sex ration of calves from artificially inseminated cows and calves obtained as a result of natural service from crossbred cows was also studied in this research work. In artificially inseminated cows 123 births were recorded, of which 56 were females and 67 were males (100♀♀:125♂♂). Crossbred cows through natural service gave birth to 109 calves, of these 50 were females 59 were males (100♀♀:118♂♂). However, the difference between male and female births was not statistically different from zero (P > 0.05) in both artificially insemination and natural service.

EFFECT OF EXOGENOUS TESTOSTERONE TREATMENT ON SEXUAL ACTIVITY OF MALE ONE-HUMPED CAMEL DURING NON-BREEDING SEASON IN PAKISTAN

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Male dromedary is a seasonal breeder, winter and early spring (Nov-March) constitute the rutting season of camel in Pakistan. It is well established and most of the scientists believe that camel breed in specific days of the year throughout world, but contrary to it some authors have suggested that camel can breed throughout the year, by proper food supply and photoperiod control. So this study was designed to evaluate the effect of exogenous testosterone on the sexual activity of male camel during non-breeding season in natural ecology of Punjab, Pakistan. Six healthy, adult (6-9 years old), male camels were given exogenous testosterone (Testoviron depot 250 mg, @ dose 10ml intramuscular) at an interval of one week for one month (August, 2010). Behavioral and physical signs were recorded on daily basis and the blood samples were recovered one hour before each injection, then three days after each injection and during autumn season. Blood samples were also collected during the months of September and October, to record the serum testosterone concentration during autumn. Serum testosterone concentrations were monitored by RIA Testosterone kit, direct IM1119 IMMUNOTECH (Beckman Coulter Company, USA). The testes of each camel were scanned during experimental period and during autumn using a B-mode real

time ultrasound scanner (Picker CS 9100-Model EUB-405 Tokyo, Japan) equipped with a 7.5-MHz linear-array transducer. Means, standard error and percentage were computed using Microsoft Excel software. One-way ANOVA (STATISTICA 6.0) was used to analyze monthly hormonal variations. The mean serum testosterone concentration (ng/ml), one day before commencement of treatment, 03, 07, 10, 14, 17, 21, and 24 days after treatment was recorded as 0.32 ng/ml, 0.72, 1.06, 2.09, 1.01, 1.23, 0.97 and 1.66 ng/ml, respectively. It was recorded as 0.29 and 0.65 ng/ml during the months of September and October. All the experimental animals showed docility in summer season. Almost all the rutting behavioral signs including; anxiety, restlessness, urine spraying, poll and salivary glands secretions, frequent diarrhoea and fighting were almost absent during the autumn season. Ultrasonic picture of testes during autumn appeared as least echogenic and reduced in size due to decrease in the interstitial contents. Present study revealed low sexual activity and serum testosterone level during non-breeding season even after the treatment of camels with the exogenous testosterone.

PHYSIOLOGICAL AND BIOCHEMICAL POTENTIAL OF SILYMARIN, GLYCYRRHIZIN AND PICRORRHIZIN ON CCl₄ INDUCED LIVER INJURY IN MICE

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Carbon tetrachloride (CCl₄) is a well-known hepatotoxin that is widely used to induce toxic liver injury in a range of laboratory animals. Physiological and biochemical potential of silymarin, glycyrrhizin and picrorrhizin was evaluated by the serum levels of some liver enzymes like AST (P = .024), ALT (P = .013) and ALP (P = .019) and total protein (P = .031) were evaluated as a marker of liver injury. Moreover another crucial biochemical parameter MDA (P = .011), one of the products of lipid per oxidation was also evaluated with three antioxidants, GSH (P = .017), SOD (P = .008) and catalase (P = .009). In conclusion, the combined effect of silymarin, glycyrrhizin and picrorrhizin showed most effective action as compared to individual or dual treatment in CCl₄ induced liver injury in mice.

ANTIDIABETIC AND ANTIHYPERLIPIDEMIC POTENTIAL OF DIFFERENT EXTRACTS OF FRUITS OF AEGLE MARMELLOS IN MICE

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This study was designed for the evaluation of antidiabetic and antihyperlipidemic effects of different extracts of fruits of *Aegle marmelos* in mice. For this purpose 25 mice were acclimatized

in the experimental rooms of the department of Pharmacology and Toxicology for one week prior to study. These animals were given food and water *ad libitum*. Food and water were withdrawn from all experimental animals for an hour before drug treatments and restarted just after administration of drug. The experiment was carried out after one week of adjustment period. Mice weighing 15-30gm were made diabetic by injecting 150mg/kg body weight of isotonic solution of alloxan intravenously in the marginal ear vein to induce hyperglycemia. Eight days after injection, serum glucose levels of all surviving mice were determined and the mice with serum glucose levels greater than 200mg/dl were considered as diabetic and were used for study. The mice were divided into 05 groups *i.e.* A, B, C, D and E containing 05 animals in each. Group A was kept as untreated control, Group B was administered glibenclamide (10mg/kg), and Group C was treated with Petroleum ether extract of fruits of *Aegle marmelos*, Group D was treated with chloroform extract and Group E was treated with aqueous extracts. All the drug preparations/extracts were given orally. Treatment was given once a day and continued for 35 days. Samples of blood from test animals were withdrawn at the intervals of 07, 14, 21, 28 and 35 days. Change in serum glucose level, lipid profile and change in body weight were tested and compared among the groups. It is clear that aqueous extract of *Aegle marmelos* fruit has potent antidiabetic and antihyperlipidemic effect in alloxan induced diabetic mice as compared to petroleum ether extract and chloroform extract.

PREVALENCE OF GYNECOLOGICAL PROBLEM IN GILGIT, GILGIT-BALTISTAN

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A cross sectional prospective study was conducted from February to March 2011 in Gilgit at District Head Quarter Hospital and Central Military Hospital. Data was collected through interviews from patients after verbal consent. A total number of 160 women were interviewed directly. Random sampling among different reproductive age group was done. The average age of patients was 30 years. Dysfunctional Uterine bleeding (17.4%) and Vaginitis (17.4%) of different types were the most common problems in this study. The prevalence of other problems such as infertility, fibroids, anemia, urinary tract infections and pelvic inflammatory diseases was found to be between 4.2% to 13%. Retroverted uterus (0.7%) and Rectovaginal fistula (0.7%) were least common. Low economic statuses (73.1% house wives), illiteracy (53.1% illiterate), carelessness about health issues, early marriages (average age: 17 years).

ANTHROPOMETRIC PROFILE ASSESSING HEALTH RISKS OF YOUNG ADULTS OF MIRPUR REGION

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Anthropometric contour has proved to be a useful index for assessing its vulnerability to degenerative diseases and their risks factors in a population. Presently in the different region of World researchers are focusing on children and young adult populations in development of the

metabolic disorders because of urbanization and changing life style. Young adult females (n: 423) of an average age of 19.11 ± 0.095 years and males (n: 241) of mean age of 18.82 ± 0.110 years volunteered for the study at various educational colleges from Mirpur region of Kashmir. Based on international body mass index (BMI) criterion in the females 20.2% were obese, 26.2% overweight, 36.2% in normal range and 17.4% were underweight. In case of the males 24.5, 32.5, 32.0 and 11.0% were assessed in obese, overweight, normal and underweight BMI. On the basis of ethnic based criterion of South Asia waist circumference was within normal range in all females including the obese and overweight categories, in the males however waist circumference was 35% and 13.5% greater than the upper limit of normal range in obese and overweight respectively depicting evident central obesity. The waist-hip ratio (WHR) was higher in overweight compare to within normal range in obese females whereas in the males obese exhibited greater WHR only showing the evident gender difference in the parameter. The results of the study are worked out for correlations and compared with reports of other populations. It is however obvious to implement community-based interventions for reducing unhealthy anthropometric profile of young population to protect against risks of serious degenerative diseases.

CHARACTERISTICS OF TYPE 2 DIABETES IN THE AFFECTED POPULATION OF QUETTA REGION

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The clinically confirmed one hundred and fifty Type 2 Diabetic patients registered at health facilities e.g. hospitals, private clinics and newly diagnosed Type 2 diabetic subjects at health camps were studied for the characteristics of Type 2 diabetes. In comparison forty nine clinically normal subjects were also studied as controls for all the parameters as in Type 2 diabetic subjects. Body mass index, waist hip ratio, systolic & diastolic blood pressure and circulatory levels of fasting & random glycemia, glycosylated hemoglobin, total cholesterol, triglyceride, high density lipoprotein cholesterol low density lipoprotein cholesterol, very low density lipoprotein cholesterol and insulin were estimated. The characteristics of the Type 2 diabetic subjects were screened for their categorization in metabolic syndrome. The general data was also analyzed in the context of the gender, age, ethnicity and specific life features. In T2DM subjects females comprised 65 % of the total diabetics and in the controls the ratio was about 30%; thus illustrating that greater number of females was found to be inflicted with the syndrome compared to males. Pushtoon population is the most inflicted followed by Punjabis and Hazara ethnically. In all significantly represented ethnic groups greater number of females were diabetic. Pushtoon and Punjabis were assessed to be inflicted with disease with life style and Hazara because of familial factors. In BMI index diabetic population in general and female diabetic population in particular was significantly overweight. Systolic and diastolic blood pressures showed the prevalence of significant hypertension and excessive random & fasting hyperglycemia in the diabetic subjects. Glycosylated hemoglobin was significantly elevated in the syndrome afflicted subjects compared to the controls. In general the diabetics and both female and male populations exhibited marked and significant hyperinsulinemia in comparison to the controls. Total cholesterol was markedly elevated in general diabetic population proportionally contributed by higher levels in the males compared to the females. Low density lipoprotein cholesterol was elevated in general syndrome afflicted population and it was

mainly contributed by significant rise in the females compared to the males. Very low density lipoprotein cholesterol was also observed increased significantly in general in the diabetics. High density lipoprotein cholesterol was found lower in all the subjects compare to the controls. Triglycerides did not demonstrate any notable effect in the comparable groups. Increase in BMI, severe hyperglycemia, marked hypertension, lowered HDL-c, raised LDL-c and most pertinent the hyperinsulinemia evidently demonstrated that investigated subjects had advanced to type2 diabetes mellitus after the development of metabolic syndrome. In certain characteristics the metabolic syndrome of the studied population in some way is found deviated and is assumed because of specific life style of these ethnic populations and living at higher altitude.

EFFECT OF JOURNEY TIME ON THE STATUS OF CORTISOL AND ACTIVITY OF ACETYLECHOLINESTERSE DURING LIVE HAULING OF ADVANCED FRY OF ROHU (*LABEO ROHITA*)

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The effect of journey time on water quality parameters, status of cortisol and activity of AChE, in advanced fry of *Labeo rohita*, during live hauling in close system for twelve hours was investigated. Fry of *L. rohita* average weight and length 2.45 ± 0.25 g and 5.14 ± 0.17 cm respectively were packed in polyethylene bags under pressurized pure oxygen, at a stocking density of 70 g L^{-1} . Before loading water temperature was 21°C , oxygen, 5.6 mg L^{-1} , pH 8.4 mg L^{-1} and total ammonia $< 0.5 \text{ mg L}^{-1}$. The percentage mortality of fry was increased with increase in journey time and 89% survival was observed at the end of transportation. The whole-body and water borne cortisol increased significantly ($P < 0.001$) at 2 hrs and then start decreasing. The second peak of water borne and whole body cortisol concentration was observed at 8 and 10 hrs of journey time while cortisol release rate showed continuous decreasing trend after 2 hrs. AChE activity showed negative relation and continuously decreased with increase in journey time. The results suggest that at this temperature and stocking density fry can tolerate about 8-10 hrs journey time.

DETERMINATION OF OXYTOCIN IN MILK, BLOOD, URINE AND FAECES OF COWS AND BUFFALOES ADMINISTERED EXOGENOUS OXYTOCIN

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Oxytocin is a peptide hormone which is synthesized in the hypothalamic neurons and released from the posterior pituitary gland. This hormone has a wide range of applications in human and veterinary medicine. To address people's concerns of exogenous oxytocin (OT) administration to lactating bovines, a study was undertaken to (a) establish an enzyme immunoassay (EIA) for OT determination in milk, (b) quantify OT in milk, blood and urine

samples administered OT, and (c) study influence of OT on digestion and gastric motility in bovines. Sensitive EIA validated according NCCLS (National Committee for clinical laboratory standard) was developed for OT in skim milk in an analytical range of 10–250 pg/mL. Milk samples collected from cows (n=50) administered either 1mL or 2mL of 10 IU OT prior to milking were investigated for the presence of OT. Intramuscular oxytocin injection caused elevated OT blood levels observed at 10 to 12 minutes while decreased levels at 5 to 10 hours, before the collection of samples. There was no significant difference among both groups with the mean concentrations of OT levels in skim milk of treated cows being 38pg/mL and 67.8 pg/mL. While OT levels in blood samples were 27 pg/mL and 62.9 pg/mL, subjected to same dosage administration, respectively. Similarly, OT levels detected in urine samples were 60.2 pg/mL and 98.38pg/ml. Oxytocin level in fecal samples were minimal 15.4 pg/mL and 31.1 pg/mL corresponding to both doses. Oxytocin act as a neurotransmitter and their receptors play a major part in the complex regulation of gastrointestinal motility and have predominant targets for drug development. The numerous receptors involved in motility are located mainly on smooth muscle cells. These results suggest that to some extent oxytocin excites the contractile stimulation of digestion and affect the smooth muscles where OTR are present and thus enhances the process the digestion in cows. Whether secreted endogenously or administered exogenously, it produces the desired effects within minutes and is metabolized rapidly into inactive products. If at all oxytocin is secreted in the milk and is ingested along with milk, it is degraded by the gut enzymes and can not reach blood circulation in biologically active form. Thus, there seems to be no harm in consuming milk from oxytocin treated dairy animals.

MEASUREMENT OF STATUS OF CORTISOL IN RESPONSE TO HUSBANDRY PRACTICES BY INVASIVE AND NON-INVASIVE METHOD

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The stress response of fingerlings of grass carp *Ctenopharyngodon idella* following exposure to husbandry practices like confinement and chasing was examined. Water borne cortisol was monitored through flow through system and validated with plasma cortisol level. Pre stress plasma cortisol level of fingerling was 6.03 ± 4.11 ng/ml that increased significantly to 77.83 ± 1.31 ng/ml respectively after 30 min of confinement. The water borne cortisol concentration and release rate also showed similar trend, increased from pre stress levels 0.68 ± 0.08 ng/L and 0.02 ± 0.005 ng/g/h to 3.16 ± 0.05 ng/L and 0.57 ± 0.03 ng/g/h respectively after 30 min confinement. Plasma cortisol level of fingerlings also increased significantly ($P < 0.001$) immediately after 5 min chasing and peaked at 4 hrs and after that it showed decreasing trend and recovered after 24 hrs. Water-borne cortisol concentration and release rate also increased significantly ($P < 0.001$), attained maximum level at 4 hrs and recovered within 24 hrs. This study clearly revealed that aquaculture practices like confinement and chasing are the stressful event for the fingerlings of grass carp *Ctenopharyngodon idella* and there is need to consider the after effects on health and welfare of fish and practices should be carried out that minimize these stressful impacts. Whilst a positive correlation among plasma and water release rate of cortisol indicate that non-invasive method is most appropriate method for assessment of cortisol levels without disturbing and sacrificing fish.

**INFLUENCE OF DIETARY PROTEIN ON SERUM CHEMISTRY AND BLOOD
HAEMATOLOGICAL CONSTITUENTS ON THE PRODUCTIVE PERFORMANCE IN
SAHIWAL HEIFERS**

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36 Sahiwal cattle of 18 months of age were used to determine the effect of crude protein (CP) (16% of dry matter basis) on haematological and serum biochemical constituents in Sahiwal heifers. The animals were randomly assigned three treatments using analysis of variance technique under completely randomized design. The heifers received green fodder (Control), 0.5 % of concentrate with green fodder and 1 % of concentrate with green fodder in diets were fed for three months, the average daily gain were improved in the heifers fed 1% of concentrate ration. The increase in CP had effect on White Blood Cell, Red Blood Cell, Haemoglobin, Hematocrit, MCV, RDW, MCH, Platelets, Lymphocytes, Monocytes and GR. While increased concentration of CP had increasing affect on Total protein, Albumin, cholesterol, urea, uric acid. The increased affect of WBC, MCH, and PLT indicated improved immunoglobulin levelin animals fed 1% concentrate ration with green fodder. Similarly, an increased CP in fed increased ADG, decreased serum glucose, increased urea and uric acid.

**ASSESSMENT OF CORNCOBS AS A SUBSTITUTE OF WHEAT STRAW ON FEED
INTAKE, GROWTH RATE AND NUTRIENTS DIGESTIBILITY IN BUFFALO CALVES**

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Nine Nili-Ravi buffalo calves (age 10-12 months, and average body weights 160±3.30 kg) were randomly divided into three groups with 3 animals in each. Three isonitrogenous (12% CP) and isocaloric (68% TDN) mixed diets were fed. Diet A consisted of 75% concentrate+25% wheat straw; diet B had 75% concentrate+13% wheat straw+12% corncobs, while, diet C contained 75% concentrate+25% corncobs. Corncobs and wheat straw were ground in a hammer mill before mixing in concentrate feed. Diets were offered as per National Research Council (NRC, 2001) requirements. All calves were offered 8-10 kg/day of available green fodders (oats, maize and millet). The study lasted for 105 days (March to July). Growth trial was followed by a digestibility and nitrogen balance trial. Results revealed no-significant difference in the intake of dry matter (DM) among all groups, however, the total body weight gain or average daily weight gain and feed conversion ratio (FCR) were better ($P<0.05$) in group C compared to group A, however group A and C were not different statistically ($P>0.05$) from group B. Average weight gain (gm/heah/day) were found to be 670, 696 and 778 in groups A, B and C, respectively. The FCR (kilograms of feed consumed per kg weight gain) were found to be 7.34, 7.49 and 6.92 in groups A, B and C, respectively. The digestibility of DM was alike among all groups, whereas the digestibility of crude protein (CP) and crude fibre (CF), were significantly ($P<0.05$) higher in group C compared to

group A, but group A and C did not vary from group B. Feed cost per kg weight gain of group C was 27.28 and 23.55% less than calves in groups A and B, respectively. It may be concluded that corncobs can beneficially substitute wheat straw in buffalo calves of one year age.

RISKS AND COMPLICATIONS ASSOCIATED WITH HYPERTENSION IN PREGNANT WOMEN VISITING DIFFERENT HOSPITALS OF LAHORE

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Hypertension is a multifactorial disease that is manifested haemodynamically by an increased total peripheral resistance affecting vital organs like liver and kidney. Hypertensive disorders during pregnancy are major cause of maternal and fetal morbidity, and it is one of the leading causes of maternal mortality. The present study was conducted to determine the risks and complications associated with hypertension in pregnant females between the ages of 21-35 years. This study was conducted on 250 female subjects including 150 pregnant females, 50 pregnant non-hypertensive females as positive control and 50 non pregnant non hypertensive females as negative controls. Demographic parameters such as BMI, blood pressure, family history of hypertension and other major disease were recorded. Blood platelet count, Liver and kidney function tests including serum bilirubin, ALT, AST, ALP, serum creatinine, uric acid, blood urea and proteinuria were assessed by chemistry analyzer (Bio rad). Serum creatinine and uric acid were elevated in pregnant hypertensive females as compared to non pregnant hypertensive females ($P > 0.05$). Serum bilirubin, ALT and AST were significantly higher in pregnant hypertensive females in comparison with the other two groups ($P > 0.05$). A significant relationship between proteinuria and hypertension was observed in pregnant hypertensive women ($P > 0.05$). For comparison between and within groups, ANOVA was applied. Our findings further support that age, BMI, blood pressure was significantly related to kidney and liver enzymes in pregnant hypertensive females.

EFFICACY OF LOCALLY PREPARED METHYLACETOXY PROGESTERONE (MAP) SPONGES ON ESTRUS SYNCHRONIZATION AND CONCEPTION RATES IN CYCLIC AND ACYCLIC NILI-RAVI BUFFALOES (*BUBALUS BUBALIS*)

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A study was performed to assess the efficacy of locally prepared intravaginal methylacetoxy progesterone (MAP) sponges for synchronization of estrus and conception rate in cyclic and acyclic Nili-Ravi buffaloes. In first experiment, 20 buffaloes were grouped into treated and controls (n: 10) and again sub-divided into cyclic (n: 4) and acyclic (n: 6) lots in each group, depending on the presence of corpus luteum on the ovaries. MAP sponges were deposited in buffaloes for 16 days. After removal of sponges, animals were monitored for estrus. Progesterone

concentrations were estimated in synchronized animals for one estrous cycle by radioimmunoassay. In general, synchronization rate in MAP-treated groups was significantly higher ($P < 0.05$) as compared to control animals (60 vs. 0%). However, it was non-significant between cyclic and acyclic buffaloes of treated groups (75 vs. 50%). At estrus, mean serum progesterone in synchronized animals was at basal levels ($< 1 \text{ ngml}^{-1}$ in cyclic and acyclic treated groups, respectively). It attained peak levels of 3.00 ± 1.80 to $3.45 \pm 0.44 \text{ ngml}^{-1}$ on d-14, then declined on d-18 and dropped to basal values on the next estrus. In the second experiment, the effect of a MAP-based treatment was compared with an 11-days apart double injection prostaglandin ($\text{PGF}_{2\alpha}$) protocol for synchronization and conception rates. The estrus synchronization and conception rates by $\text{PGF}_{2\alpha}$ treatment were non-significantly higher than MAP-based protocol (90 vs. 70% and 44.44 vs. 42.86%). The conception rate of cyclic buffaloes (50%) receiving MAP-based regimen was also non-significantly higher from acyclic ones (33%) receiving the same protocol. In conclusion, the treatment with locally prepared MAP sponges proved effective in synchronizing estrus and comparable with prostaglandin regime in terms of conception rates in Nili-Ravi buffaloes.

A HEMATOLOGICAL COMPARISON BETWEEN NATURAL AND SYNTHETIC SOURCE OF INFLAMMATION IN ALBINO RATS

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Nerium oleander, (N.O.), an ornamental shrub whose poisoning has been reported in humans and various animals, its oral doses proved fatal to animals within 1 to 24 hours. Thioacetamide (TAA) a potent hepatotoxicant induces histological and biochemical changes which leads to liver cirrhosis akin to human. The aim of this study was to stimulate acute phase response (APR) with administration of natural (N.O.) and synthetic (TAA) sources of inflammation after 24h in rats and to find out correlations among the hematological parameters. Intraperitoneal TAA, (Group I, 300mg/kg) and intramuscular N.O. leaves extract (Group II, 10 ml/kg) were administered in albino rats of about 180-200g. Control rats received injections of normal saline (n=3). The rats were kept under standard conditions with 12h 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 after 24h following TAA and N.O. leaves extract induction. Blood of the control and treated animals, was drawn through cardiac puncture and processed for the measurement of hematological parameters. Statistically significant neutrophilia ($P < 0.0001$) and lymphocytopenia ($P < 0.0001$) were noted in Group I and Group II, compared to control while leukocytopenia ($P = 0.082$) was observed in Group II. Erythrocytosis (38%, $P = 0.0006$), polycythemia (28%, $P = 0.0085$) and increase in HCT (23%, $P = 0.0038$) was noted in Group I when compared with control. Taken together these results we can conclude that the introduction of TAA and N.O. extract leads to stimulate acute phase response (APR) with more or less similar changes in hematology.

OPTIMIZATION OF PHYSICOCHEMICAL CONDITIONS TO ENHANCE THE GROWTH OF *PASTEURELLA MULTOCIDA* FOR VACCINE PRODUCTION

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Hemorrhagic septicemia (HS) is an acute disease of cattle and water buffaloes. In Pakistan it is frequently caused by B: 2 serotype of *Pasturella multocida*. Vaccination is the principal means of prevention. Routinely used vaccines are either plain bacterin, alum precipitated or oil-adjuvanted preparations. In vaccine manufacturing higher yields of bacterial cell mass are of great value in terms of process economics. Present work extends the optimization of various physicochemical conditions of culture, e.g. effect of different media compositions, pH and inoculum size. After reactivation *P. multocida* (Roberet type I; B: 2) were confirmed through polymerase chain reaction (PCR), microscopy and biochemical testing. The growth medium: casein/sucrose/yeast (CSY) broth was used for *P. multocida* growth. Different concentrations of yeast extract, casein hydrolysate, and sucrose with different inoculum sizes and PH were employed. After 48 hours incubation, the dry weight (biomass) of each culture was determined. The maximum growth was achieved in broth media containing 2% of yeast extract, 2% of casein hydrolysate, 1.2-1.4% of sucrose, when inoculum of 36 h broth culture (O.D: 73) was used at 8% of culture medium, and pH was set in the range of 7.0-8.0. The results indicate that different concentrations of media components and physical conditions have significant impact on the growth kinetics of *P. multocida*. Adaptation of optimized physicochemical conditions can make vaccine against hemorrhagic septicemia more economical.

9. TOXICOLOGY

BIOCHEMICAL EFFECTS OF METHYL PARATHION ON THE FISH (*APHANIUS DISPAR*)

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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. 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 methyl parathion (organophosphorus pesticide) on *Aphanius dispar* (killi fish). The enzymes acetyl cholinesterase (AChE), alanine aminotransferase (ALT), aspartate aminotransferase (AST) were used to assess the biochemical effect of methyl parathion in fish juveniles using enzyme analysis kit. The results depict both elevation and inhibition of enzymes in the methyl parathion treated fish as compared to control. The results revealed significant increase in alanine aminotransferase activity by (105.19%) as compared to control whereas acetyl cholinesterase (AChE) activity levels decreased (50.2%) significantly ($p < 0.05$), and also aspartate aminotransferase (AST) activity levels decreased in methyl parathion treated fish by 98.67% respectively. The alteration of biochemical processes in fish can be used as bio-indicators of the effects of pesticide in the marine environment.

ENVIRONMENTAL IMPACT OF COAL CLAY BRICK KILN EXPOSURE ON THE REPRODUCTIVE HEALTH OF ADULT MALE BRICK KILN WORKERS

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Aim of this study was to evaluate the impact of brick kiln emission on the reproductive health of adult male workers. Bricks kiln workers are directly exposed to physical hazards as well as reproductive and developmental toxicants, emitted as residue. A total of 103 people were used for this study. 58 brick kiln workers were selected from Chakbaili near Rawat and were categorized as exposed group while 45 healthy males from Islamabad were selected and were categorized as control group. Different parameters i.e. body mass index (BMI), disease prevalence, work duration, marital status, addiction were noted. Blood samples were collected from both control and exposed groups and were analyzed for hormonal determination. Serum levels of testosterone and luteinizing hormone (LH) were determined using EIA kits according to the manufacturer's instructions. BMI values in exposed group were significantly lower ($p > 0.01$) as compared to control group. Eye diseases, skin diseases, pulmonary and intestinal disorders were also high in exposed group as compared to control group. Serum testosterone and LH levels were significantly low ($p > 0.05$) in exposed group as compared to control group. Probability of primary infertility was 1% while secondary infertility was 4% in exposed group as compared to control group. These findings suggest that brick kiln workers need a better work place environment with lower occupational exposure and healthy living standards.

DNA FRAGMENTATION AS A BIO-INDICATOR FOR LONG TERM EFFECT OF RADIATIONS IN THE WORKERS OF RADIATION DEPARTMENT OF FAISALABAD HOSPITALS

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The study was carried out at hospitals of Faisalabad especially (District Head Quarter Hospital (DHQ), Punjab Institute of Nuclear Medicine (PINUM), Allied Hospital and other private hospitals. DNA fragmentation was examined by using the method of Perandones *et al.* (1993). The 100 subjects were divided equally into control (no exposure) and subjects which were indirectly exposed to radiation. A questionnaire was developed and the subjects who have at least 4-15 years of indirect exposure (duration of service) and belong to 20-50 years of age were selected. The exposure duration of workers belonging to different hospitals was non-significantly different ($P>0.05$). In DHQ the minimum age was 29 years and maximum age was 46 years with mean % DNA fragmentation \pm S.E (58.98 \pm 1.35). In Allied hospital the minimum age was 28 years and maximum age was 45 years with mean % DNA fragmentation \pm S.E (57.29 \pm 1.17). In FIC the minimum age was 24 years and maximum age was 35 years with mean % DNA fragmentation \pm S.E (56.17 \pm 1.50). In Private hospitals the minimum age was 29 years and maximum age was 46 years with mean % DNA fragmentation \pm S.E (58.43 \pm 0.82). The different age groups showed highly significant effect of % DNA fragmentation ($P<0.01$).The % DNA fragmentation showed positive and highly significant ($p<0.01$) correlation with age and exposure duration. The % DNA fragmentation at different hospitals was non-significantly different ($P>0.05$). The long term exposure of ionization radiation causes induction of DNA damage. The % DNA damage increases with increasing age and exposure duration in radiationally exposed workers. The present study showed % DNA fragmentation could be use as a biomarker for the estimation of radiological effects in occupationally exposed workers.

HEMOTOXICITY ASSESSMENT OF PETROL PUMP WORKERS

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Petrol (gasoline) supplied at filling stations contains a number of toxicants. Biomonitoring of exposure to field workers at workplaces has gained importance in the evaluation of human health hazards as occupational exposure to gasoline has proven health effects. This study reports the impact of gasoline on the health of petrol pump attendants at various filling stations at Islamabad and Rawalpindi. The study group consisted of 159 healthy, non-smoker petrol filling workers, aged 20-50 years with exposure duration ranging from 1-20 years while 100 healthy objects of the same age group but not directly exposed to petrol served as controls. To determine the health impact of gasoline exposure, clinical tests like complete blood profile, liver and renal function tests and molecular tests like DNA ladder and quantification were carried out. Results showed that workers exposed for more than ten years had significant increase in hemoglobin (Hb) ($P < 0.0001$) and red blood cells (RBC) ($P < 0.007$) when compared with the control subjects. White blood cell count ($P < 0.004$) and platelets ($P < 0.005$) were significantly lowered in refueling workers as compared to

controls. The DNA ladder assay of the workers working for more than ten years showed adduct or smear formation when compared to the control subjects. A significant decrease in DNA concentration was noticeable in exposed workers as compared to control subjects ($P < 0.003$). Lowered total serum protein content was observed in workers exposed for more than ten years as compared to controls. In liver function tests, exposure was associated with higher level of serum AST, ALT, ALP and bilirubin as compared to controls. This indicated the possibility of hepatotoxicity on exposure of gasoline. In exposure, gasoline produced a significant increase in serum creatinine ($P < 0.0001$) and urea concentration ($P < 0.008$) in exposed workers as compared to the control subjects. No significant difference was observed in serum testosterone level in exposed and non exposed subjects. The results of the present study indicate that there is a significant toxic effect of gasoline on workers for longer duration. Improved detection and prevention technologies are needed to answer environmentally related health question for petrol filling workers.

EFFECT OF FLUOROQUINOLONE ANTIBIOTIC (ENROFLOXACIN) ON BLOOD CELLS MORPHOLOGY, BLOOD CELL INDICES AND SERUM BIOCHEMICAL PARAMETERS IN GOLDEN CHICKENS

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Enrofloxacin is a broad spectrum synthetic fluoroquinolone antibiotic which is used as a routine treatment in chickens for urinary tract, skin and respiratory infections. The present study was conducted to investigate the effects of accidental overdose of enrofloxacin on blood cells morphology, hematological and serum biochemical parameters of golden chickens (*Gallus gallus domesticus*). A total of twenty healthy male golden chickens aged three months, weighing between 1.5 to 2.5 kg were divided into four equal groups each containing five birds. Group A, B and D received oral doses of enrofloxacin at the concentration of 10, 100 and 200 mg/kg body weight respectively daily for 15 days. Group C served as control and received 0.75% saline. Blood samples were collected at day 0, 8 and 15 through brachial vein puncture. Blood smears reported cell morphology. Hematology parameters included Hb, RBC count, PCV, MCV and MCHC. Essential serum parameters such as total cholesterol, TG, HDL, and VLDL were also studied. Birds were sacrificed on 15th day of the experiment. Results demonstrated no significant variation in hematological parameters except MCV levels that showed increase in the 100 mg dose group ($P < 0.02$) on 8th day as compared to the pre-dose group, while MCHC levels were reduced in 10 mg dose group ($P < 0.047$) on 8th day as compared to control group. Among serum biochemical parameters, no significant variation was observed in serum total cholesterol and HDL- cholesterol. Triglycerides levels were increased in the 10 mg and 100 mg dose group on 8th day ($P < 0.029$) and 15th day ($P < 0.029$) respectively in intragroup comparison. On intergroup comparison, triglycerides levels demonstrated increase on 8th day in the 10 mg dose group ($P < 0.018$) and reduced in 100 mg dose group ($P < 0.0001$). LDL levels increased in the 10 mg dose group ($P < 0.027$) and 100 mg dose group ($P < 0.0001$) as compared to the control group. VLDL levels were reduced on 8th day ($P < 0.027$) and 15th day ($P < 0.031$) as compared to pre-dose birds. On intergroup comparison, VLDL levels were reduced in 100 mg ($P < 0.028$) and in 200 mg ($P < 0.0001$) as compared to the control group. Morphological changes occurred in the morphology of red and white blood cells of treated birds. The current study thus provides evidence that irrational use of antibiotics in general and

enrofloxacin in particular, may lead to alteration of cell morphology, hematological and biochemical parameters but in total the drug appears.

MOLECULAR, BIOCHEMICAL AND HISTOPATHOLOGICAL EFFECTS OF ENROFLOXACIN ON GOLDEN CHICKENS

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Enrofloxacin is a broad spectrum synthetic fluoroquinolone antibiotic which is used as a routine treatment in chickens for urinary tract, skin and respiratory infections. The present study investigated the effects of accidental overdose of enrofloxacin on genomic DNA, serum proteins, testosterone concentration and vital organs of golden chicken. A total of twenty healthy male Golden chickens aged 3 months (average body wt. 2.5 to 3.0 kg) were divided into four equal groups each containing n=5 birds. Group B, C and D received oral doses of enrofloxacin at the concentrations of 10, 100 and 200 mg/kg respectively daily for 15 days. Group A was control and received 0.75% saline solution orally. Blood samples were collected at day 0, 7 and 15th day through brachial vein puncture. The serum samples were analyzed for total proteins, albumins, globulins, creatinine, uric acid, testosterone concentration and for the enzyme activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). DNA was extracted from blood and quantified and ladder assay was run. Birds were sacrificed on 15th day of the experiment and tissues were separated for histology. Result demonstrated no significant variation in serum ALT levels, while AST levels decreased ($p < 0.001$) and ALP levels also reduced ($p < 0.01$) in higher dose groups. Uric acid levels increased ($p < 0.004$) but no change was observed in serum creatinine levels. Total proteins remained constant throughout the experiment, while serum albumins ($p < 0.003$) and globulins levels ($p < 0.002$) were reduced. Testosterone concentration increased on 7th day of the experiment in the group treated with 100 mg ($p < 0.01$) and 200 mg ($p < 0.03$) but became normal at the end of the experiment. No fragmentation or damage was observed in DNA and there was no significant change in the quantity of DNA in any of the treatment groups. Histological assessment showed no obvious tissue damage. The present study suggests that enrofloxacin overdose may lead to hypoalbuminuria, impaired liver and kidney functions in the chickens. Careful use of antibiotics is therefore recommended as regards dosage and duration of administration.

ACUTE TOXICITY OF CADMIUM CHLORIDE (CDCL₂.H₂O) ON CHANNA MARULIUS AND WALLAGO ATTU AND THEIR BEHAVIOURAL RESPONSES

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The concentration of water by heavy metals is a worldwide environmental problem. Concentrations of heavy metals have been increasing in aquatic environments as a result of human activities. Various metals have the capability to induce harmful effects on living organisms. Static bioassay tests were carried out in order to evaluate LC₅₀ value of cadmium chloride for freshwater

fish, *Channa marulius* and *Wallago attu* as well as to observe behavioural alterations posed by cadmium. The LC₅₀ values of *Channa marulius* and *Wallago attu* for 96-hr were found 75.70±0.91 and 32.96±0.36 mgL⁻¹, respectively. The major behavioural alterations observed during the experiments were abnormal behaviour in the form of erratic swimming, equilibrium loss and enhanced surfacing behaviour. Fish, *Channa marulius* and *Wallago attu* showed the increase in the hyperactivity and convulsions by increasing individual metals and their mixture concentrations in the mediums. The equilibrium status, somersaulting activity and fin movements were maximum at lower metals exposure concentrations and were decreased by increasing exposure concentrations. There were no behavioural changes and deaths observed in the control medium throughout the experiment.

DETERMINATION OF LEAD LEVELS IN THE AMNIOTIC FLUID AND UMBILICAL CORD BLOOD OF WOMEN LIVING NEAR LEAD RECYCLING SMELTER

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Lead is ubiquitous metal having well known toxic effects on human health. Although metal is claimed to have been phased out on the petrochemical products yet it continues to be added to environment from variety of sources. Battery recycling being one of the major activities is major source of lead in the environment of many cities of Pakistan as huge scraps of lead acid batteries are always available for recycling. In the present work amniotic fluid and umbilical cord blood of women was examined for lead content. The blood samples were collected at the time of delivery and all of the subjects belonged to villages located adjacent to a battery recycling smelter. The results showed mean levels of lead in amniotic fluid as 89 ± 0.22, µg/dl and umbilical cord blood as 76.2 ± 0.43 µg/dl. The data on regular monitoring of blood lead levels of these subjects and changes associated with first, second and third trimesters is also presented. The lead levels of amniotic fluid correlated very well with urine lead levels of respective subjects and were also found to be significantly higher than the reference subjects. In the present work data is also presented on levels delta amino levulinate dehydratase activities of subjects.

EVALUATION OF THE EFFECTS OF NIGELLA SATIVA CRUDE SEED EXTRACT ON CONCENTRATIONS OF SOME HEAVY METALS AND ANTIOXIDANTS IN SERUM OF DRUG-INDUCED THROMBOCYTOPENIC RABBITS

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The present study was proposed to analyse the effects of thrombocytopenia on some heavy metal concentrations and enzymatic and non-enzymatic antioxidants in serum of chloroquine-induced thrombocytopenic rabbits. The crude extract of *Nigella sativa* seeds with respect of its pre- and post-treatment, was also analysed for its curative effect on disturbances in levels of studies parameters in thrombocytopenic rabbits. The significantly decreased concentrations of heavy metals; iron, nickel and cobalt, with subsequent decreased in platelet count were found in serum

form drug-induced thrombocytopenic rabbits. No significant affect of *Nigella sativa* pre-treatment (14 days) was observed on the status of heavy metals in serum of thrombocytopenic rabbits. However, the significant effect of *Nigella sativa* post-treatment was observed in sera of thrombocytopenic rabbits with significant increase in platelet count and in concentrations of heavy metals. The catalase activity and the concentration of ascorbic acid were found to be decreased in thrombocytopenic rabbits. The *Nigella sativa* pre-treatment of was found to help in normalising the levels of these antioxidants. However, the significant increase in catalase activity and in concentration of ascorbic acid was found in serum of *Nigella sativa* post-treated rabbits. The findings of the present study therefore suggest the affirmative effects of post-treatment of *Nigella sativa* seed extract in normalizing the platelet count, heavy metal concentrations and enzymatic and non-enzymatic antioxidants in blood serum.

DEVELOPMENTAL DEFECTS PRODUCED BY *IN UTERO* EXPOSURE OF SEPTRA D.S IN MICE

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During this study, an antimicrobial drug, Septra D.S was tested for its ability to induce developmental defects in mice fetuses. For this purpose, different concentrations of the drug, 0.00, 4.10, 8.33 and 16.66 µg/g B.W. were used. The doses were given orally to pregnant mothers on days 6-12 of gestation, and implants were recovered on day 18 of gestation. Morphological studies of the fetuses showed abnormalities such as hemorrhages, microphthalmia, limb deformities (forelimb micromelia, forelimbs extensions, drooping wrists, low set arms, hindlimb displacement), hygroma, kyphosis, kinky tail and abdominal cysts. Dose dependant intrauterine growth retardation was also observed. Fetal resorptions (45.83%) were noted in higher dose group. Morphometric studies of fetal body parts like head circumference, eye circumference, forelimb and hindlimb size and tail length indicated a significant ($P<0.001$) dose dependant decrease as compared to control fetuses. The fetal body weight and CR length were also reduced significantly ($P<0.001$) in all dose group. In comparison with control results obtained in this study clearly indicate that Septra D.S has the potential to cause teratogenicity and toxicity in developing mice. It is quite apparent that this drug must be used with utmost care during the period of organogenesis.

THE STUDY OF ANTI-STRESS POTENTIAL OF GEMMOTHERAPEUTICALLY TREATED HERBAL MIXTURE (*PIPER NIGRUM*, *LAVANDULA ANGUSTIFOLIA* AND *CORIANDRUM SATIVUM*) IN RABBIT

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The stress is a general negative outlook, excessive worrying, moodiness, irritability, agitation, inability to relax, feeling lonely, isolated and depressed, while to relief the anxiety and stress conditions is termed as anti-stress. This study was sought to investigate the anti-stress activity of gemmo-therapeutically treated mixture of *Coriandrum sativum*, *Lavandula angustifolia* and *Piper nigrum*. The mixture for this safe medicinal usage because coriander, lavandula and

black pepper have a great historical importance as medicinal herbs. For this purpose fresh growing herbs of coriander, lavender and black pepper were used to prepare gemmo-modified herbal mixture. Adult rabbits were selected for in vivo study. Throughout investigation period rabbits were housed in a room under normal and stressed conditions according to the groups of animals. Physical and chemical stress was applied by different methods. During whole investigation behavioral changes in rabbits were also recorded and their blood samples were taken after regular administration of drug. The anti-stress activity of herbal mixture was analyzed by measuring dopamine.

**AN INVESTIGATION OF THE PRESUMPTIVE DETOXIFYING ACTION OF
HOMEOPATHIC DRUG KALI IODATUM 30 ON CHRONICALLY MERCURY
POISONED RABBITS**

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Female rabbits were exposed to 1mg/body weight of mercuric chloride on alternate days for six weeks. Four groups (A, B, C, D) of rabbits were made; in each group 5 rabbits were placed. Group A acted as control which received no toxicant. Group B were given intraperitoneal injections of 0.2mg/b.w of mercuric chloride on alternate days and acted as poison control. Group C were given intraperitoneal injections of 0.2mg/b.w of mercuric chloride on alternate days along with 100mg/b.w of EDTA daily, orally and Group D received intraperitoneal injections of 0.2mg/b.w of mercuric chloride on alternate days along with five drops of Kaliiodatum 30 daily, orally. Nine serum biochemical parameters were studied. It was seen that mercuric chloride alone enhanced the serum urea, creatinine, GPT, GOT, bilirubin, total cholesterol, triglycerides and LDL cholesterol whereas it decreased the serum HDL cholesterol. Mean body weight was also reduced by the administration of mercuric chloride alone. It was observed that all these above mentioned disturbances in the serum were counteracted by EDTA (Ethylenediamine tetra acetic acid) and Kaliiodatum 30. It was noticed that Kaliiodatum 30 was more effective in retrieving all the nine serum biochemical parameters as compared to EDTA.

**AN INVESTIGATION OF THE PRESUMPTIVE DETOXIFYING ACTION OF THE
HOMEOPATHIC DRUG KALI IODATUM 30 ON CHRONICALLY LEAD POISONED
RABBITS**

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Lead is an important toxic heavy metal which is used in various industries and also in fuel such as gasoline. Lead interferes with drug and cholesterol metabolism and also in heme biosynthesis. Lead mimics body's essential elements including calcium, copper, magnesium, zinc and iron and thus interferes with body's natural processes. The aim of this study was to investigate into the detoxifying effect of kali iodatum 30 if any in reversing the adverse effects of lead poisoning (15mg/Kg b.w. as lead nitrate for six weeks) on liver and kidney of rabbits. The effect of heavy metal was compared using Student t-test. Serum ALT, AST and bilirubin levels of lead poisoned rabbits were increased ($P < 0.005$) in lead poisoned rabbits as compared to control however

serum ALT, AST and bilirubin levels of treated groups (5 drops of kali iodatum 30) showed marked decrease ($P<0.005$) as compared to those of lead poisoned rabbits. Serum creatinine and urea levels of lead poisoned rabbits were increased ($P<0.005$) as compared to those of control however serum creatinine and urea levels of treated groups (5 drops of kali iodatum 30) showed marked decrease ($P<0.005$) as compared to those of lead poisoned rabbits. These findings suggest kali iodatum 30 a good antidote for lead poisoning.

EFFECT OF CHROMIUM ON DNA DAMAGE IN THE BODY ORGANS OF FRESH WATER FISH, *CTENOPHARYNGODON IDELLA*

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The fish *Ctenopharyngodon idella* was exposed to different sub-lethal concentrations (20, 25, 33 and 50% of LC_{50}) of chromium for one month. After exposure, fish organs viz. gills, liver and kidney were analyzed for DNA damage by using Comet assay under controlled laboratory condition. DNA damage in all the three body organs of fish was dose dependent. The gills of fish showed significantly higher % DNA damage, cumulative tail length and genetic damage index values of $38.33\pm 29.62\%$, $93.73\pm 72.38\mu\text{m}$ and 1.36 ± 0.95 , respectively. However, fish kidney showed significantly least DNA damage of $24.33\pm 28.24\%$, cumulative tail length of $61.34\pm 69.66\mu\text{m}$ and genetic damage index of 0.92 ± 0.87 . The ranks of DNA damage in all the three body organs of fish were liver > kidney > gills. It can be concluded that the indices of DNA damage can be used as biomarkers of metallic toxicity in fresh water environments.

ACUTE TOXICITY OF WATERBORNE METALS TO MAJOR CARPS

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Acute toxicity tests, in terms of 96-hr LC_{50} and lethal concentrations of water-borne metals (Co, Cr, Pb) and their mixtures were conducted under laboratory conditions to evaluate the sensitivity of major carps viz., *Catla catla*, *Cirrhina mrigala* and *Labeo rohita*. All tests were performed under constant water temperature (30°C), pH (7.50) and hardness (225mgL^{-1}). Fish fingerlings (110-day age) were first acclimatized to laboratory conditions and then shifted to glass aquaria for toxicity experiments. During the whole acute toxicity trials, fish mortality and physico-chemical variables of water viz., carbon-dioxide, dissolved oxygen, pH, potassium, sodium, temperature, total hardness and total ammonia were checked at 12-hr intervals. Fish mortality data were analyzed through Probit method with 95% confidence interval to estimate 96-hr LC_{50} and lethal concentrations of metals and their mixture for each species of fish. Among the three fish species, *Catla catla* showed significantly ($p<0.05$) higher sensitivity to all the metals with the mean 96-hr LC_{50} and lethal concentrations of 40.16 ± 22.72 and $71.33\pm 39.98\text{ mgL}^{-1}$, respectively, followed by that of *Labeo rohita* and *Cirrhina mrigala*. Among the treatments, Co+Cr+Pb mixture appeared significantly most toxic to the fish than that of individual metals and their binary mixtures. Results suggested that metals (Co, Cr and Pb) in a mixture form showed synergistic

effects on the fish. However, there existed 2x synergistic effects for Co+Cr, Co+Pb and Co+Cr+Pb while that of Cr+Pb mixture was 3x.

**RENAL AND HEPATO-PROTECTIVE EFFECTS OF ARAND (*RICINUS COMMUNIS*)
LEAF EXTRACT AGAINST CCl₄ INDUCED HISTOPATHOLOGICAL AND
MICROMETRIC CHANGES IN MICE**

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In the present study effects of the methanolic extracts of *Ricinus communis* (Arand) were studied for reclamation of the histological and morphometric derangements of CCl₄ exposure in liver and kidney of mice. There were 3 Groups of 15 animals each. They were named as control, CCl₄ treated and CCl₄ +Ricin treated groups. CCl₄ was applied in a concentration 0.2 ml/kg in corn oil for once on day 1. In CCl₄ +Ricin treated group, saturated 50% methanolic extract of Ricinus leaves (0.2ml/ animal) was given after 24 hours of the CCl₄ treatment. Animals in control group received pure corn oil only. All animals were sacrificed after 72 hours of the first treatment to obtain liver and kidneys for histological preparations. The histological sections were digitally photographed for histopathological study and computer based micrometry. Mean cross-sectional area of the glomeruli and proximal tubules, proximal tubular wall thickness, caliber of the proximal tubule and height of the brush border showed significant ($p < 0.01$) decline in CCl₄ group to that of the control. Moreover necrosis of the kupper cell, misalignment of hepatic cord and widening of the sinusoids along with steatosis of hepatocytes was obvious in CCl₄ group. All histopathological and micrometric signs of hepatic and renal histology were found recovered and treatment of Ricinus leaf extract. Based upon the results we conclude that Ricinus leaf extract ameliorates the histopathologies of liver and kidney caused by CCl₄ exposure,

**EVALUATION OF HEPATIC FUNCTIONAL INTEGRITY ON SILYMARIN AND
PICRORRHIZIN AS PRE AND POST TREATMENT AGAINST CCl₄ INDUCED HEPATIC
INJURY IN MICE**

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The present work was designed to investigate the hepatic functional integrity on Silymarin and Picrorrhizin as pre and post treatment against CCl₄ induced hepatic injury in mice. Silymarin at the rate of 200mg/kg of the body weight and Picrorrhizin 50 mg/kg of the body weight against the hepatotoxicity induced by CCl₄. Total 12 mice were used which were divided into four groups, each group containing 3 mice. First group A served as control and all the remaining groups (B, C, and D) received CCl₄. The mice in groups C and D received Silymarin 200mg/kg and Picrorrhizin 50mg/kg as pre and post treatment respectively. Samples were taken from coccygeal vein of mice for the estimation of ALT, AST, ALP, and TP and to determine the role of antioxidant enzyme. The

result of present study show that due to toxicity induced by carbon tetrachloride, levels of ALT, AST, ALP, TP rise. Silymarin and Picrorrhizin reduce the level of these hepatic enzymes, Moreover, Pre and Post treatment with Silymarin and Picrorrhizin stimulate protein synthesis. The level of TBARS and Lipid peroxides get elevated due to induction of toxicity as a result of CCl₄. Than lipid per oxidation occurs and free radical get produced, than plant extract like Picrorrhizin that reduce lipid per oxidation, that in turn reduce free radical production, also stimulate free radical scavenging mechanism. SOD is an intracellular enzyme that is present in aerobic cell protects the cell from damaging reaction of ROS. CAT is heamoprotein. It protects the cell from accumulation of H₂O₂ by dismutating it to from H₂O and O₂. Toxicity induces as a result of metabolic conversion of CCl₄ by Cytochrome P-450 into trichloromethyl, Peroxy trichloromethyl, and chloride free radical. Level of hepatic enzyme or conventional indicators also rise. Both plant extract Silymarin and Picrorrhizin maintained the level of this hepatic enzyme within the normal level, and stimulate the regeneration of hepatocytes.

TERATOLOGICAL EFFECTS OF CYPERMETHRIN EXPOSURE AND THEIR AMELIORATIONS ON CO-TREATMENT OF VITAMIN E AND OLIVE OIL IN CHICK

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Developmental outcomes of cypermethrin and their ameliorations on olive oil and vitamin E treatments were studied in domestic chick (*Galus domesticus*). Fertilized eggs were divided randomly in 5 groups (60 each) identified as: control; Cypermethrin, Cypermethrin+olive oil, Cypermethrin+ vitamin E and Cypermethrin+ olive oil+ vitamin E groups. Twenty eggs from each group were recovered after 7 and 14 days of incubation each and the remaining 20 eggs from all groups were allowed to hatch normally or exteriorized (if not hatched) on day 21. Percent hatchability recorded was maximum in control and minimum in cypermethrin group. While the same, for the other three groups, remained in between but significantly higher than cypermethrin group. Mean body weight of the hatchlings showed an overall highly significant variation among the groups ($p < 0.0001$). Post hoc analysis showed significant difference of cypermethrin group with all other groups. Abnormalities seen in 7 days embryos include general growth retardation, reduced beak, micro-ophthalmia, microcephaly, forelimb micromelia, hind limb Amelia and micromelia. The abnormalities seen in 15 days embryos include open eyes, abdominal edema, hind limb deformities and patchy plumage. Abnormalities of the hatchlings include unilateral and bilateral axial limbic deformities, bilateral distal or digital deformities, patchy plumage, glaucoma and spina bifida. Maximum growth retardation in embryos and hatchlings was seen in cypermethrin group while some embryos in Cypermethrin+ olive oil+ vitamin E group showed agile growth. Limbic abnormalities were present in all cypermethrin treated groups however multiple abnormalities per embryo were recorded only in cypermethrin and cypermethrin+olive oil groups. Our findings suggest that cypermethrin is a potent developmental disruptor of the avian system. Cypermethrin induced oxidative stress on the unborn seems a major cause of such deformities as the same was significantly corrected with a combined treatment of potent antioxidant therapy of vitamin E and olive oil.

EFFECT OF NICOTINE ADMINISTRATION ON THE TESTICULAR HISTOLOGY IN ALBINO MICE

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Nicotine causes many dangerous effects in different levels to many species and also causes many histopathological changes in organs. In this study toxicity of nicotine was observed in mice. Animals were divided into two groups, Control and treated (1 mg/kg). Total 65 mice were taken and 5 died during acclimatization. 20 mice were taken in Control and 40 in treated group. Initial body weights were noted in Control (28.33±1.31), and Treated group (29.80±1.25) and final body weights were also noted (36.89±1.31) and (39.16±1.25) respectively. The mice were given the normal diet and their food intake was recorded. The value of the food intake in the Control group was (100.61±5.81) and in Experimental group was (96.57±5.50). After six weeks mice were dissected and testes were weighed. In the Control group the testis weight was (1.01±0.056) and in Experimental group was (0.83±0.07). this showed decrease in weight of testis in Experimental group. Section cutting of testes of both was done by following Eosine and Haematoxylin staining method. The histopathological changes were seen in the Experimental group with disruption of the seminiferous tubules and less numbers of the Leydig cells as compared to the Control group and also dark vacuoles were found in the Experimental group. Nicotine inhalation causes testicular degeneration in mammals and also causes infertility due to less production of sperms.

SUB LETHAL EFFECT OF CYPERMETHRIN ON EARLY DEVELOPMENT OF ROHU (*LABEO ROHITA*): A STUDY OF MORPHOLOGICAL PARAMETERS

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Cypermethrin is one of the most highly effective synthetic pyrethroid insecticides, isolated from chrysanthemum flower. It has extensively used in nearly all sectors/places like, agriculture, forestry, buildings, farm yard and gardens, where there is need of prevention of insects (Glickman *et al.*, 1982; Casida *et al.*, 1983). Runoff from all this cites may enter the aquatic water bodies and shows low to moderate persistence in the aquatic environment. Therefore this study was designed to investigate the sub lethal effects of cypermethrin on survival rate and some morphological aspects of early developmental stages of rohu (*Labeo rohita*). Acute toxicity test was performed on newly fertilized eggs of *Labeo rohita* by using different concentrations of CYP and calculated LC₅₀ value of CYP for 96 hrs was found to be 42.14 µg l⁻¹. Newly fertilized eggs were then incubated in the presence of sub lethal concentration *i.e.* one fifth of LC₅₀ (8.43 µg l⁻¹) of CYP in a well designed flow through system. Experiment was conducted in two phases, in the first phase; experiment was terminated after 48 hrs while in the second phase of experiment exposure of CYP was extended up to 96 hrs. During incubation, temperature was 26 ± 0.6 °C while pH and dissolved oxygen ranged between 7-8 and 4.5-6.5 mg l⁻¹ respectively. Percentage mortality and deformity increased with increased in time period in the treated group as compared to control group. The mortality percentages between control versus treated groups were 04.03 ± 1.0% vs. 19.47 ± 1.0%.

10.73 ± 1.2% vs. 27.13 ± 1.1%, 12.27 ± 2.1% vs. 29.64 ± 2.1% and 12.58 ± 1.2% vs. 34.31 ± 2.0%, during 24, 48, 72 and 96 hrs treatment period respectively. Morphologically all the embryos appeared normal in control group while deformities like eroded or elongated yolk sac, larvae with short tail, no eyes or larvae with zigzag movement was observed in CYP treated group from blastula up to fry stage. The result suggests that CYP affects the early development of fish by causing developmental fatality and deformities even if present in very less amount.

HISTOLOGICAL STUDY OF LUNGS IN NICOTINE TREATED ALBINO MICE

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Nicotine is the most toxic agent of the cigarette smoking not only for the humans but also for the mammals. The present study was conducted to observe the effects of nicotine on the lungs of male albino mice. 65 mice were selected for the experiment. They were divided into the two groups. 20 mice were in control and 40 mice were in experimental group. Five mice were died during acclimatization. The weight of both groups was recorded. The dose of 1mg/kg body weight of nicotine was given dermally to the experimental group whereas control group mice were provided with saline solution. After 6 weeks all mice were dissected. Their lungs were removed and weights were recorded. Then tissue microtomy was done and staining was completed by using hematoxylin and eosin staining. Results showed the significant change in the body weight (g) of experimental mice (32.29±1.25) as compared to control group mice (33.83±1.31). Food intake has been decreased in the experimental mice (96.57±5.50) as compared to control group (100.61±5.76) representing the significant change. Lungs weight showed the significant decrease in experimental mice group (1.20±0.004) as compared to the control group (2.05±0.008). Pulmo-somatic index has also been noted that was decreased in experimental group (3.70±0.016) as compared to the control group (6.05±0.023). Histological changes of the lungs in the nicotine treated mice showed the proliferation of the cells, damaged connective tissue network and congestion of lungs. Overall results of the nicotine treatment revealed that the nicotine is toxic to the mammals.

STUDY OF HISTOLOGICAL CHANGES IN LIVER OF DERMALLY NICOTINE TREATED MICE

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Nicotine is a primary addictive alkaloid present in tobacco. It is metabolized chiefly in the liver, and tends to cause lesions. The major pathway of nicotine metabolism in the liver of humans is the formation of cotinine. The current study was carried out in order to assess the toxic effects enforced by nicotine on liver of mice. For this, 65 adult male albino mice were taken from which 5 mice died during acclimatization period. The initial body weights were recorded. The mice were then divided into 2 groups; the control group and the experimental group. After 6 weeks of treatment with 1mg/kg nicotine dose, the final body weights were obtained. All mice were slaughtered and their livers were taken out and preserved in formalin after which they were

subjected to section cutting through microtome and staining with Hematoxylin & Eosin. The initial and final body weights of control group was (28.33±1.31) and (36.89±1.31) respectively. Similarly, for treated group the initial and final body weights were (29.80±1.25) and (35.39±1.25). Food intake was also observed to be decreased from (119.41±5.76) of the control group to (115.01±5.50) of the experimental group. The liver weight of the control group was (5.15±0.04) which also decreased to (4.74±0.12) in experimental mice. Major histological changes were widening and enlargement of sinusoids, necrosis, degenerative alterations of hepatic cords and hepatocytes, enlargement of nuclei, and fat deposition. Nicotine administration to the encountered animals induces lethal effects. For functional integrity of the organs, extreme and direct exposure to such drugs must be prevented.

EFFECT OF LAMBDA CYHALOTHRIN ON SERUM LEVEL OF ANDROGEN IN HUMAN AND LEYDIG'S CELLS IN RABBITS

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The studies were made to evaluate the effect of Lambda cyhalothrin on serum levels of androgen in human and leydig's cells in the testes of Rabbits (*Oryctolagus cuniculus*). The testosterone levels in 60% of total farmers were decreased significantly 4.77±0.28 ng/ml as compared to the levels 5.77±0.98 ng/ml sought before the exposure to lambda cyhalothrin. However, testosterone levels were also decreased in 40% farmers but the variation was insignificant 5.27±0.84ng/ml as compared to before exposure 5.39±0.89 ng/ml. The testicular weight of the rabbits exposed to lambda cyhalothrin for 10 days was reduced 3.45± 0.13 grams as compared to control group 4.11±0.06 grams. Whereas, 3.20 ± 0.08 grams as compared to control 4.11±0.06 grams exposed to lambda cyhalothrin for 15 days. Histopathological results of the rabbits exposed to lambda cyhalothrin for 10 and 15 days reveal that, 1. The seminiferous lobules have become elongated and oval. 2. Picnosis was noted throughout the lobular space. 3. Tumor formation was clearly visible in the interstitial space which has become compressed. 4. No leydig's cells were found in the animal group exposed to lambda cyhalothrin for 10 days. However, very drastic effects 1. The sheaths of the seminiferous lobules have become completely thin and somewhat broken. 2. Vacuolation and erosion was noted throughout the lobular space. 3. Leydig's cells hypertrophied leading towards tumor formation were noted in the group exposed to lambda cyhalothrin for 15 days period indicates that the effects of compound is very drastic and fatal.

OCCUPATIONAL EXPOSURE TO METALLIC DUST AND ITS IMPACT ON THE HEMOGRAM AND SERUM BIOCHEMISTRY OF WORKERS OF CUTLERY INDUSTRIES IN WAZIRABAD, PAKISTAN

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Occupation based impact of metals (Al, Cu, Fe, Ni, Pb, Sn and Zn) and resulted stress profiles as envisaged by hemogram and serum biochemistry were determined in 221 exposed

whetting workers of cutlery industries located at Wazirabad, Pakistan. Significant drop in platelets (PLT) (31%), mean platelet volume (MPV) (24%) and hemoglobin (Hb) (23%) with respect to insignificant responses of other erythrocyte indices (PCV, MCV, MCH and MCHC) suggests bone marrow suppression. The stressed profile of leukogram is expressed by leukocytosis (74%), lymphocytosis (54%), monocytosis (43%) with eosinopenia (79%) and neutropenia (64%). The serum biochemistry analyses revealed hyperglycemia (37%), hypocortisolemia (76%) and hypotriglyceridemia (60%). In addition to this, elevated activities of pseudocholinesterase (PChE) and serum indicators of hepatic and cardiac functions (alanine aminotransferase ALT, aspartate aminotransferase AST, alkaline phosphatase ALP and creatine kinase CK) suggest functional damage to the liver and/or heart. The findings of the present study describe the basis of different diseases / disorders that are arising due to potentially hazardous exposures in the cutlery industries and warrant for formulation and implication of strict legislation ensuring health of the workers.

OCCUPATIONAL EXPOSURE TO CLEANING SOLVENTS AND THEIR HEALTH HAZARDS: A PRELIMINARY SURVEY OF SCREEN PRINTING UNITS IN LAHORE, PAKISTAN

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Organic solvents are being used intensively in many industrial processes. Occupational exposures to these solvents are often deleterious to workers. In order to determine stress profiles of exposed workers in the context of organic solvents' (acetone, glycol ethers, methanol, toluene and xylene) exposure, a questionnaire based survey of screen printing units located in Lahore was conducted. Loss of memory was the key threat found among the workers and the percentage of patients suffering from this disorder reached up to 72%. The ratio of occurrence of this symptom was higher in workers of age between 40 and 50 years. Other common disorders included high blood pressure, depression, dizziness, dry skin, headaches, occupational fatigue, sore throat and vision difficulty. The percentages of patients suffering from such troubles were 47%, 53%, 56%, 55%, 49%, 56%, 30% and 38%, respectively. The overall trend of disorders increased with increase in age as well job duration. These results necessitate guidance and strict appliance of proper working station wherein efficient exhaust system(s) be installed immediately.

TOXICOLOGICAL ASSESSMENT OF ESSENTIAL OILS FROM SELECTED MEDICINAL PLANTS

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Essential oils are produced as secondary metabolites by aromatic plants, predominantly belonging to families Apiaceae, Lamiaceae, Myrtaceae and Rutaceae. The family Rutaceae has great economic importance for its numerous edible fruits and essential oils. In the current study

essential oils of seven plants of the family Rutaceae including, *Aegle marmelos*, *Murraya koenigii*, *Citrus reticulata blanco*, *Zanthoxylum armatum*, *Skimmia laureola*, *Murraya paniculata* and *Boenninghausenia albiflora* were used for their toxicological assessment. These plants have already been reported beneficial in multiple ailments therefore, the present study focused on the toxicological assessment of essential oils. Seven groups of selected essential oil treated wistar rats were established against control group (n=5) which received water for 14 days; animals were offered feed and water *ad-libitum* and treated with essential oils (400mg/kg body weight). On day 15 all the animals were sacrificed and blood samples were stored for hematological and serological study. Hematological studies revealed an elevation in TEC in animals treated with essential oils of *Murraya koenigii*, *Skimmia laureola* and *Boenninghausenia albiflora*, while an elevation in PCV and depletion in MCV was observed in animals treated with *Murraya paniculata* and *Boenninghausenia albiflora* respectively. Serological investigations demonstrated that LFT remain unaltered in all groups but a significant depletion in triglycerides and elevation in blood sodium level was observed in animals treated with *Aegle marmelos* and *Citrus reticulata blanco* essential oil. *Zanthoxylum armatum* caused a significant increase in urea level. Treatment with *Skimmia laureola* and *Murraya paniculata* induced similar changes along with elevation in blood urea level. *Boenninghausenia albiflora* affected many markers including RBC, MCV, triglycerides, HDL, LDL, urea and sodium. *Murraya koenigii* did not affect any serological parameter.

IMPACT OF CADMIUM (CD) ON JUVENILES OF TILAPIA, *OREOCHROMIS NILOTICUS* UNDER LABORATORY REARED CONDITIONS

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Cadmium (Cd) is one of the twenty three heavy metal toxicants, is widely used in Ni-Cd batteries manufacture, metal and mining industry, dentistry etc. because of its non-corrosive nature. Cadmium is released in considerable amounts through industrial effluents into soil, surface and ground water systems. These excess amounts in addition to naturally occurring levels gradually build up to toxic levels causing damage to the biota of the aquatic ecosystem. Cadmium was found to interfere with many protein and carbohydrate metabolisms by inhibiting the enzymes involved in the processes. The present study evaluates toxicity of Cadmium and its impact on Tilapia (*Oreochromis niloticus*). Fishes were collected from contaminated saline water ponds near Zeal Pak cement factory Hyderabad, Sindh. Short term tests of acute toxicity were performed over a period of 96 hours using cadmium chloride. The renewal technique was followed by exposing the fish to test solutions of different concentrations in the range of 2 mg/L to 8 mg/L. Preliminary experiments were conducted to choose concentrations that resulted in the mortality of the fish in the range of 10-90%. The toxicity experiments were then conducted using the chosen concentrations of CdCl₂ on the fingerlings (2.5±1 cm) in triplicate and the LC₅₀ determined using simple graphic (% Mortality vs. Log Concentration) method. The calculated average in 96-hr LC₅₀ is 4.5 mg/L. The feeding activity was reduced as the cadmium concentration increased. Swimming activity reduced to 10% in 2mg but increased to 60% in 4mg then it was reduced continuously as the concentration increased. The number of fish affected was increased with the increase of the cadmium concentrations. No eye abnormalities were found in 2mg and all the fish larvae were with eye abnormalities in 8mg cadmium.

EFFECT OF IMIDACLOPRID (NEONICOTINOIDS) ON SERUM LEVEL OF TESTOSTERONE IN HUMAN AND LEYDIG'S CELLS IN RABBITS

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An attempt was made to know the effects of an endocrine disrupting chemical, imidacloprid on the serum level of the testosterone in human and leydig's cells in rabbits (*Oryctolagus cuniculus*). In this context, Imidacloprid a systemic insecticide belongs to a class of chemicals called the Neonicotinoids was applied through olfaction in humans and administrated orally in animals. The farmers/ spray workers exposed to imidacloprid for minimum 3 hours showed significant decrease in circulating testosterone 4.61 ± 0.74 ng./ml. in 55% of the total persons involved as compared to the levels 5.42 ± 0.79 ng./ml. sought before exposure. Whereas 45% of the total farmers/spray workers showed insignificant decrease in serum testosterone levels 5.19 ± 1.46 ng./ml. as compared to 5.34 ± 1.67 ng./ml. before the exposure. The testicular weight in the rabbits exposed to imidacloprid for 15 days was reduced significantly 3.62 ± 0.10 grams as compared to the control group 4.06 ± 0.06 grams. The interstitial space as compared to control became widened and the number of leydig's cells was also decreased. Tumor formation in initial stage was noted in the interstitial space. The results indicate that the effect of compound is very high, but not fatal.

OCCUPATIONAL EXPOSURE TO METALLIC DUST AMONG WORKERS OF CUTLERY INDUSTRIES IN WAZIRABAD, PAKISTAN: A PRELIMINARY SURVEY

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Industrial health is a burning issue of developing countries where safety measures are practiced, if at all, at very low profile. Such a situation has been affecting health of workers employed in whetting units of cutlery industries situated in the study area. This paper reports a questionnaire based data of 100 plus workers for the first time in this regard. Risk of various suspected and prevailing infections was assessed by categorizing data with respect to age of workers as well as working durations. In general, apparent look of the workers was not too bad but the symptomatic pictures of these were amazingly the worst. As 96% of the workers were suffering from cough of which 65% were found to be the patients of persistent productive cough and 31% were those of persistent dry cough. Symptoms of bronchial / pulmonary damage were observed in 9% of the workers who were discharging blood in sputum. Dizziness, headaches, back aches and occupational fatigue were the common stresses found among workers of above forty years age. The overall percentages of these disorders were 25%, 21%, 41% and 45%, respectively. Other disorders like eye irritation, skin allergy and persistent itch were frequent in all age groups. The corresponding figures for these ailments appeared as 37%, 19% and 28%, respectively. Conclusively, the purpose of industrialists should not only minting money but they must have to spend significant proportions of their earnings towards the work safety and workers' welfare. In addition, workers' welfare societies / authorities should have a proper check and balance of the industries for keeping the workers and working conditions safe.

EVALUATION OF GENOTOXIC EFFECTS OF ENDOSULFAN IN FRESH WATER FISH, *CIRRHINA MRIGALA*

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Endosulfan, a compound of organochlorine pesticides, is used extensively in Pakistan to control insect pests in orchards and agricultural crops. An extensive usage of endosulfan has contaminated the natural freshwaters in the province of the Punjab due to bulk discharges of agricultural runoffs leading to adverse effects on the indigenous fish species, *Cirrhina mrigala*. The present study was conducted to evaluate the genotoxic effects of endosulfan in freshwater fish, *Cirrhina mrigala* using micronucleus test and comet assay. The 96-hr LC₅₀ of endosulfan for the fish was determined as 1.35±0.02 µg L⁻¹. On the basis of 96-hr LC₅₀, fish were exposed to four sub-lethal concentrations of endosulfan (10, 20, 33 and 50% of LC₅₀) with negative and positive control, separately, to investigate the DNA damage, micronuclei frequencies and other nuclear abnormalities for 30 days. The induction of micronucleus frequency and other nuclear abnormalities viz., binucleated, dumbbell, notched, blebbed and deformed nuclei of cells induced by different concentrations of endosulfan in peripheral erythrocytes of *Cirrhina mrigala* showed statistically significant differences at p<0.05. The DNA damage in terms of types of DNA damage (Type 0 – IV) from which percentage of DNA damaged cells, genetic damage index and cumulative tail length in *Cirrhina mrigala* exposed to different sub-lethal concentrations, with negative and positive control groups were also determined. The 50% of LC₅₀ exposure of endosulfan exhibited significantly higher DNA damage in terms of percentage of DNA damaged cells (90.67±2.31%), genetic damage index (3.03±0.06) and cumulative tail length (1395.91±12.88 µm) at p<0.05. This study revealed significant effects of various concentrations of endosulfan to induce micronuclei, other nuclear abnormalities and DNA damage in fish peripheral blood erythrocytes. This study also revealed comet assay and micronucleus frequency indices as the sensitive, rapid and useful methods in determining the potential genotoxicity of endosulfan in fish.

HEPATIC RESPONSES OF THREE CARP FISH SPECIES AGAINST METALS' POLLUTION IN RIVER RAVI, PAKISTAN

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A replicated 4 x 2 x 3 factorial arrangement comprising 4 sampling sites (Siphon=A, Shahdera=B, Sunder=C and Balloki=D), 2 flow seasons i.e., high (post monsoon) and low (winter) and 3 fish species (*Cirrhinus (C) mrigala*, *Labeo (L) rohita* and *Catla (C) catla*) was applied for studying bioaccumulation of cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), zinc (Zn), manganese (Mn), nickel (Ni) and mercury (Hg) in the livers of sampled fish from the river Ravi around Lahore. The order of metals bioaccumulation was Zn > Fe > Mn > Cu > Cr > Hg > Ni > Pb > Cd. While site wise order was C > D > B > A. The present investigation revealed that the toxicity of metals fluctuated significantly in fishes at all the sampling sites with season. The entire

sampling sites showed higher metal concentrations in fishes' livers during low than the high flow season. Among the fish species, Cd, Cr, Cu, Zn, Mn, Ni and Hg accumulations were highest in *C. mrigala*, while lowest in *C. catla*, excepting the Cr and Fe. Significantly elevated levels of all the investigated metals in livers of the sampled fishes from the polluted sites indicated differential response of the three fish species to the anthropogenically modified river segment and raise concerns about the alarming situation of the Ravi ecosystem and consequently the fishes and their consumers' health.

ACUTE TOXICITY OF BINARY MIXTURE OF PESTICIDES TO THE FISH, *CYPRINUS CARPIO*

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The pesticides viz. bifenthrin, chlorpyrifos and endosulfan are now extensively used for the control of agricultural pests. Large quantities of pesticide residues reaches the aquatic environment causing risk to the non-target organisms like fish by entering into the food chain and hence disturbing the ecological balance. This study reveals acute toxicity of pesticide mixtures (bifenthrin, chlorpyrifos and endosulfan) to the fish, *Cyprinus carpio* that was determined in terms of 96-hr LC₅₀ and lethal concentrations. Acute toxicity tests were performed with 180-day old fish under controlled laboratory conditions with three replications for each test dose. Physico-chemistry of the test media showed significant impacts on the tolerance limits of the fish. *Cyprinus carpio* showed significantly more sensitivity against chlorpyrifos+endosulfan mixture exhibiting the mean LC₅₀ of 0.42±0.02 µgL⁻¹, followed by that of bifenthrin+endosulfan, bifenthrin+ chlorpyrifos with the mean concentrations of 0.53±0.03 and 0.76±0.04µgL⁻¹, respectively. The mean lethal concentrations for the fish were 1.23±0.08, 0.83±0.05 and 0.70±0.04µgL⁻¹ against bifenthrin+chlorpyrifos, bifenthrin+endosulfan and chlorpyrifos+ endosulfan, respectively.

DNA DAMAGE INDUCTION IN PERIPHERAL ERYTHROCYTES OF FISH DURING CHRONIC EXPOSURE OF COPPER

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A diverse array of agricultural and industrial chemicals containing heavy metals and other xenobiotics are contaminating the aquatic environments. Metals are biologically non-degradable that can cause toxicity in animals through oxidative damage to membrane lipids, DNA and proteins. This study reveals copper induced genotoxic damage in peripheral erythrocytes of 150-day old fish, *Labeo rohita* using Comet assay and micronucleus test under controlled laboratory conditions. Fish were exposed to four different sub-lethal concentrations viz. 17%, 25%, 33% and 50% of LC₅₀ of copper, separately, for 30 days. Peripheral blood of chronically exposed fish was examined for damaged nuclei (%), genetic damage index (GDI), cumulative tail length (µm)

micronuclei frequency (%) and frequency of other nuclear abnormalities. Chronic exposure of copper to *Labeo rohita* induced DNA damage in peripheral erythrocytes of fish that varied significantly ($p < 0.05$) with exposure concentration. The DNA damage caused by copper was significantly ($p < 0.05$) higher than that recorded in negative control group. Significantly higher genetic damage in terms of percentage of damaged nuclei ($66.00 \pm 3.46\%$), genetic damage index (2.11 ± 0.15) and cumulative tail length ($205.70 \pm 0.10 \mu\text{m}$) was recorded at 50% of copper LC_{50} exposure to the fish while this damage was significantly minimum at 17%. Significantly higher micronuclei frequency of $28.51 \pm 0.11\%$ was observed at 16.71 mgL^{-1} i.e. 50% of LC_{50} of copper while it remained significantly least ($10.50 \pm 0.11\%$) due to 5.57 mgL^{-1} (17% of LC_{50}) concentration. This study reveals that both Comet assay and micronucleus test can be used as useful tools for the determination of genotoxic effects of metals on fish.

EFFECT OF LEAD ACETATE ON BLOOD BIOCHEMISTRY, LIVER AND RENAL HISTOPATHOLOGY IN RAT ALBINO (SPRAGUE DAWLEY)

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The present study was conducted to investigate the toxic effect of lead acetate (200mg/Kg body weight) for 48 hours on blood, liver and kidney of rat albino (*Sprague dawley*). The study included the estimation of enzyme activities like ALAT (alanine aminotransferase) and ASAT (aspartate aminotransferase), some of the biochemical contents like glucose, urea, total lipid, cholesterol and protein in blood and histopathological changes in liver and kidney. Lead acetate treatment did not affect the plasma ALAT and ASAT activities. Among biological contents glucose activity significantly increased ($P < 0.001$) while rest of contents such as lipid, urea, cholesterol and protein remained unaltered. There was a significant decrease ($P < 0.05$) in mean body weight between control and treated group. Among histopathological changes necrotic damage to hepatocytes, renal cortical area and nuclei disappearance was observed.

EFFECT OF CADMIUM CHLORIDE ON THE HISTOPATHOLOGY OF LIVER AND TESTIS IN MICE ALBINO (BALB/c)

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The present study was performed to assess the histopathology of liver in BALB/c mice exposed to $25 \text{ mg CdCl}_2/\text{kg b.w}$ for 48 hours. Single dose of Cd changed liver histology. Blurred trabecular structure, compact nuclear chromatin and hypertrophy in hepatocytes were observed. The color of liver was changed to slightly yellowish brown. Similarly cadmium induced severe pathological changes in the testis, including damage in seminiferous tubules and leydig cells. The mean body weight was also reduced significantly ($p < 0.05$) by injecting the heavy metal.

MERCURY INDUCED HISTOPATHOLOGICAL CHANGES AND PROTEIN ESTIMATION IN MUSCLE AND LIVER OF *LABEO ROHITA*SHUMAILA CHAUDARY¹, MALEEHA MANZOOR^{1,2}, MUHAMMAD SAMEE¹ AND FOUZIA SYED¹*Department of Wildlife and Fisheries, Government College University, Faisalabad*²*Punjab University, Lahore, Pakistan*
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The organ most associated with the detoxification and biotransformation process is liver and muscles are the specialized tissues that serves their function primarily by contraction.. Any damage to them will induce abnormalities in this process of detoxification and movement and ultimately can result in mortality of fish. The toxic effect of mercuric chloride (HgCl₂) on the structure of liver tissue and muscle histology of *Labeo rohita* were investigated. Fish collected from Government Fish Seed Hatchery, Faisalabad were exposed to four HgCl₂ doses viz. 0.10, 0.25, 0.50 and 1.00 mgL⁻¹ designated as T₁, T₂, T₃ and T₄ while T₀ was control. 14 days HgCl₂ exposure induced significant changes in liver tissue and muscle tissue in treated groups when compared to control. Mercuric chloride induced noticeable changes in the liver tissue like necrosis, aggregation of inflammatory cells between the hepatocytes, thrombus formation, nuclear hypertrophy, intravascular hemolysis in blood vessels and presence of cytoplasmic degeneration in liver tissue with respect to control showing normal histology. The degree of change in liver tissues was higher at high concentration of mercury dosage. Muscle sections treated with Mercuric chloride showed deleterious changes in tissues. Alterations in muscle structure observed were atrophy, degeneration of muscles, edema, splitting of muscle fibers. 14 days HgCl₂ exposure induced significant change in protein content in liver and muscle tissue in treated groups when compared to control. It was observed that rising amount of Mercury in the fresh water bodies coming from different sources like factories, tanneries, pharmaceuticals and different industries is providing a big threat for aquatic life and ultimately to human health.

ANALYSIS OF HEAVY METALS (Fe, Mn, Cu, Zn, Ni, Cd) IN *MEGALASPIS CORDYLA* (LINNAEUS 1758) FISH FROM THE COAST OF KARACHI.FARZANA YOUSUF, QURATULAN AHMED
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The distribution of heavy metals (Fe, Mn, Cu, Zn, Ni, Cd) was detected in the muscles, liver, kidney and gills of fifty eight (58) fishes of *Megalaspis cordyla* fish. The fish were collected seasonally (Pre-monsoon, monsoon, post monsoon) during (January, 2012-December, 2012) from Karachi fish Harbour. Our result showed the highest mean length (cm) (26.8±2.72) and weight (g) (182±47.71) were measured in monsoon season and the lowest mean length (cm) (23.8±5.64) and weight (g) (132±75.91) were measured in post monsoon season. The concentrations of heavy metals were recorded highest in liver than in muscles kidney and gills. In liver of the fish highest mean concentration of Fe (528.24±141.73 ug/g) were recorded in pre-monsoon season and the lowest mean concentration of Ni (0.23±0.17 ug/g) in kidney were recorded in post monsoon season. Iron, zinc, copper, and manganese were recorded most abundant in fish liver, kidney and gills while cadmium and nickel were recorded lowest level of mean concentration in all fish tissues. Heavy metal concentration were analyzed using inductively coupled plasma optical Emission (ICP-

OES) Spectrophotometer. The metal concentration were decrease in sequences as Fe>Zn>Cu>Mn>Ni>Cd.

HAEMATOLOGICAL STUDIES OF SILVER CARP, *HYPOPHTHALMYCHTHYS MOLITRIX* (VALENCIENNES) (CYPRINIDAE) EXPOSED TO A HEAVY METAL, LEAD (Pb)

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The present study was aimed to investigate the effects of heavy metal Lead (Pb) on blood indices of silver carp, *Hypophthalmichthys molitrix* under laboratory conditions. The fish (20 fish / treatment) was exposed to three sub-lethal doses of Pb(NO₃)₂ (T1 =6mg/l, T2=12mg/l, T3= 18mg/l) of for five weeks in 280L glass aquaria with a flow through system. The haematological indices exhibited alterations in all three treated groups. The RBCs showed sudden decrease in their number on exposure to lead than control value (88%). The final values of RBCs were 73.6% (T1), 71.2% (T2), and 62.0% (T3). Lymphocytes showed increasing trend from normal value (5.6%) and the maximum increase was 20.0% (T3) followed by 14.4% (T1) and 13.4% (T2). The fluctuation in the number of macrophage was also observed. The maximum value of macro phages was recorded as 7.8%, 8.4% and 13.8% in T1, T2 and T3. The monocytes also fluctuated and the maximum increase was 6.6% (T3), 6% (T1) and 4.8% (T2). Thrombocytes too showed change in T1 and maximum value for T3 as 3.6% at week 5. The maximum deviation from the normal values of blood indices was observed in fishes exposed to the highest concentration of lead (18mg/l). The haemoglobin level also changed over time on exposure to various treatments. The lowest Hb content was observed to be 4.143±0.241 in T3 at week 4. This study showed that lead even at lowest concentration have adverse effects on haematology parameters of the fish exposed to it.

EFFECTS ON GROWTH AND GILLS OF SILVER CARP *HYPOPHTHAIMICTHYS MOLITRIX* (VALENCIENNES, 1844) EXPOSED TO LEAD (Pb)

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In this study, effect of Lead nitrate on the growth and gills of silver carp, *Hypophthalmichthys molitrix* was studied. Seventy six *H. molitrix* were sampled from Punjab University, Research Fish Farm, acclimatized and divided into four groups. One group was served as a control and remaining three groups for treatment T1, T2, and T3. Treatments *i.e* T1 (8mg/l), T2 (16mg/l), and T3 (24mg/l) were used to determine the effect of different concentrations of lead nitrate, on weekly basis (W1 to W5), on the growth performance of silver carp. The weight loss was maximum at T3 and there was very small increase in total and fork length, under the toxic effect of lead nitrate. Similarly histopathological effects were observed on weekly basis. Severe degenerative changes were observed at high concentration of Lead nitrate *i.e* (24mg/l). Hyperplasia, desquamation, Infiltration of secondary lamellae, necrosis, fusion of secondary filaments, hyperplasia of epithelial cell, vacuolation of cytoplasm of lining epithelium, rupturing of epithelium and insignificant congestion of the blood spaces. Mortality rate was very high at 4th and 5th week in T2 and T3. The caudal fin of dead fishes became black and totally degenerated. Hence

accumulation of metals in various organs of fish may cause structural lesions and functional disturbance. Therefore, the problem of metal pollution is considered among the most serious one. It is supposed to be one of the greatest national health problems.

THE EFFECT OF CEMENT DUST EXPOSURE ON HEMATOLOGICAL, RENAL AND LIVER PROFILES OF CEMENT INDUSTRY WORKERS

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Cement dust exposure has been reported to be the major cause of various occupational health problems and long term complications. The Present study, therefore evaluates the level of ALT, AST, Creatinine, uric acid and haematological profile in subjects occupationally exposed to cement dust in 30 cement factory workers having more than 15 years of experience as compared to 30 controls of the same socioeconomic status. In haematological profile, haemoglobin, RBCs, Mean corpuscular volume, MCV, MCH and WBCs were significantly higher in exposed subjects as compared to that of controls, however there was no significant change in MCHC and Platelets. A significant elevation was also observed in ALT, AST, Creatinine and Uric acid. Higher haematological profile probably reveals that cement mill workers are exposed to metals that enhance haematopoietic system. Elevated levels of liver enzymes (ALT, AST) proves hepatic damage. Significantly higher creatinine suggests nephritic damage and higher uric acid levels may be to combat oxidative stress.

VARIATION IN TESTOSTERONE LEVEL IN IMPOSEX AND NON IMPOSEX INTERTIDAL GASTROPODS FOUND ALONG THE COAST OF PAKISTAN

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Organotin compounds are used in antifouling paints and wood preservatives to prevent fouling of marine organisms on ships and other marine structures. The leaching of these compounds has caused imposex (pseudohermaphroditism) globally in females of over 170 species of prosobranch gastropods inhabiting areas close to marinas and harbours. The investigations of imposex induction pathway indicate that tributyltin (TBT) and triphenyltin (TPT) produce this abnormality via endocrine dysfunction, interfering with the regulation of testosterone. The two gastropod species *Thais bufo* and *T. rudolphi* were analyzed from Manara rocky ledge, Clifton and Gaddani ship-breaking yard. At Gaddani the incidence of imposex was 100% while at the other two sites no such female was found. The analysis of testosterone level was carried out by using radioimmunoassay (RIA). Higher level of testosterone was recorded in imposex females than males from Gaddani ship-breaking yard. In samples of Clifton and Manara rocky ledge elevated concentrations of testosterone were noted in males. The relatively higher level of testosterone in imposex females indicates that TBT elevates free testosterone levels in imposex females.

**HISTOPATHOLOGICAL CHANGES IN GILL AND LIVER OF SILVER CARP
(*HYPOPHthalmichthys molitrix*) DURING ACUTE EXPOSURE TO A
PYRETHROID (*DELTAMETHRIN*) PESTICIDE**

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Pyrethroids belong to the most commonly used pesticides worldwide. Their massive expansion is a threat to the natural environment including the aquatic ecosystems. Although pyrethroids are rapidly degraded in soil and plants, they are extremely toxic to fish because of fish high sensitivity to them. The pyrethroid class of insecticides, including deltamethrin, is being used as substitutes for organochlorines and organophosphates in pest-control programs. This study was aimed to investigate the impact of this commonly used pesticide on different tissues as pyrethroids are absorbed by fish gills readily and then distributed to bile, liver and kidney. Fish samples (7 fish in each test group) were exposed to different concentrations of deltamethrin between 0-2 ppm for 96 h in 40 liter water tubs. Significant changes in tissues were observed after acute exposure of chemical. The very low LC₅₀ obtained for deltamethrin (0.10 ppm) indicated that deltamethrin is highly toxic to *Hypophthalmichthys molitrix*. Tissues were collected from each group with different concentration of pesticide. These tissues were processed and sectioned at 5 micron and then hematoxylin and eosin (H&E) staining was performed. Disorders of movement and breathing during acute poisoning were observed. The most common gill changes of fish treated by deltamethrin were desquamation and necrosis when compared with control gills. Liver showed degeneration of hepatocytes and pyknotic nuclei were observed in affected hepatocytes.

**EVALUATION OF LIPID PROFILE AND TOTAL PROTEINS AS A DIAGNOSTIC
TOOL FOR THE ASSESSMENT OF HEPATIC INJURY INDUCED BY
THIOACETAMIDE (TAA) IN *RATTUS NORVEGICUS***

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Thioacetamide (TAA), a proved hepatotoxic and necrotic agent has been used extensively employed in various animal models as it consistently induces fibrosis and cirrhosis akin to human liver cirrhosis. The present study was aimed with the objective to assess change in lipid profile, tissue and serum total proteins particularly albumin and blood sugar random (BSR), in acute condition after TAA injection in rats. TAA (300 mg/kg body weight) was injected intraperitoneally in Wistar rats (n=3). All the animals were anesthetized and sacrificed after equal intervals of 12h up to 24h. Blood was drawn, serum separated and livers were excised and processed for protein profile. The administration of TAA led to acute hepatic toxicity. Hypoproteinemia in tissues (P=0.0014) and sera (P=0.0423) while hypoalbuminemia (P=0.0044) were observed in serum in the experimental time points against control. Furthermore, Lipid profile and BSR were also studied. After 12h of administration hypercholesterolemia (P<0.0001),

hyperlipidemia and a rise in Low density lipoproteins (LDL) ($P=0.0003$), triglycerides and high density lipoproteins (HDL) was observed while a significant negative change in very low density lipoproteins (VLDL) ($P=0.0235$) were noted on the same study point. On the other hand hyperglycemia ($P=0.0099$) was observed after 24h, similar results was found after 12h though the changes were insignificant. Taken together these results we can conclude that liver fibrotic agent TAA intoxication led to a noticeable change in serum and tissue total protein contents specifically albumin. Furthermore, the assessment of lipid activities especially cholesterol and low density lipoproteins (LDL) as well as BSR in serum proves to be valuable tools for acute hepatic damage.

STUDY OF GILL TISSUE AND RENAL PATHOLOGY AND PROTEIN ESTIMATION IN ROHU (*LABEO ROHITA*) EXPOSED TO MERCURIC CHLORIDE

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Gills plays vital role for respiration and kidneys are very vital organs. Any damage to them will induce abnormalities in this process of respiration and waste removal and ultimately can result in mortality of fish. The toxic effect of mercuric chloride ($HgCl_2$) on the structure of gill tissue and kidney histology of *Labeo rohita* were investigated. Fish collected from Government Fish Seed Hatchery, Faisalabad were exposed to four $Mgc1_2$ doses viz. 0.10, 0.25, 0.50 and 1.00 mgL^{-1} designated as T1, T2, T3 and T4 while T0 was control. 14 days $Mgc1_2$ exposure induced significant change in gill tissue and kidney tissue in treated groups when compared to control. Mercuric chloride induced noticeable changes in the gill tissue like necrosis, dilation in blood vessels, hyperplasia, hypertrophy, hemorrhage in blood vessels and curling of filaments in gill tissue with respect to control showing normal histology. The degree of change in gill tissues was / higher at high concentration of mercury dosage. Kidney sections treated with Mercuric chloride showed deleterious changes in tissues. Alterations in kidney structure observed were severe degenerative and necrotic changes in renal tubules with focal areas of necrosis, hemorrhage, haemosiderin, and aggregation of inflammatory cells, severe degeneration of hepatic tissues and vacuolar degeneration in the epithelium of renal, tubules. 14 days $Mgc1_2$ exposure induced significant change in protein content in gill and kidney tissue in treated groups when compared to control. it was observed that rising amount of Mercury in the fresh water bodies coming from different sources like factories, tanneries, pharmaceuticals and different industries is providing a big threat for aquatic life and ultimately to human health.

ASSESSMENT OF FOETAL TOXICITY OF DELTAMETHRIN IN MICE

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Deltamethrin is one of the most toxic pyrethroids for veliebrate used extensively in pest control. The present study was conducted to evaluate the oral median lethal dose and teratogenic effects in mice foetus. The pregnant mice were exposed to single doses of 19.36, 09.7, and 04.8 $\mu g/g$ BW. These concentrations of deltamethrin were given orally to the pregnant mice on day "6" of gestation. The fetuses were recovered on day "8" of gestation. The morphological studies revealed abnormalities including sacral hygromae, microcephally, micromelia, open eyelids and.

microphthalmia, exophthalmia, cryptophthalmia, drooping Wrist, kyphosis, and short tail. In addition to these abnormalities, resorptions were also observed as the doses were increased. Morphometric analysis showed an overall reduction in body weight, crown rump length, brain and eyes circumferences and length of hind and forelimbs and tail size with the significant differences ($p < 0.001$) against control. The histological studies shows malformations including defective nasal pouch, nasal septum with atrophied inferior cochlea, defective eye, pericranial hydrocephaly, cleft palate, degeneration of jaw muscles. Tissue necrosis of brain, liver and intestine were also observed in serial as compare to control. On the basis of these findings it is concluded that the oral exposure of deltamethrin is potentially teratogenic for developing mice and is equally harmful to foetal development in humans.

**TOXIC EFFECTS OF DELTAMETHRIN ON SPLEEN AND KIDNEY OF SILVER CARP
(*HYPOPHTHALMICHTHYS MOLITRIX*) DURING ACUTE EXPOSURE**

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The contamination of surface waters by pesticides used in agriculture is a problem of worldwide importance. Pesticides also can get into the water indirectly after running off from surrounding treated crops. Pyrethroids like deltamethrin have been shown to be up to 1000 times more toxic to fish than to mammals and birds at comparable concentrations. Fish sensitivity to pyrethroids may be explained by their relatively slow metabolism and slow elimination of these compounds. During present work different groups (7 fish in each test group) were exposed to different concentrations of deltamethrin between 0-2 ppm for 96 h in 40 liter water tubs. Significant changes in tissues were observed after acute exposure of chemical. Fish were dissected and tissue samples from spleen and kidney were collected from each group with different concentration of pesticide. These tissues were processed and sectioned at 5 micron and then heamatoxilin and eosin (H&E) staining was performed. Disorders of movement and breathing during acute poisoning were observed. Deltamethrin destructive effects on kidney and spleen were observed as compared to the control fish. Changes in kidney included degeneration of renal tubules, dilatation of glomerular capillaries, degeneration of glomeruli, intracytoplasmatic vacuoles in epithelial cells of renal tubules with hypertrophied cells and narrowing of the tubular lumen. Pyknosis and necrosis were observed in spleen of treated fish as compared to control fish.

SECTION – I I**PESTS AND PEST CONTROL****SEARCHING BEHAVIOR AND PARASITISM OF *TRICHOGRAMMA CHILONIS* (ISHII) (HYMENOPTERA: TRICHOGRAMMATIDAE) IN OKRA**

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Searching and parasitism ability of host of egg parasitoid, *Trichogramma chilonis* (Ishii) (Hymenoptera: Trichogrammatidae) was evaluated in okra vegetable in a field trial at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, during the year 2010. Irrigations, hoeing and fertilizer requirements of the crop was maintained under standard agronomic practices for the whole growth period of the crop. Experiment was carried out under unsprayed conditions. Parasitoids were released into the crop from a fixed central point and evaluation of parasitism was made at a tested distances from 1 to 20 meter. Factitious host, (*Sitotroga cerealella*) eggs were counted and pasted by natural tree gum on paper cards and tagged on leaves of okra plants at various tested distances into the field. Host eggs of each treatment of different time intervals *i.e.*, 24, 48 and 72 hours were collected and placed under lab conditions for 3 days until the appearance of parasitism evidence by changing the colour of egg. Results showed that search for host by *T. chilonis* was varied according to distance and time interval and eggs were parasitized at all the tested distances ranged from 40.3 to 61.8, 42.5 to 75.0 and 22 to 38.6% after 24, 48 and 72 hours, respectively from 1 to 20 metere distance.

INCIDENCE OF RED BUG (*DYSDERCUS* SPP.) AND DUSKY BUG (*OXYCARENUS* SPP.) IN COTTON CROP

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Bt and non Bt cotton varieties were sown under field conditions in two crop seasons of the years 2011 and 2012 at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad. Occurrence and damage to cotton yield by red cotton bug and dusky cotton bug were recorded. Both the pests begin their attack during the months of July and August at squaring and flowering stage of crop and continue even in months of December and January. The highest number of red cotton bug was recorded in the month of September, 2011 (33.5/plant) as compared to 12/plant in September, 2012. The highest number of dusky cotton bug was observed in month of November, 2011 (65/plant) as compared to 73/plant in November, 2012. Damage was also recorded in plots where insecticides were used to control these pests. It was due to its concealed habit of feeding inside semi cracked bolls (dusky cotton bug) and its breeding place under fallen leaves (red cotton bug). Foliar applications were found less effective.

A STUDY OF IMPACTS OF DOSES OF VARIOUS FERTILIZERS AND CLIMATIC CONDITIONS ON PREVALENCE OF THRIPS ON *Bt.* COTTON IN MULTAN, SOUTHERN PUNJAB, PAKISTAN

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Three *Bt.* Cotton varieties *viz.*: *Bt.* Cotton – 121, VIP – 333, BR- 304 were tested for relationship of various doses of nitrogen, phosphorous, potassium fertilizers application and infestation of *Thrips tabaci* Lind. These varieties were also studied for climatic factor *viz.*: mean temperature, average humidity, precipitation and day length for infestation of the same pest on selected *Bt.* Cotton varieties. The study was conducted during June 2011 to December 2011. The crop was sowed during second week of May 2011 following RCBD replicated two times at Central Cotton Research Institute Old Shujaabad road, Multan, Punjab, Pakistan. The significant change observed by application of N1P1K1 and N2P2K2 composition of fertilizer with infestation of thrips on all three selected varieties of *Bt.* Cotton. The application of N3P3K3 fertilizer composition caused no significant change to infestation of thrips except average humidity all other climatic factors caused no significant change to infestation of thrips on these three *Bt.* Cotton varieties.

THE INFESTATION RATE OF *ORYZAEPHILUS SURINAMENSIS* (COLEOPTERA: SILIVANDAE) ON STORED FRESH AND DRY DATES OF SINDH, PAKISTAN

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The date fruits are very important food; contain a high percentage of carbohydrates as well as vitamins and minerals, but no fat and cholesterol in dates, Al-Farsi *et al* (2007). In Pakistan, Sindh is an ideal place for cultivating the different varieties of dates, so that in Sindh dates are stored in huge amount. But in Sindh stored dates are facing a critical problem of insect infestation especially by Saw toothed beetle, *Oryzaephilus*. We have reared Saw toothed beetle on three varieties of fresh dates *viz.*, Kupro, Karbalian and Fasly and two dry date varieties *viz.*, Aseel and Dadhi for three months from 01-02-2012 to 31-04-2012 to observe varietal susceptibility of dates against Saw toothed beetle. The percentage of in One hundred dates (fruit) of each variety was examined. About one hundred dates of each variety was provided to saw toothed beetles in five different jars for a period of three months. The observations were made weekly. The examination leads to determination of infestation rate in different varieties. The highest infestation rate was observed in fresh date varieties compared to dry dates. The larvae and adults both feed voraciously on dates. The adult beetles were easily entered in date fruits with their strong chewing mouth parts, that is why the infestation rate was faster on fresh dates compare to the dry dates. The moisture and sugar (carbohydrate) was more in fresh dates than dry dates. The fresh dates have also soft and tender flesh (mass), so that beetles easily infested the fresh dates. This result indicates that increase in moisture, carbohydrates and being a soft flesh of fresh dates varieties were corresponded the increase in infestation rate by stored beetles. The infestation rate showed significantly positive correlation with moisture, carbohydrates and soft flesh. It was observed that one life cycle was completed almost in one month.

HOST SEARCHING ABILITY AND PARASITISM OF *TRICHOGRAMMA CHILONIS* (ISHII) (HYMENOPTERA: TRICHOGRAMMATIDAE) IN TOMATO UNDER NATURAL FIELD CONDITIONS

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Field experiment was conducted at Nuclear Institute for agriculture and Biology (NIAB), Faisalabad, during the year 2009-2010 in tomato crop to evaluate the host searching and parasitism ability of egg parasitoid, *Trichogramma chilonis* (Ishii) (Hymenoptera: Trichogrammatidae). Standard agronomic practices as irrigations, hoeing and fertilizer requirements were adopted during the entire growth period of the crop under pesticide free conditions. Releases of parasitoids were made from a fixed central point and parasitism was evaluated at distances from 1 to 20 meter from released point. Counted eggs of factitious host, *Sitotroga cerealella* were pasted on paper cards and tagged on tender twigs and leaves of tomato plants at tested distance all around the field. Parasitism was checked after 24, 48 and 72 hours from release intervals. Results showed that search for host by *T. chilonis* was very successful and eggs were found parasitized at all observed distances. Parasitism varied with time intervals at various distances and it was 53.9 to 78.0, 69.5 to 85.7 and 24 to 50.1% after 24, 48 and 72 hours, respectively.

EVALUATION OF *BEAUVERIA BASSIANA* (BAL.) INFECTIVITY TO WHITE-BACKED PLANTHOPPER, *SOGATELLA FURCIFERA* (HORVATH) UNDER LABORATORY CONDITION

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The efficiency of formulated *B. bassiana* based mycoinsecticides to control *S. furcifera* was assessed under laboratory conditions. Five concentrations of isolate 274 (10^5 - 10^9 spores/ml and (0 control) were used to treat eggs and nymphal instars of *S.furcifera*. All spore concentration was infectious to eggs and nymphal instars. The highest mortality and reduced egg hatching percentage was recorded at high spore concentration.

BIOCIDAL EFFECT OF VARIOUS PARTS OF COMMONLY AVAILABLE PLANT PRODUCTS ON *TRIBOLIUM CASTANEUM* (HERBST) (COLEOPTERA: TENEBRIONIDAE) IN STORED PRODUCTS

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Stored products face immense losses by insects ranging from 10-40%. The major pest attacking stored products is *Tribolium castaneum* (Herbst). Both larvae and adults cause losses which result in weight losses, profuse smell, and many times setting of fungi attack. Synthetic insecticides have been used to control this pest for many decades. But the residential effects,

resistance developed in the target species and effect on non-target species made it compulsory to meet the modern day need. Botanicals offer promising results in controlling *T. castaneum* in stored grains, having a few disadvantages. The present study encompasses the use of powders of Neem seed kernals, Eucalyptus and Tobacco in flours of wheat (whole meal and maida), rice and corn; and Garlic, Ginger and Lemon grass in the grains of wheat, rice and gram pulse. Garlic, Ginger and Lemongrass were tested against the grains of wheat, rice and gram pulse. Weight loss in grams, percent weight loss and the number of dead and alive adults of *T. castaneum* was counted after four months. Grains treated with Garlic and Ginger gave least weight loss followed by that of Lemongrass. Tested adults were reduced significantly. Effect of Neem seeds, Tobacco leaves and Eucalyptus leaves was also calculated in wheat, rice and corn flours. Number of larvae, pupae and adults of *T. castaneum* were calculated. In each of the flours, Neem seeds and Eucalyptus leaves gave similar results followed by that of Tobacco. As Neem seed used showed the best results, it was tested against *T. castaneum* in wheat wholemeal flour and maida. Neem seed powder was added weight by weight (w/w) in each of the flours in five doses *i.e.* 1%, 2%, 3%, 4% and 5% w/w. Efficient results were obtained as the percentage of Neem seed powder was increased in the flour, except on 3% where the number of larvae, pupae and adults increased unexpectedly. Each of the plant powder gave potential results as grain protectant which revealed that the use of these pesticides would be a novel tragedy for the control of this pest.

**COMPARATIVE BIOLOGY STUDY OF *CHRYSOPERLA CARNEA* (STEPHENS)
(NEUROPTERA: CHRYSOPIDAE) TO NEW UNIDENTIFIED SPECIES**

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Comparative biology of predator, green lacewing, *Chrysoperla carnea* (Stephens) were studied to an unidentified specie to their different life stages at controlled temperature ($26\pm 2^{\circ}\text{C}$) under laboratory conditions. Lacewing adults of both species were cultured in equal size rearing units with standardised adult diet. All developmental and reproductive biological parameters such as larval duration at different instars, pupal duration, fecundity per female, male and female longevity. Results showed that there was a significant difference among all the observed parameters within *Chrysoperla* spp. Egg incubation period (days) in unidentified spp. was prolonged (6.0) as compared to 2.5 in *C. carnea*. Similar trend of larval (11 and 16.8 days), pupal duration (98.3 and 13.6), male longevity (32.8 and 21.0 days) female longevity (43.3 and 33.6 days) and fecundity (469.6 and 352.6 nos.), in *C. Carnea* and unidentified spp., respectively were recorded. It is concluded from the present study that biological parameters of *C. Carnea* is comparatively more significant than that of unidentified spp which may include in mass rearing.

**PRELIMINARY STUDIES ON THE PATHOLOGY CAUSED BY *MERMIS NIGRESCENS*
DUJARDIN IN ORTHOPTERA FROM SINDH**

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Nematodes *Mermis nigrescens* Dujardin, 1842 parasitize many species of grasshopper they have been reported infecting grasshopper populations upto 70%. At the present significant

parasitism of *M. nigrescens* was reported on 8 species of grasshoppers i-e *Locusta migratoria* (Linnaeus, 1758) , *Oedaleus senegalensis* (Krauss, 1877), *Poekilocerus pictus* (Fabricius, 1775), *Hieroglyphus nigrorepletus* I. Bolivar, *Anacridium aegyptium* (Linnaeus) 1764, *Cyrtacanthacris tatarica* (Linnaeus), *Choroedocus illustris* (Walker) and *Choroedocus robustus* Serville from Sindh. It was observed that incidence increased with the increase in humidity after the monsoons rains. Furthermore parasitism ratio was reported significantly higher in flooded rice fields as compared to grasslands and other dry habitats. Beside this, it was also studied that infected individual failed to develop ovaries or mature testes. This information has been collected for the first time from this region. Present study recommended that *M. nigrescens* might be proved best tool in control management of pests.

ASSESSMENT OF INFESTATION AND MANAGEMENT OF STORED GRAINS BY RED FLOUR BEETLE *TRIBOLIUM CASTANEUM*(HERBST) (TENEBROINIDAE: COLEOPTERA) IN SOUTHERN PUNJAB, PAKISTAN.

QAMAR SAEED, SYED MUHAMMAD ZAKA, MARRYAM BAKHAT AWAR AND MUHAMMAD RASHID AKRAM

Tribolium castaneum (Herbst) (Tenebrionidae: Coleoptera) is a cosmopolitan pest of many stored products. It causes a bundle of damage to the stored commodities. A number of chemical and natural insecticides have been in use against this pest for centuries. Farmers inherit this knowledge from their forefathers and researchers uncover the chemical analysis for these natural products by the results of their experiments. For this reason, a survey was conducted in the 5 districts Dist. Bahawalpur, Multan, Lodhran, Khanewal and Vehari in southern Punjab, Pakistan to inquire about the damage and control measures of stored grain pest *T. castaneum*. The key elements of this survey were to identify the problems in storage such as lack of identification of pests by people or lack of knowledge of protection of grains during storage. The data was collected from five hundred people, including 65% of house owners and farmers, 20% of shopkeepers and 15% of females (65:20:15). Metal bins, earthen bins and polyethene bags were used for the storage purpose of grains. Different control methods were applied to control this pest. From the interviewed people, it was revealed that 43% people use phosphine tablets, 26% use botanicals, 22% use cleaning method, 0.33% use mercury, and 0.12% use sodium chloride salt as their control measure while 0.29% people do not adopt any control measure. This investigation can be helpful in clearing up new platforms for research in the aspects of stored grains pests and their control measures.

SOME STUDIES ON THE PATHOGENIC MICROORGANISMS AS BIOLOGICAL CONTROL AGENTS OF *HIEROGLYPHUS* SPECIES (HEMIACRIDINAE: ACRIDIDAE: ORTHOPTERA) FROM PAKISTAN

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The genus *Hieroglyphus* is considered polyphagous and destructive pest of cash crops in Pakistan. In order to decline their population there is the need for environmental friendly alternative biological control that involve the use of natural enemies and pathogens to control pests. At the

present observation has been made on three microbial agent i-e entomopathogenic fungi, *Scelio* and *Eutrombidium* species. These are very important to reduce the grasshopper's population in field. During the present study three pathogenic fungi species i.e. *Metarthizium flavoviride* Gams & Roszypal, *Beauveria bassiana* (Bals.-Criv.) and *Aspergillus* sp. Micheli were isolated and identified with infection the following incidence rates: (53%), (35%) and (12%) respectively on *Hieroglyphus*. Moreover some species of *Scelio* are found exclusively parasitoids of *Orthoptera* eggs and significantly reduce the hatching percentage of embryo of *Hieroglyphus*. Overall parasitism ratio of *Scelio aegypticus* was reported significantly highest i.e., 36.0% followed by 33.75% and 30.16% caused by *S. hieroglyphi* and *S. mauritanicus* respectively. In addition to this, *Eutrombidium trigonum* was also reported as severe bio-control agent of grasshoppers it significantly infest the host species beings generally occurs by way of the head, pronotum, thorax under the wing-and elytron-pads, and some parts of femur of older hoppers as well as adults. *Eutrombidium* infestation mostly occurs during months of July to September when humidity percentage was reported significantly high in the field. Present study strongly recommended that these microorganisms have been considered on numerous occasions for inclusion in Integrated Pest Management (IPM) programs. This study was supported by grants received from Higher Education Commission Islamabad for Research Project (HEC No.20-1762 / R&D/10).

THRIPS TABACI (THYSANOPTERA: THIRIPIDAE) - A MENACE FOR ONION CROP IN PUNJAB (PAKISTAN).

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14 Varieties/promising lines (Faisalabad Red, Desi large, Rubina, Red imposta, Mirpur Khas, Dark Red, Early Red, Pk-1023, Posa Red, Desi Red, Phulkara, VRIO-6, VRIO-3 and VRUO-A) were screened against Thrips. Varieties, Desi Red, Desi large, Phulkara, were found more tolerable against thrips where minimum thrips population/plant recorded was 26.11, 26.83 & 28.19, respectively as compared to others. VIRIO-3 was found most susceptible where maximum thrips population/plant i.e., 52.80 was recorded. Plant extracts viz; neem leaf, datura seed, Akk leaf, garlic and Confidor (Imidacloprid 20 SL) were tested against thrips. After 7-days of observation, minimum population of thrips (10.02) was observed in plots where confidor was sprayed followed by the Akk leaf extract (11.83), Datura leaf extract (12.23) whereas neem and Garlic found the least effective resulting maximum population i.e. 14.70 and 14.60 respectively. Minimum Thrips population (15.42) was recorded from plots where hoeing was done at 10-days interval followed by 15-days interval (18.27), 20-days interval (21.31) and maximum thrips (28.58) population was recorded in plots where no hoeing was done. Among different Insecticides (Diafenfthiuron 50EC, Thiacloprid 48 SC, Acetamiprid 20 SL, Acephate 97DF, Imidacloprid 200SL, Chlorfenpyr 360SC, Blaster 72.5% WP (Imidacloprid + Acephate), Bugatti 50% SC (Imidacloprid + Diafenfthiuron) tested against thrips, Thiacloprid 48 SC gave maximum mortality% (85.04%) after 3-days followed by Bugatti 50% SC @ 200 ml/acre (84.16%), Chlorfenpyr 360SC (83.25%), Blaster 72.5% WP (81.58), Bugatti 50% SC @ 250 ml/ac (80.20), Diafenfthiuron 50 EC (79.86%), Imidacloprid 200 SL (74.75%), Acephate 97DF (74.39%). After 7-days, Chlorfenpyr 360 SC gave maximum mortality (87.62%) and the descending order of % mortality of thrips was Acephate 97DF

(86.98%) > Blaster 72.5% WP (83.65%) > Bugatti 50% SC @ 250ml/acre (82.89%) > Bugatti 50% SC @ 200 ml/acre (82.87%) > Thiacloprid 48 SC (81.17%) > Diafenfthiuron 50 EC (81.15%) > Imidacloprid 200 SL (76.40%) > Acetamiprid 20 SL (71.71%).

BIO-INTENSIVE INTEGRATED MANAGEMENT OF THRIPS, *THRIPS TABACI* (LIND.) ON BT-COTTON IN PUNJAB, PAKISTAN

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The natural resistance of Bt cotton to the thrips, *Thrips tabaci* (Lind.) through morphological and chemistry traits of cotton leaves, coupled with weather factors and finally deliberate release of natural enemies to manage thrips was assessed, on a screened resistant and a susceptible Bt cotton genotypes/ varieties. In the preliminary varietal screening trials, Twenty genotypes of Bt-cotton viz., AA-802, BH-178, MN-121, Bt-131, Bt-141, CBS-1, FH-114, FH-113, FH-4243, GM-2085, IR-3, IR-4, IR-901, IR-824, IUB-212, IUB-222, IUB-2009, Subhan-2001, Tarzan-2 and VH-259 during 2008 and based on per leaf density data of the pest, 6 genotypes (VH-259 and Tarzan showing susceptible response, BH-178 and H-4243 showing intermediate response and GM-2085 and AA-802 had comparatively resistant response) were selected, latter showing 12% HPSI. All the genotypes differed significantly with respect to physico-morphic and chemical plant characters. Hair density on midrib and vein showed positive and significant correlation with the pest density ($P \leq 0.01$), while gossypol glands on lamina, total minerals showed negative and significant correlation ($P \leq 0.05$). The pest population was not significantly correlated with weather factors during 2008, 2009 and on cumulative basis except relative humidity during 2009 and on cumulative basis which was significantly and positively correlated, r-values being 0.555 ($P \leq 0.05$) and 0.451 ($P \leq 0.01$), respectively. Relative humidity had also maximum impact on per unit change in thrips' population during 2008, 2009 and on cumulative basis which showed 14.6 percent, 22.7 and 20.7 percent, respectively. The maximum temperature contributed 11.8% role in per unit change of the pest during 2008 while rainfall shared 8.2 and 7.0% during 2009 and on cumulative basis, respectively. The effect of the 15 treatments on the population of Thrips consisted of integration of release of *Coccinella septempunctata* and release of *Chrysoperla carnea* at one week interval for six releases, spray of Neem Seed Kernel Extract (NSKE) 5% at the rate of 1500 ml ha⁻¹ at fortnight intervals for three times, Spray of spinosad 240 SL (Tracer) at the rate of 125 ml ha⁻¹ at fortnight intervals for three times and their combinations. The application of spinosad alone and in combination with other control methods proved to be the most effective treatment. The application of bio-agents viz. Coccinellid and *C. carnea* resulted in 4.72 and 4.51 thrips per leaf, respectively and did not differ significantly with each other. The application of coccinellid and *C. carnea* in combination also was less effective with 4.37 thrips per leaf and differed significantly from those of observed in all other treatments. The application of NKSE resulted in 4.00 thrips per leaf and found superior from those treatments where bio-agents were released alone and in combination. However, the maximum yield was observed where all the control methods were integrated together showing 30.976 kg plot⁻¹ seed cotton. On the basis of these interventions, the maximum cost benefit ratio was calculated to be 1: 15.19 in those plots of AA-802 where spinosad alone was sprayed and this treatment was found to be the best economical and easy to apply for the farmers. The minimum cost benefit ratio was recorded in T-5 i.e. 1: 1.02 where both the biological agents were released together. The results have been discussed in the light of these cost benefit

ratio and it was concluded that deliberate use of predators may be expensive for management of Thrips on Bt cotton.

EFFECT OF DELTAMETHRIN AND BIFENTHRIN ON MORTALITY OF *HIPPASA MADHUAE* (LYCOSIDAE: ARANEAE) UNDER LABORATORY CONDITION

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The specimens of *Hippasa madhuae* were collected from unsprayed fields in the vicinity of Sargodha. Effect of deltamethrin and bifenthrin (pyrethroid) on *Hippasa madhuae* was studied from May 2011 through August 2011 under laboratory condition in the Department of Biological Sciences, University of Sargodha, Punjab, Pakistan. For this purpose topical and residual bioassay tests were performed. Both insecticides were found to be highly toxic. The susceptibility of *Hippasa madhuae* to insecticides was sex related. Males were highly susceptible than females, although the ratio of susceptibility varies in all treatments of insecticides. Mortality was increased with increasing the concentration of insecticides. Results of residual bioassays showed that these insecticides were highly toxic to spiders even after one month of its exposure. Residual toxicity was higher for deltamethrin than bifenthrin.

EVALUATION OF INSECTICIDE RESISTANCE AMONG DIFFERENT FIELD POPULATIONS OF FRUIT FLY, *BACTROCERA ZONATA* (SAUNDERS) (DIPTERA: TEPHRITIDAE) IN GUAVA ORCHARDS

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Bactrocera zonata (Diptera: Tephritidae), is one of the most notorious pests of fruits and vegetables in Pakistan. The farmers mostly rely on chemical control to control this pest by spraying pyrethroids and organophosphate on their fruits and vegetables in Pakistan, but farmers attained unsatisfactory results due to ineffective pesticides control against this pest. The present research was conducted to determine the resistance level of 5 technical grade insecticides viz., trichlorfon, malathion, bifenthrin, lambda-cyhalothrin and methomyl against seventeen field populations of *B. zonata* in guava orchards at Sahiwal, Punjab, Pakistan i.e., 108/7R, 112/7R, 7/11L, 9/11L, 184/9L, 112/12L, 44/12L, 49/12L, 14/14L, 9/14L, 59/12L, 7/14L, 30/11L, 146/9L, 162/9L, 18/11L, 23/11L. Results showed that trichlorfon was found moderate to high resistance ratio and malathion, bifenthrin, lambda-cyhalothrin with moderate to low level of resistance in 15 populations of *B. zonata* i.e., 108/7R, 112/7R, 7/11L, 9/11L, 184/9L, 112/12L, 44/12L, 49/12L, 14/14L, 9/14L, 59/12L, 7/14L, 30/11L, 18/11L, 23/11L whereas, 3 populations 146/9L, 162/9L

and 184/9L were behaved as susceptible to above 4 mentioned tested insecticides. The methomyl was observed as susceptible to all the above 17 tested field populations of *B. zonata*.

MITIGATION OF INSECTICIDES RESISTANCE IN THE LABORATORY RESISTANT STRAINS OF FRUIT FLY, *BACTROCERA ZONATA* (SAUNDERS) (DIPTERA: TEPHRITIDAE) BY REPRODUCTION OF RESISTANT STRAINS TO NEXT GENERATION WITHOUT EXPOSURE OF INSECTICIDES

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Bactrocera zonata (Saunders) is an important pest of vegetables and fruits in Pakistan. It caused massive economic losses. The use of pesticides has been main source for controlling this pest in Pakistan, but insecticides resistance is the main issue for controlling this pest. The recent study was conducted to minimize the three insecticides viz., trichlorfon, malathion and bifenthrin resistant strains (M₁, M₂ and SWL) of fruit fly, *Bactrocera zonata* (Saunders) by reproduction to next generation without exposure of insecticides. The result showed that reproduction of resistant strains to next generation with exposure to three insecticides i.e., trichlorfon, malathion and bifenthrin insecticides had increased their resistance ratio up to 6th generation with selection pressure of insecticides. The progeny of 10th generation, which was obtained by the reproduction of resistant strain from 6th to 9th generations without exposure of insecticides, showed significant decreases in their resistance ratio as compared to 6th generation. It is suggested that if *B. zonata* field populations got resistance to any specific insecticide, that insecticide may be replaced to other insecticide for some period to overcome resistance against *B. zonata* population to that of specific insecticide.

EFFICACY OF TWO NEONICOTINOID INSECTICIDES AS SEED TREATMENT AGAINST THREE EARLY SUCKING PESTS OF COTTON CROP UNDER NATURAL FIELD CONDITIONS

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The neonicotinoid insecticides, thiamethoxam 70 WS and imidacloprid 70 WS are being used for cotton seed treatment in cotton crop to control various early season sucking pests. The residual efficacy of thiamethoxam 70WS and imidacloprid 70WS was studied as seed treatment against three early season cotton sucking pests i.e., *Thrips tabaci*, *Bemisia tabaci* and *Amrasca devastans* at their recommended doses, @ 3gKg⁻¹ cotton seed and 10gKg⁻¹ cotton seed

respectively. The trial was conducted during the year 2012 at Irshad Agriculture Farm, Sahiwal, Punjab, Pakistan. The results showed that *Thrips tabaci*, *Bemisia tabaci* and *Amrasca devastans* populations were remained below ETL up to 40 days after sowing in thiamethoxam 70WS cotton seed treated plots, while up to 50 days after sowing in imidacloprid 70WS cotton seed treated plots as compared to untreated plots. It is concluded that imidacloprid 70WS residues is more persistent than that of thiamethoxam 70WS.

**SYNERGISTIC EFFECT OF IMIDACLOPRID AND ENTOMOPATHOGENIC FUNGI
BEAUVERIA BASSIANA AGAINST SOGATELLA FURCIFERA HORVATH
(HOMOPTERA: DELPHACIDAE) IN RICE CROP**

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The white backed planthopper (*Sogatella furcifera* horvath), is a serious pest of rice. Present investigation has been carried out to determine the relative efficacy of Imidacloprid and *Beauveria bassiana* and also to check their possible synergistic effect when applied in combination, for the sustainable management of *S. furcifera*. The results indicate that population density in case of *B. bassiana* treated plots was 16.86, 13.54 and 11.94%, while in Imidacloprid treated plots was 14.45, 11.98 and 8.86% after 7, 14 and 21 days, respectively. The population density was lowest (6.82%) in *B. bassiana* + Imidacloprid treated plots, after 21 days. Similarly, minimum infestation (9.46%) was recorded where combine application of *B. bassiana* and Imidacloprid was done, followed by Imidacloprid (13.06%) and *B. bassiana* (18.52%). The results highlight a potential for pest control by combined formulation or application of *B. bassiana* and imidacloprid.

**EFFICACY OF INSECTICIDES AGAINST CITRUS PSYLLA (*DIAPHORINA CITRI*) IN
FIELD AND LABORATORY CONDITIONS**

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The Field experiment was conducted on citrus plants to check the efficacy of insecticides against citrus psylla, at Horticulture Research Institute, Jhang road, Faisalabad while lab studies were carried out at Entomological research Institute, Faisalabad. Four insecticides, polytrin-C, lambda-cyhalothrin, bifenthrin and imidacloprid applied, had almost equal effect on the population reduction of citrus psylla on all citrus plants. Trial was laid out in Randomized Complete Block Design (RCBD) having five treatments with three replications in citrus orchard, after three days of spray showed percentage control as 96.91%, 94.33%, 93.83% and 93.06% of following insecticides Polytrin-C, Imidacloprid, Bifenthrin and lambda-cyhalothrin respectively, calculated by MSTAT-C. Psylla adults were exposed to different concentrations (500, 400, 300, 200 and 100 ppm) of imidacloprid and bifenthrin, and two controlled conditions (with leaves and without leaves). Both imidacloprid and bifenthrin insecticides proved the most effective against *Diaphorina citri* with LT₅₀s of 4 and 5 hours respectively at a concentration of 500 ppm, calculated by probability test with Minitab-15.

EFFECTIVENESS OF DIVERGENT CONTROL METHODS ON INFESTATION OF SUGARCANE STEM BORER (*CHILO INFUSCATELLUS* SNELLEN)

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In order to find the most effective IPM method for the control of stem borer of sugarcane, and to improve yield contributing factors, an experiment was conducted at farmer's field located at Noor Wala 15-km Jampur road, D. G. Khan. Two sugarcane varieties (BF-162) and (CP-43/33) was sown during third week of February 2010. Experiment was laid out in Randomized Complete Block Design (RCBD) under factorial arrangement with 7 treatments including control each having three replications. The results revealed that application of all control methods in the combined form proved most effective showing minimum percent infestation of stem borer, minimum internode damage, maximum cane height, Weight/cane and maximum cane yield followed by Furadan 3G application @ 14kg acre⁻¹ (1st at germination and 2nd on earthing up) and by releases of Bio-control agent *Trichogramma chilonis* @ 30,000 parasitized eggs acre⁻¹ at 15 days Interval from April to onward and trash mulching at the time of germination.

A LABORATORY ASSESSMENT FOR THE POTENTIAL OF ENTOMOPATHOGENIC FUNGI AGAINST *TRIBOLIUM CASTANEUM* (HERBST.) (COLEOPTERA: TENEBRIONIDAE)

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The red flour beetle is a very important primary pest of stored wheat in world. To evaluate the pathogenicity of entomopathogenic fungi *Beauveria bassiana* and *Isaria fumosorosea*, immersion bioassays were performed under laboratory conditions against the adults and 2nd instar grubs of *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae). Spores were separated from fifteen days old fungus culture and subsequently four different concentrations *i.e.*, 2×10⁸, 3×10⁸, 4×10⁸ and 5×10⁸ of *B. bassiana* and *I. fumosorosea* were made in 0.05% Tween 80 solution by serial dilution. On adults, minimum mortality 12.5% and maximum percent mortality 32.5% was recorded on 7th day after the application of concentrations 2×10⁸ and 5×10⁸ spores/ml of *B. bassiana* respectively while on immatures, minimum 2.5% mortality on 5th day and maximum mortality percentage *i.e.*, 80 was obtained on 7th day after the application of concentrations 2×10⁸ and 5×10⁸ spores/ml of *B. bassiana* respectively. On the other side, on adults minimum mortality 7.5% and maximum percent mortality 22.5 was recorded on 7th day after the application of concentrations 2×10⁸ and 5×10⁸ spores/ml of *I. fumosorosea* respectively while on immatures minimum 5% mortality on 6th day and maximum mortality percentage v;z;70 was obtained on 7th day after the application of concentrations 2×10⁸ and 5×10⁸ spores/ml of *I. fumosorosea* respectively.

**SURVEILLANCE ON POPULATION DYNAMICS AND FRUITS INFESTATION OF
TEPHRITID FRUIT FLIES IN GUAVA (*PSIDIUM GUAJAVA* L.), MANGO (*MANGIFERA
INDICA* L.) AND CITRUS (*CITRUS RETICULATA* BLANCO) ORCHARDS**

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Fruit flies (Tephritidae) are serious and amongst the world's worst pests of fruits that cause enormous losses in orchards. The research studies to be carried out on fruit fly monitoring can accomplish a number of things and insure timely implementation of appropriate pest control measures. For practical field implementation two variables such as flies per trap per week and percent fruit infestation were monitored by installation of 3 Steiner traps hung on tree per hectare baited with Methyl euginol attractant (lure). Surveillance for flies per trap was done each week by counting trap catches, recording and removing any flies that have been collected in the trap and identifying the species. The percentage fly infestation for each fruit was examined randomly analyzing a total of 100 ripe fruits and number of fruits showing fruit fly oviposition marks. The results showed that the tephritids were present relatively in less numbers in citrus followed by mango fields, while more prevalent in guava trees indicating that guava orchard was the most preferred host habitat despite the presence of other fruit species as potential hosts of fruit flies. The *Bactrocera zonata* (Saunders) was the predominant species (more frequent, constant and dominant) at each experimental site. Fruit fly analysis by installation of traps on a Guava (*Psidium guajava* L. var. Gola) orchard characterized high population frequency of *B. zonata* (116.67-307.58) captured per trap per week in June to August and accounting for 8.05-18.59% infestation values recorded. Surveillance of fruit fly populations in Mango (*Mangifera indica* L. var. langra) orchard revealed that peak population of *B. zonata* (40-30 per trap per week) and fruit infestation (9.05-7.45%) were recorded in June, July & August. Monitoring of fruit fly population in Citrus (*Citrus reticulata* Blanco var. Kinow mandarin) orchard indicated that the highest density of *B. zonata* (27.83-79.58 per trap per week) and fruit infestation (3.33-3.70%) were found in October & November. All of this information gives fruit fly activity in an area, estimate of population, species composition, sex, growth stage and determining whether number of pests are increasing or decreasing to remain away from dependence upon insecticides.

**COMPARATIVE TOXIC EFFECTS OF *RUBUS FRUTICOSUS* L. AND *VALERIANA
JATAMANSI* JONES AGAINST GRAINARY WEEVIL, *SITOPHILUS GRANARIUS* (L.)**

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The poor storage conditions in developing countries provide favorable environment for the growth of stored grain pests, bio-pesticides are the best alternative of synthetic insecticides. In the present research 1-5 g of black berry, *Rubus fruticosus* L., fruit and mushk bala, *Valeriana jatamansi* Jones, rhizome powder were tested, separately against 10 grainary weevil, *Sitophilus granarius* L., in 50 g wheat grains for seven days. The phytotoxicity was the highest at five grams

(2.86±0.3), (3.83±0.4) and was the lowest at one gram (1.03±0.3), (1.20±0.2) for *R. fruticosus* and *V. jatamansi*, respectively, in comparison with the control. In residual test, the survival rate of *S. granarius* at 1 g and 5 g of *R. fruticosus* was (9.0±0.0), (9.8±0.4) and (7.0±0.4), (9.40±0.9), respectively, on 1st and 7th day in comparison with the control. The survival rate of *S. granarius* at 1 g and 5 g of *V. jatamansi* was (9.0±0.0), (9.8±0.4) and (7.0±1.2), (9.20±1.1), respectively, on 1st and 7th day in comparison with the control. In the extended effect of both plants powders, 100% mortality was for 5 g of both the powders, the lowest mortality of 36% and 54% for *R. fruticosus* and *V. jatamansi*, respectively, on the 4th day in comparison with the control. There is 5.60% and 14.92% reduction in the activity of malate dehydrogenase enzyme, in *R. fruticosus* and *V. jatamansi*, treated insects, respectively, in comparison with the control. The alpha amylase enzyme activity was 6.82% reduced and 63.63% increased in *R. fruticosus* and *V. jatamansi*, treated insects, respectively, in comparison with the control. It is concluded that plant powders have high potential in protecting wheat against insect pests. It is recommended that plant derived products may be used against stored grain pests as an alternative to synthetic pesticides due to their efficacy and safety.

COMPARATIVE TOXICITY OF INSECTICIDES AGAINST TWO LEPIDOPTERAN IMPORTANT INSECT PESTS OF CAULIFLOWER CROP

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Study was conducted to test four insecticides *i.e.*, profenofos, emamectin benzoate, λ -cyhalothrin and lufenuron against field populations of important cauliflower insect pests (*Plutella xylostella*, *Spodoptera litura*) at different larval instars during 2010-11. The field populations collected from cauliflower from Rawalpindi and Taxila farm fields was tested using leaf dip bioassay method under laboratory conditions. Emamectin benzoate was the most toxic insecticide for both insect pests. However, profenofos were the second most toxic insecticides to different larval instars of *S. litura* and λ -cyhalothrin to *P. xylostella*. High LC₅₀ values for lufenuron for this limited time exposure might be due to its slow acting as chitin synthesis inhibitor. Emamectin benzoate can be suggested as the most effective insecticides against both field populations of these insect pests along with profenofos and λ -cyhalothrin.

EFFECT OF TEMPERATURE ON THE BIOLOGICAL AND PREDATORY POTENTIAL OF *CHRYSOPERLA CARNEA* (NEUROPTERA: CHRYSOPIDAE) FED ON COTTON MEALYBUG, *PHENACOCCLUS SOLENOPSIS* (TINSELY)

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Cotton mealybug, *Phenacoccus solenopsis* (Tinsley), has become an emerging and serious threat to agriculture sector in Pakistan. It attacks a number of crops, ornamentals and fruit trees.

Different bio control agents are used for the control of cotton mealybug. Among these *Chrysoperla carnea* (Stephens) (Neurotera: Chrysopidae) is one of the most important generalist predator and the larvae of are voracious predator of soft bodied arthropods including cotton mealybug. The present study were conducted to check the effect of four constant temperatures, 20, 24, 28 and 32±1°C were evaluated on the biological parameters and predatory potential of *Chrysoperla carnea* feeding on *Phenacoccus solenopsis* at Insect pest management program , National Agricultural research centre Islamabad. The results revealed that maximum incubation period was 4.6 ± 0.13 days and minimum was 2.7±0.09 days at 20±1°C and 32±1°C respectively. The total larval developmental duration was 12.8±0.69, 10.5±0.62, 9.0±0.56 and 7.7±0.52 days at four temperatures respectively. The maximum pupal duration was 8.3±0.37 days while the minimum was 4.8 ± 0.32 days at mini and maximum temperature levels. The results indicate that temperatures have significantly effect on the developmental duration of immature stages and with increasing temperature developmental duration decrease. The larval predatory potential was 104.4±0.05, 118.5 ±1 0.04, 150.3 ± 0.05 and 107.3 ± 0.05 crawlers per larvae at four temperature levels respectively. The adult female longevity was 61.5 ±0.02, 49.4 ± 0.02, 42.3 ±0.03 and 37.2 ±0.02 days respectively. The male longevity was 29.4 ±0.02, 27.3 ±0.01, 22.8 ±0.02 and 17.2 ±0.02 days. The highest net reproductive rate (Ro) was 180.5 eggs per female at 28 ±1 °C and lowest reproductive rate was 59.62 eggs per female at 20 ± 1 °C. The mean generation time (T) was longer (61.0) at 20 ± 1 °C and shorter (42.0) days at 28±1°C. Maximum intrinsic rate of increase (rm) was (0.052) and minimum was (0.029) females per female at 28±1°C and 20 ±1°C. The population doubling time (DT) was highest (10.344) days at 20 ± 1 °C and shortest (5.757) days at 28 ±1°C. The present study exhibited that among four temperatures, 28 ±1 °C was more suitable for rearing *Chrysoperla carnea* feeding on *phenacoccus solenopsis*, owing to shorter developmental time and the best results obtained for the fertility life table parameters

STUDIES ON SEASONAL MONITORING OF FRUIT FLY, *BACTROCERA ZONATA* (SAUND.) BY USING METHYL EUGENOL TRAPS AT DIFFERENT HEIGHTS OF GUAVA TREES AT TWO DIFFERENT DISTRICTS OF SINDH

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Present study has been carried out during the year 2012 in months of June to November on an area of six acres of guava orchards in each district, including Nuclear Institute of Agriculture (NIA) Tandojam, Hyderabad and Mahota, Larkana. The observation on adult populations of fruit flies were recorded weekly through pheromone trap baited with lure toxicant mixture (95 % methyl eugenol + 5 % thiodan insecticide). There were four treatments i. e. pheromone traps installed on ground surface (T1), 1 meter (T2), 2 meters (T3) and 3 meter heights (T4). Killed male flies in traps were counted and species were identified at weekly interval. The data regarding the effect of various trap heights on trap rate of *Bactrocera zonata* and *B. dorsalis* in Tandojam (NIA) showed that weekly trap rate of *B. zonata* was highest i-e (4268.84±613.12/week) during the month of July when trap was installed at 2 m height, while it was lowest (184.55±16.08/week) in November at 2 m and 3 m trap heights. The weekly trap rate of *B. dorsalis* was highest i-e (14.36±1.55/week)

during th month of July at 2 m trap height, while lowest ($1.70\pm 0.22/\text{week}$) was recordede in November at 2m and 3 m trap height. In case of trap rate of fruit flies at Larkana district, the data indicated that weekly trap rate of *B. zonata* was highest ($5542.08\pm 587.05/\text{week}$) during the month of July when trap was installed at 2 m height, while lowest *B. zonata* trap rate ($1185.70 \pm 29.67/\text{week}$) was recorded in November at 3 m trap height. The weekly trap rate of *B. dorsalis* was highest i-e ($62.44\pm 0.75/\text{week}$) during the month of September at 2 m trap height, while it was found significantly lowest i-e ($18.30\pm 0.31/\text{week}$) in the month of November at 1m trap height.

**FIELD EFFICACY OF DIFFERENT INSECTICIDES AGAINST GRAM POD BORER,
HELICOVERPA ARMIGERA HUB. ON GRAM CROP**

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The studies on the effect of different insecticides against gram pod borer, *Helicoverpa armigera* Hub. on gram crop were carried out at pulse Section, Rice Research Institute, Dokari, during the rabi season. The Experiment was laid out Randomized Complete Block Design with four replication and eight treatments (including check). The crop was sprayed twice, the insecticides applied were Tracer 240 EC, Match EC, Shogun 1.8 EC, Lorsban 40 EC, Pirate 360 LSC, Steward 150 SC and Thiodan 35 EC. The pre-treatment observation was taken one day before application of insecticide and post-treatment observation were taken 3, 6, 10, 15 and 20 days after application of insecticides. The result revealed that on an over all mean basis the minimum pest population was recorded in Steward and Shogun treated plots with a mean population of 0.15 and 0.16 larvae per plant followed by Thiodan with mean 0.20 larvae per plant respectively.

**A NEW AND EFFECTIVE BAIT DELIVERY METHOD FOR CONTROL OF WILD
BOAR POPULATIONS**

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Wild boar infestations, during the last decade or so, have increased both vertical and horizontal throughout the country and the animal has emerged as a major agricultural pest. Environmental factors such as expansion in its habitat and non-existence of any national or provincial control programmes contributed significantly in the increase of wild boar populations. Areas which were not known of its distribution in the past are, presently, heavily infested with wild boar, i.e. Pothwar plateau, salt range, desert land of Thal, Galiat, Baklakot, and Azad Jammu & Kashmir. Various control methods such as shooting, trapping and poisoning have been tested, evaluated and recommended. Poisoning, using different toxicants, has proved to be the most successful and cost-effective method for controlling infestations to prevent damage to crops. Toxicants such as anticoagulants (warfarin, coumatetralyl and brodifacoum) and acute poisons such as sodium mono fluoroacetate (1080) or sodium fluoroacetamide (1081) are being used successfully with grains such as whole wheat and maize. However, there are multi-environmental and operational problems associated with the use of grain baits. These include primary and

secondary poisoning to non-target wildlife species, hazards to live-stock, intentional and accidental poisoning and very high operational costs. To overcome and reduce these problems a new bait delivery method, using acute poisons such as Aldicarb, has been evaluated by conducting preliminary trails. This method involves the use of poultry bird heads (broiler), and Aldicarb, 1080 and 1081 as toxicants. The toxicants are mixed with peanut butter or animal fat at the concentration of 2 and 0.03 percent w/w, respectively. This paste like mixture is inserted (2-3 g) into the buckle cavity of the poultry head. Surface and sub-surface baiting is conducted by using these treated heads. The baiting strategy includes the use of treated heads as piles (10-15 or more) or placing 3 or 4 heads at equal intervals of distance along the wild boar trails. To test this method preliminary field trails were conducted at Fateh Jang, Taxila and Kalarkahar where high infestations of wild boar were reported. Three nights baiting using 1080 and Aldicarb showed very high consumption (90-100%) of the bait. Dead bodies of wild boar, although very difficult to locate, were also recorded in the treated area. No non-target protected wildlife species were affected during the baiting. Further area based trials of this environment-friendly method are suggested as it is fairly safe, economically cheap, and can easily be adopted by the farmers.

**A STUDY OF DIFFERENT ABIOTIC FACTORS AND VARIOUS DOSES OF
FERTILIZERS ON PREVALENCE OF WHITE FLY ON Bt. COTTON IN MULTAN,
SOUTHERN PUNJAB, PAKISTAN**

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Cotton cultivation in semi-arid region of Punjab provides the great potential to offer livelihood to millions of small farmers. There are many factors which influence on cotton crop. Such factors include as length of the growing season, climatic conditions (including day length, temperature, light, humidity, rainfall, and precipitation), cultivation availability of nutrients, soil moisture and pests attack. It is believed that environmental factors exert the major influence on yield development during the season. Temperature is the main environmental factors contributing to yields in cotton. White fly population and effect of different types of fertilizers and climatic conditions were evaluated to check the infestation on the Bt.Cotton throughout the season. Impact of different doses of fertilizers and abiotic factor on white fly population were evaluated to check the infestation on the different varieties of Bt. Cotton viz: Bt. Cotton-121, VIP -333, BR-304, throughout the season. Study was conducted on the experimental area in Central Cotton Research institute Multan, Punjab Pakistan. It was observed that pest outbreak was increased during flowering and at the time of introduction of fertilizers due to increase in plant growth. It also increases due to increase in temperature and low humidity. In the beginning there was less white fly population, which increases during last week of October 2011, as the crop reaches toward maturity, white fly population increases. The data shows significant correlation with population of white fly because it seems that crop size and high temperature encourages the pest attack. During the month of August to September white fly population increase tremendously. Maximum population of white fly was calculated in last week of September and October as crop reaches towards maturity. Due to less humidity in air and high temperature and fast growth rate also favour white fly population. It decreases due to high humidity and fall of leaves in Bt. Cotton. The study was conducted during June 2011 to December 2011. The crop was sowed during second week of May 2011 following RCBD replicated two times. Significant changes were observed by applications of N1P1K1,

N2P2K2 and N3P3K3. The effect of abiotic factors like temperature and day length show significant correlation between density of white fly population. While the population of white fly decreases with increase of humidity and precipitation in the air.

EFFECT OF INSECTICIDE ON THE FUNCTIONAL RESPONSE OF *OXYOPES JAVANUS* (OXYOPIDAE: ARANEA) AGAINST APHIDS

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Present study was designed to assess the functional response of most abundant hunter species of spider *Oxyopes javanus* (Araneae: Oxyopidae) against four species of aphids viz., *Aphis gossypii*, *Macrosiphum rosae*, *Aphis nerii* and *Sitobion avenae*. Results of the study showed that *O. javanus* consume *Aphis gossypii* more vigorously than all other types of aphids. While, *Macrosiphum rosae* were consumed in smallest proportion among all the studied prey species. However, consumption rate of all aphid species increased with the increase of density. In agroecosystems, different insecticides are used to suppress the population of aphids. These insecticides not only decrease the survival rate of spiders but also dampen their predatory potential against herbivore pest. Laboratory experiments were performed to assess the effect of imidacloprid on the predatory potential of *O. javanus* against aphid population. For study specimens of *O. javanus* were exposed to different concentrations of imidacloprid (10, 20 and 30% of field rate) by spraying method. In treated spiders, the predation rate was significantly low as compared to untreated spider. The concentration of imidacloprid has inverse relationship with the killing and consumption rate of *O. javanus*. These results suggest that imidacloprid can affect the performance of spiders in the agricultural fields and should be used cautiously in the integrated pest management (IPM).

MONITORING THE POPULATION OF SUCKING INSECT PEST ON SESAME CROP

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Experiment on monitoring the population of sucking insect pest on sesame crop was carried out at the experimental area of Oil Seed Section (Agriculture Research Institute) Tandojam, during 2011. The results revealed that the population of sucking insect pests *i.e.* aphid, *Aphis gossypii* (Glover), Jassid, *Amrassca devastans* (Dist), thrip, *Thrip tabaci* (Lind), and whitefly, *Bemisia tabaci* (Genn), were observed separately and mean population was calculated weekly. The results revealed that the population of whitefly remained maximum, followed by jassid, thrip and aphid during the study period. The results further depicted that the maximum mean population (4.02 /leaf) of whitefly remained in the 2nd week of August, 2011 with minimum (0.70/leaf) in 3rd week of June, 2011. While weekly average population of whitefly was 2.41 per leaf from 3rd week of June to 2nd week of September, 2011. The data further showed that overall maximum weekly mean whitefly

population ranged between (4.02 ± 0.2155) in the 2nd week of August, 2011. The results of jassid showed that (3.69) was maximum in 1st week of August and (0.55) minimum population in 2nd week of September, 2011 with average population of (2.16) from 3rd week of June to 2nd week of September, while the overall mean population of jassid ranged (3.69 ± 0.255) in 1st week of August. While the results of thrip showed that (1.67) was maximum in 1st week of August and (0.13) minimum population in 2nd week of July with average population of (0.91) from 3rd week of June to 2nd week of September, while the overall mean population of thrip ranged (1.67 ± 0.1736) in the 1st week of August, 2011. While the results of aphid depicted that (0.93) was maximum in 3rd week of July and (0.08) minimum population in 3rd and 4th week of June with average population of (0.54) from 3rd week of June to 2nd week of September, 2011 while the overall mean population of aphid ranged (0.93 ± 0.4901) in 3rd week of July. The results further showed that the statistically analysis of data remained significant, while the correlation with temperature and relative humidity remained positive and significant in the month of July, August and September, 2011 but negative and non significant association in the month of June with the pest populations.

POPULATION DYNAMICS OF THRIPS AND WHITEFLIES AND THEIR NATURAL ENEMIES ON MUSTARD CROP IN DIFFERENT LOCALITIES OF SINDH

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The field studies were on population dynamics of sucking insect pest of thrip, *thrips tabaci* and whitefly *Bemisia tabaci* pests and their predator *Geocoris* spp on Mustard crop *Brassica campestris* L, in Tando Allahyar and Tandojam District Hyderabad Pakistan. The population dynamics of thrips and whitefly pests and their predator *Geocoris* spp were carried out at weekly intervals; twenty five plants were randomly selected from each locality the population on with the help of magnifying glass and insect sucker machine. The plants were carefully handled to avoid disturbing the pests and their natural enemies. Time of observation was 8-10 am. The results showed that on the weekly mean population per leaf of thrips and whitefly and their predator *Geocoris* spp on mustard. The thrip and whitefly population varied with different dates and phenology of plant in the field of Tando Allahyar. Initially the population of thrip was very low (3.69 ± 0.45) in the 3rd week of December, the population increased and the highest thrip population was recorded (100.62 ± 0.51) , in the 3rd week of March. Similarly the population of whitefly was very low (1.00 ± 0.25) in the 3rd week of December, the Population increased and highest whitefly population recorded as (24.77 ± 0.89) , in the 3rd week of March. Similarly the predator, *Geocoris* spp remained maximum (15.33 ± 0.31) in 3rd week of February and minimum (4.50 ± 0.25) in 1st week of January. The results Showed that on the weekly mean population per leaf of thrips and whitefly and their predator *Geocoris* spp on mustard at Crop Protection Field (CPT). The maximum thrip population was (129.46 ± 0.84) in the 3rd week of March and minimum (4.92 ± 0.55) in the 3rd week of December 2010. The maximum whitefly population was (40.85 ± 0.90) in the 3rd week of March and minimum (2.69 ± 0.21) in the 3rd week of December 2010. The predator, *Geocoris* spp population was minimum (5.00 ± 0.32) in the the 3rd week of December 2010 initial stages and reached its peak as (10.50 ± 0.31) in 3rd week of March. The temperature and humidity varied during different dates and time in all localities of Sindh. The temperature and humidity play an

important role in increasing the thrips and whitefly and their predator *Geocoris* spp population on mustard crop.

TESTING OF NEEM AND SWEET FLAG IN WAREHOUSE AGAINST *TRIBOLIUM CASTANEUM* (HERBST) (COLEOPTERA: TENEBRIONIDAE)

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Neem and sweet flag extracts in petroleum ether were tested in warehouse of NARC during 2004 for six months storage period. The Hessian cloth bags measuring 21x 35 cm (20 kilogram capacity) were used. The extracts were applied to fumigated wheat and the treatments were: i) Neem oil applied to bag @ 1000 µg/cm², ii) Neem oil applied to grains @ 1000 mg/kg, iii) Neem oil applied both to bag and grains as in i and ii, separately, iv) Neem oil + Sweet flag oil (50:50) applied to bag @ 1000 µg/cm², v) Neem oil + Sweet flag oil (50:50) applied to grains @ 1000 mg/kg, vi) Neem oil + Sweet flag oil (50:50) applied both to bags and grains as in iv and v, separately, vii) Coopex dust @ 1 g/kg, viii) Polythene enclosure, and ix) Control. The experiment was replicated thrice. The store was infested with *Tribolium castaneum*. Results revealed that among plant extracts, neem extract applied to both bag and grain was the most effective up-to 4 months showing 56.32 insects per kilogram. Neem extract applied to grain only was the best after 5 months storage having 41.03 insects per kilogram. After 6 months storage minimum number of insects (28.55) were recorded in neem and sweet flag together applied to grain and 32.69 insects in neem and sweet flag applied to both bag and grain as against 734.30 in control. Neem extract applied to bag only was also significantly better than control but not from all other extract applications, which were non-significant among each other in six months storage.

OCCURRENCE AND ABUNDANCE OF THRIP AND WHITEFLY PESTS AND THEIR NATURAL ENEMIE *GEOCORIS* SPP ON COTTON CROP AT VARIOUS LOCALITIES OF SINDH

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The field studies were carried out on occurrence and abundance of thrip pests on cotton *Gossypium arborium* L, in Tando Allahyar and Crop Protection Field (CPT)Sindh Agriculture University Tandojam economically important crop. The survey on the population fluctuation of thrips and their natural enemies were carried out at weekly intervals. Twenty five plants were randomly selected from each locality of each crop, five leaves were selected, two from bottom, two from middle and one from top and counted the population on with the help of magnifying glass. The plants were carefully handled to avoid disturbing the thrip and their natural enemies on the

plants. Time of observation was 8-10 am. The result showed that the thrip and whitefly and their natural enemies population at Tando Allahyar in cotton crop varied with different dates and phenology of plant in the beginning the thrip and whitefly population was very low, the minimum thrip population (3.00 ± 0.15) in 2nd week of April but it reached its peak during last week of July as (75.6 ± 0.78) thrips. Similarly the maximum population of whitefly (24.45 ± 55) in last week of July and Minimum (1.00 ± 0.35) 2nd week of April. Similarly the predator, *Geocoris* spp remained Minimum (1.00 ± 0.14) 3rd week of April and maximum (12.00 ± 0.85) in last week of July. Similarly the result showed that the thrip and whitefly and their natural enemies population in cotton at Crop Protection Field (CPT), initially the thrip population was minimum in the 1st week of May (15.55 ± 0.52) and reached its peak (191.6 ± 0.85) on last week of August. Similarly the whitefly population minimum in the 1st week of May (5.56 ± 0.42) and reached its peak (91.6 ± 0.85) on last week of August. The predator, *Geocoris* spp population was minimum (4.00 ± 0.60) in the starting in the 1st week of May and maximum predator population was (25.6 ± 0.59) in the 3rd week of August. The temperature and humidity varied during different dates and time in Tando Allahyar and Crop Protection Field (CPT) TandoJam localities of Sindh. The temperature and humidity play very important role in increasing the thrip and whitefly and their predator *Geocoris* spp population.

**EFFECT OF NITROGEN AND PACLOBUTRAZOL ON MANGO LEAF GALL MIDGES,
PROCONTARINIA SPP. (CECIDOMYIIDAE: DIPTERA)**

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Paclobutrazol is a growth regulator that is being used for floral induction of biennially bearing mango tree during off year. This chemical causes change in plant physiology, so may have an indirect effect on mango insect pests. Fertilizers are also known for causing change in insect populations on plants. So, present study was conducted to investigate the effect of nitrogen and paclobutrazol on mango gall midge (*Procontarinia* spp.) population in field conditions. Two factorial RCBD (4 replications) was designed with three doses of Paclobutrazol (0, 40, 50 g/plant) and three Nitrogen levels (0, 500, 1000 g/plant) on a mango cultivar: 'Chaunsa Samar Bahisht' in Mango Research Station, Shujabad. To sample 1 tree (1 treatment) infested leaves were counted from randomly selected 20 leaves (5 leaves from each side of the tree) in March-April with one week interval. The results were non-significant ($P= 0.4832$) showing mean infested leaves per sample \pm SE: 2.41 ± 0.62 , 1.70 ± 0.51 and 1.95 ± 0.55 at nitrogen doses 0, 500, 1000 g/plant respectively, but the infestation of the gall midge significantly increased with increase in dose of paclobutrazol ($P= 0.0199$) showing mean infested leaves per sample \pm SE: 1.29 ± 0.29 , 1.77 ± 0.46 and 3.02 ± 0.73 at 0, 40, 50 g/plant respectively. The results conclude that the mango midge population is unaffected by nitrogen dose upto 1000 g/plant, but increases with increase in paclobutrazol dose.

**COMPARATIVE EFFECTIVENESS OF SOME INSECTICIDES FOR CONTROLLING
COTTON THRIPS (*THRIPS TABACI* AND *SCIRTOTHRIPS DORSALIS*) ON THE
COTTON CROP**

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The efficacy of five insecticides, viz., asephate 75% SP, chlorfenapyr 36EC, Confidor 200 SL, abamectin 1.8EC and dimethoate 40EC was estimated against thrips (*Thrips tabaci* and *Scirtothrips dorsalis*) on cotton crop under natural field conditions during the year 2012 at Mazhar Shah Agricultural Farm, Chawant, Pak Pattan, Punjab, Pakistan. The results showed that asephate 75% SP was most effective after 24 hours to 72 hours then its effectiveness gaudily reduced up to 7th day while chlorfenapyr 36EC was moderate effective after 24 hours then its effectiveness increase after 72 hours and its residual effect remained up to 7th day. In case of Confidor 200SL, abamectin 1.8EC and dimethoate 40EC, the thrips population was suppressed after 24 hours while their effectiveness decrease after 72 hours up to 7th day. It is concluded that chlorfenapyr 36EC is more effective for the controlling of thrips population on cotton crop among the all tested insecticides.

**APHIDS FEEDING ON WHEAT AND REDUCTION IN PHOTOSYNTHESIS AND
CHLOROPHYLL CONTENTS**

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Wheat is the most important cereal crop in the world. Two aphid species (*Schizaphis graminum* *Rhopalosiphum padi*) damage the wheat crop throughout the world by sucking the sap and transmitting different viral diseases, thus causing economic damage. We report losses in photosynthesis and chlorophyll contents in aphid protected and unprotected conditions as small rise in net photosynthetic rate can translate into enormous increases in wheat yields. Experiments were conducted following randomized complete block design with three replicates during crop year 2012. Aphids were protected by applying an insecticide. Populations of aphids were recorded throughout season. Chlorophyll contents were recorded by SPAD-502 whereas; photosynthetic activity was recorded by IRGA, respectively. Feeding of aphids reduced photosynthetic rate, total CO₂, transpiration rate and water use efficiency in some experiments but not in all. Further studies are needed to confirm the results. If the exact time/stage of the crop is determined at which the highest reduction occurs, it might help in optimizing insecticide application timings.

**BIOCIDAL ACTIVITY OF ESSENTIAL OILS OF *ACHYRANTHUS ASPERA* AND
SASURREA COSTUS AGAINST STORED GRAIN PEST *TRIBOLIUM CASTANEUM*
(COLEOPTERA: TENEBRIONIDAE)**

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Essential oils of *Achyranthus aspera* (Puth kanda) and *Sasurrea costus* (Kust-i-shireen) were tested for their biocidal activity against one of the most dreaded pest of stored grains, *Tribolium castaneum* (Herbst). Three concentrations viz., 5, 10 and 15% were used against the above mentioned pest. The mortality data was recorded after 24, 48 and 72 hours of treatment application. The highest mean mortality was 9.46 and 6.84% in case of *A. aspera* and *S. costus* respectively, at 15% concentration after 72 hours of treatment application. Whereas minimum mortality 2.64% and 0.78% was observed at 5% concentration in case of *A. aspera* and *S. costus* respectively, after 72 hours of treatment. It was also observed that mortality was increased with the increase in concentration up to 72 hours of exposure period. The present study suggests that essential oils of *A. aspera* and *S. costus* have insecticidal properties against one of the most serious pest of stored wheat and could be employed as alternatives for chemical pesticides.

**GROWTH REGULATORY IMPACT OF EXTRACTS OF *SALSOLA BARYSOMA*,
PEGNUM HERMALA, *SAUSSUREA COSTUS* AND *NICOTIANA TABACUM* AGAINST
RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM* (HERBST) (COLEOPTERA:
TENEBRIONIDAE)**

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Growth regulatory effect of extracts of four medicinal plants *Salsola barysoma*, *Pegnum hermala*, *Saussurea costus* and *Nicotiana tabacum* was evaluated and data regarding percent pupation and percent adult emergence of *Tribolium castaneum* (Herbst) was observed. The results showed that plant extracts had significant effect on percent pupation and percent adult emergence and with increase in concentration their emergence decreases. The acetone extract of *N. tabacum* at maximum concentration (10%) showed minimum percent pupation (40%) and percent adult emergence (28.33%) followed by the plant extracts of *S. barysoma*, *P. hermala* and *S. costus*. The acetone extract of *S. costus* was proved least effective showed maximum percent pupation (88.33%) and percent adult emergence (78.33%) at lower concentration (2.5%). From these results it was concluded that following extracts of indigenous medicinal plants could be useful for suppressing the population of *T. castaneum* (Herbst).

SUSCEPTIBILITY OF DEVELOPMENTAL STAGES OF *OXYA VELOX* (FABRICIUS) (ORTHOPTERA: ACRIDIDAE) TO *ASPERGILLUS* SPECIES FROM SINDH

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The effect of infection by the fungal entomopathogen *Aspergillus* on feeding by the *Oxya velox* (Fabricius) was investigated under laboratory conditions. A significant reduction in feeding as indicated by faecal production was recorded 2-3 days after inoculation. Furthermore, a significant difference was noted in the time to death of earlier developmental stages *i.e.* (1st -4th) infected individual all died by day 3rd compared to later stages *i.e.* (5th-Adults) all the infected individual died by day 7th. Present study suggested that, *Aspergillus* played significantly role for the reduction of grasshopper's population it can be commercially used as bio-control agent.

TOXICOLOGICAL AND REPELLENT EFFECT OF *CITRULUS COLOCYNTHIS* OIL AGAINST *SITOPHILUS GRANARIUS* L.(COLEOPTERA: CURCULIONIDAE)

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Grainary weevil (*Sitophilus granarius* L.) is a stern pest of cereals and their products during storage. Present investigation was carried out to evaluate the essential oil of *Citrus colocynthis* for their possible toxic and repellent activity under laboratory conditions. The oil of *C. colocynthis* was applied at 10, 20 and 30% concentrations. The results showed that highest mortality 39.65% was observed at 30% concentration followed by 20% (24.86%) and 10% (16.67%) after 144 hours of treatment. Similarly, repellency was highest (86.46%) at 30%, followed by 74.56 and 61.34% at 20 and 10% concentration, respectively. Therefore, the essential oils of *C. colocynthis* can serve as the foundation for development of plant based bio-insecticides.

ROLE OF LEAF TRICHOME FOR INSECT RESISTANCE IN DIFFERENT GENOTYPE OF TOMATO

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Damage from insect herbivores is often times negatively associated to trichome production, and enhanced trichome production may thus be advantageous as it increases resistance against herbivores. Ten genotypes of tomato (*Solanum lycopersicum* L.) were assessed for insect resistance

as affected by their leaf trichome density. A field experiment was carried out to determine the genotypic and phenotypic differences in leaf trichome density for insect resistance. Relative resistance and fitness of genotypes were found in leaf trichome density. Leaf trichome density was positively and strongly correlated to insect resistance across populations. Results indicated that trichome density was related to insect resistance suggesting positive directional selection in seven tomato genotypes. Phenotypic variation in leaf trichome density in field indicated that genetic differences for this character in tomato genotypes would be beneficial for natural insect resistance mechanism ensuring safe environment.

**STUDIES ON WHITEFLY RESISTANCE IN RELATIVE TO HAIRINESS OF COTTON
(*GOSSYPIUM HIRSUTUM*) LEAF**

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To study the degree of whitefly (*Bemisia tabaci*) resistance versus hairiness of leaves and to know the relative importance of different aspects of hairiness, 106 strains from the genetic stock were evaluated for density of hair on the mid rib, density of hair on the lamina, and the hair length in relation to whitefly nymphs. Population per leaf as well as the leaf damage. Total partial and multiple correlation coefficient were determined. It was found that degree of whitefly resistance has definite correlation with the pilosity of the plant on the relative importance of three characteristics of hairiness studied length of hair seemed to be of prior importance, closely followed by density of hair on lamina whereas hair on the mid rib does not seem to play any importance part in impacting resistance to the pest. However, combination of hair length with high density of hair on lamina may be the best index of selected in breeding for resistance to whitefly attack.

**EFFICACY OF INDIGENOUS PLANT EXTRACTS AGAINST LARVAE OF *CULEX*
QUINQUEFASCIATUS (DIPTERA: CULICIDAE)**

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Because resistance to synthetic insecticides is more and more developed in mosquitoes of medical importance, the recent trend is to explore eco-friendly plant extracts with the potential to suppress pest populations. Investigations were made to assess the larvicidal potential of essential oils from some indigenous plants against larvae of *Culex quinquefasciatus* (Diptera: Culicidae). Results reveal that after 24, 48 and 72 h of exposure to essential oils, lemon (*Citrus limon*) caused highest mortality (66.22%, 75.13% and 90.25, respectively) followed by eucalyptus (*Eucalyptus*

camaldulensis L.) (59.83%, 70.0% and 87.01, respectively). *Culex quinquefasciatus* larvae showed highest susceptibility, both in terms of LC₅₀ and LT₅₀, to lemon (*Citrus limon* L.) essential oil after 24 h, but were highly susceptible to essential oils from musumbi (*Citrus sinensis* L.), bakhra (*Tribulus terrestris* L.), darchini (*Ricinus communis* L.), eucalyptus (*Eucalyptus camaldulensis* L.) and succari (*Citrus sinensis* L.) after 48 and 72 h.

EFFECT OF GAMMA IRRADIATION ON CITRUS PSYLLA, *DIAPHORNIA CITRI* NYMPHS AND ADULTS

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The citrus psylla, *Diaphornia citri*, is the key pest of citrus in Pakistan and quarantine pest in many parts of the world. Pakistan consequently loose export to the pest free countries as the eggs and nymphs, can be transmitted with fresh fruits and with nursery plants to the importing countries. Irradiation as a phytosanitary treatment was explored as method to prevent psylla nymphs from further development to adult stage. First and second stage nymphs infested citrus shoots and 1-2 year old infested nursery plants were irradiated in two different experiments with target doses of 40, 80, 100, 150, 200, 250, 300 Gy gamma irradiation and held for possible mortality and development inhibition. Post irradiation mortality was low for the target dose of 40-100. The 100 Gy treatment dose did not prevent adult emergence. However, emerging adults were short lived and died within next 48 hrs. The 150Gy dose prevented development and all nymphs died within 3 days post irradiation period. Doses of 200 Gy resulted in 100% mortality of nymphs within 24 hrs. Mortality of citrus psylla adults when exposed to various doses of gamma irradiation 40, 80, 100, 150, 200, 250, 300, 400, and 600, 800, 1000, 1500 Gy showed that male citrus psylla were the most radio tolerant than females. Dose of 800 Gy resulted in complete mortality of the males within five days and females within 4 days. The trend in mortality for females was almost the same except that they were comparatively more susceptible to irradiation than males.

IMPACT OF PESTICIDE TREATMENT ON SPIDER COMMUNITY OF RICE ECOSYSTEM OF PUNJAB, PAKISTAN

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A study was conducted to evaluate the effects of four pesticides viz., Lambda Cyhalothrin, Imidacloprid, Monomehypo and Cartap on the densities and diversities of the ground and foliage spiders residing rice fields of Punjab, Pakistan. Sampling was conducted from four fields randomly selected at three sites, Adaptive Research Farm, Sheikhpura; Chandrai Village, Lahore and Qaisar Ghar, Kasur using pitfall traps and de-vac vacuum insect net. One of the fields at each site was designated as the control and the rest were treated only once with a pesticide, during study period. A total of 3,332 specimens representing 12 families, 27 genera and 39 species were collected from all treated and un treated study areas. Lycosidae and Tetragnathidae were the most dominant families comprising 52 and 19 percent of the total sample, respectively. Lycosidae was represented

by four genera and 11 species and Tetragnathidae by three genera and four species. Dominant families were Salticidae, Linyphiidae, Araenidae, Oxyopidae and Thomisidae. Most dominant species of overall data were *Lycosa terrestris* and *Tetragnatha javana*. Collective contribution of these two species was 52.18 percent of the total data. Lambda Cyhalothrin, being a spray preparation, caused a decrease in the densities of spiders in the foliage specimens while having no effect on the ground specimens. Imidacloprid was found to be the most toxic in the fields, since, the densities of both the ground and the foliage spiders decrease in the treated fields. Monomehyppo caused a decrease in density of only the foliage specimens while not affecting ground specimens. Cartap had no effect on the survival of foliage and ground spiders. Spider diversities decreased in all treated fields after the application of pesticides but recovered as the season progressed due to recolonization from surrounding area. Our data indicated that caution should be exercised in the use of pesticides and IPM programs should be carefully managed due to the reason that pesticides have adverse effects on the density and diversity of spiders.

**INTEGRATED PEST MANAGEMENT OF *PHYLLOCNISTIS CITRELLA* STANTON
(LEPIDOPTERA: GRACILLARIIDAE) FROM FAISALABAD.**

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The citrus leafminer (CLM), *Phyllocnistis citrella* Stainton, (Lepidoptera: Gracillariidae) is an important pest of citrus. It attacks the newly emerging leaves of citrus. Biological agents like *Chrysoperla carnea* and *Trichogramma* spp. were used along with essential oil, Diver and Emamectin benzoate 1.9% EC to control this pest. These controlling agents were used on nursery plants planted in Postgraduate Agricultural Research Station University of Agriculture, Faisalabad in an integrated manner to control the pest more effectively. *Chrysoperla carnea* larvae and *Trichogramma* spp. was released through cards. Oil and pesticide was sprayed by spray gun in the form of mist. Experiment was laid out in Randomized Complete Block Design (RCBD). The data for mortality of leaf miner had been recorded 24, 48 and 72 hours of insecticide application and control treatment was also included. Frequency of these controlling agents was determined on the appearance leaf miner in new or old leaves in each study. The data were collected from plants regarding percentage mortality to check the efficacy of controlling agents. The collected data were analyzed statistically using standard procedures.

**STUDIES ON THE COMPATIBILITY OF NEEM OIL WITH PREDATOR,
CHRYSOPERLA CARNEA FOR THE MANAGEMENT OF APHIDS (HOMOPTERA:
APHIDIDAE) IN CANOLA (*BRASSICA NAPUS* L.)**

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The present studies were conducted to find out the compatibility of neem oil with the predator, *C. carnea* for the management of aphids in canola. Among the different treatments tested,

module consisting of neem oil 2% + *C. carnea* proved very effective in reducing the aphid population with an average of 18.6/plant. Neem oil 1% and *C. carnea* as a separate treatments also produced significant results compared to untreated check where mean per plant population of aphid was 39.3 and 41.3 respectively. Maximum seed yield (3295.1 kg/ha) was recorded from neem oil 2% + *C. carnea* followed by neem oil 1% + *C. carnea* (3219.1 kg/ha) and neem oil 2% (2809.4 kg/ha), respectively. Over all mean population of *C. carnea* was highest (0.48/plant) in plots treated with predators alone. The same was second most abundant (0.40/plant) in plots treated with neem oil 1% + *C. carnea* followed by plots treated with neem oil 2% + *C. carnea* (0.36/plant). The study manifested neem oil concentrations relatively safe to beneficial insects and suitable for use in integrated pest management program of aphids in canola.

**TOXICITY OF INSECTICIDES AGAINST LARVAE OF SPODOPTERA AND
HELICOVERPA (LEPIDOPTERA: NOCTUIDAE)**

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Toxicity of 10 insecticides was carried out against larvae of *Spodoptera* and *Helicoverpa* under laboratory conditions at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad. Leaf dip method was used to test different insecticides against larvae of *Spodoptera littoralis* and *Helicoverpa armigera*. 2nd instar larvae were fed on insecticide treated leaves. Results showed that against *S. litura*, flubendiamide, rynaxypyr, chlorfenapyr, spinetoram, lufenuron, methoxyfenozide gave 80 to 98% mortality as compared to indoxacarb, spinosad, bifenthrin, and lambdacyhalothrin (58 to 64% larval mortality). Against *H. armigera*, Flubendiamide, rynaxypyr, chlorfenapyr, spinetoram gave 89 to 95% larval mortality when compared to emamectin (76%) indoxacarb (72%), spinosad (68%), bifenthrin (65%), lambdacyhalothrin (66%) and cypermethrin (53%).

**EFFECT OF DIFFERENT FORMULATIONS OF IGRS AGAINST THE LARVAL
MORTALITY, GROWTH INHIBITION AND LARVAL DEFORMITIES OF CULEX
QUENQUIFASCIATUS MOSQUITOES UNDER LABORATORY CONDITIONS**

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Experiments were conducted in CRD using different concentration (0.01, 0.02, 0.03, 0.04 and 0.05ppm) of juvenile hormones mimics (methoprene, pyriproxyfen 10WDG, Hexaflumeron and pyriproxyfen 0.5G) separately against different larval instars of *Culex quenquifasciatus*. The granular formulation of these IGRs were pulverized to the consistency of talcum powder with a mortar and pestle and agitated for 2 hours in tap water. This suspension was used to derive final concentrations of each IGR to the range of 0.01 to 0.05 ppm in water. Batches of 25 (1st to 4th instars) larvae were added separately to the 500 ml disposable cups containing 100 ml of each concentration and 3 drops of 1% larval diet slurry was added as larval food. Controls comprised of

water and food only. Each concentration and the control were replicated 4 times and the entire assay was repeated twice. All cups were covered with fine net to prevent the escape of emerging adults. Data on larval deformities, growth inhibition, larval mortality and adult's emergence was monitored up to 21 days. Dead larvae, molted exuviae, were removed daily. Results showed that all IGRs were fatal to the first and 2nd instar with 70-90% mortality and showed significantly high inhibition (50-80%) against the 3rd and 4th instars larvae. The tested IGRs were classified in term of the tested parameters in order of Pyriproxyfen 0.5G > Pyriproxyfen 10WDG > Methoprene > Hexaflumeron. The results can be utilized in the environment friendly control techniques of *Culex* mosquitoes.

INSECTICIDAL ACTIVITY OF PLANT ESSENTIAL OILS AGAINST *T. CASTANEUM* (H). (COLEOPTERA: TENEBRIONIDAE)

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The study was conducted to evaluate the effects of three essential oils clove (*Syzygium aromaticum*), olive (*Olea vera*) and Kalvanji (*Nigella sativa*) at 2,4,6,8 and 10% including control treatment mixed with acetone as formulation against *Tribolium castaneum*. Mortality data was recorded after 24, 48 and 72 hours. *Olea vera* was the most effective against grubs of *T. castaneum* followed by *N. sativa* and *S. aromaticum* oil, having the LT₅₀ values 67.17h, 75.71h and 88.93h respectively. *S. aromaticum* was found most effective against adults of *T. castaneum* followed by *N. sativa* and *O. vera*, having the LT₅₀ values 81.11h, 96.81h and 105.33h respectively. *N. sativa* was the most effective against grubs of *T. castaneum* followed by *O. vera* and *N. sativa*, having the LC₅₀ 9.17%, 9.34% and 14.27% respectively. *S. aromaticum* was the most effective against adults of *T. castaneum* followed by *N. sativa* and *O. vera*, having the LC₅₀ 9.65%, 11.39% and 16.33% respectively. The Repellency experiments were also carried out. Maximum repellent effect was recorded in *N. sativa* (48.3%) followed by *O. vera* (47.5%) and *S. aromaticum* (46%). The overall results show that potential use of these plant essential oils in warehouses and food storage godowns is an alternate way for grain protectants.

POPULATION FLUCTUATION OF SUCKING INSECT PESTS OF BT AND NON-BT COTTON CULTIVARS UNDER VARYING ABIOTIC CONDITIONS

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The present study was performed to determine the fluctuations in population of sucking insect pests including whitefly, jassid and thrips in four Bt (FH.2015, N.121, IR.901, and FH.114) and four non Bt (FH.942, FH.941, CRSM.2007, and FH.207) cultivars of cotton crop (*Gossypium hirsutum* L), under different abiotic conditions. According to results, the maximum population of

whitefly was seen on CRSM-2007 (1.78%), followed by N.121 (1.71%) and FH-114 (1.65%). The minimum whitefly seen in cotton cultivar FH.941 (1.22%), after this on variety FH.942 (1.39%) and then cultivar FH.2015 comes where the intensity observed was (1.54%). The variation in *Amrasca devastans* intensity on tested cultivars on per leaf basis, the highest intensity of the *A. devastans* observed in cotton cultivar N.121 (1.77%) while minimum in case of FH.941 (1.07%). While in case of thrips on per leaf basis, the highest population of thrips was observed on IR 901 (2.07%) followed by N 121 (2.01%) and minimum (1.28%) in FH.941 cultivar. Correlation coefficient analysis of whitefly, jassid and thrips population against three abiotic factors viz., temperature, relative humidity and rainfall, revealed that the positive correlation of whitefly, jassid and thrips population with mean daily temperature. However negative correlation was found with rainfall and relative humidity. The pooled analysis revealed that the whitefly, jassid and thrips population was more on Bt cultivars compared to non Bt cotton cultivars. In conclusion, Bt cotton cultivars tested in the present study have been more preferable for sucking insect pests compared to non Bt cotton cultivars.

RELATIVE POPULATION OF SUCKING AND BOLLWORM INSECT PESTS ON DIFFERENT COTTON VARIETIES

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Studies were conducted to investigate insect infestation on Bt and non-Bt cotton varieties and genotypes at farmers field in Tando Allahyar during Kharif season. The seed of four cotton varieties, viz. KMG-1, KMG-2, KMG-3 and NIAB-78 was sown on May 8, 2003 in a completely randomized design with four replications. Infestation of jassid on KMG-1, KMG-2, KMG-3 and NIAB-78 was first recorded 47 days after sowing. Whitefly infestation was found on KMG-1, KMG-2, KMG-3, and NIAB-78 in the fourth week of June 2003. Thrips infestation was prevalent in the genotypes and varieties on June 24, 2003. The results revealed that highest (0.847 per leaf) population of jassid was found on Niab-78 followed by 0.787 per leaf and 0.777 per leaf on KMG-1 and KMG-2 respectively. The highest white fly population of 0.842 followed by 0.765, 0.762 and 0.757 per leaf was recorded on KMG-3, Niab- 78, KMG-1 and KMG-2, respectively. The larval population of Spotted bollworm, *Earias* spp. Indicated that maximum infestation of Spotted bollworm was recorded on NIAB-78, followed by KMG-3, KMG-2 and KMG-1.

EVALUATION OF COMPARATIVE TOXICITY OF DIFFERENT INSECTICIDES AGAINST *PERIPLANETA AMERICANA* (L.) FROM URBAN LAHORE

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The present study was designed to investigate the insecticidal efficacy of four different classes of insecticides: pyrethroids, organophosphates, phenyl-pyrazoles and neo-nicotenoids. One

representative chemical from each class was selected to compare the toxicity: deltamethrin from pyrethroids, Dichlorovinyl Dimethyl Phosphate (DDVP) from organophosphates, fipronil from phenyl-pyrazoles and imidacloprid from neo-nicotenoids. These insecticides were tested for their LC₅₀ values against *Periplaneta americana* (Linnaeus) under topical bioassay method. Fipronil 2.5% EC was highly effective at all concentrations applied, while DDVP 50% EC was least toxic amongst all. One way analysis of variance confirmed significant differences between mortality of *P. americana* (L.) and different concentrations applied ($p < 0.05$). This study revealed that locality differentiation is an important factor in determining the range of resistance between various localities, as all three localities behaved differently in terms of their levels of resistance.

DIVERSITY OF SAND FLIES (DIPTERA, PSYCHODIDAE) OF DISTRICT DIR LOWER, KHYBER PAKHTUNKHWA

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A survey was carried out from April to August 2010 in six selected localities of District Dir Lower to investigate the sand flies diversity. Eight species of sand flies viz. *Phlebotomus sergenti*, *P. major*, *P. bergeroti*, *P. hindustanicus*, *P. kazironi*, *P. salangensis*, *Sergentomyia babu* and *S. baghdadis* were identified from the study sites. *P. bergeroti* and *P. hindustanicus* are reported for the first time from Khyber Pakhtunkhwa. Among the collected species *P. sergenti* was found to be the most abundant sand fly species.

SYNERGISTIC EFFECTS OF MALATHION AND TALSTAR MIXTURES ON ADULTS OF STORED GRAIN PEST, *RHYZOPERTHA DOMINICA*

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The development of resistance against organophosphate (OP) and pyrethroid insecticides is continuously increasing. Due to this phenomenon many insecticides when used singly, are becoming ineffective against these resistant populations or their higher concentrations are required to control them which are dangerous for our environment. This study was planned to investigate the efficacy and synergistic activity of mixtures of OP and pyrethroid insecticides against the adults of Lahore population of stored grain pest, *Ryzopertha dominica* verses single pesticides. For this purpose the stock culture was collected from stored grain godowns of Lahore and working culture was maintained in departmental insectary at uniform temperature and humidity. The OP, (Malathion) and pyrethroid (Talstar) mixtures were prepared in two combinations. In first combination (the Mixture-I), the concentration of Malathion, while in the second (Mixture-II), the concentration of Talstar was kept constant. Mix-I combinations were consisted of malathion and Talstar in ratios, 10: 0.5, 10: 1.0, 10: 1.5, 10: 2, 10: 2.5. Mix-II consisted of above insecticides in 9.5: 2, 9: 2 and 8.5: 2 combinations. The adult insects were exposed to above mixtures separately in triplicate groups of 10 in each petri plate for 48 hour duration. The results showed that after treatment with malathion alone, LC₅₀ was 47.18ppm and with pure talstar it was 1.54ppm but with the mixture of both pesticides LC₅₀ was dropped down to 0.42, 0.21, 0.40, 0.32, 0.23ppm for ratios

10:0.5, 10:1.0, 10:1.5, 10:2, 10:2.5, respectively, whereas to, 1.73, 0.05 and 0.01 for ratios 9.5:2, 9:2, 8.5:2, respectively. The order of effectiveness for Mix-I was 10:1.0 > 10:2.5 > 10:2 > 10:1.5 > 10:0.5. For Mix-II efficacy order was 8.5:2 > 9:2 > 9.5:2. The findings in this study suggested that both insecticides when used in mixtures showed synergistic activity against the adults of *R. dominica* of Lahore population. It is concluded that the mixtures of these insecticides may effectively be used not only to control *T. granarium* but also against other stored grain insects.

PCR-BASED DETECTION OF APHIDS IN THE GUT CONTENTS OF ARTHROPOD PREDATORS

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Fodders (maize, sorghum, chari, shaftal and alfalfa), wheat and mustard crops were sampled round the year in the cropland of Punjab. The most abundant pest species of the cropland were aphids namely *Aphis maidis*, *Schizaphis graminum*, and *Diuraphis noxia*, which were subjected to gut analysis. Species-specific primers of these aphid species were applied on the selected predators. *Coccinella septempunctata* and *Oxyopes javanus* were positive for the consumption of *Aphis maidis* (corn aphid), *Hippodemia convergens* for *Schizaphis graminum* (wheat aphid), while *Neoscona theisi* was positive for *Diuraphis noxia* (wheat aphid). Such findings seemed to be helpful for implementation of species specific biological control against a specific pest in our agro-ecosystems.

BIOLOGICAL PARAMETERS AND PREDATORY POTENTIAL OF *CRYPTOLEAMUS MONTROUZIERI* MULSANT (COLEOPTERA: COCCINELLIDAE) FEEDING ON COTTON MEALY BUG, *PHENOCOCCUS SOLENOPSIS* TINSLEY AT THREE CONSTANT TEMPERATURE LEVELS

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The cotton mealy bug, *P. solenopsis* is a sucking pest and secretes honey dew which results in sooty mold growth, which reduces photosynthesis process and resulted in huge economic loss. Different bio control agents are used for the control of cotton mealy bug among these *Cryptoleamus montrouzieri* Mulsant commonly known as mealy bug destroyer is one of the most important predator for the control of mealy bug. The present study on the biological parameters and predatory potential of *Cryptoleamus montrouzieri* Mulsant (Coleoptera: Coccinellidae) feeding on Cotton mealy bug were evaluated at three different temperatures *i.e.* 24, 28 and 32 ±1°C with 65 ±5% Relative humidity and 16:8 (L:D) photoperiod under growth chamber at Insect pest management program, NARC Islamabad. The results revealed that maximum incubation period

was (4.16 ± 0.24) days at 24 ± 1 °C, while minimum was 2.84 ± 0.14 days at 32 ± 1 °C and the maximum hatching percentage (92%) was observed at 32 ± 1 °C. The total grub durations were 12.7 ± 0.91 , 11.9 ± 0.68 and 8.56 ± 0.84 days with predatory potential of 231.33 ± 0.02 , 283.55 ± 0.03 and 268.57 ± 0.05 crawlers per grubs at three constant temperatures respectively. The maximum pupal duration was 5.47 ± 0.44 at 24 ± 1 °C and minimum was 3.84 ± 0.41 days at 32 ± 1 °C. The adult female longevity was 91.2 ± 0.01 , 74.8 ± 0.01 and 57.0 ± 0.01 days while male longevity was 82.2 ± 0.01 , 60.6 ± 0.01 and 38.5 ± 0.02 days respectively. The female fecundity rate was 137.1 ± 0.07 , 235.1 ± 0.08 and 166.2 ± 0.09 eggs per female respectively. The life table parameters indicate that maximum generations time (T) was 91.2 at 24 ± 1 °C and minimum was 57.0 days at 32 ± 1 °C. The maximum population doubling time (DT) was 13.11 at 24 ± 1 °C and minimum was 8.05 days at 32 ± 1 °C. The intrinsic rate (rm) was maximum (0.0374) at 32 ± 1 °C and minimum (0.0231) 24 ± 1 °C. The present study exhibited that among three temperatures, 32 ± 1 °C was more suitable for rearing *Cryptoleamus montrouzieri* feeding on *phenacoccus solenopsis*, owing to shorter developmental time and the best results obtained for the fertility life table parameters. The results further indicate that with increasing temperature developmental duration significantly decrease.

**DEVELOPMENTAL PERIOD OF *ENCARSIA* SP.? *CIBENSIS* LOPEZ-AVILA
(HYMENOPTERA: APHELINIDAE) IN WHITEFLY *BEMISIA TABACI* GENNADIUS
(HOMOPTERA: ALEYRODIDAE)**

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Mass rearing of parasitoids is needed for inundative releases of parasitoids which are applied for control of insect pests in the field. *Encarsia. sp.? cibensis* Lopez-Avila is described as nymphal parasitoid of whitefly *Bemisia tabaci* Gennadius. For rearing of *Encarsia. Sp.? cibensis* the culture of whitefly was maintained in Insectary-Biological Control Labs. National Agricultural Research Centre (NARC), Islamabad. Whitefly rearing was studied by comparing some biological parameters of whitefly on three host plant species *i.e.* cotton, brinjal, and tomato at 27 ± 1 °C and 50-55% R.H. The biology of *Encarsia sp.? cibensis* was studied in the laboratory at 25 ± 1 °C and 50-55% R.H. The parameters studied were incubation period, larval, pupal, and adult developmental duration, and sex ratio. Males were distinguished from females by the occurrence of two meconial pellets in the female and four in the male under microscope. The results showed that maintenance of whitefly culture was more efficient on brinjal plants as compared to tomato and cotton. The life cycle of whitefly was completed in shorter duration 19.8 ± 0.19 days on brinjal as compared to 20.53 ± 0.28 days on tomato and 23.05 ± 0.23 on cotton plants. The observed male to female sex ratio was 1:1.6 on brinjal and 1:1.5 on cotton and 1:1.5 on tomato. Average incubation duration of *Encarsia .sp? cibensis* in whitefly nymph was 2.10 days, larval duration 6.6 days, pupal duration 5.5 days. Total duration for adult emergence was 14.20 days. These studies enabled us to rear this important parasitoid successfully under laboratory conditions. This study will help for mass production and releases in the field.

EFFECT OF THREE LIGHT REGIMES AT THREE VARYING CONSTANT TEMPERATURES ON THE LIFE-TABLE PARAMETERS OF BROWN LACEWINGS *SYMPHEROBIUS FALLAX* (HEMEROBIIDAE: NEUROPTERA)

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The brown lacewings are general predators. All the stages predate on soft body scales mealybugs and mites. Life-table studies were carried out describing survival, reproduction and development of *S. fallax* at three different varying constant temperatures and photoperiods. The studies were conducted in the lab in cold incubators controlled at 20, 24, and 28°C. The effects of three photoperiods *i.e.* 8L:16D, 12L:12D and 16L:8D termed as short, medium and long day at three different temperature as already mentioned above were studied on all the stages. The intensity of light was 7.5 watts/M². The temperature affected the life table parameters. Survival was positively temperature-dependent. The age specific fecundity was not affected by photoperiod. Sex ratio was also not affected by temperature. Three different photoperiod regimes could not produce diapause symptoms in egg, larvae and pupae of *S. fallax* at 20, 24, and 28°C. Any difference in the development in different photoperiod regimes could be due to accumulation effect of heat of day-length affect.

SUSCEPTIBILITY OF *MUSCA DOMESTICA* (DIPTERA: MUSCIDAE) TO λ -CYHALOTHRIN AND CHLORPYRIFOS IN SARGODHA DISTRICT

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The present study was conducted to evaluate the susceptibility of *Musca domestica* to λ -cyhalothrin and chlorpyrifos in district Sargodha. We also calculated and compared LC₉₀ and LT₉₀ of both insecticides. Flies from all populations were found susceptible to tested concentrations of both insecticides. The toxicity of chlorpyrifos was higher compared to λ -cyhalothrin as it caused higher mortality at lower concentration and in less time. It is concluded that there is no resistance against λ -cyhalothrin and chlorpyrifos in *M. domestica* in the study area.

INSECTICIDE RESISTANCE IN *BACTOCERA ZONATA* (DIPTERA: TEPHRITIDAE) IN DISTRICT, SARGODHA, PAKISTAN

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Present study was designed to investigate the role of non-specific esterases, glutathione-S-transferases and monooxygenases in insecticide resistance in *Bactocera zonata*. Flies were collected from Gauva orchids of Ajnala, Sargodha. For biochemical estimation of insecticide

detoxifying enzymes, flies were exposed to the selected insecticides (*i.e.*, Trichlorofon, Malathion and λ -cyhalothrin) for one hour and then shifted to clean jars. The activity of insecticide detoxifying enzymes in the survivors and control were compared. The activity of insecticide detoxifying enzymes was higher in survivors compared to the susceptible flies (control). The activity of beta esterases, glutathione S-transferases and monooxygenases of Trichlorofon and Malathion treated flies did not differ. However, both groups showed higher activities of detoxifying enzymes compared to the control. Higher activities of insecticide detoxifying enzymes in survivors compared to the control groups indicate the involvement of these enzymes in insecticide resistance.

SCREENING FOR RESISTANCE TO BIRD CHERRY OATS APHID SPECIES IN WHEAT

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A number of aphid species attack wheat crop worldwide. Among these, the bird cherry-oat aphid, *Rhopalosiphum padi* (L.) is one of the most serious pests. Direct yield reductions in wheat due to aphid infestation may range from 10-50%. Seedling bulk test on bird cherry-oat aphid in National uniform wheat yield trials (NUWYT) during 2011-12 and 2012-13 were conducted in Insect Pest Management Programme, NARC, under laboratory conditions. When the seedlings were about 5 cm high, aphids were released on them, and after 10-15 days of infestation when senescence and lodging started in seedling, the damage to each entry was visually recorded from 0 to 9 damage scale (DR), where, 0 represented healthy and 9 represented dead seedling. The wheat entries were classified as highly resistant (DR, 0-1), resistant (DR, 2- 3), moderately resistant (DR 4 - 6) and susceptible (DR 7-9) according to the extent of damage. During 2011-12, out of 24 entries, no entry was found to be highly resistant. Seven entries (V-076422(N1), V-088132(N2), WRIS-12, NR-399, V-08203, NR-400 and V-7/2011) were resistant, 10 were moderately resistant and 7 were susceptible. During 2012-13, out of 35 wheat entries, 11 were found resistant (WL-8169, NN-GANDAM-1, DN-84, TW--96009, V-09082, V-10306, V-09136, V-09087, NR-399, FSD-2008, and SEHER-06), 21 were moderately resistant and 3 (NR-408, SD-998 and AAS-2011) were susceptible. In case of antixenosis test of NUWYT-N during 2011-12 and 2012-13, the test entries were planted in a circular pattern; about 3 cm from the edge, 50 apterous aphids were released on the soil in the centre of the pot. The plants and aphids were covered with plastic cage the tops of the cages were covered with muslin cloth. There were also two muslin covered ventilation holes in the sides of the cages. After 24, 48 and 72 hrs, all the plants were observed and the numbers of aphids (adults, winged, wingless and nymphs) present on each plant were counted. During 2011-12, out of 24 entries, 3 entries (NR-379, V-088132(N2) and V-05BT014) were least preferred, eighteen were moderately preferred and 3 were highly preferred. During 2012-13, out of 35 entries, 3 entries were found least preferred (V-07096, Nia-Sunehri and NN-Gandam-2), 28 entries were moderately preferred and 4 highly preferred entries were SEHER-06, NR-400, PR-103, and TW-96010. Above studies conclude that resistant or moderately resistant entries to *Rhopalosiphum padi* (L.) should be incorporated in breeding programme to minimize aphid infestation and maximize crop yield.

**BIOLOGICAL MANAGEMENT OF *FUSARIUM* WILT OF TOMATO USING
TRICHODERMA HARZIANUM AND *PAECILOMYCES LILACINUS***

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Tomato is an important horticultural crop of Pakistan. Its production is threatened by a number of biotic factors. Among these *Fusarium oxysporum* f.sp *lycopersicae* cause severe yield reduction in Pakistan. In the present study two beneficial fungi *Paecilomyces lilacinus* and *Trichoderma harzianum* antagonists to plant pathogenic fungi, alone or in combination were evaluated for the management of *Fusarium oxysporum* f. sp. *lycopersici* wilt complex. The three fungi were individually examined for variations in their growth and sporulation and their mutual interactive effects (dual culture method) on potato dextrose agar medium at 28°C. The pathogenic behavior of *F. oxysporum* f. sp. *lycopersici* in susceptible tomato Moneymaker was studied and an integrated approach consisting of *P. lilacinus* and *T. harzianum* was evaluated in pots in a glass-house as well as in field. Among the three fungi, the growth and sporulation of *T. harzianum* was faster (8.8 cm in 7 days, after 7 days respectively) than *P. lilacinus* (6.8 cm in 7 days and 14 days, respectively) and *F. oxysporum* (4.9 cm in 7 days and 18 days, respectively). In dual culture studies, the combinations of *P. lilacinus* with *T. harzianum* or *F. oxysporum* did not exhibit any mutual inhibition/suppression, while the combination of *T. harzianum* + *F. oxysporum*, resulted in 42% inhibition of *F. oxysporum*. The results of pot and field experiments indicated that combined application of *P. lilacinus* and *T. harzianum* not only caused more reduction in tomato wilt but also enhanced plant growth parameters than their alone application. Our findings revealed the potential of *P. lilacinus* and *T. harzianum* against *Fusarium* wilt of tomato.

**EFFECT OF HOST AGE ON THE PARASITIZATION OF PUPAL PARASITOID
*DIRHINUS GIFFARDII***

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Mass production of natural enemies has been a mean of improving bio-control agents for extensive orchard agriculture. Among bio-agents, *Dirhinus giffardii*, is solitary parasitoid, suppressing a number of fruit fly species in horticultural crops. The *D. giffardii*, is an end parasitoid which completes its preimaginal development inside the host pupae. We investigated the effect of pupal age on the parasitization of *D. giffardii*. Two hour old to nine days old pupae of melon fruit fly *Bactrocera cucurbitae* (Coquitt) were offered to *D. giffardii* every day. Results revealed that as host age increased the parasitization rate decreased. However, *D. giffardii* parasitized the 2 hour old pupae upto the emergence of flies. However, maximum parasitization was observed on three days old pupae followed by 4th and 5th day pupae. Moreover, fresh pupae upto four days yielded more females as compared with older ones. These findings could be helpful in defining more optimum age for the mass rearing of *D. giffardii* for use in integrated pest management (IPM) programmes for various orchards.

**MANAGEMENT OF PEACH FLAT-HEADED BORER IN STONE FRUIT ORCHARDS
AT KPK**

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The climate of Khyber Pakhtunkhwa is very congenial for the production of stone fruits such as peach, plum and apricot. Peach flat-headed borer infestation was recorded in all the three different districts of Khyber Pakhtunkhwa. During both surveys, maximum mean gummosis was recorded in plum *i.e.*, 90% in Nowshera & Peshawar and minimum in Swat (46.03%). In peaches, 91.48% gummosis was recorded in Peshawar followed by Nowshera (83.39%) and Swat (66.68%). In apricot, (85.97%) gummosis was recorded in Nowshera followed by Peshawar (80.12%) & Swat (54.49%). Maximum mean gummosis was found in Nowshera followed by Peshawar and Swat. During survey, maximum exit holes were observed in plum *i.e.*, 83.0% at Peshawar followed by Nowshera (65.1%) and Swat (37.1%). In peaches, maximum exit holes were recorded in plum orchards *i.e.*, 81.95% at Peshawar followed by Nowshera (60.77%) and Swat (40.77%). In apricot, maximum exit holes were recorded in plum *i.e.*, 63.17% at Peshawar followed by Nowshera (44.93%) and Swat (36.96%). Maximum exit holes were found in Peshawar followed by Nowshera and Swat. During surveys, predator and parasites associated with peach flat-headed borer was not found in all stone fruit orchards at different districts of Peshawar, Nowshera and Swat. Relative peak population of peach flat-headed borer (1st generation) was recorded in April *i.e.*, 24.60 adults/plastic strip trapper at Peshawar followed by Nowshera (15.30) and Swat (9.00). Second generation was emerged in July *i.e.*, 15.20 adults/ PST at Peshawar followed by Nowshera (11.40) and Swat (7.10). Peak population of 3rd generation was recorded in October *i.e.*, 7.80 adults/PST at Peshawar followed by Nowshera (6.60) and Swat (3.00). In Bordeaux mixture, Nurelle-D and Thiodan @ 75 ml/10L showed synergistic effect in reducing borer infestation of gum points and exit holes after 30 days during dormant season. Direct spray of Fyfenon, Curacron and Thiodan @ 50 ml/10L at the time of adult emergence time was found effective for the control of adult Peach flat-headed borer.

EFFECT OF FEMALE AGE ON PARASITIZATION POTENTIAL OF *TRICHOGRAMMA CHILONIS* ISHII (TRICHOGRAMMATIDAE: HYMENOPTERA) ON *EARIAS VITELLA* FAB. UNDER LABORATORY CONDITIONS

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Determination of reproductive rates of natural enemies is important for assessment of their potential as biological control agents. Reproductive rates depend on biological parameters like size, age, experience, and egg load of female parasitoids. Female age is considered to influence clutch size decisions in parasitoids. The present study was therefore designed to look at the age related response of one to seven day old female parasitoid *Trichogramma chilonis* Ishii on parasitization of *Earias vitella* under laboratory conditions. Results showed that maximum numbers of eggs were parasitized by three day old females (14.64 ± 0.38 , Mean \pm SE) and least by seven day old females (5.18 ± 0.55 , Mean \pm SE). Maximum progeny was produced by four day old

females (12.86 ± 0.48 , Mean \pm SE) and maximum number of female progeny was produced by two day old female parasitoids (8.46 ± 0.41 , Mean \pm SE). There was significant effect of wasp age on parasitization potential and fecundity. The knowledge obtained may be useful for designing strategies for management of *E. vitella* infestation.

RISK ASSESSMENT STUDIES OF INSECT AND WEEDICIDE RESISTANT COTTON ON CHICKS

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The study was conducted to evaluate the risk associated with double gene Bt cotton (Cry1Ac+CryIIA) and weedicide resistant cotton with Cp4EPSPS gene (Glyphosate resistant Gene) on hen. The study comprises of three groups each with thirty hens of age 15day. One group was fed with Bt protein in the form of grinded leaves and seeds mixed with commercial diet at the ratio of 33% Bt of total given diet. Similarly second group was also supplemented with Glyphosate at the ratio of 33% of total given diet while third group was nourished with normal commercial diet. After 120 days of treatment at this rate four Chickens from each group were sacrificed blood samples were taken and vital organs were cut and processed for histological and biochemical analysis. Microtome was used for section cutting, and then sections were stained with Hematoxyline and Eosine. Data obtained from histological and biochemical analysis revealed that no difference was observed in each group fed with transgenic diet as compared to control. Protein isolated from the serum samples was also analyzed using SDS-PAGE. Results showed absence of Bt and glyphosate protein at their required level when evaluated with protein prestained molecular weight ladder. No amplification was observed in any group with gene specific primers of Bt and Glyphosate gene. From this study it is clear that transgenic cotton containing Bt and glyphosate resistant genes are safe for organism like hen.

REPRODUCTIVE PATTERNS IN THE HOUSE RAT (*RATTUS RATTUS*) AND THE NORWAY RAT (*RATTUS NORVEGICUS*) IN URBAN RAWALPINDI, PAKISTAN

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Rodents are the most abundant and successful group of mammals, probably, due to their small size, short reproductive cycle and quite varied dietary habits. The house rat, *Rattus rattus* and the Norway rat, *Rattus norvegicus* has been declared as the most destructive pest species in many indoor environments of the world, including Pakistan. In the proposed research study, both the species of rats were live trapped from different indoor habitats of Rawalpindi urban and were brought to the laboratory of the Department of Zoology, PMAS Arid Agriculture University, Rawalpindi. The morphometrics of the captured specimens were recorded and the animals were sexed and autopsied. In case of females, reproductive parameters such as sign of lactation, presence/absence of teats, ovary size, uterine horn, number of embryos found in the reproductive tract, their measurements and the litter size was recorded. While in case of males, position and weight of testes was recorded. A high reproductive activity in *Rattus rattus* female was found in

September to November; in case of the Norway rat, *Rattus norvegicus* it was high in June - July and during the month of December. While males of both the species showed a high reproductive activity throughout the study duration. The proposed study is expected to help in identifying the reproductive patterns in both the commensal rodent species for suggesting their management strategies.

**INSECTICIDAL ACTIVITY OF *NICOTIANA TOBACCUM*, *PEGNUM HERMALA*,
SASSUREA COSTUS AND *SALSOLA BARYOSOMA* AGAINST *TROGODERMA
GRANARIUM* (EVERTS)**

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Present study was carried out to evaluate the biocidal activity of plant extracts against *Trogoderma granarium* (Everts) at 5, 10, 15 and 20% concentrations, after three exposure times; 24, 48 and 72 hours, under laboratory conditions. Plant extracts were obtained by rotary shaker using acetone as solvent. According to the results, *Nicotiana tobaccum* showed maximum mean mortality 6.36%, followed by *Salsola baryosoma*, *Pegnum hermalla* and *Sassurea costus* with mortality values 5.02, 4.70 and 3.67% at 20% concentrations after 72 hours of treatment application. In the light of this study, it was suggested that, these plant extracts would be a promising alternatives of conventional synthetic insecticides, if tested at concentrations higher than those used in this experiment.

**EVALUATION OF DIFFERENT INSECTICIDES AGAINST RICE LEAFFOLDER
CNAPHALOCROCIS MEDINALIS (GUENEE) UNDER FIELD CONDITIONS**

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Rice Leaffolder is considered a sporadic insect pest of rice in Pakistan. It can causes up to 30 % to 40 % leaf infestation and 30% yield loss. A field trial was conducted at Rice Research Institute Kala Shah Kaku Punjab Pakistan in which efficacy of certain insecticides viz., Proaxis 60CS (gamma-cyhalothrin), Karate 2.5 EC (lambda-cyhalothrin), Hoopoe 4G (cartap hydrochloride), Capstar4G (cartaphydrochloride), Padan4G (cartaphydrochloride), Belt480SC (flubendiamide), Virtako40WG (Thiamethoxam+chlorer), Coragen20CS (chlorantraniliprole), Pilot4G (cartap hydrochloride) was checked against rice leaffolder *Cnaphalocrocis medinalis* (Guenee). The experiment was laid out in RCBD with three replications. Insecticides were applied at economic threshold level (ETL) and observations were recorded after seven days of insecticide application. All the insecticides effectively controlled the pest and increased grain yield was achieved as compared to control. Belt 480 Sc (1.12 %) was the best amongst all followed by Proaxis60CS (1.28%), Padan4G(1.53%) Capstar4G (1.70%), Karate 2.5 EC (1.83%) hoopoe 4G (1.91%), Pilot4G (2.37) Virtako 40WG (2.37%), coragen20CS (2.59%) and control (5.83%). Highest paddy yield was achieved in case of Belt 480 Sc (3.33 t/h) followed by Proaxis60CS (3.18 t/h), Padan4G (3.02 t/h), Karate 2.5 EC (3.01 t/h), Capstar4G (2.99 t/h), hoopoe 4G (2.90 t/h),

Virtako 40WG(2.87t/h),Pilot4G (2.75 t/h),coragen20CS(2.57t/h) and control (2.21 t/h).

**REPELLENT EFFECT OF SOME MEDICINAL PLANT EXTRACTS AGAINST
KHAPRA BEETLE, *TROGODERMA GRANARIUM*, EVERTS (COLEOPTERA:
DERMESTIDAE)**

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Khapra beetle, *Trogoderma granarium* (Everts) is one of the world's most destructive pest of whole and ground cereals, oilseeds, dry fruits, copra and other stored products. Repellent effect of acetone extracts of four medicinal plants viz; *Nicotiana tobaccum*, *Pegnum hermala*, *Sassurea costus* and *Salsola barysoma* was assessed against larvae of *T. granarium*. The extracts of these plants were applied at 5, 10, 15 and 20% along with one untreated check for each treatment. The results revealed that maximum mean repellency (55.33%) was shown by *N. tobaccum*, followed by *S. barysoma* (52.33%), *P. hermala* (51.33%) and *S. costus* (46.67%). It was also observed that there was increase in repellency with the increase in concentration. The application of these plant extracts can protect stored grains against the attack of *T. granarium*.

**MORPHO-PHYSICAL AND CHEMICAL PLANT CHARACTERS AFFECTING
POPULATION FLUCTUATION OF WHITEFLY IN SUGARCANE**

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The present study was conducted in three farmer's fields located in Rahim Yar Khan, Bahawalpur and Faisalabad districts to assess morpho-physical and chemical plant characters, which affect on population density of whitefly. The selected genotypes differed significantly in all the morpho-physical plant characters except moisture percentage in the leaves. The sugar contents viz. POL, brix, CCS and fiber percentage also had significant difference among genotypes. Cane girth, leaf area and hair density on leaf sheath resulted negative and significant correlation with the pest population. Whereas, plant height and thickness of leaf sheath showed positive and significant correlation. Non-significant correlation was recorded between moisture contents and pest population. Cane girth had negative and significant impact on the pest population fluctuation and contributed maximum role i.e. 35.2%. Leaf area and plant height were the 2nd most important factors, revealed 19.2% and 18.9% respectively, role in population fluctuation of the pest. All the regression equations were found to be fitted the best. Nitrogen, potassium, magnesium and zinc contents had positive and significant correlation with the pest population while phosphorus showed negative and significant correlation. Nitrogen percentage in the leaves of sugarcane plants showed positive and significant impact with maximum role i.e. 96.3% in population fluctuation of the pest. The other factors like phosphorus, potassium, manganese and zinc exerted negligible role in population fluctuation of whitefly in sugarcane crop. CCS in sugar samples had positive and

significant correlation while fiber percentage showed negative and significant correlation with the pest population. CCS showed positive and significant role with 49.2% impact while fiber contents in the samples of sugar had negative and significant role with 25.8% impact on the population fluctuation of whitefly.

**EVALUATION OF RESISTANCE IN MUNGBEAN GENOTYPES TO BRUCHIDS
(CALLOSOBRUCHUS MACULATUS)**

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Preliminary research work was initiated with an objective to identify bruchid resistant mungbean genotypes and incorporation of resistant genes in local high yielding well adopted mungbean genotypes with joint collaboration Mungbean Breeding Group at NIFA. Bruchids (Pulse beetle) (*Callosobruchus maculatus*) (F.) are the principal post-harvest pest of mungbean and other stored pulses. In storage, the adult female lay eggs directly on seed coat. The newly hatched larva bore through the egg shell and penetrates seed coat, continue to feed and complete their development inside the seed. After completion, the insects emerge as adult beetles leaving behind a hole at the exit point. Bruchids infestation causes reductions in the weight, seed viability, sale ability and infested grains unfit for human consumption. The alternative to chemicals and other control measures is to develop bruchids resistant genotypes under storage conditions. Culture of bruchids beetle was maintained on bold mungbean grains at $28\pm 2^{\circ}\text{C}$ and $70\pm 5\%$ relative humidity. Insect of uniform age males and females were collected separately by isolating mungbean grains in small transparent glass test tubes mouth plugged with cotton. 1015 pairs of newly emerged adults were collected within 24 hours and released in glass/plastic jars containing sound grains of mungbean. The jars were covered with muslin cloth to facilitate aeration. The stock culture maintained was utilized for conducting the experiment. Twelve mungbean genotypes were evaluated to ascertain their resistance to bruchid beetles. The resistance of mungbean genotypes to bruchids was evaluated on the basis of oviposition on grains, adult population developed from eggs laid, grain infestation, developmental period and % grain damage. The results indicated that none of the tested genotypes was completely immune to bruchid attack. Out of 12 genotypes, three genotypes *i.e.* 12-7-012-20, 12-7-012-28 and 12-7-012-17 had the lowest grain damage *i.e.* 11.66%, 13.33% and 19.43% respectively indicated tolerant to bruchids. Highest grain damage 83.88% was recorded in genotypes 12-7-012-30 with maximum oviposition (60) and adult progeny 83.88%. The remaining genotypes indicated susceptibility to bruchids.

**INTEGRATION OF CONTROL METHODS IN SUPRESSING THE POPULATION OF
WHITEFLY ON SUGARCANE**

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The study was conducted to determine the relative effectiveness of different control methods (cultural, biological, chemical and botanical) singly and in their possible combinations for the control of sugarcane whitefly on resistant (HSF-242) and susceptible (CPF-243) genotypes of

sugarcane under field conditions in Punjab, Pakistan. Following randomized complete block design with three replications. The whitefly population (nymphs + Adults) were recorded at fortnightly interval from each locality. The whitefly population were recorded significantly different ($P \leq 0.01$) among dates of observation, treatments and between genotypes. In the treatment 15, maximum control of whitefly was recorded e.g. 1.57/leaf and 3.49/leaf with population reduction of 72.45% and 65.68%, in addition the cane yield was recorded 114311.6 and 122446.0 Kg/ha over control *i.e.* 24.27 and 20.17% in resistant (HSF-242) and susceptible (CPF-243) genotypes, respectively. Whereas 2nd instars larva per leaf of *Chrysoperla carnea* was recorded less effective to control whitefly e.g. 4.40/leaf and 8.22/leaf with reduction in whitefly population *i.e.* 22.80% and 19.17% and with minimum cane yield 92410.8 and 106967.6 kg/ha with minimum increase over control *i.e.* 0.46 and 4.97% in resistant and susceptible genotypes, respectively. The cost benefit ratio was recorded highest for the Imidacloprid 200 SL @ 568 ml/ha e.g. 1: 4.25 and 1: 8.06 and the lowest for the treatments where predator was released e.g. 1: 0.07 and 1: 0.90 in susceptible and resistant genotypes, respectively.

COMPARATIVE TOXICITY OF ORGANOPHOSPHATE AND NEEM OIL AGAINST MUSTARD APHID

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In vitro experiments were conducted in the Toxicology Laboratory, Pesticide Research Institute, SARC, PARC, Karachi. Serial dilution ranging from 0.00625-1.62% of imidacloprid diluted in water and emulsion of Neem oil ranging from 0.5-20% using detergent were used for leaf dip method on aphid. Five cm² leaf disc of mustard plant were dipped in each concentration for 10 seconds and then air dried and place on filter paper impregnated with 0.5 ml water in petri-dishes of 9cm of diameter. In each experiment five treatments of different concentrations including check and control were taken in three replicates. Twenty non-winged aphid were release on treated leaf disc and mortality data were recorded after 24 hours of treatment. At low concentrations 50-80% mortality was recorded in case of imidacloprid and the same mortality were recorded in high dosed of Neem oil. The mortality data were subjected to Abbot's formula and LC₅₀ s were calculated.

MONITORING OF SPOTTED BOLLWORM, *EARIAS VITTELLA* (LEPIDOPTERA: NOCTUIDAE) BY USING SEX PHEROMONES AND LIGHT TRAPS AND IMPACT OF RAINFALL ON ITS POPULATION

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Spotted bollworm *Earias vittella* is major lepidopteron pest of cotton crop throughout the world specially tropical and subtropical regions such as Ethiopian Palearctic and Oriental (Indo-Pak). Present study of adult moth population by using sex pheromones and light traps was carried

out during cotton season 2006-2009 from June to November. Four locations of Hyderabad division such as Saeedabad, Hala, Tando Allahyar and Jamshoro were selected for study. One hectare area was selected in each location. Our observations showed that *Earis vittella* was widely spreaded in Hala, Saeedabad, Tando Allahyar and Jamshoro during cotton season throughout the studied period (2006-2009) but maximum population was found in Hala, about 31.27 (mean of total captured moth was 1500), and minimum number of adult moths were captured in Jamshoro, about 12.79 (mean of total captured moth was 622). Environmental changes especially rainfall left the negative effect on the population of adult moths. The efficacy of sex pheromones was much more effective than light traps against *E. vittella* during whole study period (2006-2009). Maximum adult moths were captured during the month of September among all locations. Present study will help the growers for bollworm scouting specially their emergence and peak population and mating disruption.

ASSESSMENT OF DAMAGE CONTROL CAUSED BY *PSITTACULA KRAMERI* USING A MECHANICAL REPELLENT AT MATURE AND POST HARVEST STAGES OF WHEAT AND MAIZE

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The mature and post-harvest losses can be tenacious in unguarded situations. Flocks of rose ringed parakeet (*Psittacula krameri*) attack the sheaves of wheat and maize causing economic losses. Avian pesticides are commonly used to control depredations, but this approach is not eco-friendly. An alternate of this approach is the use of mechanical methods for management of avian pests. In the present study Wind Powered Hawk Eye Rotator (WPPER) were used to inhibit loss caused by *Psittacula krameri* (Rose ringed parakeet) at mature and post harvest stages of wheat and maize. Study was carried out in the cultivations of University of Agriculture, Faisalabad from April through May, 2009. The infestation was reduced to 47% and 51% in maize and wheat respectively. It was evident that the use of mechanical repellent was beneficial to reduce crop damage and maintain the agro-ecosystem sustainability.

PREDATOR-PREY INTERACTIONS OF SOME SELECTED SPECIES IN WHEAT IN FAISALABAD, PAKISTAN

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In agro-ecosystems predator-prey interactions are of great importance and insects as largest group play an important role to manage crop pests. Theoretically, these interactions influence the structure and dynamics of an agro-ecosystem. The predator-prey interactions were determined on the basis of numerical superiority of a predator and its prey in fields of selected crop. For this purpose sampling was carried out for a period of two years. Analysis of the variety of predators and

preys (which in most of the cases were also the pests in wheat) showed that *Formica* spp. 1, *Camponotus* spp. *Solenopsis invicta*, *Oxychillus alliarius*, *Formica* spp2, *Dolichoderus taschenbergi*, and *Clubiona obesa* were the dominant predators. While *Armadilidium vulgare*, *Megomphix hemphilli*, *Armadilidium nasatum* and *Pangaeus bilineatus* were dominant preys in order of their abundance in the field. Polynomial regression analysis revealed that *A. vulgare* was the preferred prey of *Formica* spp 2, *C. obesa*, *Camponotus* spp., *Formica* spp. 1. *M. hemphilli* was the preferred prey of *C. obesa*, *O. alliarius* and *Formica* spp.2. *A. nasatum* was predated by *D. taschenbergi*, *Formica* spp. 2 and *C. obesa* while *P. bilineatus* was most the most preferred prey of *D. taschenbergi* and *S. invicta*. Such types of theoretical models are very helpful in designing Integrated Pest Management (IPM) programs.

PREDATOR-PREY RATIOS OF MACROINVERTEBRATES IN WHEAT AND SUGARCANE AGRO-ECOSYSTEMS

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The present study on selected species of coleopteran, hymenopterans, arachnids and hemipterans was conducted to evaluate prey-predator ratios (pip) based on the logistic abundance of species in wheat-weeds and sugarcane-weeds agro-ecosystems in Faisalabad district. Theoretically, these interactions have significant impact on the structure and dynamics of an agro-ecosystem. Polynomial regression was applied on the relative abundance of selected predators with each of the prey species. In wheat-weeds agro-ecosystem coleopterans and hymenopterans predators showed more $R= 0.70$ with aphid species and *P. perpusilla*, whereas in sugarcane agro-ecosystem coleopterans, arachnids and only single species of hymenopterans showed more $R= 0.70$ with aphid species and *P. perpusilla*. By using such inferences species specific biological control can be applied against targeted pests of the wheat and sugarcane agro-ecosystems.

PREVALENCE AND CONTROL OF ASIATIC CITRUS PSYLLID AND HUANGLONGBING IN PAKISTAN

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Citrus stands first in area and production among the world's tree fruits. In Pakistan a citrus fruit is the most important fruit crop grown on the area of 160,000 hectares with production of 1.8 MMT annually. Citrus fruit is grown in all four provinces of Pakistan but Punjab produces over 95% of the crop because of its favourable growing conditions. Among the insects pests, which infest and cause heavy losses to the citrus, citrus psylla (*Diaphorina citri* Kuwayama [Hemiptera: Psyllidae]) is the most destructive and consequently the most important of all the insect pests of citrus in Pakistan. Asiatic citrus psyllid was recognised as a major pest of citrus in subtropical and tropical Asia, initially in India and then elsewhere in the region. The available evidence suggests that it is indigenous to the Indian subcontinent (Hollis, 1987) and has spread from this region to

other citrus-producing regions of the world. Both the nymphs and adults of the citrus psylla suck the cell sap with the help of their sharp piercing mouth parts and cause curling and defoliation of leaves and flowers and die back of branches from tip to downward, as a result of all these happenings premature dropping of fruits occur. Citrus psyllid population is negatively correlated with relative humidity. In Pakistan, during January the mean temperature is relatively lower as compared to other part of year, and it is known that the few nymphs of psyllid were present in January and maximum population is usually observed in the months of September and November. Ahmed *et al.* (2004) observed that the population of *D. citri* peaked twice a year, *i.e.* in the month of April and August. The build-up of population appears to be dependent upon the fresh vegetative growth in spring (March) and summer (September). Growth of sooty mould fungi on honeydew excreted by nymphs leads to discoloring of foliage and fruit, and lead to reduction of photosynthesis (Wang *et al.*, 2001). However, the status of the psyllid as a pest in orchards is due to it being the vector of Huanglongbing. Huanglongbing (HLB), whose name in Chinese means "yellow dragon disease", was first reported from southern China in 1919 and is now known to occur in next to 40 different Asian, African, Oceanian, South and North American countries. In Pakistan, the disease has been reported on sweet orange trees grafted on sour oranges. ELISA and electron microscopy of infected trees confirmed the identity of the disease in Pakistan. The causal organism of the disease is transmitted by *Diaphorina citri* (Catling, 1970). *D. citri* has been reported on citrus in Pakistan and it is also responsible for greening disease (Catling, 1970). The agent is a phloem-restricted, non cultured, Gram negative bacterium causing crippling diseases denoted "greening" in South Africa, "mottle leaf" in the Philippines, "dieback" in India, "vein phloem degeneration" in Indonesia. The HLB bacterium causing disease in Asia belongs to the genus *Candidatus Liberibacter*. As far as chemical control of citrus psylla is concerned, Boulahia *et al.* (1996) found that Confidor (imidacloprid), Fipronil (thiocyclam) and the mineral oil significantly reduced the pest population as compared to untreated plants. Mixtures of various insecticides have also been evaluated and found effective against citrus psylla. Rezk *et al.* (1996) concluded that a mixture of vertimec (abamectin) and mineral oil was the most effective combination for the control of citrus psylla followed by a mixture of methomyl (Lannate) + dimethoate, methomyl alone and dimethoate. Ictara, (thiamethoxam) and Cascade (Flufenoxuron) were found to reduce pest population. Two to three sprays of these insecticides at 10-15 days interval were found to be effective against citrus psylla. Rezk *et al.* (1996) concluded that methamidophos, dimethoate and imidacloprid had almost equal effect on the population reduction of citrus psylla on the three species of the citrus (Kinnow, Feutrell's early and Musambi).

POPULATION DYNAMICS OF FRUIT FLY (*BACTROCERA DORSALIS*) LARVAE AND PUPAE IN CITRUS AND GUAVA FRUITS IN SARGODHA REGION, PAKISTAN

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Since last ten years the oriental fruit fly, *Bactrocera dorsalis* (Hendel) has become widespread and economically important pest of fruits in different regions of Pakistan specially Sargodha. The purpose of present study was to investigate the population dynamics of fruit fly

(*Bactrocera dorsalis*) and to observe the effect of temperature and humidity on the population of fruit fly in different months (November to February) in Sargodha region. Five plants (Citrus and Gauva each) were selected randomly to collect infected fruits to check the population dynamics of fruit flies in five gardens located at different locations. The infected samples were collected by an interval of 9-15 days during whole study period. After 10-12 days of incubation period, the infected samples of citrus and guava were exposed in the laboratory to investigate the population of *B.dorsalis*. Population fluctuation in fruit flies was observed in infected Citrus and Guava samples. In guava fruits, a significantly higher population of larvae and pupae were observed as compared to citrus fruits. It was noted that population of fruit flies increased with increase in temperature and decrease in humidity. It was concluded that the maximum population of fruit fly in both fruits was observed in the month of November with least humidity (50%) and highest temperature (21°C), whereas minimum population was observed in the month of January at maximum humidity (63%) and minimum temperature (11°C).

DIVERSITY AND ABUNDANCE OF APHIDS OCCURRING ON DIFFERENT CULTIVATIONS OF FAISALABAD

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Aphids are small and soft bodied insects, which are phytophagous in nature, their infestation is a major cause of reduction in crop yield. In Pakistan, losses in crop yield due to aphids exceed more than 70 percent. Present study was conducted to determine the species diversity and abundance of Aphids in the cultivations of Faisalabad, during the study period extending from November 2010-April 2011. Results revealed that thirteen species of the aphids were recorded from Faisalabad during study period. *Myzus persicae* was the most abundant species followed by *Brevicoryne brassicae*, *Lipaphis erysimi*, *Schizaphis graminum*, *Sitobion avenae* and *Aphis nerii*. These species were found throughout the study period, while species, least in number were *Macrosiphum rosae* and *Macrosiphum euphorbia*. Highest diversity of aphids was evidenced in *Brassica* from mid- February to March and lowest in the month of November. Wheat was infested by four species of aphids, while infestation of single species in each following crops Tomato, Tobacco, Cucumber, Chili and Citrus was found.

HOST-PARASITOID RELATIONSHIP AMONG FAMILY BRACONIDAE (HYMENOPTERA) AND SELECTED LEPIDOPTERANS IN CROPLAND OF FAISALABAD, PAKISTAN

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Lepidopterans (Caterpillars) are serious pest of many crops. Braconids are the 2nd most diverse group of these beneficial insects, cosmopolitan in nature and keep check on caterpillars by

parasitizing them. Identifying and conserving these biological control agents is a basic implementation while relying least on chemicals so as to reduce their impacts on ecosystem. In this paper host-parasitoid relationship is calculated by using linear regression analysis. For this purpose Host/ Parasitoid ratios were calculated from monthly abundance data. Selected crops like Fodders, Vegetables, Brassica and wheat were sampled round the year and data was pooled to get overall monthly abundance of species. Six abundant species of Braconids (*Cotesia vestalis*, *Cotesia flavipes*, *Heterospilus* spp., *Cotesia congregata*, *Cotesia plutella*, *Heterospilus eurostae*, and seven abundant species of Lepidopterans (*Pieris brassicae*, *Trichoplusia ni*, *Helicoverpa zea*, *Helicoverpa armigera*, *Spodoptera exigua*, *Pseudoplusia includens*, *Spodoptera litura*) were selected for finding their correlation. *Pieris brassicae* showed best probable association with *C. vestalis*, *C. flavipes*, *H. spp.* *C. plutella* exhibit close relationship with *H. zea*, *H. armigera* while *C. congregata* did not show association with any member of the Lepidopteron under study. Such findings are very helpful in devising biological control programs in fields.

MYOTIS FORMOSUS AND MINIOPTERUS SCHREIBERSII, TWO NEW BAT SPECIES RECORDED FROM PAKISTAN

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Fifty four bat species belonging to eight families and twenty six genera have been reported from Pakistan. Information regarding chiropteran fauna of Pakistan is mainly based literature and museum surveys and as such little field efforts have been made to make a countrywide survey of bats. We surveyed Malakand Division to explore the bat fauna of this area from 2010-2012 and recorded Hodgson's bat *Myotis formosus* (Hodgson, 1835) and Schreiber's long-fingered bat *Miniopterus schreibersii* (Kuhl, 1918). *Myotis Formosus* was captured from Wach Khwar (N34° 58.104' E72° 28.270') in Swat district, Mattak (N34° 59.066' E72° 02.970') in Dir district and Barcharait Daim (N34° 33.737' E71° 44.872') in Malakand district. The Schreiber's long-fingered bat was captured from Fatehpur (N35° 04.345' E72° 29.502'), Bahrain pul (N35° 12.418' E72° 32.963'), Fizagat (N34° 47.586' E72° 23.672') and Fish hatchery (N35° 08.439' E72° 32.917') in Swat district and from Barcharait Daim (N34° 33.737' E71° 44.872') in Malakand district. This paper documents external body, cranial and bacular measurement of the two newly recorded bat species.

BIODIVERSITY AND DYNAMICS OF MACROINVERTBRATE POPULATIONS IN WHEATWEEDS AGRO-ECOSYSTEM IN PUNJAB (PAKISTAN)

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The study was conducted in wheat-weeds agro-ecosystem along the peripheral cultivation belt around Faisalabad (30° 31.5 N and 73° 74 E; 184.41 m a b s 1). This study documents diversity

and dynamics of foliage macro-invertebrates sampled from edges and centers of wheat weeds agroecosystem. A total of 4,228 foliage of macro-invertebrates belonging to phylum Arthropoda (92.41%) and Mollusca (7.59%) were recorded. Diptera (27.65%), Hemiptera (27.58%), Cokoptera (18.21%) and Hymenoptera (7.36%) were dominant while Orthoptera, Neuroptera, Lepidoptera and Araneae collectively formed (11.62%) arthropods. Mollusca constituted only of pulmonate gastropods (7.59%). The overall diversity was ($H' = 3.36$) that varied seasonally at statistically significantly level. Maximum diversity was recorded in autumn ($H' = 3.57$) that was followed by spring ($H' = 3.23$) and winter ($H' = 3.09$). In contrary to the general findings, Diversity of macro-invertebrate populations in the center ($H' = 3.363$) was significantly higher than the edges ($H' = 3.299$) of the fields ($p = <0.001$).

MACROINVERTEBRATES DIVERSITY AND PHYTOCHEMICAL POTENTIAL OF AGRICULTURAL WEEDS OF FAISALABAD DISTRICT

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The present study was conducted in the agroecosystem of Faisalabad district, to assess the distribution, abundance and diversity of macroinvertebrates inhabiting on weeds as well as these weeds were evaluated for their phytochemicals potentials. A total 430 specimens were captured, constituting of 9 order, 45 families and 84 species on six weed species *viz.* *Convolvulus arvensis*, *Cronopus didymus*, *Chinopodium murale*, *Euphorbia helioscopia*, *Rumex dentatus* and *Parthenium hysterophorus*. Of which *Parthenium hysterophorus* (3.737) and *Euphorbia helioscopia* (3.715) were comprised more diverse macroinvertebrates. Phytochemical potential of weeds revealed that flavonoids, tannins, alkaloids, terpenoids, saponins, batannins and cardiac glycosides were recorded in all weed species. It is concluded that weeds naturally grown in the agroecosystem not only support vast diversity of macro invertebrates but also constituting a variety of chemicals, which are used to manufacture medicines.

ECOLOGICAL STUDIES OF FOLIAGE SPIDER FAUNA IN CITRUS ORCHARD

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The present study showed the diversity and characteristics of foliage spider fauna occurring in the citrus orchard of horticulture research area, university campus. Jarring method was used for spider collection. The faunistic investigation yielded 80 species of spiders, belonged to 15 families and 36 genera. Salticidae was the dominant family consisted of 22 species belonged to 7 genera. At species level, *Marpissa pseudodacoratus* (9.71%), *Neoscona bengalensis* (6.65%) and *Oxyopes azhari* (6.62%) were the dominant species. Guild structure scrutiny revealed ten feeding guilds

namely, orb web builders, jumping, ground dweller, foliage dweller, scattered line weavers, mesh web spinners, sheet web builders, plant dwellers, ambushers and wandering spiders. Orb web builders and jumping spiders were the dominant feeding guilds representing --% and 35.76% respectively of total collection. During the study period richness and diversity peaks were recorded in March (3.04), June (3.08) and November (2.92). Comparison of yearly data showed that both the monthly samples size ($F=4.65$; $d.f.=11$; $P<0.01$) as well as yearly sample size ($F=4.94$; $d.f.=1$; $P<0.05$) of two years were significantly different. The number of species in monthly samples were statistically different ($F=3.32$; $d.f.=11$; $P<0.05$). The PCA revealed that the monthly samples had generally the scattered distribution on principal component coordinates. Among the environmental factors, temperature and relative humidity had a significant effect on population distribution.

EFFECTIVENESS OF SOME INSECTICIDES AGAINST OKRA SHOOT AND FRUIT BORERS, (*EARIAS SPP.*) ON OKRA, *ABELMOSCHUS ESCULENTUS* (L). MOENCH CROP

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Okra (*Abelmoschus esculentus* L. Moench) is one of the most important and commonly grown vegetables all over the Pakistan. This crop is attacked by a number of phytophagous insects, but okra shoot and fruit borers, (*Earias* spp.) are the most important one, which attack severely on okra crop at the vegetative and fruiting parts. This pest is commonly control by different insecticides. The current study was conducted to check the effectiveness of 5 insecticides viz., spinosad 240SC, bifenthrin 10% EC, deltamethrin 2.5 EC, lambda- cyhalothrin 2.5 EC and Coragin[®] 20SC against shoot and fruit borer, (*Earias* spp.) during 2012 at Rana Agricultural Farm, Shah Pur Obha, Shujabad, Multan. The results showed that spinosad 240SC and Coragin[®] 20SC were most effective against *Earias* spp. after 72 hours to 7th days among the all tested insecticides followed by deltamethrin 2.5 EC and bifenthrin 10% EC and lambda- cyhalothrin 2.5 EC.

BIODIVERSITY AND CRANIALOGY OF RODENTS (FAMILY MURIDAE) INHABITING DESERT AREAS OF UMERKOT AND MITHI DISTRICT OF SINDH

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A total number of 60 specimens including 44 male and 16 female of Muridae family, of genus *Meriones* of rodents were collected from the desert area of Umerkot and Mithi district in the months December 2011 to October 2012. Muridae family is a one of the largest family among all the

families of the mammalian population, where the studies of Genus meriones of Muridae family initiated to determine the presence of exact species inhabiting the studies areas. The data includes various parameters i-e body length body weight, length of tail, length of hind limb and basalcondyle, zygomatic arches, mestodont, interorbital, diatema, mandible, Superiormolar, inferiormolar. The present work also includes external morphological studies such as variation in fur colour.

EFFECT OF POPULATION DENSITY ON THE FECUNDITY OF *CALLASOBRUCHUS CHINENSIS* REARED ON CHICK PEA UNDER LABORATORY CONDITION

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Callasobrochus chinensis Linnoeus (Coleoptera, Bruchidae) is a serious pest of chick pea (*Cicer arietinum* L.) in stored condition. It is a cosmopolitan species found in Asia and Africa. In order to study the fecundity the initial stock of insect were collected from Grain storage Laboratory, SARC and reared in Toxicology laboratory of Pesticide Research Institute, PARC Karachi on chick pea, obtained from the local market of Karachi. 100 grams of chick pea was taken in glass jars covered with muslin cloth tied with rubber band. The experiment was conducted in three replicate and each replicate containing 5 jars and in each jar 1, 2, 3, 4, and 5 pairs of insects were released. After 10 days of interval insects were removed and number of eggs laid on each grain was counted. The average eggs laid by insects 1, 2, 3, 4 and 5 pair were recorded to be 37, 3, 41, 54 and 114 respectively. Similarly after same interval windows on each grain was also counted. Oviposit ion was found to be directly proportional to number of female indicating that that the male had more chances to mate more than one female. It was recorded that only 61% eggs were hatched and only 37% were found to be pupated. In this experiment only on the average only 10% damage of grain was recorded irrespective of the number of pairs present in the jars.

ABIOTIC FACTORS EFFECTIVENESS ON POPULATION DYNAMICS OF *OXYCARENUS LAETUS* (KIRBY), (HEMIPTERA; LYGAEIDAE) ON COTTON FROM AARI AND SURROUNDING OF GINNING FACTORY AREAS FROM TAUNSA SHARIF

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Population dynamics of dusky cotton bug (*Oxycarenus laetus* Kirby) on selected genotypes (BT-121 and CIM-496) in relation to abiotic factos. Maximum population of dusky cotton but was observed per boll (after opening of bolls) and whole plant during end of Novembe on BT-121 and CIM-496 cotton respectively at AARI Faisalabad. The pest population was recorded and the meteorological data regarding weather factors were collected from Meteorological Department (ARRI), Faisalabad. Dusky cotton bug was also collected from four ginning factory areas at Taunsa

Sharif, District Dera Ghazi Khan. Dusky cotton bug was found throughout the year but in April and early June, it was only found near the ginning factories. While maximum average population of dusky cotton bug per plant was around the distances of 100 meters and lower population was observed at 200 and 300 meters than 100 meters of ginning areas. The BT-121 was much susceptible to bug population as compared to CIM-496. The maximum population was observed near ginning factory areas than other places. Temperature has significant positive correlation while relative humidity and rain fall showed a negative impact on bug population

DIETARY HABITS OF THE BARN OWL (*TYTO ALBA*) IN AN AGRICULTURAL FIELD OF CENTRAL PUNJAB

SHAINA ABDUR-RAB AND HAMMAD AHMAD KHAN

Department of Zoology and Fisheries, University of Agriculture, Faisalabad.

The barn owl (*Tyto alba*) is a well-known bird of prey throughout the world, specifically feeding on rodents, mainly rats and mice. The owl therefore serves as natural biological controlling agent in the crops. Conservation of this owl, therefore, should be propagated in Pakistan, for an effective management of rats and mice, rather than using the toxicants in the sustainable agro-ecosystems, therefore preventing a potential impact of poisons on the plantations. The study therefore designs to explore diet constituents of the barn owl in an agricultural farm, to assess its food preferences and diet composition. Weekly visits (seven days) will be conducted in the evening to figure out the pellets of the barn owl, collate them and after being placed in the envelope, number them. Pellets will be dried for three days. Pellets will be weighed and disseminated into small pieces and the prey items will be critically checked mainly the skulls and the bones of small mammals, amphibians, some insects and plant materials to indicate the food preference of the barn owl. The observed and checked specimens will be identified with reference to the available reference models. The data will be collated and statistically analyzed through suitable statistics, to interpret the results in a meaningful manner. Present studies focus on knowing the food habits and dietary constituents by the analysis of pellets of the barn owl, collated from the main roost in the agricultural farmland. In the recent past, relentless toxicants use has led to the undesirable situation in the country, with incorporation of unwanted chemicals in the animal food web. In view of this, conservation of barn owl assumes to be an important factor in the ecological communities to overcome such predicaments.

SECTION – I I I
E N T O M O L O G Y

**REVISION OF THE WORLD SPECIES OF *MUSTHA* AMYOT & SERVILLE
(HEMIPTERA: PENTATOMIDAE: PENTATOMINAE: HALYINI) BASED ON
MORPHOLOGICAL CHARACTERS**

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Mustha Amyot and & Serville (1843) is one of the richest Palaearctic genera of the tribe Halyini, with ten species described: *baranovi* Kiritshenko 1952, *gigantea* Horvath 1906, *incana* Stål 1876, *izmirensis* Memon & Ahmed 2009, *longispinis* Reuter 1890, *morgani* Horvath 1906, *serrata* Fabricius 1974, *spinosula* Lefebvre 1837, *spinosus* Ahmed & Kamaludin 1984 and *vicina* Hoberlandt 1995. The genus is widely distributed throughout the world, in; Pakistan, Afghanistan, India, Tajikistan, Iran, Egypt, Syria, Israel, Jordan, Turkey, Eastern Europe and South Africa. Out of the ten species, six had been described by 1952, and only three species have been added in the genus during the last fifty five years: *spinosus* (Abasi and & Ahmad 1971) from Pakistan, *vicina* Hoberlandt 1995 from Iran and *izmirensis* Memon & Ahmed 2009 from Turkey. The characters of each taxon used in this work are based on the observation of holotypes, paratypes and those given in the literature. Presently genus *Mustha* is revised with its ten world known species distinguished from all other genera of tribe Halyine by the presence of a set of unique characters: that is the presence of very distinct spines on the lateral margins of the entire body (head, pronotum, abdomen), and also on the posterior margin of the eighth paratergite of the female genitalia. The geographical distribution of all *Mustha* species along with key of ten species is given; a brief discussion on the relationship of genus *Mustha* with two closely allied genera *Phircodus* and *Orthoschizops* is also discussed.

**PREVALANCE OF *Aedes aegypti* MOSQUITO AND OCCOURANCE OF DENGUE
CASES IN HYDERABAD REGION**

ATTAULLAH ANSARI, NASREEN MEMON, BHOJO MAL AND JUMA KHAN

Department of Zoology University of Sindh, Jamshoro

Mosquitoes are among the best known group of insects because of their importance to men as vectors of some of the most distressing human diseases such as Dengue, Malaria, West Nile Fever and Yellow Fever. Dengue is the most rapidly spreading viral disease transmitted to human being by the bite of female *Aedes* mosquitoes. Presently we have surveyed four Talukas of Hyderabad district (Qasimabad, Hyderabad city, Hyderabad rural & Lateefabad) and Jamshoro for prevalence of *Aedes aegypti* mosquito. Out of these four Taulkas, only Hyderabad city (old Hyderabad city) was found positive to *Aedes aegypti* and Jamshoro city was also found positive. This is the first record of *Aedes aegypti* mosquitoes from Hyderabad city and Jamshoro. The specimens of mosquitoes were randomly collected with the help of insect net and by sucking tube

(Aspirator) from different fields of above mentioned localities during June 2011 to July 2012. We have also collected Dengue cases data from Liaquat University Hospital Hyderabad from June 2011 to July 2012. Total 97 confirm Dengue cases were reported in the year 2011 but no case was reported in the year 2012 till July. During present study it was observed that the population of *Aedes aegypti* mosquito in Hyderabad is increased specially during and after monsoon season i.e in the month of July, August, September, October and November. Peak season for Dengue fever in year 2011 started after monsoon period in the month of September 47 cases were reported followed by October 44 and November 2 cases were reported.

NEW RECORD OF TWO SPECIES OF GENUS *PAPILIO* LINNAEUS (LEPIDOPTERA, PAPILIONIDAE) FROM DISTRICT SANGHAR, SINDH

BHOJOO MAL MANGHWAR, NASREEN MEMON, ATTAULLAH ANSARI AND
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The genus *Papilio Linnaeus* (1758) is one of the biggest genera of family Papilionidae. It comprises about 200 species throughout the world. They are found in all continents of the world except Antarctica and maximum number of population is found in tropical areas. They are commonly called Swallowtail, because the posterior part of hind wings look like tail. They look very beautiful because of the colourful scales on their wings. During present study, 150 specimens were collected from different localities of district Sanghar, from September to December 2012. Identification has been done by the colouration of fore wings, mouth parts, external and internal male and female genitalia and by observing paratypes. Out of 150 specimens two species of genus *Papilio* namely *Papilio demolus* and *Papilio polytes* were identified. These species were compared with their closely allied species.

REVISION AND NEW RECORD OF GENUS *SPHAEROPHORIA* LEPELETIER AND SERVILLE (DIPTERA, SYRPHIDAE) FROM QUETTA, BALOCHISTAN, PAKISTAN

JUMMA KHAN TURK, NASREEN MEMON, DILDAR ALI SOLANGI, BHOJOO MAL AND
ATTAULLAH ANSARI

Department of Zoology University of Sindh, Jamshoro

Syrphid flies are commonly known as hover flies or flower flies. Genus *Sphaerophoria* belongs to tribe syrphini. They have cylindrical abdomen and are considered beneficial flies. The adults' flies are important pollinator, while the larvae are predator in nature, these are aphidophagus too. Presently we have surveyed Quetta and their adjoining areas i.e. Pishin, Dusht and Kuchlak and more than 245 specimens were collected during March 2012 to November 2012. The species were identified on the basis of external morphological characteristics specially the coloration such as marking on abdomen, tibiae and wings venations and also male and female internal genitalia. During this period we identified two species of genus *Sphaerophoria*, *S. ruepelli* Wiedemann (1830) and *S. taeniata* Meigen (1822). It has been a new record of these species from surveyed localities of Quetta, Balochistan, Pakistan.

**IMPACT OF MATING ON LONGEVITY OF MALES OF RED COTTON BUG
DYSDERCUS CINGULATUS (HEMIPTERA: PYRRHOCORIDAE)**

NAHEED SOOMRO, NEELAM BANO, SAMINA MALIK, TAHIRA JABEEN AND
 KARIM DAD PITAFI

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Dysdercus cingulatus (Red cotton Bug) is the major pest of cotton plant. Cotton yield for the last few years has been reduced which may be due to the heavy pest infestation. During the present research investigations were made to study the impact of mating on longevity of males of red cotton bugs *Dysdercus cingulatus*. The observations were carried out on sex starved males of *Dysdercus cingulatus* under laboratory conditions. The results indicate that unmated males live longer than mated males, a confirmation for transferring some vital resources other than the sperm and seminal fluids required to enhanced longevity of males.

FURTHER INCIDENCE OF *SCHIZODACTYLUS* FROM SINDH PAKISTAN

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At the present 3 species of *Schizodactylus* i-e *S. monstrosus* (Drury), *S. Minor* (Ander) and *Schizodactylus?* have been collected from the River Indus Hyderabad. These insects appear exclusively carnivorous in habits and mainly feed on different types of ground darkling dung beetle. In this paper their distribution and morphological account has been discuss in detail.

**A NEW SPECIES OF GENUS *CALLOGRYPHUS* SJOSTEDT (GRYLLIDAE:
 ORTHOPTERA) FROM SINDH, PAKISTAN**

MALIK SAMINA, N.M. SOOMRO, T.J. URSANI, BALOCH NAHEED AND M.S. WAGAN

Department of Zoology, University of Sindh, Jamshoro

The female of *Callogryllus saeedi* n.sp, is described for the first time from Sindh, Pakistan.

**DISRIBUTION AND INCIDENCE OF SHORT HORNED GRASSHOPPERS FAMILY
 ACRIDIDAE OF PUNJAB**

NAHEED BALOCH AND M.S.WAGAN

Department of Zoology, University of Sindh, Jamshoro

During the survey of short horned grasshoppers (1995-1997) in various districts of the Punjab collected material was sorted out in 58 species of grasshoppers. Of these 20 belong to Locustinae (= Oedipodinae), 10 to Gomphocerinae, 7 to Eypreocnemidinae, 5 to Acridinae, 4 to Oxyinae, 3 to Cyrtacanthacridinae, 2 each to Euthyminae (=Hemiacridinae), Calopteninae, Truxalinae, 1 each to Dericorythinae, Tropidopolinae and catantopinae. During the present study 5

Species namely, *Ochrilidia jagoi*, *O muzafferi*, *Sphingonotus akbari*, *S. hussaini* and *Aulacobothrus punjabensis* are described as new. Eight species are recorded for the first time from this area. These are *Dericorys tibialis* (Pall) *Choroedocus illustris* (Walker), *Cataloipus cognatus* (Walker), *Mioscirtus wagneri rogenhoferi* (Saussure), and *Gelastorhinus semipictus* (Walker) *Phlaeoba tenebrosa* Walker, *Duroniella laticornis* (Krauss) and *Oxypterna afghana* Ramme. The incidence of adult grasshopper species in the various districts of the Punjab during 1995, 96 and 97. The most abundant species was *Aiolopus thalassinus thalassinus* forming (16.69%) of the total number of all the grasshopper species Collected, followed by *Duroniella laticornis* (11.51%), *Aulacobothrus luteipes* (8.56%) *Spathosternum prasiniferum prasiniferum* (8.29%) *Heteracris littoralis* (7.64%); and *Acrida exltata* (7.26%).

**OCCURRENCE OF *TRILOPHIDIAANNULATA* (THUNBERG) (OEDIPODINAE,
ACRIDIDAE, ACRIDOIDEA, ORTHOPTERA) FROM PAKISTAN**

BARKAT ALI BUGHIO, RIFFAT SULTANA AND M. SAEED WAGAN
Department of Zoology, University of Sindh, Jamshoro, Pakistan

The present study was carried out on external morphology, Phallic complex and distributions of species *Trilophidiaannulata* (Thun berg). This orthopteran is considered as major pest of crops, vegetables, orchards and grasslands in Pakistan. As well as Whenever its Population may increased at different localities. The number of insects reach at an peak level in April to end of September until the cold December comes. Characteristics of taxonomic importance, Such as the Structure of pronotum, number of antennal segments, number of tibial spines and features of Phallic complex was studied to determine the distribution and status of pest in agricultural fields. The species distribution and occurrence at the district level are also considered.

**TAXONOMIC STUDY AND DISTRIBUTION OF CONOCEPHALINAE (ORTHOPTERA:
TETTIGONIOIDEA) ENSIFERA OF PAKISTAN**

WAHEED ALI PANHWAR, RIFFAT SULTANA AND MUHAMMAD SAEED WAGAN
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The representative of family Tettigonioidea are important pests of agriculture crops forest, fruits, orchards, berry shrubs and grasses while many species are ecologically associated with forest biocenoses, damaging trees and shrubs. During the present study a total of 321 specimens of Conocephalinae were collected and sorted out into 3 species namely: *Conocephalus* (*Anisoptera*) *maculates* (Le Guillou, 1841), *Euconocephalus incertus*, Walker 1869 and *Euconocephalus pallidus*, Redtenbacher, 1891, pertaining to 2 tribes viz: Copiphorini and Conocephalini. At the present two distinguish color forms in tegmina and wings of *Conocephalus* (*Anisoptera*) *maculates* was observed in some specimens tegmina and wings are white in color and without spots whereas in majority of specimens tegmina are dark in color and having spots. These 3 collected species are widely distributed all occurring all over the country, but *E. pallidus* less in population as compared to *E. incertus*. This study was supported by grants received from Pakistan Science Foundation Islamabad for Research Project No PSF/S-SU/BIO (423).

**TAXONOMIC STUDIES ON VARIOUS TRIBES OF SLANT- FACED GRASSHOPPER
(ACRIDINAE) OCCURRING IN SINDH**

QURBAN ALI MEERANI, RIFFAT SULTANA AND MUHAMMAD SAEED WAGAN

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At the present, a total of 1728 specimens of Acridinae pertaining to 4 genera representative of 4 tribes viz: Acridini, Truxalini, Phlaeobini and Duroniella were collected. The complete description of 7 species namely: *Truxalis eximia eximia* Eichwald, *Truxalis fitzgeraldi* Dirsh, *Acrida exaltata* (Walker), *Acrida gigantea* (Herbst, 1794), *Phlaeoba tenebrosa* (Walker), *Phlaeoba infumata* Brunner, 1893 and *Duroniella laticornis* (Krauss) is provided along with photographs, drawing- lines, measurements of different body components, complete description of species, list of important host plants and their distribution at districts level. Further, phallic complex of these 7 species has been studied in detail for the first time from this region.

**GRASSHOPPERS (ACRIDOIDEA: ORTHOPTERA) OF KOTDIJI KHAIRPUR, SINDH,
PAKISTAN**

FAKHRA SOOMRO AND M. S. WAGAN

Department of Zoology, S.A.L University, Khairpur

The short horned grasshoppers of Kotdiji historical site of Khairpur District of Sindh are studied for the first time. The material was collected from the mountainous, desert and agricultural land of Kotdiji fort, Ubhan Shah and localities from September 2011 to June 2012. Following species were identified: Family Acrididae: Subfamily Oxyinae: *Oxya hyla hyla* Audinet-Serville, 1831, *O. fuscovittata* (Marschall, 1836), *O. bidentata* Willemsse, 1925, *O. velox* (Fabricius, 1787); Subfamily Hemiacridinae: *Hieroglyphus perpolita* (Uvarov, 1932), *H. orzivorius* Carl *Spathosternum prasiniiferum* (Walker, 1871); Subfamily Tropidopolinae: *Tropidopola longicornis longicornis* (Fieber, 1853); Subfamily Catantopinae *Diabolocatantops innotabilis* (Walker, 1870); Subfamily Cyrtacanthacridinae: *Anacridium rubrispinium* (B. Bienko, 1948), *Cyrtacanthacris tatarica* (Linnaeus 1758); *Schistocerca gregaria* (Forsk. 1775); Subfamily Calliptaminae: *Acorypha glaucopsis* (Walker, 1870), *Sphodromerus undulatus undulatus* (Kirby, 1914); Subfamily Eyprepocnemidinae: *Eyprepocnemis alacris impicta* (Uvarov), *Choroedocus robustus* (Walker, 1870), *Heteracris littoralis* (Rambur, 1838), *H. adspersa* (Redtenbacher, 1889), *H. notabilis* Uvarov, 1942; Subfamily Oedipodinae: *Acrotylus humbertianus* Saussure, 1884, *A. longipes subfasciatus* B. Bienko, 1948, *Aiolopus thalassinus thalassinus* (Fabricius, 1781.), *A. thalassinus tamulus* (Fabricius, 1798), *A. simulatrix simulatrix* (Walker, 1870), *Gastrimargus africanus sulphureus* B. Bienko, *Hilethera aeolopoides* (Uvarov, 1922), *Locusta migratoria* (L. 1758), *Mioscirtus wagneri rogenhoferi* Saussure, 1888, *O. rosescens* Uvarov, 1942, *O. senegalensis* (Krauss, 1877), *Scintharista notabilis pallipes* Uvarov, 1941, *Sphingonotus savignyi* Saussure 1884, *S. rubescens rubescens* (Walker, 1870), *Sphingonotus* spp. Subfamily Acridinae: *Acrida exaltata* (Walker, 1859), *Duroniella laticornis* (Krauss, 1909), *Phlaeoba tenebrosa* (Walker, 1871), *Truxalis eximia eximia* (Eichwald, 1830), *T. fitzgeraldi* Dirsh, 1950; Subfamily Gomphocerinae: *A. luteipes* (Walker, 1871), *Chorthippus indus* Uvarov, 1941, *Crucinotacris decisa* (Walker, 1871), *Leionotacris bolivari* (Uvarov, 1921), *Ochrilidia geniculata* (I. Bolivar, 1913), *O. gracilis gracilis* (Krauss, 1902), *Oxypterna afghana* Ramme, 1952, *Gonista rotundata* Uvarov, 1933, *Gelastorhinus semipictus*

(Walker, 1870) Dericoythidae: Dercorythinae *Dericorys tibialis* (Pallas), Pyrgomorphidae: *Chrotogonus trachypterus trachypterus* (Blanchard), *Pyrgomorpha Bispinosa deserti* (B. Bienko), *P. inequalipennis* Bolivar; *Attractomorpha acutipennis blanchardia* (Kirby) *Poeciloceris pictus* (F.) The grasshopper species are classified according to their life forms as Terricoles, Graminicoles, Herbicoles and Arboricoles.

**PRELIMINARY STUDIES ON THE IMMATURE STAGES OF OEDIPODINAE
(ACRIDIDAE: ORTHOPTERA) FROM SINDH**

SAJJAD ALI LARIK AND RIFFAT SULTANA
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Oedipodinae are considered very important from economic point of view they generally occur in wide range of habitat and destroyed the valued agriculture crops. At the present 328 specimens of Oedipodinae were collected from the different regions of Sindh. Collected specimens were sorted out into six instars. Further, there was significant variation have been observed in the morphological characteristics of the instars.

**DESCRIPTION OF ONE MALE NEW SPECIES OF PHILODROMUS SINDHICA
(ARANAE: PHILODROMIDAE) FROM SINDH, PAKISTAN**

T.J. URSANI, N.M. SOOMRO, MALIK SAMINA AND M.H. SOOMRO
Department of Zoology, University of Sindh, Jamshoro

The male of *Philodromusindhica* n.sp. is described for the first time and its genitalia is studied.

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

BILAL SAEED KHAN,* MUHAMMAD HAMID BASHIR, KHURAM ZIA, MUHAMMAD
ASIF QAYYUUM AND ZAHID MEHMOOD SARWAR
Department of Agric. Entomology, University of Agriculture, Faisalabad
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Mites belonging to the family stigmatidae are well known predators against the phytophagous mites and small soft bodied insects and oftenly collected from aerial plant parts or soil leaf litter. A survey was conducted to explore the predatory mite fauna of Punjab, a new predatory record of genus *Agistemus* was collected from 283/Layyah on bittergourd (*Momordica charantia*). Specimens mounted on the glass slides and drawings of different body parts were made. These specimens were compared with the already described species. Ceremonial description, illustration of main body parts, host range and comparison remarks are also given. Sixteen (16) paratype females were collected from Jhang and T.T. Singh on bittergourd and cotton crop. All

specimens were deposited in the Acarology Research Laboratory, Department of Agri. Entomology, University of Agriculture, Faisalabad, Pakistan.

**POPULATION DYNAMICS AND BIOLOGY OF SECONDARY DENGUE VECTOR
MOSQUITO *Aedes albopictus* SKUSE (DIPTERA: CULICIDAE)**

MUHAMMAD SARWAR

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Mosquito *Aedes albopictus* Skuse has an important epidemiological role as an arboviruses vector and is known to be an efficient carrier of human dengue fever virus. Sampling for *A. albopictus* was done at least once a week during the whole season and achieved through the use of nets and ovitraps by counts of larvae and adults. The presence of *A. albopictus* was noted heterogeneous through out the year and population monitored reached maximum proliferation in post moon soon season in the month of October due to favorable conditions after occurrence of heavy rain fall. Vector proliferation increased gradually from January to May and then a sharp decline confirmed in June and July. Considering the whole seasonal dynamic observations, the peak breeding activities were detected during the months of September, October and November as a result of heavy rain from July to August. The lowest density was found in the months of December, January and February due to low temperature. In this effort, mosquitoes were reared and observed for their progression through different life stages by means of laboratory experiment. The development of *A. albopictus* was assessed from egg to adult emergence under $25\pm 2^{\circ}\text{C}$ temperature and $75\pm 5\%$ relative humidity associated with L: D 12: 12 h. A blood meal of Balb/ C mouse was offered to vector daily. The eggs hatched into larvae in about 3 days. The growth durations of first, second, third and fourth instar larvae lasted for 1.3, 1.2, 1.7 and 2.8 days, respectively (larvae start to form pupae in 7 days). The fourth instar larvae time was longer than the others stages, while the pupation time was longer than the fourth instar larvae growth period. Adult's emergence occurred in 3 days after pupation (pupal development time). The overall total immature development period from egg laying to adult emergence was 13 days, under laboratory condition. The average lifespan of male and female was between 25 and 30 days, respectively.

**HOST RANGE, NEW LOCALITY RECORDS AND SEASONAL DISTRIBUTION OF THE
ONLY *Bombus* POLLINATOR, *Bombus haemorrhoidalis* SMITH IN
MARGALLA AND MURREE HILLS, NORTHERN PAKISTAN**

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Bombus haemorrhoidalis, the only *Bombus* pollinator species was collected at monthly interval from different non-agricultural and agricultural flora of Margalla and Murree hills of Pakistan during 2011. Twenty three plant species of thirteen plant families were observed visited for nectar and pollens with seven new localities ranging from 542-1986 m altitude. The most

commonly visited plant family was Asteraceae. More population of males and daughter queen was observed from July to September. Prevalence of queen, workers and males differ from parks to forests and with altitude. More activity of workers was observed in early and late day hours to avoid harsh climate of June to August. A brief taxonomic note with comparative morphological variations of with *Bombus terrestris* is also presented.

REVISION AND NEW RECORD OF GENUS *SPHAEROPHORIA* LEPELETIER AND SERVILLE (DIPTERA, SYRPHIDAE) FROM QUETTA, BALOCHISTAN, PAKISTAN

JUMMA KHAN TURK, NASREEN MEMON, DILDAR ALI SOLANGI, BHOJOO MAL AND ATTAULLAH ANSARI

Department of Zoology University of Sindh, Jamshoro

Syrphid flies are commonly known as hover flies or flower flies. Genus *Sphaerophoria* belongs to tribe syrphini. They have cylindrical abdomen and are considered beneficial flies. The adults' flies are important pollinator, while the larvae are predator in nature, these are aphidophagus too. Presently we have surveyed Quetta and their adjoining areas *i.e.* Pishin, Dusht and Kuchlak and more than 245 specimens were collected during March 2012 to November 2012. The species were identified on the basis of external morphological characteristics specially the coloration such as marking on abdomen, tibiae and wings venations and also male and female internal genitalia. During this period we identified two species of genus *Sphaerophoria*, *S. ruepelli* Wiedemann (1830) and *S. taeniata* Meigen (1822). It has been a new record of these species from surveyed localities of Quetta, Balochistan, Pakistan.

FORAGING BEHAVIOUR OF *APIS DORSATA* FAB. ON *GREWIA ASIATICA* L.

MUHAMMAD FAHIM KHALID*, SHAFQAT SAEED, SYED MUHAMMAD ZAKA, FARHAN MAHMOOD SHAH AND MUHAMMAD KASHIF RABBANI

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Apis dorsata Fab. is native to southern Asia and is one of the major pollinators of phalsa (*G. asiatica* L). Current study was conducted to observe the foraging activity of *Apis dorsata* on *G. asiatica* at different day times. To find the contribution of insects in pollination, 20 branches with unopened flower buds were selected randomly, tagged and number of buds on these branches were counted. Out of these branches, 10 were veiled with nylon mesh bags and 10 were kept open. After fruit setting, the covered branches were unveiled and number of fruits were counted on open branches as well. Diurnal dynamics of the bee population were measured by counting the number of visitors on a floral branch in five minutes and 7 branches were observed in an hour. The numbers of flowers bloomed (yellow colored) on that day were also counted on these branches. Foraging behaviour of 25 *A. dorsata* individuals were observed on *G. asiatica* in different day hours. Meanwhile, their pollen baskets were also observed (either these carry pollen grains or not). Results showed a significant difference between percent fruit setting on covered and uncovered branches were 22.60% and 59.60% respectively. The frequency of the *A. dorsata* did not show significant correlation with the number of the bloomed flowers on branches ($P= 0.965$) which

shows the attraction of *A. dorsata* towards individual flowers. *A. dorsata* was active in the field from 9:00 AM to 5:00 PM and highest visiting frequency of *A. dorsata* was from 1:00 PM to 2:00 PM (2.6 visits/minute/branch) indicating a higher nectar reward at this time. At anthesis (9:00-10:00 AM) 100% bees were found collecting pollens and nectar as well from the flowers, covering the flower stigma with the venter of body and their pollen baskets were full of pollen grains. As anthesis started to decline (10:00-11:00 AM) the bee population gradually shifted their activity of pollen collection to only nectar collection at 12:00-5:00 PM, from the nectar chamber (do not visit stigma during this period). It is concluded that the visits of *A. dorsata* to stigma is in coincidence to the anthesis and stigma receptivity of *G. asiatica* which may be due to co-evolution.

FAUNASTIC STUDY OF HALYINE SPECIES (HEMIPTERA, PENTATOMIDAE, HALYINI) OF SINDH, PAKISTAN

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According to available literature a large number of species have been added to the tribe Halyini from time to time and also place of different species changed from one genus to other by different workers, new records are also explored time to time. During this study, specimens of the tribe Halyini were collected from different districts of Sindh provinces of Pakistan and compared with available literature and preserved material including Holotypes and paratypes and prepared and modified keys of genera and species of tribe Halyini.

REVISION AND NEW RECORD OF SPECIES OF FAMILY COCCINELLIDAE FROM DIFFERENT LOCALITIES OF SINDH

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Ladybird beetle of family Coccinellidae comprise of nearly 5000 described species worldwide. They have hemispherical body with small head & short legs with dorsally red, orange, black or yellow colour with black, white or yellow spots. Majority of ladybird beetle are predators while few are phytophagus. Only few record of Coccinellidae found in Pakistan, with no specific record from Sindh. Nearly 1000 specimens were collected from Dec. 2011 to Dec. 2012 from different localities of Sindh. Four species namely *Psyllobora viginitiduopunctata*, *Cheilomenes sexmaculata*, *Coccinella septempunctata*, and *Coccinella undecipunctata* were identified by observing internal genitalia of male & female ladybird beetles. The study was focused to collect, identify & compare the specimen from different areas of Sindh.

ECOLOGICAL SURVEY OF *ANOPHELES CULICIFACIES* IN DISTRICT HYDERABAD AND DISTRICT TANDO MOHD KHAN OF SINDH

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Mosquitoes are slender and relatively small insects, some of the mosquito species transmit diseases such as malaria, dengue and yellow fever. Presently we have done ecology of *Anopheles* in District Hyderabad and Tando Mohd Khan, from January to June 2012. During present study we identified *Anopheles culicifacies* as a dominant vector species in both districts. We observed that Tando Mohd Khan possessed high ratio of mixed infection of malaria (*P. vivax* and *P. falciparum* both found in the blood of patients), Because of permanent breeding sites such as irrigation channels and rice fields. On the other hand Hyderabad district has low rate of mixed infection of malaria. We found that District Hyderabad possessed large number of temporary breeding places such as open stagnant water pools, small and large puddles and depression in the ground in taluka Qasimabad of district Hyderabad.

NEW RECORD OF TWO SPECIES OF GENUS *TRIBOLIUM* MACLEAY, (COLEOPTERA, TENEBRIONIDAE) FROM DISTRICT HYDERABAD, SINDH

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Tribolium Mac Leay (1895) includes 36 described species which make serious damage to flour and store grains, these species are cosmopolitan as well and destroy domestic flour as well as shop flour to great extent. We have collected 239 specimens from different localities (shops, houses, godowns) of Hyderabad district including rural and urban areas. Identification has been done by the colouration of fore wings, mouth parts, external and internal male and female genitalia and by observing paratypes. Two species of *Tribolium* namely *Tribolium castaneum* and *Tribolium confusum* are pre dominantly reported as major pests.

SOCIAL BEHAVIOR OF WASP (*VESPA VELUTINA* LEPELETIER, *POLISTES FLAVUS* CRESSON) AND STRUCTURAL DESIGN OF THEIR NEST

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In the present study social behaviour and structural design were carried out of two wasp species: hornet wasps (*Vespa velutina* Lepeletier) and the paper wasp (*Polistes flavus* Cresson) from the different localities of the District Mansehra, Pakistan. Nest of *V. velutina* was completely closed except one opening for entry or exit with 1-10 steps layers with hexagonal cells inside the

nest. Nest of *P. flavus* was found among bunches of leaves of tree 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 the nest materials showed Ca highest while K with the lowest amount with descending *V. velutina*: Ca>K>Al>Mg>Si while in *P. flavus* nest Ca>Mg>Si>Al. All species of wasps showed strong defensive behaviour, If disturb, they try to protect their larvae in their nest. When they felt their nest was no longer safe, they start to shift their larvae from the nest to safe place picked in their limbs.

**IDENTIFICATION AND DESCRIPTION OF ONE NEW SPECIES OF GENUS
FORCELLINIA (ACARIDAE: ACARI) FROM PUNJAB**

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Food is the basic necessity of life and almost each individual on this planet need optimum dietary nutrients for its survival. But the population of the world is growing higher and higher resulting in number of issues regarding production and storage of food. Mites of Family Acaridae are the major pests of all type of stored grains. They are the major concerning factor in reducing food quality as well as causing huge nutritive problems. Mites of the genus *Forcellinia* are important pests and they have been reported from different commodities in different areas of the country. One new species *Forcellinia chounawalaensis* was collected from Chounawala (Hasilpur) from Wheat (*Triticum aestivum*) grains. The hypopi were separated from the grains by using Berlese's Funnel. Permanent slides were prepared in Hoyer's medium. The type was deposited in Acarology Research Laboratory, Department of Agri. Entomology, University of Agriculture, Faisalabad

**EFFICACY OF SOME PLANT EXTRACTS IN ETHANOL AGAINST STORED GRAIN
MITES OF FAMILY ACARIDAE INFESTING WHEAT**

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Mites are the major pest of stored produce; particularly wheat is greatly affected by these mites during storage. They are minute Arthropods and microscopic in size. They are widely distributed throughout the world and damage almost all types of stored grains. In this research the efficiency of methanolic extracts of leaves of commonly found plants i.e. Neem (*Azadirachta indica* L.), Kanair (*Phoenix carensis*), Amaltas (*Casia fistula*) and Dhatura (*Datura Stramonium* L.) was carried out against stored grain mites of family acaridae in laboratory under CRD. Different concentrations of 8%, 4%, 2%, 1% and 0.5% in acetone were used for each plant extract. The data was recorded at weekly intervals for 28 days. Amaltas proved more effective as compared to other plant extracts with 91% mortality after 28 days with LC₅₀ value 0.11% solution. Kanair caused

85% mortality, Neem 88% and Dhatura 74% mortality. The LC₅₀ values decreased with the passage of time for all the methanolic extracts. Dhatura was least effective with 74% mortality after 28 days having LC₅₀ value of 0.24% solution after 28 days. Amaltas was found more effective for the management of these mites.

**STUDY OF BIOLOGY AND BREEDING HABITATS OF *Aedes* MOSQUITOES
ESPECIALLY DENGUE VECTORS IN SOME URBAN AREAS OF DISTRICT
PESHAWAR**

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A survey was conducted to determine the preferred habitats of mosquitoes breeding in artificial or temporary habitats. Particular emphasis was made on dengue vector *Aedes aegypti* in different urban localities of Peshawar. Monthly sampling of mosquito immature was conducted from variety of breeding habitat *i.e.* tires, plastic cans, metal drums, discarded receptacles, mud pots, ceramic pot, fountain and bamboo sections. Further processing was carried out in laboratory including mosquito rearing, preservation and species identification. Meteorological data of sampling localities and physicochemical parameters of water (pH, turbidity, colour and odour) were also determined. A total of three genera of mosquitoes were recovered; *Aedes*, *Culex* and *Anopheles*. Genus *Aedes* was the predominant genus constituted 83% of the total collection, represented by *Aedes aegypti* and *Aedes albopictus*. *Ae. aegypti* was the most dominant, abundant and constant species comprised of 993 female and 1106 male mosquitoes. Peak mosquito abundance was recorded in October (1276) followed by Nov (501) while least number (72) was observed in June. No mosquito species was collected from December to March and May. Tires, mud pots and ceramic pot inhabited three species each whereas no mosquito species was found in bamboo sections. *Aedes aegypti* was reported from four habitats; tires, plastic cans, metal drum and ceramic pot. Tires were the most productive breeding habitats accounted for 78.5% of the total immature collection. Mud pots and fountains were equally productive though the later yielded only *Anopheles stephensi*. *Aedes aegypti* and *Ae. albopictus*, potential vectors of dengue have both been encountered in the present study. For effective vector control tires, mud pots, plastic cans and metal drums should be among the habitats of focus in dengue epidemiology.

**SPECIES DIVERSITY PATTERN OF MOSQUITOES WITH PARTICULAR EMPHASIS
ON CULICINE BREEDING IN CERTAIN HABITATS OF PESHAWAR**

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A study about the breeding habitats of mosquito, including ecology, abundance and effect of physicochemical characteristics on mosquito immature, in some targeted areas of Peshawar was carried from June, 2011 to May 2012. The habitats selected for this survey were Construction Pond, Waste water channels, Agricultural drains, Percolating water, Animal track Hoof prints, Duck pond and Water filled Mud pots comprising both temporary and permanent habitats. A total of 3760 adult mosquitoes comprising 1974 females and 1786 males were recovered from total 8137 immature collected. These mosquitoes belonged to thirteen species *viz.* *Cx. quinquefasciatus*

(72.63%), *Cx. tritaenorrhyncus* (8.324%), *Cx. theleri* (0.346%), *Cx. bitaeniorhyncus* (0.771%), *Cx. vishnui* (1.41%), *An. stephensi* (3.83%), *An. fluviatilis* (1.862%), *An. culicifacies* (4.92%), *An. pulcherimus* (0.053%), *An. annularis* (0.239%), *Ae. albopictus* (5.479%), *Ae. unilineatus* (0.027%) and *Culiseta longiareolata* (0.106%) were recorded. Some 10% mosquitoes were mounted on slides for larval identification that were successfully identified reporting four Culicine species *i.e.*, *Culex quinquefasciatus*, *Cx. tritaenorrhyncus*, *Cx. theleri* and *Cx. pseudovishnui*. *Culex quinquefasciatus* was found to be dominant and frequent, recorded from all positive habitats. Monthly distribution of mosquitoes occurrence report tri-modal distribution with peaks in June, November and March. *Culex tritaenorrhyncus* was the second most abundant species collected from five habitats with dominant status and moderate distribution. The highest mosquito abundance was observed at polluted waste water and agriculture drains Larvae of *Aedes* mosquitoes were found only in mud pots and *Culiseta* mosquitoes in waste waters.

SPECIES COMPOSITION OF SAND FLIES (DIPTERA: PSYCODIDAE) FROM NORTH WAZIRISTAN AGENCY

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There is great medical importance of sand flies as a significant vector of cutaneous leishmaniasis. Since recent reports on the cutaneous leishmaniasis from North Waziristan Agency, the present study is aimed to examine the species richness and composition of sand flies in selected localities of North Waziristan Agency. We collected about 3000 specimens of sand flies from the remote areas of North Waziristan Agency during March to October 2012. The study areas included three tehsils namely Miran Shah, Mir Ali and Shawal. The voucher specimens of sand flies were fixed in berlese media and identified up to specific level using updated keys. A total of 15 species are being reported from the study area with largest abundance of *Sergentomaya baghdadis*.

DISTRIBUTION AND ECOLOGY OF MOSQUITO FAUNA AND DESCRIPTION OF ITS HABITATS IN DISTRICT SWABI

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No published information is available regarding the mosquitoes of District Swabi. This study is an attempt to provide a comprehensive knowledge about the species composition and seasonal variations of mosquitoes and their possible breeding habitats in four selected villages of the District. So the present survey was conducted in different villages of district Swabi like Kalu Khan, Yarhussain, Nawakali and Main Swabi. Varieties of habitats were surveyed from June, 2011- May, 2012 including both permanent and temporary habitats. Special emphasis was given to polluted waters laid on sides of Mardan –Swabi road, mosquito fauna of which was compared with other habitats like grassy ditches, edges of streams and rivers, and different container habitats (tins, cans, water storage pots etc). Sampling was done monthly from each site. The larvae and pupae were reared in Laboratory and adults were preserved in tubes with silica gel. A total of 3246 specimens were collected belonging to 13 species *i.e.* *Ae. albopictus*, *An. annularis*, *An. fluviatilis*,

An. subpictus, *An. stephensi*, *Ar. subalbatus*, *Cx. bitaneorhyncus*, *Cx. psuedovishnuui* and *Cu. longiareolata*. These species belong to five genera i.e. *Aedes*, *Anopheles*, *Armigeres*, *Culex* and *Culiseta*. The most dominant species was found to be *Cx. queneqefascitus* (2671) followed by *Cx. tritaeneorhyncus* (478). Peak abundance was found in the month of July (622) followed by March (620), while the least No. of mosquitoes were found in the month of February (47). No mosquito specimen was recorded in the months of December and January. Since specific mosquitoes require special habitats so the different environmental parameters like temperature, humidity, rainfall and pH of water were also recorded to understand the relationship between habitats and occurrence of mosquitoes belonging to different taxonomic groups an essential requirement for efficient control strategies.

ON THE SAND FLIES DIVERSITY (ORDER: DIPTERA, PSYCHODIDAE) OF DISTRICT DIR LOWER, KHYBER PAKHTUNKHWA

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The present study was carried out from April to August 2010 in six selected localities of District Dir Lower to investigate the sand flies diversity. Eight species of sand flies viz. *Phlebotomus sergenti*, *P. major*, *P. bergeroti*, *P. hindustanicus*, *P. kazironi*, *P. salangensis*, *Sergentomia babu* and *S. baghdadis* were reported from the study sites. *P. bergeroti* and *P. hindustanicus* are thus far by reported for the first time from Khyber Pakhtunkhwa. *P. sergenti* was found to be the most abundant sand fly species.

A NEW RECORD OF HOVERFLIES TWO SPECIES OF GENUS ERISTALINUS (DIPTERA: MILESINAE) FROM HYDERABAD SINDH, PAKISTAN

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Genus *Eristalinus* Rondani, 1845 is one of the largest group of the insect known as flower flies or hoverflies some times syrphidflies about 600 species (Vockeroth, 1969) have been described in all over the world, having many sub genera and species belongs order Diptera, family syrphidae, sub family Eristalinae, so member of insect, genus *Eristalinus* found in all over the world fall in to several small group. This "natural groups" of hoverflies insect Genus *Eristalinus* are look like very beautiful color with shiny abdomen and very important in both positive and negative ways adult are pollinators. However, larvae of these flies are aquatic rat-tailed maggots are rarely pest Occasionally larvae appear in alarming numbers in dung pits or animal waste lagoons. Even so, they pose little threat to man or animals. Collection was brought during November 2012 to end of January 2013 from different field of Hyderabad division about 200 specimens were collected. Two species of genus *Eristalinus* are recognized on the basis of spotted eyes are present on both species *E. aneus* the body is black thorax is also entirely black *E. megacephalus* these species are look like *helophilus* because some stripes are present on thorax. We were study many "specimens of male and female species of *E. megacephalus*, *E. aneus* and their genitalia was drawn in glycericrine after clearing boiling potassium hydroxide (KOH) for the period of 4-5 minutes and

then washing in distilled water all the terminology used to identify different parts of genitalia follows Hippa et al. (2001). This is a new record of these species for the first time from Hyderabad Sindh, Pakistan.

**COMPARISON OF NESTING AND SOCIAL BEHAVIOURS OF PAPER WASP,
POLISTES JLAIVUS (CRESSON) (HYMENOPTERA: VESPIDAE) AND HONEY BEE,
APIS MELLIFERA L. (HYMENOPTERA: APIDAE)**

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The social insects, paper wasp, *Polistes jlavus* (Cresson) and honey bee, *Apis mellifera* L. have the great importance for us because they act as pollinators, predators and environmental indicators. In the present study, comparison of nesting and social behaviours of *P. jlavus* and *A. mellifera* were carried out from district Mansehra, Pakistan. The papery nest of *P. jlavus* was found among bunches of leaves of trees with 1-5 flat step layers containing hundreds of hexagonal cells in one sided hanging downward. The waxy hive of *A. mellifera* was pouched like containing double sided hexagonal cells one for eggs while other for storing food. The X-ray diffraction (XRD) was used for elemental analysis of their nest/hive building materials showed that the highest amounts of calcium (Ca) was found in both nest/hive, however, magnesium (Mg) in *P. jlavus* while silicon (Si) in *A. mellifera* were the lowest amount. Social behaviour of *A. mellifera* showed strong defensive behavior (than *P. jlavus*), i.e., pseudo attack, subsequent erratic flight, wing buzzing, mandibular pecking, abdominal pumping and abdominal twisting. By disturbing them, they try to protect their larvae in the nest with higher defensive behaviour. Parental care was also highly developed in *A. mellifera* than *P. jlavus*. The knowledge about their nesting and social behaviours help us dealing them in routine and commercial purposes.

PLANT - POLLINATOR INTERACTION IN PEACH FRUIT ORCHARD IN ISLAMABAD

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Plant - pollinator interaction eco system has important role in the activity of pollinators. To investigate this, an experiment was laid in peach fruit orchard, NARC, during 2012. The observations were taken from 29.2 2012 to 15.3 2012 on daily basis during the main flowering period in the selected orchard. Observations were taken at 9 am, 12 noon and 3 pm. The pollinator insects visiting flowers were observed for 10 minutes on each flower and counted. The time spent by each species was also noted. The average maximum temperature during this period was 22°C and average minimum 10 °C. Weeds flowering in the orchards were *Medicago polymorpha* (Fabaceae), *Wisteria sinensis* (Fabaceae), *Sonchus oleraceus*. (Asteraceae) and *Fumaria officinalis* (Papaveraceae). Fruit plant was of *Prunus persica* (Rosaceae). The number of pollinators was comparatively higher on *Sonchus* (54) followed by *Wisteria* and *Medicago* (37 each), *Fumaria* (25) and *Prunus* (15). It shows that comparatively more pollinators visited weeds rather than peach

plants. Furthermore on *Fumaria*, *Sonchus* and *Medicago*, syrphids were comparatively higher, on *Wisteria* bumble bees and on *Prunus* honey bees. Sitting time was comparatively more of butterflies (25 seconds) followed by moths (18) syrphids (7) on *Sonchus*. All pollinators spent less time on *Medicago* (1-3).

POPULATION OF INSECT POLLINATORS IN APPLE ORCHARDS AT MURREE

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Pollinators are essential for qualitative and quantitative production of flowering plants whether cropped or wild. Due to habitat degradation it is feared that their population is declining. Murree area was having a lot of Apples in the past but now its cultivation is negligible in this area. The population of pollinators was measured in Apple orchards in different locations of Murree hills by scanning method. The observations were taken in 2nd, 3rd and 4th week of April, 2012. Flowers were scanned by visual observation by noting the visits by these pollinators on randomly 100 flowers. It was done by walking in between the rows having two counters in two hands. Flowers were counted in counter in one hand and pollinator in other one. It was observed that Apis group and Syrphus group were dominant pollinators in this area. The total number of pollinators were comparatively lower (207/100 flowers) at lower elevation of 3,260 ft and somewhat higher (225) at 4,900 ft and maximum at an altitude of 5,240- 5,660 ft. As altitude increased to 6600 ft they declined (244). It shows that elevation beyond 5,660 is not much favorable for these pollinators. Other parameters like wild plants or intercropping could not be related to the populating of these pollinators.

WASP FAUNA (HYMENOPTERA: VESPIDAE) OF GILGIT, BALTISTAN

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This study was conducted to explore the vespidae fauna under family Vespidae of Gilgit-Baltistan. Specimens were collected from different areas of Gilgit-Baltistan during the years 2010-2011. The study revealed 14 species in 8 genera belonging to three sub-families that are occurring in Gilgit-Baltistan *i.e.* Subfamily Polistinae which was the diverse group in the area with 5 species in 2 genera including *Parapolybia escalerae*, *Polistes gallicus*, *P. indicus*, *P. rothneyi carletoni* and *P. waltii*. Subfamily Eumeninae was represented by 5 species in 4 genera, including *Delta dimidiatipenni*, *D. viatrix*, *Symmorphus crassicornis*, *Allorhynchium a. argentatum* and *Euodynerus fastidiosus*. The Vespinae was represented by 4 species including *Vespa orientalis*, *V. velutina*, *Vespula germanica* and *V. vulgaris*. A brief introduction to the morphology and distribution of Vespidae is given. Among these collected specimens, six species were new to Gilgit Baltistan. One species *Delta viatrix* is a new record for Pakistan. Keys to the subfamilies, genera and species have also been provided.

FAUNISTICS OF HAWK MOTHS (LEPIDOPTERA: SPHINGIDAE) FROM PAKISTAN

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Hawkmoths were collected during nationwide surveys and identified. Specimens from Plant Protection Department which were now housed in the National Insect Museum at National Agricultural Research Centre, were identified and included in the current study. Data were also collated from the literature records. This study represents the first account of hawkmoth fauna of entire country and reports 52 species in 30 genera.

REARING OF *COCCINELLA UNDECIMPUNCTATA* LINNAEUS (COCCINELLIDAE: COLEOPTERA) ON DIFFERENT NATURAL AND ARTIFICIAL DIETSMARRYAM BAKHATAWAR, QAMAR SAEED, SYED MUHAMMAD ZAKA AND
MUHAMMAD RASHID AKRAM

Biological control of insect pests of various crops is being adapted in many of the regions of the world. Predators and parasitoids are being reared in labs on natural and artificial diets. *Coccinella undecimpunctata*, being a voracious predator of many of the insect pests including aphids and mealybugs, has to be reared artificially in laboratories for mass release. In this research, *C. undecimpunctata* is reared on three natural diets and thirteen artificial diets. The natural diets included mustard aphids (*Lipaphis erysimi*) cotton mealybugs (*Phenacoccus solenopsis*) and *Sitotroga* eggs, while the artificial diets included honey, egg yolk, egg albumin, whole egg content mix, honey, boiled yolk, boiled albumin and their mixtures in the total of thirteen diets. Newly hatched larvae and 5-6 days old larvae (pre-fed on *L. erysimi*) of *C. undecimpunctata* were fed on these diets. Young larvae of *C. undecimpunctata* successfully developed on natural diets, whereas, half-grown larvae (pre-fed on *L. erysimi*) developed on natural as well as five artificial diets consisting of egg albumin as a constituent. Neither young nor half-grown larvae completed development on the remaining eight diets (figure 1). *L. erysimi* was the most preferred food with maximum survival of young ones (76.6%) and half-grown (90.0%) larvae followed by *Sitotroga* eggs and mealybug larvae. These findings can lead to the discovery of new artificial diets for the *C. undecimpunctata* for the mass release in fields in order to biologically control a number of pests for an eco-friendly pest control system.

THE ROLE OF *CHILO INFUSCATELLUS* (LEPIDOPTERA: PYRALIDAE) IN REDUCING CANE SUGAR RECOVERY IN DIFFERENT SUGARCANE VARIETIES IN SINDHMUJAHID KHAN, RIFFAT SULTANA AND AZRA ANJUM
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The response of three varieties *i.e.* Thatta-10, SPF-234 and CPF-237; against *Chilo infuscatellus* Snellen by analyzing the cane sugar recovery percentage, were studied at Cane Chemical Testing Laboratories of Faran Sugar Mills Ltd., Mehran Sugar Mills Ltd. and Matiari

Sugar Mills Ltd, during December 2011. The chemical analysis of sugarcane showed that infestation of *C. infuscatellus* significantly reduced the sugar recovery percent in infested cane, collected from the cane yards of Faran Sugar Mills Ltd., Mehran Sugar Mills Ltd. and Matiari Sugar Mills Ltd. The recovery percent difference was significantly higher in Thatta-10 (-0.83%, -2.19% & -1.95%) compared with CPF-237 (-0.6%, -1.82% & -1.77%) and SPF -234 (-0.51%, -1.24% & -1.47%) respectively. Whereas, the overall recovery percent difference was noted as -0.65%, -1.75% and -1.73%; from Faran Sugar Mills Ltd., Mehran Sugar Mills Ltd. and Matiari Sugar Mills Ltd. It suggests that Thatta-10 showing significant difference.

**HISTOLOGY OF THE ALIMENTARY CANAL OF *POEKILOCERUS PICTUS*
(FABRICUS) (PYRGOMORPHIDAE: ACRIDOIDEA: ORTHOPTERA)**

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Poekilocerus pictus (F.) is one of our most common grasshoppers and has been taught as classic insects in the most of universities of Pakistan and India. As no account of histology of *P. pictus* is available in the structure of alimentary canal here for the first time the histology of the various organs of alimentary canal of this insect was studied and compare with other known species of the genus *Poekilocerus*. During the present study significant differences were recorded in the numbers of oesophageal and crop ridged seemed to be specific taxonomic values.

**FOUR NEW RECORDS AND A KEY TO THE SPECIES OF GENUS *ANTROCEPHALUS*
(HYMENOPTERA: CHALCIDIDAE) FROM KHYBER PAKHTUNKHWA, PAKISTAN**

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New recorded species of genus *Antrocephalus*, viz. *Antrocephalus mitys*, *Antrocephalus ryukeunsis*, *Antrocephalus peechiensis* and *Antrocephalus lugubris* have been identified and described. Key to the species is also provided along with illustrations of necessary characters.

**STUDIES ON DETERMINATION OF THE RELATIONSHIP BETWEEN ABUNDANCE
OF DIFFERENT GROUPS OF SOIL MITES AND ABIOTIC FACTORS IN
AGROECOSYSTEM**

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Soil mites community is often considered to be a useful bioindicators of ecosystem conditions and changes. The abundance and structure of soil mites community is influenced by the climate and edaphic factors. These factors are important in regulating the soil mite diversity, population dynamics and their significant role as decomposers and predators. This study was

conducted to determine the changes in seasonal population abundance of soil mites in an agroecosystem in relation with abiotic factors in the field plots of cotton, maize and wheat. Populations of different groups of soil mites were sampled on fortnightly basis by examining soil cores during each crop season at Square No. 3 Entomological Research Area, University of Agriculture Faisalabad. Composite soil samples were taken and mites were extracted using an indigenously prepared model after Macfayden-Tullgren apparatus. The abundance of soil mites was then correlated with different abiotic factors. No significant differences were found in soil mite abundance across all field sites. Total 751 soil mite individuals were extracted out of which 50 % belonged to Oribatida followed by 15 % Prostigmata, 13% Ungrouped, 12 % Astigmata, 6% Mesostigmata and 5% Gamasida. Average abundance per month was highest in summer months as compared to autumn, winter and spring. Lower rank abundance modelling revealed that the oribated mites contribute the most to soil mite fauna. Analysis of variance of abundance of sampling sites showed that abundance was significantly different ($p < 0.05$) except Maize and Wheat. Shannon diversities were not significantly different. All the soil mite groups occurred frequently in the samples. Cluster analysis revealed 3 categories of mites according to abundance *i.e.* highly abundant, moderately abundant, least abundant. Soil temperature showed positive correlation with abundance, while Relative humidity was strongly negatively correlated with the abundance. Soil moisture, Soil pH and rainfall showed weak negative correlations while no correlation was observed between soil organic matter and abundance. Principal Component analysis showed that there is no specific correlation between all group composition and sampling period from April-Oct has entirely different species composition than the sampling period from Nov-Feb. Correspondence analysis showed that distribution of relative abundance of all the groups is quite dissimilar across the sampling period. Redundancy analysis showed that pH, soil moisture and organic matter are highly correlated with each other, the major group Oribatida showed strong negative correlation with relative humidity. Monte-Carlo Permutation test was performed with 499 permutations to check the significance of variables. Backward stepwise multiple linear regression was performed which resulted in R^2 statistic of 77 % of the model that selected Relative humidity, Soil temperature and soil organic matter in the final model. No serious auto correlation was found between the residuals after Durbin-Watson statistic was applied. Results revealed that to study the soil mite community it is necessary to increase the sample size, classify groups to lower ranks and carry out studies across multiple seasons.

DETERMINATION OF THE RELATIONSHIPS AMONG ABUNDANCE OF SOIL ARTHROPODS, EDAPHIC AND CLIMATIC FACTORS IN AN AGROECOSYSTEM

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Soil arthropods are an important component of agricultural ecosystems, contributing significantly to biodiversity, ecosystem structure and function. Many abiotic factors influence the diversity and abundance of soil arthropods in agroecosystems. These factors are important in regulating the seasonal patterns of arthropods diversity, their population dynamics and their significant role in improvement of soil structure and function. The purpose of this study was to investigate soil arthropod abundance in relation to a blend of soil and environmental factors. Another aim was to determine the difference in abundance and diversity of soil arthropods in different crop fields and seasons. Five crops sugarcane, cotton, wheat, berseem fodder and citrus orchard were sampled through tullgren extractions and pitfall traps on fortnightly basis. Composite

soil samples were taken and microarthropods were extracted using an indigenously prepared model after Macfayden-Tullgren apparatus. Significant differences were found in soil arthropod abundance across different sampling months and crops. Total 13673 soil arthropod individuals were collected out of which 41% were collembola followed by 16% hymenoptera, 13% acarina, 10% myriapods, 6% coleoptera, 5% orthoptera, 4% aranae. Average abundance was highest in summer months as compared to winter. Richness was higher in cotton crop representing 32 taxa while it was lowest in citrus orchard representing 19 taxa. Shannon diversities was also higher in cotton and it was lowest in Berseem. Lower rank abundance modelling revealed four groups that were mostly dominant in all crops the Collembola (Hypogasturidae), The Acarina (Mites), The Hymenoptera (Formicidae ants) and the Myriopoda (the millipedes). Analysis of variance of abundance in all crops was significantly different except sugarcane and citrus ($p < 0.05$). Shannon Diversities were also significantly different in all crops except cotton and sugarcane. Most of the soil arthropods occurred frequently in the samples except Gryllotalpidae, Carabidae and Curculionidae which occurred randomly. Cluster analysis revealed four categories of soil arthropods according to abundance *i.e.* highly abundant, moderately abundant, Least abundant and rare. Principal component analysis revealed that sampling period of April-June in Berseem crop is the major source of variation in Axis 1 explaining 80 percent of the total variation in data. Soil temperature and soil organic matter showed strong positive correlation with abundance. While relative humidity was strongly negatively correlated with the abundance. Soil moisture, soil pH showed weak correlations while no correlation was found between rainfall and abundance. Canonical correspondence analysis revealed soil organic matter, soil temperature, soil pH and relative humidity as the major source of variation in axis 1 representing about 50 percent of the total variation and axis 2 about 25 percent in the abundance counts data. Backward stepwise multiple linear regression was performed resulting in R^2 statistic of 65% of the model which selected relative humidity, soil pH and soil organic matter in the final model. No serious auto correlation was found between the residuals after Durbin-Watson statistic was applied. Results suggested that soil arthropods through their response are useful indicators of environmental variations *i.e.* climate change, soil quality and anthropogenic variations such as different cropping systems, chemical use and soil cultivation.

**NINE NEW RECORDS OF (COLEOPTERA: SCARABAEIDAE: SCARABAEINAE)
FROM NORTHERN AREAS OF PAKISTAN**

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Himalayan Mountains ranging 1500 m to 6000 m. The area is diverse and virgin for its flora and fauna. Little work is being done to explore insect community. To record some data, an extensive survey of Himalayan region of Pakistan was conducted during 2006-2012 and recorded nine new records of Coleoptera: Scarabaeidae: Scarabaeinae four new records of genus *Onitis*, one species of genus *Euoniticellus*, three species of genus *Onthophagus* and one species of genus *Heliocopris*.

**REDESCRIPTION OF A CAYSTRINE SPECIES *CAYSTRUS OBSCURUS* DISTANT
(PENTATOMOIDEAE: PENTATOMINAE) WITH NEW CHARACTERS OF GENITALIA
AND THEIR PHYLOGENETIC IMPORTANCE**

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Distant (1901) described *obscurus* in the preoccupied generic name *Odius* Stål which was transferred to *Neodius* Bergroth in (1891), followed by Distant (1902) and Oshanin (1906). Finally Bergroth (1908) transferred in Distant species into Stål's genus *Caystrus*. Later Distant (1918) and then Hsiao *et al* (1977) followed this Bergroth action. Ahmad (1980) recorded this species from Hyderabad, Sindh and next year keyed this taxon with its complete distribution within Pakistan. Its metathoracic scent auricle and genitalial characters remained however unknown. For description and illustration of metathoracic auricle the techniques of Zahid and Ahmad (2007) were generally followed and for male and female genitalia those of Ahmad (1986) and Ahmad and McPherson (1990 and 1998) were generally followed. These genitalial characters are presented and compared with related *Caystrus* species and their phylogenetic importance are highlighted.

**A PRELIMINARY REPORT ON THE ECONOMICALLY IMPORTANT
GRASSHOPPERS (ORTHOPTERA: ACRIDOIDEA) OF KHYBER PAKHTUNKHWA**

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The majority of the people of Khyber Pakhtunkhwa are connected with agricultural profession. With the increasing knowledge in the agriculture sector, efforts are continuously incremented to protect the pastoral economies from insect pests. Grasshoppers are well known pests of cash crops and grasses and reported frequently as minor pest on medicinal and other wild plants. Basic knowledge of the biological resources is an imperative to control crop damages and saving socio-economic values of agricultural communities. The present study reviews the grass hopper fauna of Khyber Pakhtunkhwa. Data from museum collections and field surveys in various parts of Khyber Pakhtunkhwa reveals the rich diversity of grass hoppers in this province.

**EFFECT OF TEMPERATURE ON LIFE HISTORY AND POPULATION GROWTH OF
THE LEAFMINER *SCAPTOMYZA FLAVA* (FALLÉN) (DIPTERA: DROSOPHILIDAE)**

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The leafminer, *Scaptomyza flava* (Fallén) is found in many parts of the world including Africa, East and West Siberia, Europe, Afghanistan, Uzbekistan, north-west China, Mongolia, Japan, Iran, North America, Australia and New Zealand. It is an important pest of brassicas, peas

and gypsophila, and has also been recorded from the plant families including Fabaceae, Caryophyllaceae, Tropaeolaceae, Solanaceae and Amaryllidaceae. Temperature is a key abiotic factor determining the development, survival and population size of insects. Here we studied the influence of four constant temperatures (15, 20, 25, and 30° C) on life history and population growth of *S. flava* on Chinese cabbage in the laboratory. This study was conducted in the Institute of Natural Resources, Massey University, New Zealand, at four constant temperatures (15°, 20°, 25° and 30°C). Different bioassay rooms were set to carry out these experiments with each room having one of the mentioned temperatures with 16 h light: 8 h dark, and 60 ± 5% R.H. The results showed that the developmental periods for egg, larval and pupal stages, and adult longevity decreased with the increase in temperature. The adult emergence rate was higher at 20 and 25°C than the other test temperatures. Similarly, higher fecundity occurred at 20 and 25°C. Life table of *S. flava* showed that the innate capacity for increase (r_m), the net reproductive rate (R_0), and the finite rate of increase (λ) reached the maximum at 25°C with values of 0.1318, 36.00, and 1.141, respectively. This study offers valuable insight on the damage potential of *S. flava* within different temperature regimens and on its mass-rearing technique in laboratory for the use in biological control programs.

A NEW STATUS FOR CAYSTRINE TAXON *CAYSTRUS MINOR* LINNAVUORI (STAT. NOV.) (PENTATOMIDAE: PENTATOMINAE) WITH EXPLORATION OF NEW CHARACTERS OF METATHORACIC SCENT AURICLE AND MALE AND FEMALE GENITALIA AND ITS PHYLOGENETIC RELATIONSHIP WITHIN *CAYSTRUS* STÅL.

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Caystrus basalis minor was described Zaire in the democratic republic of Congo, as a subspecies by Linnavuori (1972) and later keyed by him. Presently its new status is recognized, separated from *Anaropa basalis* Schouteden (1909). Although Linnavuori (1972) described and recognized *C. basalis* as a nominative subspecies but did not give description of its metathoracic scent auricle nor at separated and recognized if with complete illustration of its genitalial features with those of *C. basalis minor* Linnavuori. Presently these new characters are explored using the techniques of Zahid and Ahmad (2007) and those of male and female genitalial characters using the techniques of Ahmad (1986) and Ahmad and McPherson (1990 and 1998). *C. minor* Linnavuori is not only presently separated from *C. basalis bsalis* Schouteden on the basis of these presently explored characters but also separated and phylogenetically analyzed within other related species of *Caystrus* Stål.

AGE RELATED FECUNDITY IN *CHRYSOPERLA CARNEA* (STEPHEN): IMPLICATION AND EFFECTIVENESS IN MASS REARING

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Egg-laying habits are distinctive in insect species. Among *Chrysoperla Spp.* the eggs are

deposited at night, singly or in small groups; one female produces some 100–200 eggs. Eggs are placed on plants, usually where aphids are present nearby in numbers. The stable age distribution is defined as the schedule of fractions each age class represents in the ultimate population, during developmental time of adult affect the age-specific fecundity of *Chrysoperla Spp*. An experiment was conducted to study the Age related fecundity in *Chrysoperla carnea* (Stephen): Implication and Effectiveness in Mass Rearing at a constant $25\pm 2^{\circ}\text{C}$ temperature with 70% RH (relative humidity) in the laboratory. Same age mature adults of *C. carnea* were kept for egg laying purpose on weekly basis. The female laid maximum eggs in the 2nd week after emergence, as compare to other weeks 1st, 3rd, 4th, 5th, and 6th whereas, 2nd week resulted in maximum larval, pupal survival, adult emergence and sex ratio. While female laid minimum eggs in the 7th week after emergence. The larval, pupal survival, adult emergence and sex ratio were also decreased as compare to other treatments 1st, 3rd, 4th, 5th, and 6th weeks.

A SURVEY OF SUBTERRANEAN TERMITE (ISOPTERA) FAUNA AND ITS POPULATION DIVERSITY IN DISTRICT BAHAWALPUR

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A capturing-identification survey was conducted in district Bahawalpur aiming to evaluate the population diversity of subterranean termite fauna of this area. A total six species of termites, *Psammotermes rajasthanicu*, *Coptotermes heimi*, *Odontotermes obesus*, *Microtermes unicolor*, *Microtermes mycophagus* and *Eramotermes paradoxalis* were recorded. Using Simpson scale indices the overall diversity was found to be 68%. Furthermore maximum diversity was recorded in August (64%) and minimum in November (0.8%).

STUDIES ON POPULATION DIVERSITY OF BEETLES FAUNA IN DISTRICT BAHAWALPUR

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The present studies were carried out aiming to investigate the population diversity of beetles fauna in district Bahawalpur. Five different type of habitats namely, Interdunal sandy area, Interdunal clayey area, Sand dunes, Grassy plots and Area inside-outside residencies were sampled using 50 pitfall traps which were operated on weekly basis. A total of 507 specimens representing five families Carabidae, Tenebrionidae, Scarabaeidae, Coccinellidae, Elateridae and twelve genera *Anthia*, *Brachinus*, *Scarites*, *Calosoma*, *Blaps*, *Tentyria*, *Pimelea*, *Onthophagus*, *Tomarus*, *Thyce*, *Coccinella* and *Agriotes* were recorded. Overall diversity was 86% and 88% on Simpson scale and Shannon scale respectively. The highest similarity index (0.66) was found between the site Interdunal sandy area and Sand dunes.

INVESTIGATING THE ROLE OF MORPHOLOGY IN BUTTERFLIES' ABILITY TO RESPOND TO CLIMATE CHANGE

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Climate is gradually warming and species are responding to these changes by shifting their distribution ranges polewards and to higher altitudes. Several species of butterflies have expanded their range northwards in response to climate change. There is evidence that evolutionary changes have occurred during those range shifts. Individuals from expanding populations show a greater investment in dispersal morphology, sometimes, traded off with a lesser investment in reproduction. The main aim of this research work was to relate the morphological traits with dispersal ability of butterflies in Britain by testing the hypotheses that individuals from margin sites have heavier thorax weights than those from the core sites, hence more dispersive. Whereas these have lower abdomen weights hence investing less in reproduction. The morphological data was collected on three range expanding species in the UK since 1995 *i.e.* *Aricia agestis*, *Anthocharis cardamines* and *Pyronia tithonus*. The heavy thorax mass was considered a proxy for dispersal investment and lower abdomen mass was considered a proxy for lesser investment in reproduction. The butterfly samples were dissected and dry mass was obtained by drying samples at a constant heat of 60°C for 24 hours. The morphological data was compared with the respective control species *i.e.* *Polyommatus icarus*, *Pieris napi* and *Maniola jurtina*. Using three, two and one-way ANOVA approaches, the dry mass data was analyzed for a total of 272 individuals of *Aricia agestis* & *Polyommatus icarus*, 106 individuals of *Anthocharis cardamines* & *Pieris napi* and 110 individuals of *Pyronia tithonus* & *Maniola jurtina*. The most representative results of *Aricia agestis* due to large sample size and statistical power indicate that the individuals in the recently colonized sites are heavier than in the permanently resident sites with heavier thoraxes and abdomens, suggesting the trade off with dispersal ability may not be with reproduction. More sample size is required to draw conclusive results in the cases of *the two other range expanding species*. Further research is recommended to understand the complex interactions between morphological traits and ecological parameters.

SEASONAL VARIATION IN POPULATION DENSITY OF VARIOUS NYMPHAL INSTARS OF COTTON LEAFHOPPER *AMRASCA DEVASTANS* (DISTANT) WITH REFERENCE TO VARIOUS LIMITING FACTORS

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Cotton leafhopper *Amrasca devastans* (Distant) (family: Cicadellidae) infests a wide range of crops including *Solanum melangena*, *Hibiscus esculentus* and many other vegetables. All the developmental stages of species are injurious to crops. Infestation may be very prominent and characterized by leaf curl, stunted growth and leaf burn symptoms. Afzal & Ghani (1953) suggested that attacks of leafhoppers not only reduce the number of bolls but also damage the quality of cotton. Younus (1976) stated reduction in plants height with the increase in leafhopper population. Ghouri (1976) stated that two leafhoppers per leaf are considered as the economic damage threshold of this pest. Baloch *et al* (1980) and Ahmed (1985, 1983 a & b) studied that the

population of leafhopper in lady finger and on brinjal also reduced the per acre yield of these vegetables. The sampling was started from May 2005 & continued up to the end of August 2006. On each sampling date one to two persons worked for one hour usually before sunset on every 5th days. All nymph stages and adults of cotton leaf hopper were recorded by using leaf turn method following Ellsworth *et al.* (1995) and Naranjo *et al.* (1996) and were analyzed statistically through MINITAB 1996. High density of immature insects (1st instars) reduced along with ages and minimum in adult stage were observed during May 05 to Jul 05, later on the overall population showed gradual decline although heavy in immature from Nov 05 through Feb 06, probably associated with the low temperature and very dry climatic conditions. The declined population in Aug 05 found to be associated with the heavy fungal infestation in the field, which was found to be a limiting factor in distribution of pests in the field. The heavy density of insect pests was again recorded during May 06 to Jul 06 with the arrival new crop. It rises along with the growth of plants. The drastic decline in population during Aug 06 was found to be associated with heavy rain and marks of splash on the leaves of plant were also found to induce mortality among the insects.

STUDY OF MORPHOMETRIC CHARACTERS OF *APIS DORSATA* HONEY BEE SPECIES FROM DISTRICT NANKANA AND NAROWAL

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The selected honeybee species for this study is *A. dorsata* commonly known as “dommna” in Pakistan. The aim of investigation was to determine the range of variations of some morphological traits of Pakistani *A. dorsata* honeybee species to characterize and differentiate these honey bee species populations found in various areas of Punjab province of Pakistan geographical basis. This data could as provide us an indicator for production of honey bee colonies in future in these areas. Samples of 30 worker bees of *A. dorsata* species were randomly collected from eight different locations from district Nankana and 6 from district Norowal. Morph metric measurements were made on 20 characters. No of abdominal segments (6), no of antennal segments (10), no of simple eye (3), length of hind wing (9mm) were found similar in worker bees of both areas. No of humuli vary 18-32 and 19-30, and proboscis length (6-7mm) in Nankana and Norowal districts, respectively. Cubital index ratio for right wing ranges from 7.4-8.4, left wing 7.5-8.4 from Nankana, 8.6-8.92 of left and 9.25-10.11 of right wing from Norowal districts, respectively.

DISTRIBUTION AND ECOLOGY OF *AEDES AEGYPTI* (DIPTERA: CULICIDAE) IN PAKISTAN

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During the last few years dengue has emerged as a major vector borne disease in Pakistan. In this survey *Aedes aegypti* which had been considered to be eradicated from almost the whole of Pakistan was collected from 13 different cities. The mosquito has not only started reinvading previously occupied areas (Karachi, Peshawar and Lahore) but is also spreading to new areas (Attock, Haripur, Hasanabdal, Taxilla, Rawalpindi, Gujranwala, Sheikhpura, Faisalabad, Multan

and Hyderabad). It is breeding in almost all types of artificial containers especially in tyres. Although it is mostly using outdoors containers for oviposition the mosquito was found breeding indoors in households. Water storage activity in the south of Pakistan makes domestic artificial containers the preferred habitat for *Ae. aegypti* to oviposit. This study suggests that effective control of *Ae. aegypti* in Pakistan is possible by adopting control methods, including biological control but in particular by involving the local community. This would reduce the use of insecticides that may have serious implications to human health.

**BIODIVERSITY AND SPECIES COMPOSITION OF CARABIDAE (COLEOPTERA)
FROM DISTRICT POONCH, AZAD KASHMIR**

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The present study to explore the fauna and biodiversity of family Carabidae (Coleoptera) was conducted in different localities of District Poonch. The localities were selected by keeping in view all the parameters necessary for the measurement of Biodiversity. The identification of collected specimens of family Carabidae was carried out up to species level with the help of latest available literature and keys. The rank lists from each sampled locality were prepared and a collective rank list along with the list of taxa collected from all localities was also prepared. Diversity, abundance, richness and equitability were calculated. A total of 144 specimens of family Carabidae were collected. Five species belonging to 3 sub-families under 3 genera were identified. These sub-families are Licininae; Carahinae, Brachininae and the species are *Carabus caschmirensis*, *Chlaenius quadricolor*, *Pheropsophus sobrinus*, *Chlaenius laticollis*, and *Chlaenius hamifer*. *Carabus caschmirensis* was the most abundant species. It was followed by *Chlaenius quadricolor*, *Pheropsophus sobrinus*, *Chlaenius laticollis*, and then *Chlaenius hamifer*. The maximum abundance of family Carabidae was recorded from Rawalakot and the minimum abundance was recorded from Datot. The highest diversity of the family Carabidae was recorded from Rawalakot and the lowest diversity was calculated from Alisojal. The maximum richness of the family Carabidae was recorded from Rawalakot and minimum was recorded from Datot and Alisojal and the highest equitability of the family Carabidae was recorded from Rawalakot and lowest was recorded from Alisojal.

**DIVERSITY AND RELATIVE ABUNDANCE OF GROUND RUNNING FAUNA IN
SUGARCANE AND WHEAT CROPS OF FAISALABAD**

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Current study focused on the diversity and relative abundance of ground running fauna in sugarcane and wheat crops of Faisalabad was conducted for a period of 6 months from November 23, 2009 through April 13, 2010 throughout the cultivation period. A total of 13417 specimens

were collected from the sugarcane and wheat. The specimens were identified and grouped into 14 orders, 26 families and 44 species. Out of a total of 44 species, 15 species of arthropod predators and 3 species of vertebrate predators, 16 species of prey/pest, 7 scavenger and 2 decomposer species were sorted based on the literature regarding their feeding habits in the crop field. A total of 4532 specimens belonging to 38 species of various invertebrates and vertebrates were sampled from the sugarcane crop whereas 8885 specimens belonging to 38 invertebrate and vertebrate species were collected from wheat crop. The wheat was more successful having 8885 specimens belonging to 38 species. Among predators, *Pheropsophus hispanicus* was maximum with 169 specimens followed by *Clubiona phragmitis* with 112 specimens. *Tullbergia granulata* was highly abundant among different pest species with 3162 individuals followed by *Oniscus asellus*, *Onychiurus hortensis*, *Armadillidium vulgare* and *Sminthurus viridis* with 2806, 2553, 904 and 715 specimens respectively.

**MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF ACRIDID
GRASSHOPPERS (ACRIDIDAE: ORTHOPTERA) FROM POONCH DIVISION, AZAD
JAMMU AND KASHMIR, PAKISTAN**

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The present study was conducted to remove the identification conflicts between grasshopper species of the family Acrididae (Orthoptera) on the basis of morphology and DNA barcoding. Grasshoppers representing 26 species of the family Acrididae were collected from different habitats and host plants from Poonch division of Azad Jammu Kashmir, Pakistan. Specimens were identified taxonomically and barcoded by sequencing cytochrome c oxidase (COI) barcode region. Barcodes of 19 morphological species were successfully obtained, and the sequence data was used to separate the species by Neighbor-Joining cluster analysis. Barcode data successfully discriminated 18 species and the two species, *Patanga japonica* and *P. succincta* could not be separated from each other as they shared the barcode sequence and clustered together on the Neighbor-Joining (NJ) tree. Morphologically specimens of *Eyprepocnemis shiriaki* were identified to one species but barcode data revealed that in addition to *Eyprepocnemis shiriaki*, two more species of the genus *Eyprepocnemis* are present in the region. Similarly, on the basis of morphological characters, in subfamily Catantopinae presence of two species, *Catantops erubescens* and *C. brachycerus*, was determined but the barcode data suggest the presence of one more species in the region from the genus *Catantops*. The findings show the usefulness of barcode data in discriminating the grasshopper species and also suggest that barcode data can be reliably used for developing barcode reference library for species identification through sequence matches.

**STUDIES ON THE POPULATION OF FRUIT FLY USING THE SEX PHEROMONES
(METHYL EUGENOL) IN BER PLANTATION AT SARC EXPERIMENTAL FIELD,
KARACHI**

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Fruit fly, *Carpomyia vesuviana* Costa (Dipterex: Tephritidae) infestation was recorded on Ber plantation cultivated in SARC experimental Field at university campus Karachi (Anwar *et al.*, 2012). In order to develop the control strategy the population of fruit fly was monitored through he used of pheromones traps. The successful control of fruit flies based on pheromones traps were reported to be used for monitoring the male and female (Dominiak and Nicol, 2010) besides cultural practices and other methods (Alam *et al.*, 1999). The ber plantation was irrigated through drip irrigation system and the distance between each row and plant to plant was kept 20 feet. Traps were installed at the height of 2-4 feet. The fruit flies collected in traps were counted after 10 days. The population data were recoded from the month of October to February, 2012-13. A total 422 number of male fruitfully were collected in the month of October and identified as *C. vesuviana* Costa as compared to 201 in the month of November followed by 89 in December and gradually decreased to Zero population during the months of January and February.

SECTION – IV
PARASITOLOGY

PATHOLOGICAL CHANGES IN THE CECA OF INFECTED CHICKEN (*GALLUS DOMESTICUS*) NATURALLY INFECTED WITH NEMATODE PARASITE (*HETERAKIS GALLINARUM*) FROM HYDERABAD 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 form of eggs as well as meat. In developing countries these are mostly keeping in the free range scavenging system where they eat 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 nematode parasite (*Heterakis gallinarum*) in ceca of chicken. 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. Tissue samples from infected ceca were fixed in Bouin's fluid for 24 hours. Using routine histological techniques, 6-8 micron thick sections were prepared and stained with hematoxylin and eosin. These serial sections were mounted in Canada Balsam. The slides were observed under light microscope. Histopathological findings revealed sever damage of cecal architecture, necrosis of lamina propria, destruction of glands and muscular layer.

SOIL TRANSMITTED HELMINTH INFECTIONS AMONG DIFFERENT AGE GROUPS OF CHILDREN IN KARACHI-PAKISTAN

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Soil-transmitted helminth infection is transmitted through the eggs present in the freely passed faces on the soil. *Ascaris lumbricoides*, a giant roundworm, *Trichuris trichiura* and *Ancylostoma duodenale* are responsible to cause Ascariasis, trichuriasis and hookworm infection respectively in human without the exception of male or female and adult or children. The adult worms inhabit in the intestine of human where they produce thousands of eggs each day. In the area of deficient facilities of sanitation, ova of helminthes contaminated the soil; these eggs are directly polluted the vegetables, fruits, cooked or uncooked foods and water. Eggs are ingested by children to put infected hand in their mouths whereas hookworm 3rd stage larvae penetrate through the skin, frequently entering the body through the hands, feet, arms, or legs or by walking barefooted on the ground, soil or grasses. The present study was carried out in the five districts of Karachi and its suburbs. Total 250 Children of different age groups below two years, between 2-12 years and above 12 years also visiting Laboratories, clinics and hospitals were occupied in the study. Current

Investigation revealed that among 250 children of different age groups just 183 (73.2%) children were found infected with *A. lumbricoides*, *T. trichiura* and Hookworm. Total rate of helminthes infection was 14.8% in the below two years of children whereas 41.6% infection was recorded between 2-12 years and above 12 years children was infected in relation to 16.8%. Overall rate of *A. lumbricoides* infectivity was recorded 29.7% amongst below two years while 58.65% infection was traced between 2-12 years and 40.47% infection was found in above 12 years of children. Entire rate of *T. trichiura* infection was 45.94% in the children below two years while 25% in the children of the age of between 2-12 years along with 26.19% infection within above 12 years of children. Intact rate of Hook worm infection amongst below two years was 24.32% even as between 2-12 years was 16.34% and above 12 years it was 33.33. Comparative study reveals that the *Ascaris* infection was more prevalent than *T. trichiura* and Hook worm infection. The Children age of between 2-12 years was more tainted as compared to below two years and above 12 years children. The current study revealed that the Socio-economic status was not a significant factor except sex and age groups. Comparative parasitic study showed that by the time prevalence of soil transmitted helminthes *A. lumbricoides* infection was predominant. A contribution of health education program should be essential on main concern and implemented to control parasitic infections.

***PSEUDOSTRIGEA* SP. (TREMATODA: STRIGEIDAE) FROM A NEW HOST *ARDEOLA GRAYII* IN DISTRICT HYDERABAD, SINDH, PAKISTAN.**

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Examination of Fifty five *Ardeola grayii* from District Hyderabad, Sindh, Pakistan. Revealed nine trematodes from the small intestine of a four birds. Morphometric studies specify that the trematodes belongs to the genus *Pseudostrigea* sp. Yamaguti 1933 reported first time from the new host *Ardeola grayii* and new locality in District Hyderabad, Sindh, Pakistan. The genus is characterized by having: 1) Prominent pseudo suckers 2) fore body cup shaped, 3) Hind body cylindrical, recurved dorsally, 4) Bursa with terminal opening and muscular ring at base, 5) genital cone well delimited from surrounding parenchyma 6) Vitellaria confined to hind body.

ENTEROPARASITIC INFECTIONS AMONG SCHOOL CHILDREN AND SOME UNIVERSITY STUDENTS IN PESHAWAR

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The present survey was carried out to determine the prevalence of intestinal parasites in school going children and some University students of Peshawar. During the study, 200 faecal samples were examined from December 2010 to July 2011 at Different schools and some

University students of Peshawar city. Stool analysis was performed by direct microscopic examination. Out of 200 subjects examined, 23 (11.5%) were found harboring helminth parasites. *Hymenolepis nana* was more prevalent (8%) in whole survey as compared to other helminths e.g., *Ascaris lumbricoides* (1.5%), *Enterobius vermicularis* (1.5%) and *Trichuris trichiura* (0.5%). Statistically no significant difference ($\chi^2=6.88$, $df=3$, $\alpha=0.05$) was found in the infection rate in different age groups. Among Protozoa the commonest and non-pathogenic protozoan *Entamoeba coli* were more prevalent (66%), than *Entamoeba histolytica* (32%) and *Giardia lamblia* (17.5%). Statistically there was no association ($\chi^2=6.528$, $df=3$, $\alpha=0.05$) of protozoan parasitic infection with age. The frequency of helminth parasite infections is lower in Peshawar city may be due to improved socio-economic status, health education, and comparatively good sanitation system.

NEODIPLOSTOMUM SP. (TREMATODA: DIPLOSTOMOIDEA, POIRIER, 1886) FROM ARDEOLA GRAYII IN DISTRICT HYDERABAD SINDH PAKISTAN

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During investigation of helminth parasitic infection in *Ardeola grayii* in District, Hyderabad, Sindh, Pakistan. The birds autopsied in the laboratory for collection of internal helminth parasites. Fifty five hosts were examined at random intervals, out of which three were found infected with twelve minute trematode specimens were recovered from the small intestine of *Ardeola grayii*. The specimens were mounted permanently according to standard procedures. A detail study was conducted and belonging to genus *Neodiplostomum* sp. Railliet, 1919. The genus is characterized by having: body divided into two portions fore body and hind body, uniform distribution of vitelline follicles, Suckers are prominent and well developed, holdfast organ is well developed. Dissimilar and asymmetrical tandem testes. Ovary pretesticular thin shelled large size of eggs. Prominent bursa copulatrix sub terminal.

EPIDEMIOLOGY OF GASTROINTESTINAL PARASITES OF LAYER POPULATION IN DISTRICT FAISALABAD, PUNJAB, PAKISTAN

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Layer (*Gallus domesticus*) production is an integral part of the poultry industry in Pakistan due to its high protein contents. Among various threats, endo-parasites pose huge economic losses in the form of poor growth, low weight gain and decreased egg production in poultry. In present study, a period prevalence (Mar 2012-Feb 2013) of gastrointestinal (GI) parasites and its associated

risk factors was determined in the layer population of district Faisalabad. To this end, the live and/or dead birds brought to the Central Diagnostic Facility of the Faculty of Veterinary Science, were screened for gastrointestinal parasitic fauna through standard protocols. Relevant information regarding associated risk factors was recorded on a pre-designed questionnaire. Overall prevalence of GI helminthes and protozoa was recorded as 26.05% (260/1996) and 11.32% (113/1996), respectively. The prevalence was highest in laying birds followed in order by brooding birds and growing birds. Commercially raised birds, conventional housing system, manual method of feeding and watering, crumb-fed birds, soiled floor, were found having higher positive statistical association with the GI parasitism. This data will not only be helpful for the poultry farmers to modulate farming practices but also for the policy and decision makers to control the nuisance to ultimately increase productive capacity of the layer population in the district.

RICKETTSIOSIS: A THREAT TO HUMAN AND ANIMAL HEALTH IN PAKISTAN

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Tick-borne rickettsiosis, caused by obligate intracellular bacteria of family Rickettsiaceae, causes a febrile illness in host. Arthropods (*i.e.* ticks, mites, fleas, louse, etc.), once infected, remain reservoirs for their entire life through capability to maintain infection horizontally and vertically; hence, increasing zoonotic significance of rickettsial diseases. Mortality (10-40%) owing to rickettsiosis in humans has been a major concern especially in un-treated cases. Although, actual economic losses are yet to be known but availability of contributory factors of rickettsiosis *i.e.* vector burden, warm climate, available host and spreading factors such as travelers, rodents and transport of infected/infested animals forecast a threat to animal and human health as a result of rickettsiosis in Pakistan. Rickettsia is divided into two biotypes viz; Spotted Fever Group (SFG) and Typhus Group (TG); the former more pathogenic and prevalent. Receptors for Rickettsiae are located on endothelial cells where they multiply and induce toxin-mediated cytopathic effects inside the host (animals or humans); edema, hypotension, local ischemia, and necrosis with disseminated intravascular coagulation. These effects are reported to be ascribed by host immune response to *Rickettsial* activity *i.e.* interferon- γ secreted by T-cells and Nk cells, and TNF secreted by macrophages. Tools such as ELISA, PCR, IFA, RFLP, are encouraging in diagnosis and screening of host and vector populations for rickettsia. In spite of tremendous advancements in the field of rickettsiology and parasitology, rickettsiosis is still prevalent round the globe. Emergence of new species, wide range of vectors/host, and development of resistance remain challenges towards switching off the rickettsiosis. The international scenario of rickettsiology challenges scientists to explore it's all possible areas including; screening of vectors and hosts, species identification, treatment and control of rickettsiosis, as the reported associated factors may perpetuate the infection in animal and human populations of Pakistan. The first step towards screening livestock and vector populations has been initiated in the Department of Parasitology, University of Agriculture, Faisalabad with a financial assistant of USAID under Pakistan-US Science and Technology Cooperation Program, to determine the abundance of rickettsiae in host and vector populations.

INFECTIVITY OF *HYALOMMA* TICK VECTORS FOR *THEILERIA ANNULATA* IN PUNJAB, PAKISTAN

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The tick-borne theileriosis is amongst major vector-borne threats in livestock population of Pakistan. A complex of tick-theileria-host interaction makes it successful to maintain an infection in the environment. The present study was planned with an objective to quantify the abundance of Theileria infective tick vectors parasitizing selected bovine population of Punjab, Pakistan. A total of 900 male and 900 semi-engorged female *H. a. anatolicum* were collected, identified and dissected to procure their salivary glands (SG) for microscopic examination using Methyl Green Pyronin (MGP) and Geimsa stains. Sporozoites of theileria were only observed in type-III acini. The prevalence, level of infection and profusion of *Theileria annulata* infection were higher in female ticks than male ticks. The ticks collected from cattle had a higher prevalence, abundance and intensity (15.15%, 5.38 and 35.53, respectively) of *T. annulata* infection than the ticks collected from buffaloes (9.58%, 1.74 and 18.13, respectively). Hot and dry climate was found highly favorable for the development of *T. annulata* sporozoites in *H. a. anatolicum* ticks as the prevalence, abundance and intensity of *T. annulata* infection were higher in ticks collected from non-riverine areas (tehsil T.T. Singh and Gojra) than riverine one (tehsil Kamalia). This study not only confirms the importance of *H. a. anatolicum* ticks as vectors of *T. annulata* but also alarms the livestock population at risk of theileriosis in the area. Further research on isolation of sporozoites from vectors could be continued which can serve as candidates for immunological studies. Vector screening can be applied on larger scale to determine the areas at risk of bovine tropical theileriosis in Pakistan.

EPIDEMIOLOGY AND CONTROL OF SMALL RUMINANT FASCIOLOSIS IN DISTRICT SARGODHA, PUNJAB, PAKISTAN

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Fasciolosis, caused by *Fasciola (F.) hepatica* and *F. gigantica*, causes a serious threat for livestock in district Sargodha. In this study, we determined a descriptive epidemiology, economic significance of small ruminant fasciolosis (SRF) in animals slaughtered in the abattoirs of district Sargodha, Pakistan. *In vivo* fasciolicidal efficacy of commercially available compounds was also determined using randomized complete block design at Livestock Experimental Station, Khizerabad, Sargodha, Punjab, Pakistan. Microscopically, screened faecal samples (n=390) revealed 158 (40.51%) positive animals for fascioliasis. The prevalent species included: *Fasciola (F.) hepatica* (35.64%) and *F. gigantica* (8.21%). Mixed infection was noted in 13/390 (3.33%) subjects. Prevalence of SRF was significantly higher in females (42.25%) than males (39.52%) ($\chi^2=$

24.31, $P < 0.05$), adults (51.20%) than young ones (33.98%) ($\chi^2 = 17.96$, $P < 0.005$), emaciated animals (63.63%) than fatty (43.75%), average (43.22%) and thin (32.12%) animals ($\chi^2 = 2.64$, $P < 0.005$). Fasciolosis was estimated to cause 0.036 and 0.177 million US dollars direct (liver condemnation) and indirect (carcass depreciation) economic losses, respectively during January to June, 2012. *In vivo* fasciolicidal efficacy of oxyclozanide was found the most promising as compared to triclabendazole and levamisole in decreasing order of efficacy as indicated by the faecal egg count reduction test on day 21st post treatment. The results provided useful statistical figures on the frequency distribution of SRF and its economic significance. Moreover, data of *in vivo* fasciolicidal trial provided the most efficacious compound for the treatment of SRF in the district.

EPIZOOTOLOGY OF TICK-BORNE THEILERIOSIS IN CATTLE POPULATION OF DISTRICT KHANEWAL

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Cattle theileriosis is a protozoan haemoparasitic disease, caused by the member of family *Theileridae* (Apicomplexa; Piroplasmida), whereas *Theileria (T.) annulata* is the causative agent of tropical theileriosis. Ticks of genus *Hyalomma* act as a vector in the parasitic transmission of *T. annulata* from one animal to another. In this study, a cross-sectional survey has been conducted in cattle of district Khanewal in order to determine the epidemiology of cattle theileriosis through conventional optical microscopy of Giemsa-stained blood films. Overall prevalence of theileriosis was calculated as 25.94% (386/1488). Association of cattle theileriosis with different factors has also been studied using pre-designed questionnaire. The prevalence of theileriosis was higher in calves than adults and females than males. Within breeds, Friesian was at highest risk followed in order by cross-bred and Sahiwal. Rainy season was found optimum for the disease prevalence followed in order by summer, autumn, spring and winter. Higher prevalence of theileriosis has been recorded in tethered animals than free moving, closed housing system than semi-close and open housing systems. Poor hygienic animals were more at risk than those having good hygienic measures at farms. Animals kept on un-cemented floor were found more prone to theileriosis than that of animals kept on partially cemented and cemented floor. The data will not only be helpful for the dairy farmers to modulate farming practices but also for the policy and decision makers to control the nuisance in the livestock population of the district.

INCIDENCE OF HAEMATOPINUS TUBERCULATUS (PTHIRAPTERA: ANOPLURA) IN BUFFALO POPULATION DURING WINTER 2012

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Pediculosis, caused by members of order Pthiraptera, is a global nuisance infesting all domesticated animals including buffaloes. Louse infestation causes poor physical condition,

unthriftiness, anemia, anorexia, restlessness, discolored greasy hair, weight loss (up to 25-30 kg), and decreased milk production (15-25% per animal per year). This paper describes probably the first report of *Haemotopinus (H.) tuberculatus* (buffalo-sucking louse) infesting buffalo (*Bos bubalus bubalis*) population of district Faisalabad. A total of 400 buffaloes were randomly selected and examined for pediculosis through naked eye examination in peri-urban areas of district Faisalabad during winter 2012 (November to January). Collection of lice was done using fine forceps in a preservative (10% formalin) for taxonomic identification through conventional procedures using stereoscopic microscope. Of 280 adult lice samples examined, 68 were identified as *H. tuberculatus* (24%). Pathogenic effects, vector role and economic losses attributable to the major dairy crop in Pakistan are also discussed in this report. The ecological and physiological profile of *H. tuberculatus* detailed in this report will help the scientific community to plan control measure against this nuisance. Further research to explore the features of this important member is required on larger scale to determine its epidemics in Pakistan. This manuscript may provide useful tools to the smallholder dairy farmers as first aid control against a highly prevalent louse in buffalo population of the area.

EPIDEMIOLOGY OF TICK INFESTATION IN SHEEP POPULATION OF DISTRICT MUZAFFARGARH, PUNJAB, PAKISTAN

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Sheep (*Ovis aries*), due to its multipurpose rearing, serves as an economic support for smallholder farming community especially in the resource-poor areas of Punjab, Pakistan. Among various threats, ticks (Acari: Ixodidae) pose direct as well as indirect losses to sheep industry. The present study reports a period-prevalence of sheep tickosis and its associated determinants with respect to host (age, species, sex, and breed) and environment (geographical area and climate) in district Muzaffargarh. To this end, a total of 10800 sheep from 4 tehsils (strata) were randomly selected to conduct a questionnaire-based survey. Prevalence of ovine tick infestation was found 41.5% (4482/10800) with a predominant tick species of *Hyalomma anatolicum anatolicum* (23.3%), followed by *Rhipicephalus sanguineus* (18.2%). Breed wise prevalence was observed highest in underscript sheep (67.1%), followed in order by Lohi (45.6%), Thalli (38.6%) and Kajli (27.8%). Internal and external ears (97% and 89% respectively) were the most preferred sites of ticks in sheep. Highest tick prevalence was recorded in the month of July (45.56%) and lowest in December and January (2.89% each). Quantitatively, ewe-lambs were more burdened, although not statistically different. Prevalence of ticks was far less ($P < 0.005$) in settled farms (36.3%) as compared to that of nomadic herds (66.6%). In addition, mixed farming system (53.8%), open housing (46.1%) and uncemented floor (42.6%) favored propagation and settlement of ticks on their sheep-hosts. The results of this study provide a statistical correlation between husbandry practices and quantitative tick burden. In a broader outline, results provide key points following those farmers can achieve optimum productive output through reduced risk of ticks and tick-borne infections in sheep population of district Muzaffargarh.

**AN APPRAISAL OF ELISA FOR THE DIAGNOSIS OF CATTLE FASCIOSIS IN
DISTRICT SARGODHA, PUNJAB, PAKISTAN**

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Bovine fasciolosis has been a serious threat for livestock productivity round the globe. Previous studies indicated that district Sargodha is at risk of highest prevalence of fasciolosis. To this end, 480 cattle were randomly selected for collection of faecal samples and optical microscopic examination for identification of eggs of *Fasciola (F.) hepatica* and/or *F. gigantica*. The overall prevalence of fasciolosis was 39.38% (189/480). The prevalence of *F. gigantica* (27.05%) was higher ($P < 0.05$) as compared to *F. hepatica* (4.61%). Serological diagnosis of anti-*Fasciola* antibodies (Ab) was determined in serum samples of 480 cattle using commercially available kit as per manufacturer's recommended protocol (DRUG DIAGNOSTICA, Marburg, Germany), which revealed a total of 285 (59.37%) samples found positive for anti-*F. gigantica* Ab. This report provides successful appraisal of the commercial ELISA kit and recommends for its use in the national veterinary diagnostic laboratories and research labs in Pakistan. However future research on comparative sensitivity and specificity of commercial kit with that prepared using indigenous strains of *Fasciola* may be conducted for provision of more specific and cost-effective tool for diagnosis of fasciolosis in Pakistan.

**SLAUGHTER-HOUSE BASED EPIDEMIOLOGY OF BOVINE FASCIOSIS IN
DISTRICT SARGODHA, PUNJAB, PAKISTAN**

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Bovine fasciolosis is of serious concern regarding livestock productivity especially in the water-logged areas of Punjab, Pakistan. Among these, district Sargodha is the most favorable for growth and propagation of intermediate hosts (snails) of *Fasciola*. Present study was planned with the objective to determine the descriptive epidemiology of bovine fasciolosis in slaughter house of district Sargodha during a period of one year. Out of 661 cattle and buffaloes screened, 144 were found infected with adult flukes (*Fasciola gigantica*). The distribution of fasciolosis was found higher in buffaloes (24.18%; $\chi^2 = 14.57$) than cattle (18.77%). In buffaloes, 2-4 years age-group was found the most susceptible to fasciolosis (37.03%; $\chi^2 = 19.76$; P -value=0.001), followed in order by 4-6 years (26.67%), >6 years (13.33%) and <2 years (10%). In cattle, prevalence was found highest in age group 2-4 years (29.34%; $\chi^2 = 15.39$; P -value=0.001) followed in order by 4-6 years (19.80%), >6 years (9.09%) and <2 years (6.67%). Sex was not found having positive or negative statistical association with the abundance of bovine fasciolosis, although, numerically, infection was slightly higher in males than females. The results provided useful data on association of risk factors with bovine fascioliasis.

A STORY OF THE BRAIN EATING AMOEBIA

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Amoebae (Protozoa: Sarcodina), the unicellular eukaryotic microorganisms having cosmopolitan distribution, are capable of causing wide range of diseases in animals and humans. Among these *Naegleria (N.) fowleri* is an amphizoic, thermophilic, free-living amoeba isolated from water resources across the globe causing rare but fatal disease in humans commonly known as Primary Amoebic Meningoencephalitis (PAM). Humans get infection during utilization of water infected with *N. fowleri* e.g. swimming, bathing, etc. The amoeba enters through nose and travels through the nasal passages to reach the brain where it settles and grows by eating brain tissues. The clinical signs include: change in taste and smell, rhinitis, malaise, fever, headache, nausea, vomiting, neck rigidity, encephalitis, photophobia, confusion, delirium, seizures, coma and death. The disease is diagnosed through routine examination of cerebrospinal fluid under the microscope for the presence of trophozoite of *N. fowleri* and through the PCR. Prognosis of this fatal disease is not good and no drug of choice is available in the market for the satisfactory treatment except Amphotericin B. The only preventive measure is to handle water with caution e.g. use of chlorinated water should be ensured. Since the first human case reported from Australia in 1965, approximately three hundred cases of PAM have been reported from the entire continent which includes a total of 39 people died of this disease during the last four years in Karachi, Pakistan. The situation alarms the scientists for conducting fruitful research in this area for providing breakthrough solutions for public health in the tropics and sub-tropics.

**ANTHELMINTIC ACTIVITY OF AN INDIGENOUS PRESCRIPTION CONTAINING
WATER EXTRACT OF *TRACHYSpermum AMMI*, *NICOTIANA TABACUM* AND
*ADHATODA VASICA***

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Sheep industry suffers from massive economic losses due to heavy infection with gastrointestinal nematodes and development of anthelmintic resistance has worsened the problem. Medicinal plants, therefore, have been focused as potential candidates for their use as anthelmintics. Objective of this study was to evaluate the anthelmintic activity of an indigenous prescription containing water extract of *Trachyspermum ammi*, *Nicotiana tabacum* and *Adhatoda vasica*. Aqueous extract was prepared by soaking 5 kg seeds of *T. ammi* and 5 kg leaves, each of *A. vasica* and *N. tabacum* (grinded together) in 60 L of water for one month. After that, the plant material was heated at 50 °C till 90% of the total volume was evaporated and material was filtered through four layers of muslin cloth. For evaluation of in vitro anthelmintic activity two bioassays i.e. Adult motility assay (AMA) and Egg hatch assay (EHA) were performed and by using *Hamonchus contortus* as test organism. In vivo anthelmintic activity was evaluated by faecal egg count reduction test (FECRT) in sheep naturally infected with mixed species of helminthes. A good

anthelmintic activity was observed in all the bioassays with a dose dependent response. In egg hatch assay LC₅₀ (95% CI) was calculated as 23.3% (18.4-28.6) while in AMA LC₅₀ after 12 hour of exposure was calculated 6.85% (4.48-9.05%). Likewise, dose dependent response was also observed when sheep were drenched with different level of extract. Maximum reduction (70.93%) in egg count was observed in group treated with single dose of plant extract @ 8 ml/kg body weight. The plants considered in this study and used in ethno-veterinary system of Pakistan have a potential to be used as anthelmintic.

ANTIHYPERLIPIDAEMIC EFFICACY OF *PENNISETUM GLAUCUM* BRAN IN ALBINO RATS

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This study examined antihyperlipidaemic activity of *Pennisetum glaucum*, bran in albino rats. Healthy adult albino rats (250) of either sex were randomly divided into six groups each comprised of 45-50 rats. *Pennisetum glaucum* seed bran was fed to hyperlipidaemic rats at dose rate of 2, 4 and 6g/Kg body weight to treated groups for 15-60 days. Simvastatin was given at 0.6mg/Kg. Blood samples were taken at 0, 15, 30, 45 and 60 days. Cholic acid, cholesterol and other lipid profile parameters were analyzed. *Pennisetum glaucum* bran at 6 g/Kg was able to produce significant (P<0.05) increase in HDL- cholesterol and fall in other lipid profile parameters on post treatment days 30, 45 and 60. On post treatment day 60, *P. glaucum* bran at dose rate of 6g/ Kg also reduced total cholesterol in liver and increased faecal bile acid secretion. The results of present study suggest that *P. glaucum* bran at dose rate 6g/Kg and Simvastatin, 0.6mg/Kg body weight, were equally effective in treating hyperlipidaemia in albino rats. Moreover, the potency of *P. glaucum* for stimulating faecal bile acid secretion in rats may safely be conceived, at least, as a part of mechanisms for its antihyperlipidaemic efficacy.

REDESCRIPTION OF *THE LUTZTREMA (LUTZIELLA) MICROACETABULARAE* ROHDE, 1966 TREMATODE OF THE FAMILY DICROCOELIDAE (LOOSS, 1899)

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A Known species *Lutztrema (Lutziella) microacetabularae* Rhode, 1966 is being described for the first time from Pakistan. This species is characterized by having body long and slender, oral sucker subterminal, Acetabulum smaller than oral sucker lying in anterior third of the body. Pharynx small, esophagus prominent which become gradually wider and bifurcates in to two rudimentary caeca. Testes symmetrical at the level of posterior margin of acetabulum separated by

uterine coils., Cirrus pouch median, pre-acetabular, genital opening some distance behind pharynx. Receptaculum seminis behind ovary. Ovary submedian, post-testicular, Laurer's canal present. Vitellaria lateral from the level of testes to a short distance behind middle of the body. Uterus occupies most of the hind body, eggs small, oval, numerous. It is being reported from the rat (*Rattus rattus* L.) from Swat.

**A NEW TREMATODE OF THE FAMILY DICROCOELIDAE (LOOSS, 1899) ODHNER,
1910 IN THE GALL BLADDER OF RAT (*RATTUS RATTUS* L.) FROM SWAT,
PAKISTAN**

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A new species of the genus *Lutziella* (Rohde, 1966) Yamaguti, 1971 is described here and named *Lutziella swatensis* n.sp. from the gall bladder of rat (*Rattus rattus* L.). The new species is characterized by having body flat, smooth, longer than broad with maximum width at the level of the ovary, oral sucker with weak musculature; pharynx small; esophagus long; caeca of irregular shape which bifurcate on the anterior border of the ovary in to unequal portions; acetabulum weakly muscular, post-testicular; testes lobed; cirrus pouch somewhat median, small in size containing winding seminal vesicle, prostatic complex and short ejaculatory duct. Genital pore median. Ovary post testicular, submedian; seminal receptacle overlapping ovary; laurer's canal present. Vitellaria follicular extending on each side from almost the level of testes to anterior portion of posterior half of the body. Uterus filled with eggs occupying most of the body; eggs oval rather small, numerous, brownish in colour; excretory vesicle tubular with terminal pore.

**GENERIC CHARACTERIZATION OF SYPHACIA SPECIES OF THE RAT (*RATTUS RATTUS*) AND MICE (*MUS MUSCULUS*) OF AGRICULTURAL IMPORTANCE FROM
SWAT, K.P.K, PAKISTAN**

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In order to determine the prevalence and intensity of helminth parasitic infections in rodents of agricultural importance the present study was taken out. A total of 269 rats (*Rattus rattus*) and 81 mice (*Mus musculus*) were trapped. These rodents were anaesthetized and dissected. The helminth parasites of the class Nematoda, Cestoda and trematoda were identified. Amongst nematodes *Syphacia* species was identified to generic level and characterized by having: small

sized worms, mouth bounded with three lips, no definite buccal capsule, esophagus with posterior bulbar enlargement and intestine without diverticulum. Esophagus with a small pharynx by having posterior bulb containing three Valvar apparatus, female tail short subulate, eggs elliptical, large and asymmetrical places these parasites in the family Oxyuridae Cobbold, 1864. Possession of cervical alae, mouth with three distinctive lips, absence of buccal capsule, esophagus with pharynx, and with a distinctive posterior bulb containing a valvar apparatus, female with short but subulate tail, uterine branches but opposed not extending as far as anus, eggs asymmetrical places them in genus *Syphacia* Seurat, 1916. Male of this species has not been reported therefore cannot be finalized as to the species level.

TAXANOMIC CHARACTERIZATION OF ASPICULURIS SPECIES IN THE RATS (*RATTUS RATTUS*) AND MICE (*MUS MUSCULUS*) OF SWAT, K.P.K, PAKISTAN

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During the investigation of rodent born helminth parasitic infections in Swat, K.P.K, Pakistan a total of 269 rats (*Rattus rattus*) and 81 mice (*Mus musculus*) were trapped in different agricultural fields. These rodents were anaesthetized and dissected. Nematodes, Cestodes and trematodes were isolated. Amongst nematodes *Aspiculuris* species was identified to generic level and characterized by having: cuticle transversely striated, Broad cervical alae abruptly terminating at the level of esophageal bulb while narrow lateral flanges run to the posterior extremity. Esophagus some what club-shaped with well developed bulb containing Valvar apparatus. Male tail conical has one pair of pre anal papillae and several pairs of post anal papillae. Female tail subulate and Vualva in the middle of the body the present species is taken in to the genus *Aspiculuris* Schulz 1924.

NEW CAMALLANID NEMATODE SPECIES PARASITIZING FRESHWATER FISH OF PANJGUR (BALOCHISTAN) PAKISTAN

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During studies of fish helminthes, a new species of camalianid nematode belonging to the subfamily Camallaninae Yeh, 1960b is described from the swim bladder of fish, *Channa orientalis* Bloch and Schneider, 180 I caught off the Gwarko River at Panjgur (Balochistan). The present new species *Camallanus boomkeri* n. sp. differs distinctly from other congeneric species parasitizing freshwater fish hosts mainly in the number and structure of the buccal capsule ridges and in the caudal end of both sexes. In addition, the present species could be distinguished from most of its congeners by the 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 the shape of eggs and tail tips.

**RHABDOCHONA HASPANI NEW SPECIES (THELAZIOIDEA: RHABDOCHONIDAE)
FROM STREAM FISH (ACTINOPTERYGII KLEIN, 1885) OTSIBI DIVISION, PAKISTAN**

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A new parasitic nematode *Rhabdochona (Rhabdochona) haspani* n. sp. is described on the basis of the specimens recovered from the intestine of *Cyprinion watsoni* (Day, 1872) collected from freshwater bodies of Sibi Division, Balochistan province, Pakistan. The new specimens are clearly different from all the previously reported species of *Rhabdochona* Railliet, 1916 by the relevant length of esophagus, shape and sizes of spicules (0.24-0.34 and 0.071-0.076), distance of nerve ring, deirids and excretory pore from the anterior extremity and, in the females by measurements of eggs (0.013-0.035), morphology of vagina, position of vulva and excretory pore. The new materials are also characterized by the presence of 8 anterior prostomal teeth and by having 13 pairs including 8 preanal and 5 postanal caudal papillae in males. This nematode is the first of the genus to be reported from Sibi, Balochistan.

**APPRAISAL OF MERCURY AND NICKEL CONTAGION IN CATTLE MEAT AROUND
FAISALABAD, PAKISTAN**

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Land applications of sewage mire and sewage water gradually increase the toxic concentration of heavy metals in soil and these are increasingly up taken by the plants, vegetables and afterwards relocate into the food chain causing severe damage to both animals and human health. Mercury and nickel contamination in meat and other edible tissues is a matter of great concern for food safety and human health. These metals are toxic in nature and even at relatively low concentrations can cause adverse effects. In present study, mercury and nickel residual concentrations in meat have been determined in cattle in two areas, each consisted of three sites, along the main sewerage drains of Faisalabad city. Meat samples included those of arms, ribs and thigh. Meat samples, 20 gms each, were collected in small polythene bags. Prior to meat collection, the fat was removed. The samples were kept frozen at -4 °C until analysis. It was found that levels of mercury and nickel (mg/Kg) in arms (0.117±0.003) (2.373±0.034), rib (0.086±0.003) (2.335±0.030) and thigh (0.131±0.002) (2.370±0.033), of cattle was much higher as compared with the maximum residual limits (MRLs) available in the literature. While there is no significant difference found in the uptake of mercury and nickel during winter and summer season. Drastic measures are suggested to detoxicate and treat both the industrial and domestic effluents which are intended to be used for agricultural purpose.

NEMATODES ASSOCIATED WITH APPLE SEEDLINGS IN BALOCHISTAN

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A survey of nematode fauna associated with apple seedlings was studied in detail. Apple seedlings with symptoms of root-knot disease were smoothly uprooted from pots stored at 15°C in the laboratory for nematode isolation. The roots were dissected to remove adult females. The perineal pattern were made to identify the species under stereoscopic microscope *Meloidogyne incognita* was the nematode encountered in most soil samples collected from the six nurseries. Matrix of Bray Curtis similarity of nematodes in six nurseries of apple was used. The project is being funded by Pakistan Science Foundation (PSFINSLP/S-PARC) 187.

ASSOCIATED RISK FACTORS AND MANAGEMENT PRACTICES INFLUENCE ON BABESIA PREVALENCE 'FU CATTLES OF DISTRICT TOBA TEK SINGH, PUNJAB, PAKISTAN

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The present study was conducted to assess the impact of associated risk factors and management practices on Babesia prevalence in cattles of district Toba Tek Singh, during 2009-2010. A total 68 (14.11%; CI=11.21-17.43) out of 482 cattles were recorded positive for Babesia infection. Babesia was recorded more prevalent during July-2009 (25.64 %), whereas during December-2009 and January-2010 none of the case was recorded. The breed, age and gender of cattles represented significant differences ($P<0.05$) for Babesia infection, whereas body condition of cattles were found non significant difference for associated risk factors. Among management practices, feeding, housing system, floor type and herd size were found significant differences ($P<0.05$) for Babesia prevalence, while watering system found non-significant.

PREVALENCE OF PROTOZOAN PARASITE, TOXOPLASMA GONDII (NICOLLE AND MANCEAUX) IN SHEEP, OVIS ARIES L. AT DIR LOWER, MALAKAND DIVISION, PAKISTANTARIQ MEHMOOD² AND FARZANA PERVEEN^{1*}¹*Department of Zoology, Shaheed Benazir Bhutto University (SBBU), Main Campus, Sheringal, Khyber Pakhtunkhwa, Pakistan;*²*Hazara University, Garden Campus, Mansehra, Khyber Pakhtunkhwa, Pakistan**E-mail: farzana_san@hotmail.com

Sheep, *Ovis aries* L. are important in the epidemiology of protozoan parasite, *Toxoplasma gondii* (Nicolle and Manceaux) infection. Toxoplasmosis is a worldwide distributed zoonosis with clinical impact in the inborn fetus and in the immune suppressed individual. The present study was conducted to investigate the prevalence of *T gondii* in sheep at Dir Lower, Malakand division by

using latex agglutination test (LAT). A total of 1000 *O. aries* of both sexes were randomly selected and blood samples were collected. The overall prevalence ratio recorded was 10%. *Toxoplasma gondii* was more prevalent (28.57%) in male *O. aries* than female (8.6%). In zonewise distribution, toxoplasmosis in descending order was: Maidan (13.63%) > Munda (12.5%) > Ouch (11.76%) > Talash (9.09%) > Timergara (6.45%). In healthy *O. aries* the occurrence of toxoplasmosis was 9.21% while in infected it was 12.5%. Toxoplasmosis had the highest prevalence (33.33%) in the age group of 43-48 months. Results regarding the relationship between physical status and *T. gondii* showed that in physically normal *O. aries* the toxoplasmosis was 8.75% while in weak, lethargic and lean *O. aries* the occurrence was 15%. It can be concluded that good management practices, education and awareness of the sheep keepers is of prime importance in reducing *T. gondii* infestation in *O. aries*.

EFFECTS OF INDUCERS OF SYSTEMIC ACQUIRED RESISTANCE ON REPRODUCTION OF ROOT KNOT NEMATODES IN TOMATO

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The potency of the inducers as a systemic acquired resistance (SAR), for reduction of *Meloidogyne* spp. was investigated in tomato. The chemicals *i.e.* Salicylic acid (SA), Jasmonic acid (JA) and Benzoic acid (BA) were used. Moneymaker seedlings were drenched for six hours in these inducers with three rates from stock solutions (5.0, 7.0 and 10.0 mg/ml⁻¹) to evaluate the effect of different levels of these treatments for both pot and field trials. A field experiment was conducted at the Experimental Research Area, Department of Plant Pathology, University of Agriculture Faisalabad in a randomized complete block design with split plot arrangement in infested field plot size of 125 m² during 2011-12. Two days after transplantation, 5,000 eggs of root knot nematodes were inoculated on pot plants and field was naturally infested. Nematode reproduction was assessed after four weeks. Data on yields was recorded on maturity and nematodes development parameters like number of females, number of galls per gram, number of nematodes/100 ml of soil and egg masses was recorded after harvest. All inducers showed the considerable reduction of nematode population but Jasmonic acid caused more reduction as compared to Salicylic acid and Benzoic acid at 10.0 mg/ml⁻¹. Treated plants exhibited 30–35% reduction of RKN population as compared to untreated plants. The results of this study proved that inducers of systemic acquired resistance would be helpful in the management of root knot nematodes under field conditions.

BIOACTIVITY OF ENTOMOPATHOGENIC NEMATODES FOR PLANT PROTECTION

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To provide enough food to the people, major challenge is to protect plants from insects causing major damage. Environment concerns about chemical insecticides serve as a strong

impetus for the development of biological control agents or biopesticides. However, it is a global desire to reduce their use and to have a non polluted and healthier environment. It is recognized that the use of these highly toxic insecticides is one of the major sources to the environmental and food pollution. These nematodes and their symbiotic bacteria have been demonstrated to be non toxic to vertebrates. Successful control has been made with Pakistani isolates of entomopathogenic nematodes. Successful application depends on a variety of factor. To developing and improving bio control programs for plant protection we have to adopt strain selection, strain improvement, enhance nematode production and application and incorporation of basic research. Entomopathogenic nematodes have unique host seeking and reproductive strategies and have anatomical characteristics of biodiversity that encourage consideration of their application in plant protection.

IN-VITRO ANTHELMINTIC INVESTIGATION OF FRUITS OF SOME ETHNOBOTANICALLY IMPORTANT TREES OF PUNJAB, PAKISTAN

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The present study is the investigation of an ethnopharmacological effect *i.e.* anthelmintic activity of fruits of three important traditionally used trees, *i.e.* *Pterospermum acerifolium* Linn. *Diospyros malabarica* Kostel. and *Putranjiva roxburghii* Wall. The crude extracts of fruits, were obtained in polar and non-polar solvents *i.e.*, petroleum ether, chloroform, methanol and water, and evaluated for their *in vitro* anthelmintic activity and compared with standard medicine, *i.e.* Levamisole (positive control). The criterion was the apparent mortality of a worm *Haemonchus contortus* on the visual basis. Change in the color of the dead worms was an indicator parameter of the fact that the extracts might have damaged the skin of the worms or after transcutaneous penetration into the body has disrupted the circulatory system by causing constriction of blood vessels. The aqueous and chloroform fruit of *P.acerifolium* and petroleum ether extract of *D.malabarica* showed maximum wormicidal potency (2:00hrs) against *H. contortus*, while other extracts presented varied degree of potency (3:00 to 4:00 hrs), almost comparable to the standard medicine, *i.e.* Levamisole that had the potency of 4:00hrs.

ASSESSMENT OF GASTROINTESTINAL PARASITES IN PIT LATRINE SAMPLES IN OSHKANDASVALLEY, GILGIT-BALTISTAN, PAKISTAN

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Intestinal parasitic infections represent a global public health problem. Especially in developing countries, numerous environmental and socioeconomic factors have been identified to

be main causes for the continued persistence of gastrointestinal infections. The present study was carried out to assess the intestinal parasitic load (*Ascaris lumbricoid*, *Tricuris tricuris*, *Giardia lamblia* and *cryptosporidium*) in the rural village of Oshikhandass, Gilgit-Baltistan, in pit latrine samples. The samples collected were analyzed in monthly intervals (October 2011 to February 2012) by NaCl₂ saturated solution flotation technique. Analysis revealed that the month-wise load of parasites ranged as: October *Ascaris lumbricoid* (16.67-33.34), *Tricuris tricuris* (2.66-4.00), *Giardia lamblia* (3.33-16.66) and *Cryptosporidium* (6.66-20.66); November (16.667-40.000), (8.6667-16.6667), (0-10.000) and (9.3333-22.333); December (16.00-26.33), (0-4.66), (6.66-16.66) and (6.66-16.66); January (16.66-50.00), (5.00-6.66), (1.66-8.33) and (6.66-10.00) & February (25.00-56.66), (6.66-23.33), (3.33-16.66) & (6.66-20.00) respectively in one gram of sample. This study showed *Ascaris lumbricoid* to have the highest load among the targeted parasites.

NEW HOST RECORDS FOR TWO TREMATODES OF FRESH WATER FISHES OF SINDH

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A new host and locality is recorded for the metacercarial from *Euclinostomum* gastrocaecum Bilqeess, 1972 and is reported here. Nineteen metacercariae were collected from the liver of fish *Channa (O) striatus* of Manchar lake, Sindh which is a new host and locality record. Originally this form was described from a related fish species *Channa (O) marulius* of Haleji lake of Sindh. Morphometric variations were observed in the number of lateral branches of caeca and sizes of various structures. Similarly, Nine specimens of *Phyllodistomum ritai* (Khan, 1985 & Bilqeess, 1990) were collected during the present studies from the Urinary bladder of the fish *Heteropneustes fossilis* of Haleji lake, Sindh. This trematode is originally known from the fish *Rita rita* of Kalri lake. The present fish is a new host record. Morphological variations were also observed.

STUDY OF PARASITIC FAUNA IN SOME TROPICAL FRESHWATER ORNAMENTAL FISH IMPORTED TO PUNJAB, PAKISTAN

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This study was designed to investigate the parasitic infection in some freshwater tropical ornamental fish imported to Punjab. A total of 178 freshwater ornamental fish were examined. *Carassius auratus* (L.) (n=60), *Poecilia reticulata* (Peters) (n=70) *Poecilia sphenops* (Valemiemmes) (n=38), *Xiphophorus maculatus* (Gunther) (n=50) and *Xiphophorus helleri* (Heckel) (n=38) were analyzed. *C. auratus* 75% were parasitized, 48% with monogenoidea (trematoda), 24% with *Trichodina nigra* (Ciliophora), 15% with *Ichthyophthirius multifiliis*

(Ciliophora), 11% with *Argulus foliaceus* (Copepods) and 2% with *Lernea cyprinacea*, (Copepods). 20% *P. reticulata* were infected, 42.8% with Monogeneoidea, 33.2% with metacercariae of *Cryptocotyle* spp. 20% with *T. nigra* and 4% with *Chlidonella* sp. 34 % *P. sphenops* were parasitized, 55.5% with monogenean, 39.8% with metacercaria of *Cryptocotyle* spp, 4.7% with *T. nigra*. 40% *X. maculatus* were parasitized, 50% with metacercaria of *Cryptocotyle* spp, 39.6% with monogenean, 6.1% with *T. nigra*, and 4.3% with *Epistylis* spp. 39% *X. helleri* were parasitized, 42% with monogenean, 39.8% with metacercariae of *Cryptocotyle* spp, 8.9% with *T. nigra*, 6.1% with *Tetrahymena* spp and 3.2% with *Piscinoodinium pillulare* (Dinoflagellida). Whitish to yellowish cysts in the skin, loss of mucus, slight haemorrhage at the base of dorsal, pectoral and caudal fin were observed in heavily infected fish but the clinical signs of the infected gills were, lamella base of the gill damaged, destruction of secondary gill lamella, hyperplasia, displacement of secondary lamella and shorting of the gill lamella observed because of the parasitic load on the gills. Monogenean parasites load on *C. auratus* was significantly ($p < 0.04$) high but intensity of metacercariae of *Cryptocotyle* spp was significantly highest ($P < 0.04$) in *X. maculatus*.

BENEFICIAL NEMATODES USED AS BIOSAFE-AGENT FOR PLANT HEALTH

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Beneficial nematodes (entomopathogenic nematodes) have great potential for the biological control of many important pests and have been successfully used in agricultural systems. Agriculture is oldest profession of mankind and mainstay of Pakistan's economy. It supports directly and indirectly 70% of population for their sustenance and contributes 80% of foreign exchange earnings. Among the insect pests red palm weevil of dates, fruit fly of mango and vegetables and armyworm of cotton are the major pests throughout the country where these fruit trees and crops are grown. These nematodes offer an alternative to chemical insecticides for a number of insect pests. They possess many qualities and have broad host range, can be easily mass produced, possess the ability to seek out their host, kill their host rapidly and are safe for plant, human and environment. Pathogenicity and efficacy trials of indigenous EPN isolates have also been done successfully with positive and promising results. EPNs cultured in *Galleria mellonella* L., stored in distilled water at 5°C and were kept at room temperature for 24 hours before use. EPN spray @ 1000 juveniles/100 ml water were assessed for mortality percentage of insects. There are a number of insect pests viz., American bollworm, spotted bollworm, pink bollworm, lemon butterfly, brinjal fruit borer, pod borer, white grub, red palm weevil, mango mealy bug, pulse beetle, sugarcane shoot borer, sugarcane top borer, rice stem borer, lesser army worm, rice weevil, red flour beetle, cigarette beetle, subterranean termite, which are causing serious damage to our cash crops like cotton, sugarcane, fruits and vegetables, resulting in great economical losses to the agricultural production. The insect pests of different crops were found susceptible to infective juveniles of *S. pakistanense*, *S. asiaticum* and *H. indica* EPN species. The intensity of infestation varied with the species and life stage of the insects. There is a dire need to focus further research on these EPN isolates to explore and exploit their potential as an alternative to pesticides in Pakistan, especially in IPM programmes.

DESCRIPTION OF NEW ACANTHOCEPHALAN *CETTORHYNCHUS SONNERATII* N.SP. (ACANTHOCEPHALA: CENTRORHYNCHIDAE) FROM GREY JUNGLEFOWL, *GALLUS SONNERATII* (AVES: PHASIANIDAE) IN SINDH PROVINCE OF PAKISTAN.

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During helminthological research on birds in Khairpur District of Sindh province of Pakistan, a total of seven Grey Junglefowl, *Gallus sonneratii* were captured from different localities of Khairpur. Birds were anesthetized and dissected at Department of Zoology, Shah Abdul Latif University, Khairpur, Sindh, Pakistan. During examination of gut contents and visceral organs, a total of 76 specimens belonging to genus *Cetrorhynchus* Lühe, 1911 were collected from large intestine of hosts. Present species have aspinose, cylindrical, pseudosegmented body. Proboscis globular, armed with 24 longitudinal rows of 9 hooks both in male and female. Proboscis receptacle cylindrical. Neck very short. Lemnisci longer than proboscis receptacle. Testes tandem, oval in shape, situated in anterior fourth of body. Cement glands long, cylindrical, not well defined from their ducts. Genital pore terminal. Eggs oval to elliptical with polar prolongations; middle shell of eggs without polar prolongation. Present species differs from its close allies in body shape, number of longitudinal rows of hooks, number of hooks in each row, size of hooks and measurement of different organs. Grey Junglefowl, *Gallus sonneratii* is new host record for the genus *Cetrorhynchus* Lühe, 1911. New species is named after species name of the host.

MOLECULAR PHYLOGENY OF ENTOMOPATHOGENIC NEMATODES (*STEINERNEMA* AND *HETERORHABDITIS*) AND THEIR SYMBIOTIC BACTERIA (*XENORHABDUS* AND *PHOTORHABDUS*)

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Entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae, with their symbionts bacteria of the genera *Xenorhabdus* and *Photorhabdus* respectively, are able to infect and kill many insect species. Based on the sequences of the ITS, D2-D3 rDNA regions and 12SmtDNA gene, most of the isolates form monophyletic group with *bicornutum-ceratophorum-riobrave* and *carpocapsae-scapterisci-tami* groups. Phylogenetic analyses of the 16S rRNA gene from symbiotic bacterial isolate of entomopathogenic nematode (EPNs) from Pakistani strains were also carried out. Corresponding nucleotide sequences of other representatives of *Steinernema* available in GenBank were aligned using Clustal X1.83. The phylogenetic relationship of *Steinernema*, *Heterorhabditis* and their symbiotic bacteria were reconstructed based on ITS rDNA using maximum parsimony (MP) and neighbour-joining (NJ) of sequences data were done using PAUP* v 4.0b10. *Caenorhabditis elegans* (EU131007) was applied as outgroup during calculation of the trees based on ITS and 12S mtDNA sequences; *Cervidellus alutus* (AF331911), together with *Panagrellus redivivus* (AF331910), were used as outgroups for calculation of the trees based on D2-D3 sequences.

AN OUTLOOK OF NEMATOLOGY IN PAKISTAN

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Nematodes are the most numerous multicellular animals on earth. A handful of soil will contain thousands of the microscopic worms, many of them parasites of insects, plants or animals. Free-living species are abundant, including nematodes that feed on bacteria, fungi, and other nematodes, yet the vast majority of species encountered are poorly understood biologically. Nematodes are the most abundant and diversified group in the animal kingdom. Recently significant progress has been made in Pakistan about genomics, molecular biology biochemistry and commercialization of nematodes. This international global research effort has significantly contributed to the basic research and commercialization of these groups of nematodes. The systematic studies carried out so far on plant parasitic, free-living marine, soil nematodes and insect parasitic nematodes resulted in a total of 754 species having 222 new species and 531 new records. Nematode fauna consists of 117 new species and 282 new records of plant parasitic nematodes; 56 new and 151 new records of free-living soil nematodes; 47 new and 91 new records of free-living marine nematodes and 2 new and 7 new records of entomopathogenic nematodes (EPN's). The total nematode fauna comprised of 239 genera, 62 subfamilies, 83 families, 21 super families, 18 suborder, 11 orders and 2 classes.

HOST STATUS OF CHILI AND BELL CULTIVARS AGAINST *MELOIDOGYNE INCOGNITA*

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Chili and bell peppers (*Capsicum frutescens*) cultivars were evaluated for resistance to *Meloidogyne incognita* in a completely randomized design in a greenhouse trial. There were fourteen chili cv. Gola Peshawari, C-19, 15-2010, 11-2010, C-68, Sanam, 27-2010, Tata Puri, C-302, C-73, C-72, 28-2010, 5-2010 and 18-2010 and five pepper cv. F1 (pahuja seeds), Yolo wonder, orible, CKD and Capastreniou. Each plant was inoculated with freshly hatched 5000 eggs of *M. incognita* and its response was evaluated 60-d post inoculation. Host suitability was categorized on the basis of reproduction factor [(Pf/Pi) where Pf =final nematode population/Pi=initial nematode population], root galling severity 0 to 9 scale, root galling and egg masses indices on a 0 to 5 scale, J2 per g of root and eggs per root system. The evaluation revealed C-19 chili genotype as non-host / resistance with zero, reproduction factor, root galling and egg mass indices, and root gall severity. Eight genotypes Sanam, Gola Peshawari, 15-2010, 11-2010, C-68, Tata Puri, C-302 and 28-2010 were categorized as good hosts / susceptible and all the other five including 27-2010, C-73, C-72, 5-2010 and 18-2010 as fair host /moderately resistant ($5.0 \geq Pf/Pi > 1$). Two bell pepper genotypes Orible and F1 (pahuja seeds) were listed as poor host ($1 > Pf/Pi > 0$). Genotype Yolo Wonder and Capistano graded as fair host /moderately resistant] ($5.0 \geq Pf/Pi > 1$) and genotype CDK-1000 as good hosts/susceptible ($Pf/Pi > 5.0$).

A COMPARATIVE STUDY OF PREVALENCE OF ENDOPARASITES IN THE UNGULATE SPECIES IN CAPTIVITY OF LAHORE ZOO AND JALLO PARK

K. EJAZ, AND B.N. KHAN, G. JOSHUA, S.B.SHAMS

The present study was carried out to assess the prevalence of gastrointestinal helminthic infections by comparing the ungulate species of Lahore Zoo and Jallo Park. For this purpose a total 576 fecal samples of animals housed in Lahore Zoo and Jallo Park were collected, and analysed for a period of twelve months from November 2011 to October 2012 including urial (n=9), nil gai (10), mouflon sheep (n=47) from both the areas. Faecal floatation and sedimentation testing techniques revealed that 85.06% of the total faecal samples were positive for endoparasites. Six species were identified from this study which was *Dipylidiumcanium*, *Monieziaabendeni*, *Haemonchuscontortus*, *Ostertagiaostertagi*, *Oesophogostomum columbianum*, and *Dictyocaulus viviparous*. Mc master's technique showed that the highest egg count was determined in urial of Jallo Park and the lowest egg count was in blue bull of Lahore Zoo. It was concluded that nematodes remained high in sheep species *i.e.* urial and mouflon sheep of both the areas. However, in blue bull, prevalence of cestode is higher than nematodes. Animals of Jallo Park showed the maximum prevalence of endoparasites (94.4%) as compared to the animals of Lahore Zoo (75.6%). Parasitic infection was highest during the summer season and lowest during the winter months.

INTERACTION BETWEEN NEMATODE INOCULUM DENSITIES AND PLANT AGES ON GROWTH AND YIELD OF CUCUMBER AND REPRODUCTION OF MELOIDOGYNE INCOGNITA

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Among the most damaging root-knot nematode species, *Meloidogyne incognita* is one of the major constraints to cucumber (*Cucumis sativus* L.) in the vegetable-growing areas in Pakistan and other countries of the world. In the present studies the relationships between a geometric series of six initial population densities of *M. incognita* in the soil and growth and yield parameters and nematode infestations were investigated on cucumber. Cucumber plants of 2-, 3- and 4-week ages were inoculated with different nematode densities and observations were recorded 9 weeks after inoculation. Reductions in growth and yield parameters by nematode densities were calculated over controls. All inoculum densities and ages of plants at the time of inoculation influenced growth and yield of cucumber. It was observed that all inoculum levels caused significant reductions in these parameters and were found to be negatively correlated with the latter. On the other hand, ages of plants at inoculation had positive correlations with these parameters at each inoculum level. The inoculum levels of *M. incognita*, plant ages and their interactions also had significant effects on number of galls, egg masses and rate of nematode build up. The production of galls and egg masses was found to be positively correlated with the inoculum levels and plant ages. However, rate of nematode build up decreased with an increase in the inoculum density and appeared to be negatively correlated with inoculum densities and, on the contrary, was found to be positively correlated with plant ages. The results demonstrated that *M. incognita* has the potential to severely

impair the growth of cucumber and by delaying early exposure of the latter to nematodes can significantly abate yield losses.

A COMPARATIVE STUDY OF ENDOPARASITES OF DEER SPECIES IN CAPTIVITY OF LAHORE ZOO AND JALLO PARK.

R. KAMRAN, G. JOSHUA AND B.N. KHAN, S.B.SHAMS

The present study was carried out to assess the prevalence of gastrointestinal helminthic infections by comparing the deer species of Lahore Zoo and Jallo Park. For this purpose 392 fecal samples were collected from November 2011 to May 2012 including chinkara (n=16), hog deer (65), spotted deer (n=44) and fallow deer (n=8) from both the areas. Faecal floatation and sedimentation techniques revealed that 95.66% of the total faecal samples were positive for endoparasites. Six species were identified from this study which was *Dipylidiumcanium*, *Monieziaabendeni*, *Haemonchuscontortus*, *Ostertagiaostertagi*, *Strongyloidessterocoralis* and *Ascarislumbricoides*. Mc master's technique showed that the highest egg count was determined in spotted deer of Jallo Park and the lowest egg count was in fallow deer of Lahore Zoo. It was concluded that nematodes remained high in deer species of both the areas. However, the animals of Jallo Park showed the maximum prevalence of nematodes as compared to the animals of Lahore Zoo.

CHARACTERIZATION OF OKRA GERMLASM FOR RESISTANCE TO 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 and cause a lot of hazards to both man and livestock and inflict injury to the environment. Notable among the alternatives is the use of resistant cultivars 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 test cultivars were inoculated with 3000 freshly hatched second stage juveniles of *M. incognita*. The nematode caused reductions in various growth parameters of all the cultivars to varying levels over their respective controls. None of the cultivars was found immune, highly resistant or resistant. The cultivar 'Punjab Selection' was found to be highly susceptible as maximum galls (> 100) were recorded on the roots of this cultivar. The cultivar also showed maximum reductions in growth parameters. The cultivars Selection-31 and Okra Sindha were found to be susceptible (71-100 galls). In the same way, the cultivars Sabz Pari, Super Star, PMS-

55 and PMS Beauty appeared as moderately susceptible (31-70 galls) and reductions in growth parameters of these cultivars were comparatively less severe as compared to those observed in the case of susceptible and highly susceptible cultivars. Five cultivars viz. Sanam, Dikshah, Arka Anamika, Ikra-1 and Ikra-2 (11-30 galls) were rated as moderately resistant as these cultivars showed less damage by the nematode as compared to susceptible and moderately and highly susceptible cultivars and their use could provide a useful tool to control root-knot nematodes.

DESCRIPTION OF SPINY HEADED WORMS (ACANTHOCEPHALAN) FROM FRESH WATER FISHES OF SINDH, PAKISTAN

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Nine male worms collected from the intestine of freshwater fish *mastacembelus armatus* from Haleji Lake, Sindh, Pakistan are being described. The present species, when compared with the previously reported species, differs in several morphological characters such as body size, size of proboscis hooks, shape of the body, size of bursa and presence of two bursal glands. On the basis of the above morphological variations as well as morphometric difference it is concluded that the worm is new to science and therefore designated as *Neoechinorhynchus macrorchis* n.s.p. The species name refers large testes located in the posterior body region. Nine acanthocephalan specimens were recovered from the intestine of *Catla catla* which when compared with literature indicated a new acanthocephalan species, *Neoechinorhynchus brayi*. The new species is characterized by having medium sized stout body; lacunar system prominent; proboscis small with three circles of hooks, six in each circle, anterior hooks larger, posterior small; proboscis receptacle small; lemnisci unequal; male genitalia situated near the posterior end of the body and the bursa with subterminal pore and two bursal glands. Species name is in honour of renowned parasitologist from British Museum (NH) London.

DIPLOTRIAENA SP. FROM COMMON MYNA (*ACRIDOTHERES TRISTIS*) IN HYDERABAD DISTRICT, SINDH PAKISTAN

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Five birds, common Myna (*Acridotheres tristis*) were purchased from birds market, Hyderabad. The birds were examined in the parasitological laboratory for collection of helminth parasites. Four out of five mynas were found infected with nematodes, the nematodes were found in a bunch form in the body cavity. The nematodes were studied alive under a binocular, later these

were fixed in hot 70% alcohol and stored in a mixture of glycerine and alcohol 70%. A detailed study was conducted. The specimens were identified as belonging to the genus *Diplotrinaena* Railliet et Henry 1909. 45 males and 40 female specimens were collected and studied in detail: Male: Long and slender worms. Cuticle slightly striated. Anterior end comparatively more attenuated and attains width gradually. Mouth opening simple, without lips, surrounded by minute lateral and median pairs of minute papillae. Esophagus is long ending into club-shaped structure. The esophagus is provided with three-forked chitinous structures on each side of its anterior end. Tail ends into a broadly ended end. Caudal alae are absent. Females: Body longer and stouter than males. Body cuticle slightly striated. Mouth structure rater same as in male with esophagus and trident structures. Genital opening in anterior body region or esophageal region. Eggs double walled, thick shelled, oblong or oval in shape.

**PREVALENCE OF ECTO- AND ENDO-PARASITES IN BLUE ROCK PIGEON
(*COLUMBA LIVIA*) AT JALLO PARK, LAHORE**

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The present study was conducted to record relative abundance (%) of ecto-parasites (chewing lice) and egg per gram (EPG) of endo-parasites in Blue Rock Pigeon (*Columba livia*) in captivity at Jallo Wildlife Park, Lahore. For this purpose, 116 birds (63 adult male and 53 adult females) were randomly examined. Ecto-parasites were collected, preserved, stained and identified. All birds were found infested with different species of ecto-parasites. A total of 2898 lice (1194 female, 1324 male, 380 nymph) were identified with relative abundance of female lice 41.20%, male with 45.72% and nymph with 13.11%. The most abundant lice was *Columbicula columbae* with relative abundance 2566 (88.42%) following *Physconelloides zenaidurae* (0.1%), *Menacanthus stramineus* (1.58%), *Menacanthus pallidulus* (0.13%), *Menpon gallinae* (1.92%), *Lipeurus caponis* (0.96%), *Lipeurus tropicalis* (0.06%), *Goniodes gigas* (0.06%), *Goniodes dissimilis* (0.89%), *Goniocotes chrysophalus* (0.51%), *Goniocotes colchichi* (0.24%), *Goniocotes gallinae* (2.06%), *Colpocephalum zebra* (0.03%), *Colpocephalum turbinatum* (0.65%), *Coloceras damicorne fahrenheitzi* (0.03%), *Chelopistes meleagridis* (0.24%), *Cuclutogaster heterographus* (0.86%), *Bonomiella columbae* (0.41%), *Camanulotesbi dentatus* (0.06%) and *Hohor steillalata* (0.06%). Average number of lice recorded, were maximum on female pigeon as compared to male pigeon and this variation was statistically significantly different. Mean number of adult female and adult male pigeon were 27.750 ± 14.682 and 20.550 ± 11.598 , respectively. During this study, it was noted on the average, each bird was loaded 53.7 ± 8.1 , on adult female was 61.8 ± 12.4 and on male was 45.7 ± 10.4 . For endo-parasitic studies, fresh fecal samples of each pigeon were collected and endo-parasites were identified. A total of 21175 EPG were recorded. All the samples of faecal matters were loaded with the eggs of 6 types of helminthes and 2 types of protozoans namely, *Capillaria columbae* (3875), *Capillariaan nulata* (1775), *Capillaria aretusa* (1725), *Capillaria longicollis* (575), *Syngamus trachea* (1050), *Heterakis gallinae* (850), *Coccidian spp.* (8325), *Eimeria spp.* (2025). *Coccidian spp.* was dominant with relative abundance (39.31%). Mean EPG endo-parasitic load was 140.3 ± 18.0 , on adult female was 143.8 ± 18.0 and on male was 136.8 ± 26.6 respectively. From this study, it was concluded that it is the need of time for extensive study

on ecto- and endo-parasites of blue rock pigeon so that, these can be preserved from diseases caused by these agents which ultimately affect the health and reproductive performance of these birds.

A SPECIES *DISPHARYNX* (NEMATODE: ACUARIIDAE) FROM *PASSER DOMESTICUS* IN HYDERABAD, SINDH, PAKISTAN

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For the presence of helminth parasites, 56 House sparrow, *Passer domesticus* examined and only one was found harboring 10 Acuariidae worms. Worms from intestine of the host were removed with soft brush, killed in hot 70% Ethanol and preserved in Alcohol-glycerol solution. These specimens have tricuspid mouth with small lateral lips; buccal capsul long cylindrical transversely striated. Esophagus consisting of two parts. Valva in posterior fourth of body. Eggs are thick-shelled, embryoned. Drawings have been made with camera lucida for detailed study. However, *Passer domesticus* is new host record for the genus *Dispharynx*.

PARASITIC INFECTION IN IMPORTED ORANDA FISH, *CARASSIUSAURATUS* L

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Thirty three oranda fish, a variety of goldfish *Carassius auratus* were obtained from commercial supplies in Lahore and were observed for parasitic and fungal infections. Various organs such as fins, gills and the skin were observed by standard parasitological procedures. The slides were prepared and observed under microscope. Eight slides of gills of fish were made and observed. Five ectoparasites were found infecting the fishes. These parasites include, *Dactylogyrus* sp., *Gyrodactylus* sp., Crustacean parasite *Argulus* sp., *Trichodinasp.* *Ichthyophthariusp.* Gills were the most infected area among all the sites. The mean intensity of these parasites was 20.27, 6.1, 3.7, 3.0 and 6.67 respectively. The gill infection by th~ *Dactylogyrus* sp. showed mild to very serious pathological conditions. The secondary lamellae were partly and even completely eroded and damaged. For observation of fungal infection, the material from various parts of the fish such as; caudal fin, dorsal fin, pectoral fin, gill, abdomen, were inoculated on three different media, Malt Extract Agar (MEA), Sabouraud Dextrose Agar (SAD), Potato Dextrose Agar (PDA) in petri plates. These plates were incubated for seven days at 27-30°C and different fungal colonies were observed in the plates with naked eye. The fungal colonies were of white, grayish black, green and red, pink color. The control plates showed no growth. Slides were prepared and stained with trypan blue in lactophenol and photographed. *Aspergillus* sp., *Mucor* sp.; *Rhizopus* sp. were identified to be present on the fishes. Although these fishes look healthy but have fungal infections. Good husbandry care must be taken while maintaining fish at pet shop. This is done by providing good quality feed and having suitable water quality in the aquarium.

PARASITIC FAUNA OF SHUBUNKIN FISH, *CARASSIUSAURATUS* L. IMPORTED TO LAHORE

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The present study was aimed to investigate parasitic infection of imported shubunkin, a variety of goldfish, *Carassius auratus*. A total of 30 fishes were examined of which 17 were healthy and 13 fishes showed infection. Five species of parasites were extracted and identified which belong to 3 groups; monogenean, *Dactylogyrus* sp. *Gyrodactylus* sp.; protozoan, *Tricodina* sp., *Ichthyophthirius* sp. crustacean, *Argulus* sp., *Argulus* sp. had low prevalence (6.66%) and mean intensity (1.0). The fishes were heavily infected with *Dactylogyrus* sp. (prevalence 63.3%, mean intensity 22.31) and *Gyrodactylus* sp. (prevalence 46.6%, mean intensity 27.07). The gills were severely damaged due to high infection in secondary lamellae. The fins also showed heavy infection and the tips of fins were eroded. The infection by *Tricodina* sp., *Ichthyophthirius* sp. was low 20% and 16~6% respectively. The probable way of transmission of these parasites might be through their escape into imported fish consignment to Lahore. May be the developmental stages of the parasites has been ignored by the consignment dispatchers. Imported fishes must be strictly checked at landing sites in Pakistan to stop movement of these parasites into the country.

PARASITIC FAUNA OF IMPORTED COMET FISH, *CARASSIUSAURATUS* L.

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Thirty comet fishes in 7 different badges were studied for parasitic infection. The fishes were examined by standard parasitological procedure. Five types of parasites were observed to infected these fishes. The organs of fish such as gills, fins and skin were infected. The gills were heavily infected by *Dactylogyrus* sp. The gill filaments were damaged from tips and base. There was hyperplasia on gill filament. The prevalence of *Dactylogyrus* sp. was 86.6% and mean intensity 19.96. *Ichthyophthirius* sp. infection was also on gills and fins. *Argulus* sp. was observed on the fins, head and caudal fins of the fishes. *Argulus* sp. infection was low (16.66%). *Gyrodactylus* sp. was also found on the fins and its infection was 33.3%. *Tricodina* sp. was found on only on one fish on the caudal fin. The parasitic infection on comet seems to be carried from country of origin of the fish. This is an example of the transfer of parasites from one country to another country and it must be checked and stopped at government level to avoid the risk of spread of diseases into our aquatic environment.

SECTION - V**FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY, MARINE BIOLOGY****1. ECOLOGY AND ENVIRONMENTAL POLLUTION****ECOLOGICAL STATUS OF NALTAR LAKE, GILGIT, BALTISTAN, PAKISTAN**JAMILA BAIG¹, A. N. NAQVI¹, BABAR KHAN² AND SEEMA WAFEE³¹ *Department of Biological Sciences, Karakoram International University, Gilgit, Pakistan*² *World Wide Fund for Nature, Pakistan, Gilgit 15100 Pakistan*³ *Department of Chemistry, Karakoram International University, Gilgit, Pakistan*

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The present research was carried out to evaluate the ecological status of a high altitude lake (Chembari Lake, Naltar) in Gilgit, Pakistan. Four composite samples (total 20) were collected on the basis of accessibility to in-let zone, littoral zones and out-let zone of the lake. The samples were collected by two persons (first person hold D-kick net facing second person that help to disturb substrate by kicks). Physico-chemical analysis of the water samples revealed pH values from 7.5 to 7.9, turbidity (-5 to -9 mg/l), temperature (7°C to 20° C) and electrical conductivity (166 to 178 µS/cm). Microbiological studies showed *Escherchia Coli*, *Pseudomonas proteous*, *Salmonella* and *shigella* were amongst major bacterial communities. A total of 3863 macro invertebrate of 11 taxa were counted with Diptera-Chironomidae (45.27%) and Annelida-Tubificidae (25.08%) being the most abundant of invertebrate community followed by Arachnida-Hydracarnia (7.19%), Diptera-Ceratopognidae (06.93%), Nematoda (06.29%), Ephemoptera-Baetidae (05.12%), Trichoptera-Limnophilidae (1.99%), Plecoptera-Chloroperlidae (01.21%), Hirudinea (0.62%) ,Dytiscidae (0.20%), and Coleoptera-Hydrophilidae (0.05%) at different collection sites.

MORPHOMETRIC STUDY OF SALT RANGE SHEEP IN POTWAR PLATEAUMUHAMMAD IMRAN KHAN¹, IMRAN MOHSIN¹, MUHAMMAD ABDULLAH¹, KHALID JAVED¹, NISAR AHMAD¹, MUHAMMAD MUKHTAR², KHURAM SHAHZAD¹ AND HAMID MUSTAFA*¹¹ *Department of Livestock Production, University of Veterinary and Animal Sciences, Lahore*² *Barani Livestock Production Research Institute, Kharimuruit.*

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Eight body measurements were taken on 50 Salt Range sheep aged 0-14 months within the Potwar Plateau. The body measurements were wither height (WH), body length (BL), rump length (RL), rump height (RH), rump width (RW), shoulder width (SW), face length (FaL) and heart girth (HG). The aim was to investigate the variance structure and provide an objective description of the body shape (conformation) of the sheep within the first years of life using a cluster analysis. This was possible sequel to the application and permission of the Pearson's correlation coefficient. Applying the traditional use of wither height for size estimation, the animal measured

55.83±4.81cm. Other body measurements for that age group were: BL=49.37±4.50, RL=20.1±1.12, RH=60.18±6.06, RW=10.90±1.24, FL=35.62±2.29, SW=13.40±1.45, FaL=17.47±1.82, TL=36.72±2.71 and HG=65.98±4.30cm. Variability was generally high within body measurements. Correlations among body dimensions were positive and significant ($P < 0.05$, 0.01 ; $r = 0.40-0.99$). The factor solution from Principal component analysis (PCA) produced two clusters after a promax rotation of the transformation matrix. The first and second principal components explained 67.6 and 11.03% of the generalized variance in body measurements and gave approximately equal emphasis to each variable. The first component contained measurements that are closely associated with bone growth (FL, TL, FaL, RH, WH and BL) while the second one appeared to produce dimensions that are relatively less associated it (RW, SW and RL).

**MORPHOLOGY, POPULATION DIVERSITY AND AQUATIC ENVIRONMENT OF
BUFO STOMATICUS (LUTKEN, 1864), (ANURA: BUFONIDAE) DISTRICT LARKANA,
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During Field survey of amphibian fauna in District Larkana Sindh, in the months of March, April and May 2011, *Bufo stomaticus* (Lutken, 1864), (Anura: Bufonidae) from village Baka Pur was observed to possess a distinct and unusual morphological characteristic of a dark brown patch ventrally in the mid of palm of each forelimb. The whole Population of this variable species was determined to include 299 adult members. Of *B. stomaticus*, all recorded to have such type of morphological variation while no other amphibian species of this area possessed that. The physico-chemical parameters of its habitat including pH (7.18±0.47), conductivity IIS/cm (1909.22±1247.38), Total dissolved solids (1166.33±819.15), alkalinity (113.4±11.24), dissolved oxygen (6.7±0.533), chloride (378.78±259.25), calcium (194.25±101.90), potassium (119.70±133.02), magnesium (88.42±30.17), iron (11.42±5.90) and Chemical oxygen demand (53.94±21.34) were analyzed for the survival of this variable species in its specific area and determined as stable.

**BIODIVERSITY OF SNAILS FROM THE AGRO-ECOSYSTEM OF TEHSIL
FAISALABAD CITY**

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The present study was carried out to know the number, diversity and the living strategies of snails in the Tehsil Faisalabad city. The snails were collected randomly from the cultivated *i.e.*; wheat, sugar cane, Barseem, Tomato fields, and from the Water Course. As these crops were different varied diversity and abundance of the snails were observed. A total 12989 specimens of the snails were collected from the five different habitats and were identified on the basis of morphometric characteristics into 15 species of 11 genera belonging to 8 families and the species are: *Ariophanta bistrialis ceylanica*, *Ariophanta bistrialis ceylanica*, *Ariophanta bistrialis taprobanensis*, *Ariophanta bistrialis*, *Ariophanta solata*, *Ariophanta belangeri bombayana*,

Oxychilus draparnaudi, *Monacha (Monacha) catiana*, *Cernuella (Cernuella) Virgata*, *Oxyloma elegans*, *Physa fontinalis*, *Zootecus insularis*, *Zootecus insularis*, *Pupoides* spp., *Cecilioides acicula*. The snail abundance and diversity in the various habitats varied with the months and with the habitat. March showed the maximum diversity with respect to the occurrence of the different genera than that of the other months. May showed the least diversity for the snail fauna. Genus *Ariophanta* (10843) was the most frequent genus occurring throughout the sampling period from March to July. *Zootecus*, *Physa* and *Oxychilus* were also present in good numbers. *Cecilioides acicula* (33) was the least frequent species. The areas covers by the smaller vegetations, leaves, litters and the moist habitats were bearing the large number of snails. Maximum numbers of snails were found from Barseem crop field. The second was wheat followed by the Sugarcane, water courses and the least from tomato vegetable fields. In the present study: a total of 12989 snails representing 8 families, 9 genera and 15 species of snails were found from Tehsil Faisalabad city, the moist, sub-shadow habitat where temperature is low is preferred by the snails to live in, abundance and diversity of the snails varied with months so their diversity can be correlated with the temperature, humidity and the rainfall, this beguiling fauna is largely being discounted in Pakistan and there is a very broad scope of study in this regard.

**MORPHOMETRICAL AND ANATOMICAL ANALYSIS OF INDIAN PANGOLIN
(*MANIS CRASSICAUDATA*) INHABITING POTOHAR PLATEAU, PAKISTAN**

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Indian pangolin (*Manis crassicaudata*) is a "Near Threatened" species occurring at localized places in the country including the Potohar Plateau. The current study reports morphological and anatomical measurements of twelve specimens of Indian pangolin (*Manis crassicaudata*) collected from Potohar plateau. Body weights of the specimens ranged between 0.78 kg to 20 kg while body lengths from 41.6 cm to 147.3 cm. A strong positive correlation of body mass was found with body length ($r^2 = 0.89$), snout length ($r^2 = 0.93$) and ear length ($r^2 = 0.94$). Similarly, body length showed positive correlation with its body girth ($r^2 = 0.85$), snout length ($r^2 = 0.075$) and ear length ($r^2 = 0.77$). Maximum numbers of scales were found on trunk region of the animal ranging from $n=123$ to $n=160$ in different specimens. Scales were of different sizes on different body regions, smallest sized scales were those on the head region (0.2cm to 3.5cm) while largest on the trunk region (1.2cm to 4.5 cm). Numbers of scales on the head region differed significantly from those on the trunk ($p < 0.01$) and tail ($p < 0.05$) regions. Similarly, scale numbers on forelimbs showed significant difference ($p < 0.05$) from those on the hind limbs. Sizes of scales on head, trunk and tail regions also differed significantly ($p < 0.05$). Anatomical measurements included weights and measurements of different visceral organs including those of digestive and reproductive systems. The relative tongue weight (RTW) and relative tongue length (RTL) were found 0.2% and 37%, respectively of the total body weight and length.

HABITAT PREFERENCE AND FOOD HABIT OF RED-HEADED MERLIN (*FALCO CHICQUERA*) IN DISTRICT CHAKWAL, PAKISTAN

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Nine falcon species are reported from various parts of Pakistan; some of these species are residents, some winter visitors, some vagrants and others passer migrants. However, very little studies exist about the ecology of falcon species in this particular region. The current study, therefore, investigated habitat preference and food habit of the Red-headed merlin (*Falco chicquera*) in district Chakwal, Pakistan. Five potential sampling sites were selected through surveillance surveys. Each selected study site was visited once in a month interval for a period of eighteen month. Falcon species were observed by direct sighting using a binocular and for further identification photograph was taken and species was confirmed by field guide and available literature. Point Cantered Quarter Method was used to quantify the tree species and quadrat method was used to quantify the shrubs and herbs in the study sites. Line transects were established in the selected study sites for collecting information regarding the perching, roosting and nesting preference of the Red-headed merlin. Data was analysed in terms of density, and frequency of trees, shrubs and herbs. Importance Value Index (IVI) of tree species in all study sites was also calculated. For the investigation of food habit, regurgitate and prey remains was calculated very carefully from the perching and roosting sites and each sample was analysed, segregated and frequency and volume was find out. The frequency of bones of birds and small mammals was found to be 100 per cent whereas feathers of birds were found to be 93.55 per cent.

AEROSOL MONITORING AND ENVIRONMENTAL HEALTH STATUS IN PAKISTAN

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The depraved air quality is one of the serious problem related to our environmental health. After considering different researches we analyzed that the environmental condition of Pakistan is declined since last 3 to 4 decades at a very high rate. The manufacturing sector that contribute 17.9% of GDP of Pakistan consumes 16 percent of total oil consumption and create 285 ton CO₂, 162 ton NO₂, 378 ton SO₂ and 4400 ton particulate matter. Other emission of non-conventional toxic pollutants such as cadmium, mercury, PCBs and other tenacious organic pollutants have very little information that also have tendency to accumulate in animal's body. But it was observed that tanneries located in Kasur and Sialkot was discharging effluents with Chromium concentration ranging between 182 to 222 mg/liter against standard limit 1mg/liter. Unplanned urbanization and industrial growth created huge problems. All the big cities situated near river or coasts dump their liquid and solid wastes directly into water bodies. Approximately Karachi dumps its 600 million ton sewage daily into sea. Lahore dumps about 200 million ton liquid and 100 million ton solid waste in River Ravi. Considerable concentrations of toxic heavy metals were found in aquatic

fauna samples collected from associated waters near the cities. Air pollutants including the particulate matter, NO₂, SO₂, CO₂ and Pb, remain high 10 to 50 percent of the year and 2 to 3 times have much high concentration in our environment as compared to the standard level given by World Health Organization. Particulate matter is most concerning in this regard. The air quality is worsening mainly due to inept burning of fuel, emissions from industrial and non-industrial sources, high concentration of particulate matter and emission from automobile and non-automobile lead. The studies reveals that if the concentration of PM₁₀ increased by 10 microgram per cubic meter it can increase 1 percent daily deaths. While most of our cities have 2 to 3 percent higher PM₁₀ concentrations than the safe limits, while fine and ultrafine respirable particles concentrations studies in urban and rural environments have also revealed the same consequences. Air pollution has significant effects on exacerbation of, allergy and other respiratory diseases including asthma, bronchitis, nasal congestion, neurological dysfunction. Humidity and severe drought also increase the level of air pollutants. The ten years aerosol analyses indicate that significantly higher concentration of aerosols and rise in dense foggy days was observed in the eastern parts (Punjab) of Pakistan during the period 2008-2010. These trans-boundary aerosols enhanced the fog in Punjab and upper Sindh. Their impacts are not only threatening the environment but also for the human health and other living organisms in Pakistan. Fuel consumption is rising 200 percent with each decade adding in Pakistan that it has been assessed that by poor quality fuel burning, Pakistan is adding 550 metric tons per year of lead emissions by automobiles that costs about \$500 million per year in related health care costs. An estimated share of carbon emission in Pakistan is by industry (45.1%), transport (27.1%), residential sector (22.3%), and the commercial sector (5.6%), while the responsible sources for these emissions were oil (56%), natural gas (35.7%) and coal (8.3%). As the world release annually 23 billion ton of CO₂ into the air, Pakistan accounts for only 0.4% that attributes to, increase the earth temperature and threatening the health and habitat of animals, plants and human beings. Approximately 70 species a day are extirpating forever in the world, and rate of extinction and loss is accelerating. Just one nation can never be responsible for the problem and needs an integrated approach to contribute and restore all the habitats and ensure the stability of wide-ranging health of natural ecosystems.

MONITORING OF AIR BORNE PARTICULATE MATTER ARISING FROM DIFFERENT FUEL TYPES IN RURAL HOUSES

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PM_{2.5} is a major component of indoor air and is generated from a variety of sources. Cooking has been identified as an important source of fine particulate matter and not only the cooking methods but the types of fuel used for cooking are also important contributors of PM_{2.5}. This study was carried out to determine the concentration of particulate matter in kitchens and living rooms of rural houses of Theeng Morr (Ellhabad), Kasur where the type of fuel was LPG and biomass. Three houses were monitored for forty eight hours each using a real time aerosol monitor. The fuel type in house # 1 and 3 was Liquefied Petroleum Gas while wood was in use in the second

house. Monitoring was done in both the kitchen and living room simultaneously. There was no smoker in house # 1 while both Huka (smoking pipe) and cigarette were smoked in house # 2. Cigarette was also smoked by one member in House # 3. The average concentrations of PM 2.5 in the kitchens of the three houses were observed to be 464.518 $\mu\text{g}/\text{m}^3$, 844.999 $\mu\text{g}/\text{m}^3$, and 262.358 $\mu\text{g}/\text{m}^3$ respectively while in the living rooms of the respective houses, the fine particulate matter concentrations were 442.486 $\mu\text{g}/\text{m}^3$, 1121.207 $\mu\text{g}/\text{m}^3$ and 415.953 $\mu\text{g}/\text{m}^3$. It is noteworthy that in kitchens where LPG was in use, PM2.5 concentrations are much lower as compared to house # 2 where wood and cow dung was burnt. Moreover, in the two houses where smoking was carried out, the living rooms were more polluted than the kitchens since it is also an important source of fine particulate matter indoors. None of the observed values were in compliance with the WHO limit of 25 $\mu\text{g}/\text{m}^3$ indoors and the IAQ of these study sites poses an alarming situation. Unfortunately indoor air quality is not considered an important policy issue in Pakistan and no guidelines are present for indoor air quality. It is therefore necessary to carry out more relevant studies to characterize the indoor air quality in Pakistan.

ASSESSMENT OF INDOOR AIR QUALITY (PM_{2.5}) WITH SEASONAL VARIATIONS IN LAHORE, PAKISTAN

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Particulate matter is one of the major pollutants indoors and is responsible for a variety of respiratory problems. Monitoring of fine particulate concentration was carried out in different residential micro-environments of Lahore, Pakistan beginning from January, 2012 to January 2013. Two DustTrak (TSI model 8250) were run simultaneously in the kitchens and living rooms of the selected study sites and each house was monitored once for seventy two hours. The mean concentrations of PM_{2.5} were observed to be higher in both the kitchens and living rooms. Cooking, cleaning, smoking as well as outdoor sources were observed to contribute significantly to the indoor air quality. Seasonal variation in particulate concentration was observed to be more pronounced. The concentrations were elevated during the winter season (555.203 $\mu\text{g}/\text{m}^3$ in kitchen; 530.125 $\mu\text{g}/\text{m}^3$ in living room.), decreasing in spring (254.379 $\mu\text{g}/\text{m}^3$ in kitchen; 184.09 $\mu\text{g}/\text{m}^3$ in living room) and reaching its lowest levels in summer season (176.324 $\mu\text{g}/\text{m}^3$ in kitchen; 164.649 $\mu\text{g}/\text{m}^3$ in living room). The concentrations increased again as the weather began to grow colder during the autumn (395.042 $\mu\text{g}/\text{m}^3$ in kitchen; 445.0 $\mu\text{g}/\text{m}^3$ in living room). The increased concentrations in the winters and fall can be attributed to decreased ventilation and increased space heating apart from other sources. There is less mixing of air and the pollutants do not disperse as readily as in the summers. The windows remain open during the summer and spring resulting in infiltration of air from outdoors and decreased PM_{2.5} concentrations due to mixing of air. However, it should be noted that all the concentrations were well above the WHO guidelines of 25 $\mu\text{g}/\text{m}^3$. More detailed studies need to be carried out to determine the sources and health impacts of particulate matter indoors as well as how to reduce its levels in Pakistan.

ASSESSMENT OF SPATIAL DISTRIBUTION OF CHROMIUM IN TANNERY EFFLUENT CONTAMINATED SOIL AND ITS BIOACCUMULATION IN ASSOCIATED PLANTS

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Chromium is a potential recalcitrant pollutant at waste disposal sites adjacent to leather industries. Screening of agricultural lands in perspective of chromium distribution in soil along the distance from tanneries as well as its bioaccumulation in vegetation growing over it is necessary for agricultural management at contaminated sites. The present study reports extent of chromium distribution spatially in soil along the distance away from tanneries. Replica soil samples (0-20cm) were collected from land area adjacent to industrial sector in city Kasur and a total of seven different spots were chosen every 200m starting from the tanneries. Eight different species of plants *Desmostachya bipinnata*, *Sahadora oleoides*, *Acacia milotica*, *Avena sativa*, *Brassica compestris*, *Chenopodium murale*, *Peganum harmata* and *Fumaria* sp. were collected from the land area for analysis of bioaccumulated chromium in root, shoot and leaves. Chromium concentration in the soil was observed in the range of 600-4956ppm. Lowest concentrations were assessed in the soil samples nearest to leather tanneries while the sites 0.6-1 km away from industrial sector was found highly contaminated with chromium levels of 4800-4956 ppm. Dominant species was Cr (III) representing 58% of the total chromium. Analysis of chromium in plants showed highest chromium accumulation in *Brassica compestris* with 300ppm in roots to 50ppm in leaves while lowest accumulation (4.2ppm) was observed in roots of *Avena sativa*. Among the eight plants studied chromium bioaccumulation was in an order of *Brassica compestris* < *Peganum harmata* < *Sahadora oleoides* < *Chenopodium murale* < *Fumaria* sp. < *Desmostachya bipinnata* < *Avena sativa* < *Acacia milotica*. The order of Cr concentration in different plant parts was root<leaves<shoot. Thus these data suggest immediate attention towards the consumption of vegetables and cereals raised in such soils and bioremediation of the land areas adjacent to the leather industrial sector for healthy agricultural practices.

AIRBORNE PARTICULATE MATTER (PM_{2.5}) IN THE CLASSROOMS OF UNIVERSITY OF THE PUNJAB, LAHORE

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Air pollution is a major concern in Pakistan where both indoor and outdoor pollutants pose certain risks to human health. Accumulation of particulate matter (PM_{2.5}) in classrooms has not yet been studied comprehensively in Pakistan. This research was conducted to monitor the concentration of particulate matter in classrooms of University of the Punjab, Lahore, using a direct reading instrument, DUSTTRAK Aerosol Monitor (TSI model 8250). Sampling for PM_{2.5} concentrations was carried out in different classrooms (indoor samples) while outdoor samples were also taken simultaneously. Three classrooms were selected as indoor study sites for sampling according to the high (> 50), medium (25-50) and low (<20) number of students. A single location

(rooftop of zoology department) was selected for ambient air monitoring. Two instruments were run continuously for twenty four hours in both indoor and outdoor sampling sites. The average concentrations of PM_{2.5} in three classrooms were observed to be 672 μm^3 , 282 μm^3 and 74 μm^3 where as the average level of PM_{2.5} in the ambient air was found to be 998 μm^3 , 324 μm^3 and 120 μm^3 respectively. The number of students was observed to be an important contributing factor towards the concentration of particulate matter in the classrooms. However no association was observed between the indoor and outdoor air quality since many factors are responsible for the concentration of particulate matter in the indoor and outdoor air. It is important to note that all the observed concentrations were much higher than the WHO standards.

PHYTOCHEMICAL CONSTITUENTS OF WEEDS-BASEHNE STUDY IN MIXED CROP ZONE AGROECOSYSTEM

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The Qualitative phytochemical analysis is a significantly important to identify the chemical compounds present in the medicinal plants. The root, stem and leaves of seventeen weed species growing in agro-ecosystem viz., *Chenopodium murale*, *Convolvulus arvensis*, *Rumex dentatus*, *Parthenium hysterophorus*, *Euphorbia helioscopia*, *Cronopus didymus*, *Brassica compastris*, *Solanum nigrum*, *Sonchus oleraceus*, *Poa annua*, *Ricinus communis*, *Anthem graveolens*, *Cynodon dactylon*, *Melilotus indicus* and *Malvastrum coromandelianum* collected from peripheral cultivation belt of Faisalabad district were analyzed for their phytochemical constituents. The result of the study revealed the presence of phytochemicals viz., alkaloids, saponins, tannins, steroids, Phlobatannin, terpenoids, flavinoids and cardiac glycosides in different parts of weeds. The root and stem of these weed species were found rich in phytochemicals, which are used to manufacture medicines and drugs for various ailments.

INTRASPECIFIC VARIATION IN MALE FLOWER NUMBERS IN A DIOECIOUS FIG TREE

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Fig trees of the genus *Ficus* (Moraceae) and their associated obligate pollinator wasps (Agaonidae) represent one of the classic examples of species-specific and highly coevolved plant-insect mutualisms. In dioecious fig tree species, ovules in male figs produce adult female wasps that carry pollen to other trees. This is achieved through protogyny, with male flowers maturing at the time of wasp maturity. Some fig wasp species transport pollen and pollinate actively, others transfer pollen passively. Active pollination is thought to have probably evolved just once and have reverted back to passive pollination many times. Because active pollination is much more efficient, fewer male flowers are needed and anther-ovule ratios are considered to be a good

predictor of pollination mode. Here we describe the range of anther-ovule ratios in figs of a dioecious fig tree *Ficus montana* known to be pollinated actively. Contrary to expectations, figs on different plants showed a wide range of anther-ovule ratios that encompassed almost the entire range described previously for both active and passively pollinated fig species in general. This fig tree species appears to be one where evolutionary shifts in pollination behaviour might be facilitated.

IMPACT OF COMPOST (CITRUS PEEL WITH ROCK PHOSPHATE) ON GROWTH AND YIELD OF MAIZE

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The rock phosphate (RP) and citrus Peel (CP) composts of different ratios and at incubation time were synthesized in vessel composter. Moreover phosphorus (P) release and effects on maize growth and yield were evaluated under field conditions. The highest value of P (18.49 ppm) released was recorded after 70 days application of RP-CP compost (incubated at 35°C for RP-CP 1: 1) and the rate constant revealed that P released in two phases (Phase I and phase II). The growth and yield of maize crop were recorded significantly enhanced by the application of RP-CP compost over the fields receiving NPK and zero (untreated) fertilizers. The cost-benefit analysis also confirmed the utility of RP-CP compost, hence it is recommended as a useful alternative fertilizer for crops.

GENOTYPIC DIFFERENCES AMONG TOMATO GENOTYPES FOR NITRATE ASSIMILATION

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Fifteen tomato (*Lycopersicon esculentum* Mill.) genotypes were assessed for their nitrogen use efficiency in a controlled environment to determine the genotypic and phenotypic variation on the efficiency of nitrogen utilization and fruit yield. Variation of nitrogen utilization and assimilation in different genotypes was found as foliar NO₃⁻ application. Highest foliar NO₃⁻ concentration, protein contents and organic nitrogen contents were found in 12770,7015,12585,703217859 These genotypes also has direct effect on fruit yield per pant. Intermediate nitrogen utilization was found in genotypes 17863,17862,17867,17869 Which is correlated with low yield per plant. Hence, foliar application of nitrogen would enhance the production potential of tomato genotypes.

VARIATIONS IN WOOD DENSITY, SPECIFIC LEAF AREA AND DECOMPOSITION RATE AMONG VARIOUS PLANT SPECIES ALONG ALTITUDINAL GRADIENT IN PAKISTAN

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Present study was planned to find out the altitudinal variations in wood density, specific leaf area and decomposition rate of sixty plant species. Twelve sites were selected in six different types of forests which include tropical thorn forest, dry temperate forest, dry temperate *Cedrus* forest, sub alpine *Pinus wallichiana* forest and *Pinus roxburgii* forest. All these sites were selected along accessible elevations *i.e.* 300 m, 700 m, 1200 m, 1250 m, 1450 m, 1500 m, 1550 m, 2200 m, 2250 m, 2285 m, 2300 and 2700 m. At each site 5 species of shrubs were collected during April to June, 2010. The specific leaf area varied significantly among all the twelve sampling sites and the same was observed along the altitudinal gradient being narrower at lower and broader at higher elevations. However the relation of wood density and decomposition rate with altitude was nonsignificant. Among forest types, tropical species showed greater wood density than the rest of the five forest types. Decomposition rate was found inversely proportional to specific leaf area.

HEAVY METAL TOXICITY IN GUAVA (*PSIDIUM GUAJAVA*) IRRIGATED BY HUDIARA DRAIN IN DISTRICT LAHORE

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The aim of this study was to investigate the toxicology of heavy metals in fruits irrigated by Hudiara drain. So a survey was conducted along the whole length of Hudiara drain to assess the heavy metals accumulation in producers. Different samples of fruit (Guava) were collected at three sites (Lallo, Mohlanwall and Khurdpur) from the tree in 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 fruit (guava). The heavy metal concentration shows a significant variation, when compared to permissible international standards. The concentration of heavy metals (Ni, Mg, Al, K, Ca, Ti, Cr, Mn, Fe, Ni and Zn) at Lallo was (1448 ppm, 1255 ppm, 927ppm, 33362ppm, 836 ppm, 0, 0, 0, 104ppm, 0, 106), at Mohlanwall was (4413 ppm, 5050 ppm, 1503ppm, 63124ppm, 21287 ppm, 0, 0, 0, 264ppm, 92 ppm and 120ppm) and at Khurdpur was (32151 ppm, 22996 ppm, 16240ppm, 25818ppm, 37820 ppm, 1210 ppm, 241ppm, 240 ppm, 3636ppm, 0, 145ppm) in fruit respectively. The concentration of heavy metals tends to increase from first to third site gradually. It is concluded that Hudiara drain is highly polluted by the addition of untreated industrial effluents and city sewage and heavy metals tend to accumulate significantly high in fruits irrigated by Hudiara 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.

**ASSESSMENT OF SELECTED PERSISTENT ORGANIC POLLUTANTS (POPS) IN
MACROBRACHIUM LAMARREI FROM STREAMS OF SIALKOT DISTRICT,
PAKISTAN**

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Specimens of *Macrobrachium lamarrei* were collected from the Nullah Aik and Palkhu, Southern tributaries of River Chanab, Sialkot District, Pakistan and were analyzed for 11 Persistent Organic Pollutants (POPs) viz Beta-HCH, Lindane, Heptachlor, Heptachlor Exoepoxid, Heptachlor Endoepoxid, Dieldrin, DDD, DDE, Endrin, 2, 4-DDT and 4, 4-DDT. Five pesticide residues were detected in composite sample of *Macrobrachium lamarrei*, only at upstream: B-HCH, Lindane, DDD, 2,4DDT, 4,4DDT and their concentrations were calculated 77.46(µg/kg), 81.94(µg/kg), 40.88(µg/kg), 334.63(µg/kg) and 417.21(µg/kg) respectively on a dry weight basis. Rest of the POPs residues were not found in Prawn. The results were compared with international guideline values for pollutant concentration in prawn. Contamination level of studied POPs was found beyond the recommended limits for human consumption. The study highlighted its usefulness in the managements of POPs in study area and its catchments.

2. FRESHWATER BIOLOGY AND FISHERIES

WATER FOR LIFE

MOHAMMAD NASIM SIDDIQI

Karachi

Life originated in water, exists on earth due to presence of water. Biologically, water is life line for all processes and functions of plants and animals. From molecular level to large ecosystem – aquatic and terrestrial are sustained due to unique properties of water. Hydrosphere covers $\frac{3}{4}$ of earth surface. In spite of its apparent abundance water availability for sustaining life of animals, human and plants has become a global problem. A hydrocrisis is already there many lead to hydrodisaster. It is feared that global wars in next century may be on water disputes. Water related problems are physical, change of climate and an anthropogenic and Pakistan is victim of all problems. Mitigation is urgently needed when it is written a child is dying some where due to water borne diseases.

ICHTHYOFAUNAL SURVEY OF THE RIVER RAVI STRETCH FLOWING ALONG THE JHOK RESERVE FOREST, PAKISTAN.

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Present study was conducted to assess the current status of ichthyofauna of the river Ravi. For the purpose, a stretch of the Ravi flowing along the Jhok Reserve Forest was selected and analyzed quarterly for consecutive four years from 2009 to 2012. The overall fauna of endemic and exotic fish (*Cyprinus carpio*, *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix*) was equally dominated in this segment with exceptions of significant declines in the populations of *Bagarius bagarius*, *Channa marulius* and *Xenentodon cancila* within a four years observational period. Declines of the fish species indicate alarming levels of river pollution and the biodiversity decrement. Overall fish catches were higher in the months of September and October.

SOME STUDIES ON LIMNOLOGICAL ASPECTS OF DRAINS WATER NEAR LAHORE

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In this study, some limnological parameters (physico-chemical parameters) were measured from the drains water samples collected from five different localities near Lahore. EC, TDS, Salinity, Temperature, DO, and pH were determined on site using portable meters. Rest of the parameters were estimated in laboratory. The estimation of heavy metals (Cr, Cu, Ni, Mn and Pb) were determined in the polluted water using atomic absorption spectrophotometer at GCU Lahore. Highest pH 8.6 was recorded at Site 2 (H.D Mohlanwal) and lowest at Site 3 (Ravi upstream near

BattiChowk). DO with highest value 2.84 mg/l was recorded at Site 1(Sunder downstream Ravi) while lowest at Site 2(H.D Mohlanwal) with value of 0.38 mg/l. Highest salinity with 0.6 mg/l was observed at Site 2(H.D Mohlanwal) while lowest at Site 1(Sunder downstream Ravi). Highest TDS 643 mg/l was observed at Site 4 (DubbanPura near motorway) and lowest at Site 1(Sunder downstream Ravi) with value of 185 mg/l.Highest value of EC was recorded at Site 2 (H.D Mohlanwal) 1466 μ S/cm and lowest at Site 1(Sunder downstream Ravi). Concentration of copper was found at Site 4 (Dubbanpura near motorway)> Site 3 (Ravi upstream near battichowk) > Site1 (Sunder downstream Ravi) > Site 2 (H.D Mohlanwal) > site 5(Shadiwal near motorway). Chromium pattern was Site 5> Site 4 > Site 2> Site 3> Site1. Nickel was below detection limit in all sites. Manganese concentration was found as Site 3 > Site 5 > Site2. Lead concentration was observed as Site 3 > Site 1 > Site 2 > Site 4. The heavy metal estimation and limnological results of all 5 sites showed statistically (ANOVA) non-significant differences among all 5 sites.

MACRO-INVERTEBRATES OF SHEOSHER LAKE (DEOSAI) ALONG WITH PHYSICO-CHEMICAL PARAMETERS

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The research study was carried out to determine the Macroinvertebrates of Sheosher Lake along with physico-chemical parameters (Sheosher Lake, Deosai) in Gilgit, Pakistan. Four sampling sites were selected (littoral sites) keeping in view the accessibility of lake. A total of 787 macro invertebrates of 05 taxa were recorded. Among macro invertebrates Annelida-Tubificidae (62.38%) were the most dominant group followed by Diptera-Ceratopogonidae (21.09%), Diptera-Chironomidae (10.03%), Trichoptera-Limnophilidae (04.32%) and Hirudinea (02.16%) respectively. Physico-chemical analysis of the water samples revealed pH values from 7.1 to 7.3, turbidity (-5 to -6 mg/l) and temperature (1°C to 6°C) respectively.

CONDITION FACTOR OF THREE CARP SPECIES CULTIVATED UNDER SAME CONDITIONS

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To study length weight and condition factor relationship of *Cirrhinus (C) mrigala*, *Labeo (L) rohita* and *Catla (C) catla*, nine specimen of each species were collected from Punjab University Fish Research Form, Lahore, Pakistan. The mean total length and wet body weight were measured as 39.92 \pm 3.66cm and 645 \pm 194.71g, 37.49 \pm 3.62cm and 633 \pm 182.01g and

37.01±3.1947cm and 624±167.23g for *C. mrigala*, *L. rohita* and *C. catla*, respectively. Total length and wet body weight of the sampled specimens did not differ significantly ($P>0.05$). While condition factor showed highly significant difference ($P<0.001$) among the sampled species. Log transform regression was used to study the growth. Growth coefficient (b) of *C. mrigala* (b=3.24), *L. rohita* (b=3.14) and *C. catla* (b=3.18) indicate positive allometric growth pattern (weight gain more rapidly as compared to the cube of the length) in these carp species. The results indicated growth of the fish species in the normal range and suggest for implication of intensive culturing strategy for increase in yield.

**SEASONAL VARIATION OF PHYSICOCHEMICAL PARAMETERS IN
AQUACULTURE FISH PONDS AT FISHERIES RESEARCH AND TRAINING
INSTITUTE (FRTI) MANAWAN, LAHORE**

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Water is the core constituent in the life of all biological species. Aquaculture is the science and art of culturing aquatic organisms encompasses activities in water. Aquaculture mostly depends upon water. For better aquaculture products such as fishes etc. grow in water and depends upon water's quality. Water quality constitute it's all biological, physical and chemical parameters. There are a number of water quality parameters to boosts its production. Physicochemical parameters are known to effect biotic component of aquaculture. Several physicochemical parameters have been studied in intensive fish culture which are interrelated with each other. In the present study the physicochemical parameters included are pH, Dissolved Oxygen (DO), Temperature, Free Carbon Dioxide (CO₂), Salinity, Conductivity, Total Dissolved Solids (TDS), Hardness, Calcium Hardness, Magnesium Hardness, Chlorides, Nitrates, Phosphates, Total Phosphorus, Carbonate Alkalinity and Total Alkalinity which show variation and correlation among themselves seasonally. By managing these, water quality can be improved for better aquaculture yield.

**PRODUCTION OF MONOSEX (MALE) NILE TILAPIA, *OREOCHROMIS NILOTICUS* L.
THROUGH HORMONES AND EVALUATION OF THEIR GROWTH PERFORMANCE
IN AQUARIA AND CIRCULAR TANKS**

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The present research was conducted to determine the optimal dose rate of 17 -alpha methyl testosterone (MT) .for the production of monosex all male Nile tilapia, *Oreochromis niloticus* L. and evaluate the best growth performance of hormone treated fish. For this purpose, two similar experiments in two different environment, in indoor glass aquaria and outdoor circular tanks were conducted, using four different concentrations of hormone (20, 40, 60 and 80 mg MT/kg diet) administered orally to 4 days post hatched fry at the rate of 10% of their body weight for 28 days.

At the end of hormone treatments period fishes were weighed and calculated their average body weight gain. After treatment period of 28 days fish was fed normal diet without hormone for two months, and then dissected for sex evaluation. Results showed that all the groups that received hormone MT showed sex reversal with a significantly higher male ratio than female as compared to the control. The results of control treatment showed a normal ratio of *O. niloticus* with a 60% and 55% male in indoor glass aquaria and outdoor circular tanks and the female ratio were 40% and 45%, respectively. In sex reversal in indoor glass aquaria, treatment T3 (60 mg MT/kg of diet) showed the highest percentage of male at 82.5% and in outdoor circular tanks 85% male was obtained on same treatment. The highest gain body weight was recorded 0.1377 and 0.1387 g in indoor aquaria and outdoor tanks respectively at the treatment rate of 60 mg MT/kg of feed. Statistical analysis of data showed a highly significant difference ($p < 0.01$) for all the treatments.

MATERNAL EFFECTS OF L-ASCORBYL-2-POLYPHOSPHATE ON SURVIVAL AND GROWTH PERFORMANCE OF EARLY FRY OF SILVER CARP, *HYPOPHthalmichthys molitrix*

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The aim of this study was to investigate the maternal effect of L-ascorbyl-2-polyphosphate on the survival and growth performance of early fry of silver carp (*Hypophthalmichthys molitrix*). Both male and female broodfish were fed with graded level of vitamin C (APP₀, APP₂₅₀ and APP₅₀₀) supplemented diet for 70 days. After feeding trial on their respected diet, fish were bred through induced spawning and their progeny were obtained through reciprocal crosses. Early fry from each cross were stock in aquaria and fed live feed for 30 days and their % survival and weight gain was observed. After experimental period, significantly ($P < 0.05$) higher % survival and weight gain was observed in fry obtained from cross in which female compared to male broodfish were fed with graded level of APP. There was positive linear relationship between % survivals, growth rate of fry to the dietary APP in female broodfish. This study clearly confirms the view that AA is transferred via eggs from female broodfish to hatchling then to fry and affects their % survival and growth performance.

STUDIES ON THE ECTOPARASITIC OF GULFAM (*CYPRINUS CARPIO*) FROM CARP FISH HATCHERY DISTRICT BADIN, SINDH, PAKISTAN

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The study has been carried out over period of six month from August 2011 to January 2012 to find out magnitude of parasitization in different fish species cultured at carp fish hatchery district Badin with special reference to Gulf am fish. A sample of 100 fishes belonging to 10 species of fish was examined for parasite. The fish *Cyprinus carpio* commonly called as

Gulfam was highest in number 44 in the collection followed by *Labeo rohita rohu* 20. Of the 10 fish species examined, 05 species of fish were found to be infected by parasites. The highest infection rate (66.6%) was observed in *Cyprinus carpio* and lowest (11.7 %) in *Cirrhinus marigala*. The study showed that all fish species found infected with ectoparasite included Argulus, leaches and lernaea.

PROXIMATE COMPOSITION OF MEAT OF *CIRRHINA MRIGALA* CAPTURED FROM TRIMO HEAD

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Present research work was designed to study the proximate meat composition of *Cirrhina mrigala* that was captured from trimo head. Seven specimens of *Cirrhina mrigala* of three different weight categories designed W₁ (601-900 g), W₂ (901-1200 g) and W₃ (1201-1500 g) were captured from trimo head with the help of gill net. Maximum moisture contents (74.91±0.01) were measured underweight category W₁. From this research work it is observed that the moisture contents decreased as the weight of fish increased. Maximum protein contents (22.66±0.03) were measured in W₃ weight category. Protein contents were increased as the weight of fish increased. Maximum fat contents (2.67±0.02) were measured in W₃ weight category. Carbohydrates maximum percentage (0.65±0.01) was observed in W₃ weight category. Carbohydrates contents were found in lesser amount as compared to moisture, protein, lipids and ash. Ash contents (2.09±0.01) were observed in weight category W₃ that remain higher compared to other weight categories.

EFFECT OF PESTICIDAL RESIDUE ON RNA/DNA RATIOS ON GROWTH OF *LABEO ROHITA* FINGERLINGS

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The threat of rapidly shrinking genetic base and fast depleting biodiversity can be attributed to the indiscriminate use of pesticides that have unintended effects on all sort of aquatic life and the environment too. The present study was designed to evaluate the potential hazards of pesticides on fish (*Labeo rohita*) muscle in terms of alteration in RNA/DNA ratios and conducted in the department of Wildlife and Fisheries, GC University, Faisalabad. Nucleic acid (DNA and RNA) extraction and estimation was done in control and treated with two concentrations of Profenofos (P1-0.62ppb, P2-1.25ppb) and Triazophos (T1-1.50ppm, T2-2.50ppm) fish specimens using calf thymus DNA and bovine liver RNA for standard curves. RNA/DNA ratio was calculated to determine the rate of growth. It was observed that as the treatment preceded the concentration of DNA increases that might be due to carcinogenic and mutagenic effects of pesticides. Where as decrease in RNA concentrations were observed as the treatment preceded further that might be due to alterations in turnover of macromolecules involved in protein synthesis. In case of both treatments, it was observed as the time of exposure of pesticides was increased; the RNA/DNA ratio (µg/ml) was decreased after the end of first and second week treatments (1.028±0.05 to

0.918±0.03 for P1, 0.93±0.06 to 0.878±0.02 for P2, 0.966±0.03 to 0.903±0.03 for T1 and 0.861±0.03 to 0.808±0.01 µg/ml for T2, respectively) indicating negative effect on growth. So, pesticides that are considered beneficial for production of better yield of different crops are actually posing a great potential hazard for aquatic life as well as humans.

EFFECTS OF pH ON RELEASE OF PHOSPHORUS FROM ROCK PHOSPHATE AND PRESS MUD (FROM SUGAR MILLS) USING THE BUFFERING SYSTEM FOR MAINTAINING pH OF THE SYSTEM

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An attempt was made to study the efficiency of two natural fertilizers *i.e.*, rock phosphate and press mud (of sugar mills) in releasing available phosphorus in the form of orthophosphate in water as a function of three different pH ranges *i.e.*, 6.1-7.0, 7.1-8.0 and 8.1-9.0 at different temperatures against the time factor involved in indoor experiments. The experiments were conducted in two phases *i.e.*, during winter season (at low temperature) and summer season (at high temperature) for two months time duration period each. The data incurred from both the winter & summer experiments revealed the same sequential results *i.e.*, maximum release of available phosphorus was observed in glass experimental tanks maintained at pH level 6.1-7.0 followed by those maintained at 7.1-8.0 and 8.1-9.0, respectively. However, the phosphorus release during high temperature (summer) was more as compared to the low temperature (winter) for both the fertilizers. Another interesting observation made during the entire experiment was that the press mud of sugar mills released the available phosphorus at a fairly high rate as compared to the rock phosphate. Water quality was also continuously monitored during the entire period of the experiments. Thus, rock phosphate & press mud of sugar mills can prove to be an alternative and cheaper source of phosphorus fertilizer technology for enriching soils in fish ponds in both intensive and semi-intensive fish farming for efficiently enhancing fish production.

FEEDING HABITS, LENGTH-WEIGHT RELATIONSHIP AND RELATIVE CONDITION FACTOR (KN) OF ARGYROPS SPINIFER AND SPARIDENTEX HASTA (FAMILY: SPARIDAE) IN PAKISTAN

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Feeding habits, length-weight relationship and relative condition factor *Kn* of two demersal fish species *Argyrops spinifer* and *Sparidentex hasta* were examined. Fish samples were collected from the commercial landing site, Karachi Fish Harbour, during August 2012 to February 2013. The total length (TL) and total weight (TW) were recorded for *A. spinifer* (TL= 12.4-35.0 cm, TW= 45-804g) and *S. hasta* (TL= 16.0-36.9 cm, TW= 85.5-607g). Following the standard procedures dietary components of each fish species were recorded and expressed as percentage of numerical

composition (CN), gravimetric composition (CW) and frequency of occurrence (F). The prey items in the gut of the both fish species varied in number, weight, and frequency of occurrence. The prey items of smaller size constituted the major bulk, while the large size prey items were in smaller numbers. The food contents in the stomach of *A. spinifer* were 58% crabs, 32% fish and 10% shrimp/lobster, whereas *S. hasta* appear to feed mainly (99%) on the fish and only (1%) seaweed was also found in the gut of this species. The estimations of the length-weight relationship was achieved using the formula $W = aL^b$ which was transformed logarithmically ($\log W = \log a + b \log L$) for the combine sex. The length-weight equations for the two species examined are as follows: *A. spinifer* (n = 272) $W = -4.61 + 2.97$ (95% CI of b = 2.90 - 3.04), $r^2 = 0.95$ *S. hasta* (n = 101) $W = -3.02 + 2.27$ (95% CI of b = 2.05 - 2.49), $r^2 = 0.80$ The values of b indicated allometric relationship between length and weight of the *A. spinifer* and *S. hasta* and are positively correlated ($r^2 > 0.80$). Estimated monthly condition factor (Kn) in *A. spinifer* was 0.14 - 0.629; lowest values were recorded in August and highest in December. Whereas in *S. hasta* highest Kn (0.71) estimated in the month of October and lowest (0.12) in August. The data obtained in the present study are useful in the stock assessment and fisheries management of these important fish species being landed in Pakistan.

THE EFFECTS OF DIFFERENT DIETS ON PONDS WATER QUALITY AND GROWTH PERFORMANCE OF MAJOR CARPS WITH RESPECT TO RNA/DNA RATIO

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A three month feeding trial was conducted in circulating aquaria to determine the effects of three different fish diets (animal, plant and their mixture as crude protein source 35%) on ponds water quality and growth performance (RNA/DNA ratios) of Major carps (*Catla catla*, *Cirrhinus mrigala* and *Labeo rohita*). Fingerlings were collected from ponds and transported to the circulating aquaria of Fish Seed Hatchery, Faisalabad. The physico-chemical parameters were studied by following the method of A.P.H.A. (1998). It was observed that feed has statistically significant ($P > 0.05$) effect on all the parameters *i.e.*, pH, DO, alkalinity, water hardness, chloride, calcium and total dissolved solids except the temperature of different ponds. Temperature ranged between 17.22 - 23.39°C whereas pH, DO, alkalinity, water hardness, chloride, calcium and total dissolved solids ranged between 8.71- 9.56, 4.79 - 5.37 mg/L, 600.5 - 936.7 mg/L, 65.83 - 194.44 mg/L, 249.6 - 481.4 mg/L and 160.0 - 298.9 mg/L and 1745.8 - 2597.2 mg/L, respectively. The data was statistically analyzed with the help of ANOVA (2 factor CRD and 2 Way). In case of morphometric characteristics it was found that the weight, fork length and feed conversion ratio (FCR) values in both the different fish species and fish diets showed non-significant difference ($P > 0.05$). It was found that survival rate of different fish species was 83.33%, 87.78% and 75.56 % for animal, plant and mixed diets ponds, respectively. Growth performances was calculated with RNA/DNA ratios by isolating DNA and RNA from each fish species from each feed treatments (Schriender, 1957). The DNA, RNA concentrations and RNA/DNA ratios according to different feed stuffs showed highly significant difference ($P < 0.01$). When interaction of species and feed for DNA, RNA and RNA/DNA ratios was observed it also showed highly significant difference ($P < 0.01$).

EFFECTS OF GREEN HOUSE ON THE GROWTH & SURVIVAL OF FINGERLING MAJOR CARPS DURING THE WINTER SEASON REARED UNDER POLY CULTURE SYSTEM

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This study was performed in four earthen ponds to check the wintering effects on growth and survival of *Catla catla* fingerlings in poly-culture system. Two ponds without green house were designated as Treatment-1(T₁) and two ponds covered with green house were designated as Treatment-2(T₂). The mean temperature, DO and pH throughout the experiment were found as 18.40 ± 20°C, 3.86 ± 17 mgL⁻¹ and 8.25 ± 0.16 under T₁ and 24.6 ± 11°C, 2.43 ± 0.09 mgL⁻¹ and 8.22 ± 0.16 under T₂, respectively. No significant difference was found between initial weights of carps in both treatments. The MANOVA results revealed that there was statistically significant difference (p<0.01) between two treatments for their response to fish growth. The post-hoc test revealed that weights of carps *i.e. Labeo rohita, Cirrhinus mrigala* and *Catla catla* were significantly higher in T₂ (with green house) as compared to T₁ (without green house) at p < .05. Survival rate (%) of the *Labeo rohita, Cirrhinus mrigala* and *Catla catla* calculated by total counting at the end of the experiment was 65, 57 and 21% under T₁ and 69, 74 and 72% in T₂, respectively.

CULTURE OF TOR MACROLEPIS (INDUS GOLDEN MAHSEER) WITH INDIAN AND CHINESE CARPS UNDER SEMI-INTENSIVE POND POLY CULTURE

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Semi-coldwater Indus golden mahseer (*Tor macrolepis*) fingerlings were cultured with rohu, mrigal, catla, silver carp and grass carp under semi-intensive pond composite fish culture at Fisheries Research & Training Institute Manawan, Lahore to evaluate its growth performance, survival and effect on the growth of other cultured carps in warm waters of central Punjab. The experiment was conducted in six earthen ponds (0.05 ha each) for 75 days. 2000/ha fish fingerlings were stocked in each pond using two treatments in triplicate. Treatment-1 (T₁) stocked with only major carps *viz.* rohu 30%, mrigal 15%, catla 25%, silver carp 15% and grass carp 15% while treatment-2 (T₂) with mahseer 20% and major carps *viz.* rohu 26%, mrigal 11%, catla 21%, silver carp 11% and grass carp 11%. Fortnight application of manure (cow dung) and inorganic fertilizers (DAP and Urea) were given throughout the experiment. Feed containing 25% crude protein was applied to fish, daily (once a day) @ 3% of live body weight both in T₁ and T₂. Sampling was done after every fortnight to record the data and at the end of experiment; all the six ponds were completely harvested to evaluate species wise survival growth and production. To statistically analyze the data, multivariate analysis of variance (MANOVA) and separate univariate ANOVAs were applied. Intra-species growth of rohu, catla and grass carp varied significantly (P < 0.05) among both treatments while of mrigal and silver carp did not differ statically in spite of slight variations. Increase in total length showed the same trend. The physico-chemical parameters remained within acceptable range for warm water fish culture.

EFFECTS OF BUFFER CONTROLLED VARIOUS pH RANGES ON AVAILABLE PHOSPHORUS RELEASE FROM ROCK PHOSPHATE AND PRESS MUD OF SUGAR MILL IN AQUATIC SYSTEMS

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An indoor comparative study was conducted to evaluate the efficiency of two fertilizers *i.e.*, apatite rock phosphate and press mud of sugar mills, to release phosphorus in the form of orthophosphate in water at three different buffer controlled pH ranges *i.e.*, 6.1-7.0, 7.1-8.0 and 8.1-9.0. The pH was controlled in the required ranges through the addition of requisite quantities of acetic acid and sodium acetate buffer. The experiment was conducted in two phases *i.e.*, during winter and summer seasons for a period of eight weeks in each phase. The objective of this attempt was to ascertain the optimum pH range for better phosphorous release from the use of these fertilizers. The impact of temperature and fertilizer dose was also determined to observe their correlation with the release of orthophosphates. The data incurred from both the winter & summer experiments revealed the same sequential results. However, the phosphorus release during the summer season was significantly higher than that released during the winter season for both the fertilizers. The maximum release of available phosphorus was observed in glass aquaria maintained at pH level 6.1-7.0 followed by those at 7.1-8.0 and 8.1-9.0, respectively. It was also recorded that amongst the two fertilizers, press mud of sugar mills released the available phosphorus at a significantly high rate as compared to the rock phosphate. Water quality was also continuously monitored during the entire period of the experimentation. Various physico-chemical parameters like water temperature, dissolved oxygen, pH, electrical conductivity, total alkalinity and total hardness were also analyzed to notice the variations with respect to changes in pH ranges as well as with the alteration of doses and types of fertilizers. From this research experiment, it has been concluded that maximum release of available phosphorus occurred from press mud of sugar mills in experimental treatment maintained at pH level of 6.1-7.0, during summer season. However, both rock phosphate & press mud of sugar mills proved to be an alternative and cheaper source of phosphorus as compared to the inorganic fertilizers that can be used to enhance the fish production both under intensive and semi-intensive fish farming system.

EVALUATION OF EXTRUDED AND NON-EXTRUDED FEED FOR ITS IMPACT ON GROWTH PERFORMANCE OF FINGERLINGS OF MAJOR CARPS

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The study was performed in six cemented tanks to check the evaluation of extruded and non extruded feed for its impact on growth performance of fingerling of Major carp. In three cemented tanks, extruded feed was used which were described as T₁ R₁, T₁R₂ and T₁R₃. In other set of three cemented tanks non-extruded feed was used which were mentioned as T₂R₁, T₂R₂ and T₂R₃. After 2nd month the MANOVA results revealed that there was statistically significant difference in growth (weight) of major carps for the two treatments (F=170.12, p=.000). The post-

hoc test revealed that weight of carps *i.e.* Thaila & Rohu were significant higher in T1(Extruded Feed) as compared to T2(Non-extruded Feed) at $P < .05$. Significant differences for the same species *i.e.* Thaila & Rohu were also observed after 3rd month at $p < .05$. While the weight of three species *i.e.* Rohu, Thaila & Mori differed significantly at the end of 4th month at $p < .05$.

PESTICIDE RESIDUES IN FLESH OF *LABEO ROHITA* COLLECTED FROM RIVER RAVI BETWEEN ITS STRETCHES FROM SHAHDARA TO BALLOKI HEADWORKS, PUNJAB-PAKISTAN

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Levels of DDT, DDE, Endosulfan, Endosulfan Sulfate, Carbofuran and Cartap were determined in the flesh of *Labeo rohita* collected from ten sampling sites of River Ravi between its stretches from Shahdara to Head Balloki by Gas Chromatograph equipped with electron capture detector (GC-ECD) method. All fish samples were found contaminated with varying concentrations of DDT, DDE, Endosulfan and Carbofuran while, Endosulfan Sulfate and Cartap were not detected. Levels of all pesticide residues were below the maximum residue limits (MRLs) for food standards. Pesticide concentrations in the fish flesh were ranged from 0.256 to 0.274 for DDT, 0.205 to 0.231 for DDE, 0.086 to 0.103 for Endosulfan and 0.168 to 0.185 $\mu\text{g g}^{-1}$ for Carbofuran. The results show that the pesticide concentrations in the fish flesh decreased in the order: DDT > DDE > Carbofuran > Endosulfan. After Degh fall and After Hudiara nulla Fall River sampling sites were found with highest pesticide concentrations. Findings of this study concludes that stretch of River Ravi from Shahdara to Balloki Headworks has a potential for polluting and rendering the water unfit for aquatic life so, constant monitoring programs are needed to be initiated to reform the present situation.

ESTIMATION OF BLOOB BIOCHEMISTRY OF *S. PLAGIOSTOMUS* DURING PRE SPAWING, SPAWING AND POST SPAWING PERIODS

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Blood biochemical parameters are often used to evaluate the health status and spawning period of fish. Specimens with different weight and length were randomly collected through the year, from Jan, 2012 to Dec, 2012 and examined biochemically. The gonads of *S. plagiostomus* were examined for its protein, glucose, total lipids, total cholesterol, and DNA / RNA ratio, as well as for urea contents during three different phases of pre-spawning, spawning and post-spawning. The level of total Protein, RNA and total lipids content were found lower in pre-spawning season (37.57 ± 6.13 , 58.7 ± 6.09 and 17.97 ± 2.31), respectively and significantly increased from pre-spawning to spawning period (84.20 ± 27.43 , 161.87 ± 30.7 and 35.58 ± 2.18) and declined significantly in post-spawning period (33.14 ± 7.60 , 58.15 ± 6.09 and 13.95 ± 2.52) (mg/g) at the 0.05 level. However, the level of serum cholesterol and glucose in gonads were highest during the pre (60.81 ± 6.29 , 60.59 ± 5.36) and post spawning periods (70.42 ± 4.75 , 57.28 ± 28.14). It was

observed that with the decrease in Gonado Somatic Index (GSI) values, cholesterol and glucose level also decreased (30.14 ± 3.57 , 29.142 ± 6.79). Similarly, it was observed that during the summer blood urea decrease (1.604 ± 0.01) and increase in winter (5.244 ± 1.40) in both sexes. It was significantly different at the 0.05 level. However, not any significant difference was observed in the DNA level during the whole reproductive cycle.

CHEMICAL AND MINERAL COMPOSITION OF *LABEO ROHITA* AND *WALLAGO ATTU* INHABITING RIVER INDUS IN MIANWALI DISTRICT

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Fishes are world widely accepted as a source of high quality protein, minerals and a rich source of poly-unsaturated fatty acids (PUFAs). In Pakistan white meat especially fish meat gained an important place in human diet due to its taste, nutritional quality and convenient price. This study was carried out to investigate the chemical composition and mineral profile of two fish species namely, *Labeo rohita* and *Wallago attu* inhabiting the River Indus in Mianwali District, which are indigenous and widely consumed in the study area. The study followed a 2 x 3 factorial arrangement by involving 2 fish species, each with 3 weight categories ($W1 \leq 1.0$, $W2 \leq 1.5$ and $W3 \leq 2.0$ Kg). Fishing was performed with the help of local fishermen. Sixty samples of each fish species were selected from three weight categories on the basis of their routine weights at which they are caught and sold in the study area. The chemical composition of fish muscles were assessed by following AOAC (1990). Selected minerals (Na, K, Ca, Mg, Fe, S, P, Zn, Mn, Ni, Pb, Cr, Hg, Cu, Cd) in fish muscles were analyzed by a Varian Vista-MPX CCD simultaneous ICP-AES (Varian Inc, Australia) machine located at Newcastle University, UK. Mineral concentrations were calculated as mg kg^{-1} wet weight. Minitab 16 software was used to test the main effects of fish species (Sp), weights (W) and their interaction for each mineral for significance at $P < 0.05$. The mean mineral contents in fish muscles were also compared with their permissible levels of European Commission (EC, 2006) and World Health Organization (WHO, 1986). It was investigated that crude protein content in *wallago attu* was 79.60% while in *labeo rohita* was 74.92%. Fat was reported as 18.67% and 13.33% in *labeo rohita* and *wallago attu*, respectively. While ash and total carbohydrates were reported as 4.95% and 4.46% and 1.46% and 2.60% in *labeo rohita* and *wallago attu*, respectively. The mean metal concentrations were either below the detection limit (BDL) or well below the permissible levels of EC or WHO for food fish. Generally metal concentrations increased with increased weight of fish. The order of mineral concentration was found as $P > Ca > K > Mg > Na > S > Fe > Zn > Mn > Cr > Pb > Ni > Hg$ in *Labeo rohita* and $P > K > Na > Mg > S > Ca > Fe > Zn > Cr > Pb > Ni > Mn > Hg$ in *Wallago attu*. Cadmium and copper were not detected in any of the fish species analysed. Levels of mineral elements in fish species were within the limits of international standards. Smaller fishes were high in Protein contents and low in fats and minerals, so these should be preferred by the consumers. As toxic minerals were well below the permissible levels of food fish so these fish species are safe for human consumption. This study have shown these Indus fish species as good source of nutrients to the consumers.

STOMACH CONTENTS ANALYSIS OF GOLDLINED SEABREAM *RHABDOSARGUS SARBA* (FAM: SPARIDAE) FROM THE COASTAL WATERS OF KARACHI, PAKISTANSHABIR ALI AMIR^{1,2*}, PIRZADA J. A. SIDDIQUI¹, SHAHNAZ RASHID³ AND SAFIA MUSHTAQ¹¹*Centre of Excellence in Marine Biology, University of Karachi- Pakistan,*²*Pakistan Museum of Natural History, Garden Avenue, Shakarparian, Islamabad-Pakistan*³*Institute of Marine Sciences, University of Karachi-Pakistan.***Email: shabiramir@yahoo.com*

The food and feeding habits of sparid bream, *Rhabdosargus sarba* was investigated by examining the stomach contents of 504 fishes. These fishes were collected on monthly basis from fish landing site "Ibrahim Hydri" Karachi, Pakistan during May 2010 to April 2011. The sparid breams are demersal fishes usually found in shallow coastal waters of about 3 m to deeper offshore waters of 50 m. The collected specimens of *R. sarba* ranged between 13cm to 44cm in length while weighing from 38 to 1164 grams. The analysis of stomach contents revealed that the diet of *R. sarba* is mainly composed of marine benthic invertebrate organisms including mollusca (bivalve, cephalopod, gastropod), crustacean (decapods, stomatopods, isopods, amphiphods) and polychaetes while small amounts of fish remains (scales and some parts of fish body) and filamentous algae were also observed in their diets. Filamentous algae were mainly present in the fish stomachs during winter season. Index of Relative importance (IRI) of prey items consumed by *R. sarba* was investigated for the main items as per their diet contents. This study also exposes *R. sarba* as an opportunist feeder where its main food items composes of invertebrates mainly the molluscan and crustacean.

PHYTASE SUPPLEMENTATION IMPROVES GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY OF *LABEO ROHITA* FINGERLINGS FED ON CANOLA MEAL-BASED DIETSSYED MAKHDOOM HUSSAIN^{*1,2} AND MUHAMMAD AFZAL²¹*Department of Zoology, Wildlife and Fisheries, Government College University, Faisalabad, Pakistan*²*Department of Zoology and Fisheries, University of Agriculture, Faisalabad, Pakistan*** Corresponding Author's email address: drmakhdoom90@gmail.com*

Effect of phytase supplementation to canola meal-based test diets to assess the optimal dose required for higher growth performance and nutrient digestibility coefficients of *Labeo rohita* fingerlings (avg. wt. 7.04g) through a sixty days indoor feeding trial. An extruded reference diet was prepared having 30% crude protein and 4% gross energy. The experimental diet having approximately similar caloric and protein values was formulated by mixing 70% reference diet and 30% canola meal. This experimental diet was further divided into seven test diets and were sprayed by graded levels (0, 250, 500, 750, 1000, 1250 and 1500 FTU kg⁻¹) of phytase enzyme. Chromic oxide was used as inert marker in reference and test diets. Quadratic relationship was observed between growth, nutrient digestibility parameters and phytase levels. Phytase supplementation at 750 FTU kg⁻¹ followed by 1000 FTU kg⁻¹ effectively enhanced most of the growth and nutrient digestibility parameters as compared to reference diet, control (0 FTU kg⁻¹) and other levels of phytase supplementation. It is suggested that performance of canola meal based diets can be

improved by supplementing 750 FTU kg⁻¹ level in practical diets of *Labeo rohita*. Our results suggest that phytase supplementation to canola meal-based diets at 750 FTU kg⁻¹ level is adequate to release chelated nutrients for better growth performance of *Labeo rohita* fingerlings.

IDENTIFICATION AND CHARACTERIZATION OF PATHOGENS IN FISH IN DIFFERENT AREAS OF PUNJAB

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In the present study, 152 diseased fish were studied. The study of various fish farms revealed some cases of diseases and infection. The majority of cases (68%) have abdominal dropsy, Lernaeciasis and Anoxia. All culture-able fish species of Pakistan, *Labeo rohita*, *Cirrhinus mrigala*, *Catla catla*, *Hypophthalmichthys molitrix* and *Ctenopharyngodon idella* were found to be infected with different diseases. *Catla catla* in this study was found to be least affected species. The most important reason for the occurrence of fish diseases is the addition of organic manure and decayed food into fish pond. The diseases in fish can be prevented through recent improved and management techniques.

ROLE OF PHYTASE SUPPLEMENTATION IN IMPROVING MINERAL DIGESTIBILITY FOR *LABEO ROHITA* FINGERLINGS FED ON CANOLA MEAL-BASED DIETS

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A feeding trial was conducted for 60 days to evaluate the effect of microbial phytase on mineral digestibility of *Labeo rohita* fingerlings fed on canola meal based diets. The experimental diet was consisted of 70% reference diet and 30% test ingredient (canola meal). Seven test diets were prepared by spraying graded levels (0, 250, 500, 750, 1000, 1250 and 1500 FTU kg⁻¹ diet) of phytase to canola meal-based experimental diet to assess the optimal dose required to achieve best performance in terms of mineral digestibility coefficients. Chromic oxide was incorporated as indigestible marker in the diets. Phytase supplementation significantly enhanced the mineral digestibility of *Labeo rohita* fingerlings at 750 followed by 1000 FTU kg⁻¹ level as compared to reference diet. The results of our study suggested that phytase supplementation to canola meal-based diet at 750 FTU kg⁻¹ level is optimum to release sufficient chelated minerals for *Labeo rohita*.

SENSORY EVALUATION OF VARIOUS VALUE ADDED FISH PRODUCTS DEVELOPED FROM FRESH WATER FARMED CARPS

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Public carp fish consumption demand is low due to presence of intramuscular bones. Production of boneless value added fish products is a useful strategy round the globe that enhances consumer acceptability and reduces post-harvest losses. During preset study eight value added products developed from carp species namely fish fillets, fish balls, fish nuggets, fish kebabs, fish rolls, fish sandwiches, minced fish and fish roe sandwiches were prepared to determine consumers' acceptability. Sensory attributes used for sensory evaluation were appearance, odor, texture, flavor and overall acceptability. Five point hedonic scale methodology was used for evaluation of acceptability of value added fish products, 1 very bad; 2 bad; 3 middle; 4 good and 5 very good. Sensory panel was comprised of 10 evaluators. Overall acceptability of all the eight fish products in decreasing order was minced fish (%), overall acceptability was highest for minced fish (98.5%), fish roe sandwiches (91.5%), fish nuggets (86.5%), fish kebabs (86%), fish sandwiches (84%), fish balls (83.5%), fish fillets (83%), fish rolls (72.5%). The results were encouraging and prospect for market of these fish products is very bright.

CRYOPRESERVATION OF MILT OF *LABEO ROHITA* AND *CIRRHINUS MRIGALA*

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Cryopreservation of milt is not only important for successful breeding but also for the conservation and genetic improvement of fish resources. The success of cryopreservation of carps milt depends on extender solution, milt quality, choice of cryoprotectant and cooling/thawing conditions. These studies reveal that sperms of both *Labeo rohita* and *Cirrhinus mrigala* can be successfully preserved for future use on demand. During these studies a protocol was developed for the cryopreservation of milt of *Labeo rohita* and *Cirrhinus mrigala*. This paper describes comprehensive study of various aspects of milt cryopreservation such as milt collection, sperm count in milt, sperm viability assessment, extender composition, cryoprotectant equilibrium conditions, live dead ratio and effect of thawing duration on cryopreserved milt. The milt of both carps *Labeo rohita* and *Cirrhinus mrigala* was collected in separate petri dishes containing diluents (of extender and cryoprotectant). Modified extender -C was used to increase the sperm viability. DMSO (dimethyl-sulfoxide) was used as the cryoprotectant. Volume of milt used was 3 ml of each above mentioned carp species. After addition of diluent it became 20 ml. This high ratio of milt and diluent was due to high concentration of milt. 70-80% sperms of both species were motile during pre-thaw evaluation. Sperm count in *Labeo rohita* was 1300 million sperms/ml while in *Cirrhinus mrigala* it was 1100 million sperms/ml. Sperm live/dead ratio was 70:30%. Polyvinyl straws were

kept in cooling chamber at 5°C for 2 hours. Straws were then cryopreserved in liquid nitrogen. Straws were thawed at 25°C for 30 seconds and a post-thaw motility of sperms was 50-55%.

EFFECT OF SALT CONCENTRATION ON DIFFERENT FISH SPECIES AT AMBIENT TEMPERATURE

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In this study effect of different salt concentrations on *Catla catla* and *Hypophthalmichthys molitrix* for different time periods were studied at ambient temperature. *Catla catla* and *Hypophthalmichthys molitrix* with average weight of 350g were collected from fish ponds C block Ravi campus, Pattoki. There were three treatments with different salt concentrations 20%, 25%, 30% and 0% control. Effect of different salt concentration was also studied for different time periods of 10, 20 and 30 days. Sensory evaluation of salted fish was conducted for different sensory attributes such as color, flavor, texture and appearance. Overall acceptability was highest for 25% salt concentration. Results showed significant difference in chemical composition and consumer acceptability at different salt concentrations. At 30% salt concentration fish species showed lowest moisture content, longer shelf life but unacceptable among consumer due to high salt contents. Results showed best salt concentration for *Catla catla* and *Hypophthalmichthys molitrix* is 25% for the time period of 20 and 25 days.

EFFECTS OF PHYTOADDITIVES ON GROWTH AND BLOOD CHEMISTRY OF JUVENILE *LABEO ROHITA*

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Effects of phytoadditives (onion and garlic) were investigated on growth performance and blood chemistry of *Labeo rohita*. Fish were divided into four groups viz a control and three treatments with different garlic & onion levels (T₁, T₂, T₃) with two replicates each. Fish were housed in fiberglass tanks with 126.05mg/liter stocking density and studies were continued for 76 days. There were 4 diets with 25% protein (control), 30% protein with garlic 3% (T₁), 35% protein onion 3% (T₂) 40% protein level garlic 5% + onion 5% Fish were fed @ 5% wet biomass of fish. Initial average weight of control group T₁, T₂, and T₃, was 28.53 g, 29.46, 33.60 and 34.76 respectively. Physico-chemical parameters pH, temperature and Dissolved oxygen were checked daily. Nitrate, phosphate tests and survival and body weights were measured regularly on demand. Survival rate was significantly higher in control compared to all treatments but growth was high in Group 4 with garlic + onion and 40% C.P level. In organoleptic test color, flavor, juiciness, oiliness and overall acceptability were significantly higher in Group 4 with combination of both additives and tenderness was high in control group. WBCs were high in Group 4 and Group 3 and Red blood cells were significantly high in Group 3 fed on garlic-supplemented diet.

BREEDING AND EMBRYONIC DEVELOPMENT OF *CIRRHINUS MRIGALA*

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Cirrhinus mrigala breeds naturally in rivers during monsoon season. In confined conditions it is induced spawned by the administration of ovaprim or its analogues. Nevertheless conditions of induction are quite variable from place to place so is the dosage of ovaprim. The present attempt was to standardize the breeding protocol of this species under prevailing environmental conditions at Ravi Campus Pattoki. Spawning was observed after 9 ½ hours after ovaprim injection at 29^oC. Unfertilized eggs were about 2.5mm in diameter. The egg diameter increased after swelling in water. The fertilized eggs were non-adhesive and transparent with diameter ranging from 4.6 mm to 5.0 mm. 29^oC was Found Best for maximum Egg Release, Fertilization and Hatching of the eggs of *Cirrhinus mrigala*. Blastodisc stage was observed after 17 minutes of fertilization leading to 32cell stage after 1 hour of fertilization (29^oC). Incubation period was 16 hours. The eggs of *Cirrhinus mrigala* showed rapid development and larvae started to move in the egg after 13 hours of incubation. Hatching completed in 15 hours. The hatchlings were transparent and were 2.9 mm to 3.2 mm in length. The hatched larvae received their nourishment from the egg yolk until development of mouth. Absorption of yolk sac completed in about three days.

**HAEMATOLOGICAL CHARACTERISTICS OF FISH ARE INDICATORS OF ITS
PHYSICAL STATUS**

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The studies were designed to investigate the possible variation in various hematological indices of different cultureable fish species. Five fish species comprising of three Indian and two / Chinese carps were stocked in earthen ponds of half acre each. Stocking was done following the standard stocking species and combination ratios. Studies were started in August and ended at the end of October. During this period all the fishes were fed 30% protein containing diet formulated by combination of locally available feed ingredients. Feed was offered daily at the rate of 2% of live fish body weight at 9:00 AM and 4:00 PM regularly. In addition to that inorganic and organic fertilizers were also added regularly following the existing procedure and protocol. During this period Temperature, DO and pH were regularly monitored at risk hours to avoid any set back if it is expected in the very near future. Studies were continued for three months. Experimental protocol including feeding, water quality monitoring were uniform throughout the study period. Both weight and length of fish were measured at the outset of experiment for growth comparison gained during experimental period. At the end of experimental duration fish were harvested and weighed. Ten sample of each fish species under study were randomly selected from the bulk stock and brought to the hatchery for further assessment of envisioned blood chemistry parameters. Each fish was

euthanized and heparinized syringe was inserted in caudal fin and required volume of blood was drawn and transferred to vacutainer containing EDTA (anticoagulant). After collection of required volume of blood fishes were released into respective ponds. Blood was analyzed for Hb (Haemoglobin), RBC (Red Blood Cells), WBC (White Blood Cells), Thromb (Thrombocytes), PCV (Packed Cell Volume), MCV (Mean Cell Volume), MCH (Mean Cell Haemoglobin), MCHC (Mean Cell Haemoglobin Concentration), LCT (Leucocrit), and DLC (Differential Leucocytes Count). It was observed that Hb remained uniform among all the blood samples collected from different fish species. RBCs followed the same trend. Significant differences were observed when compared with different fish species. PCV, MCV, MCH though differed considerably among the species but majority showed uniform concentrations. Monocytes however, differed significantly when compared among species. It was interesting to note that there were no basophiles in any fish species. When these parameters were statistically correlated several of them showed negative correlation with each other while some were strongly positively correlated.

EFFECT OF INCLUSION OF *CONOCARPUS* IN FEED OF *LABEO ROHITA* AND ITS EFFECT ON GROWTH, HEMATOLOGY AND ORGANOLEPTIC QUALITIES OF FLESH

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Studies were designed to investigate the effect of inclusion of *Conocarpus erectus* (Proximate analysis; moisture 74.84; protein 12.57%; fats 5.33%; ash 24.18%; crude fiber 12.68% and phosphorus 0.69%). In *Labeo rohita* feed as one of the ingredients on growth, level of WBCs in blood and organoleptic qualities of flesh. Fish were housed in fiber glass circular tanks. There were two treatments and a control with two replicates in each. Control diet contained 25% protein and was composed of conventional ingredients. 25% of the total ingredients were replaced with *Conocarpus*, a fast growing terrestrial plant in diet of treatment 1. Level of *Conocarpus erectus* was further enhanced in diet for treatment 2 where its inclusion in total diet was 50%. Feed was offered to fish @5% of its body weight. Initial and final weight of *Labeo rohita* fingerlings was 44.74, 34.24 and 13.88g and 63.58, 44.05 and 23.42 g in Control, T₁ and T₂ respectively. T₂ showed significantly higher growth (68%) than control (42%) and T₁ (28%). Organoleptic test (color, flavor, juiciness, oiliness and overall acceptability) were observed in the range of 6 to 8 in control and T₂ but in T₁ values ranged from 6-9, respectively. White blood cell count was 15.73, 18.40 and 15.48 million cells in control, T₁ and T₂ respectively. Survival rates and relative level of protection against challenge infection were significantly higher in T₁ and T₀ fed with *Conocarpus*-supplemented diet at different level of crude protein than T₂ treatments. WBCs levels were non-significant and same was the case with infection levels and survival rates. Studies reveal that up to 50% of the feed can be replaced with normal feed from conventional ingredients without affecting growth and organoleptic qualities of fish ultimately significantly reducing feed prices.

EFFECT OF ARTIFICIAL FEED AND FERTILIZATION ON GROWTH AND BODY COMPOSITION OF GENETICALLY IMPROVED FARMED TILAPIA (GIFT)ANAM ZAHID¹, NOOR KHAN¹, MUHAMMAD ASHRAF¹, SANA ASHRAF², HUMA ZAHRA¹, NIDA ISMAT¹, SAJID MAHMOOD¹ AND WAQAS ALI¹¹*Department of Fisheries & Aquaculture, University of Veterinary & Animal Sciences, Lahore.*²*Department of Wildlife & Ecology, University of Veterinary & Animal Sciences, Lahore.*

The study was conducted to evaluate the effect of artificial feed and fertilization on growth and body composition of genetically improved farmed tilapia (GIFT) in earthen ponds. A total of 160 fish were stocked in each pond. In pond 1 supplementary feed containing 30% crude protein, cow dung and poultry manure was applied while in pond 2 only supplementary feed with 30% crude protein was given at the rate of 4% fish body weight. The study was conducted for three months. The results of the study revealed that fish gained significantly higher ($p < 0.05$) weight gain in Pond 1 as compared to pond 2. The standard growth rate (SGR) and feed conversion ratio (FCR) also showed significantly higher ($p < 0.05$) values in pond 1 as compared to pond 2. In proximate analysis of fish only crude protein and lipids showed significantly higher ($p < 0.05$) value in pond 1 as compared to pond 2. The physico-chemical parameters were found in acceptable range except DO (2.23-3.87 mg/l); pH (6.88-7.05), temperature (25-26°C), conductivity (1.0-1.9 $\mu\text{S/cm}$), chloride (110-230 mg/l), total hardness (130-298 mg/l) TDS (654-1054 mg/l) and alkalinity (120-460 mg/l) in both treatments. It is concluded that GIFT strain grow much better with the combination of organic manure (cow dung and poultry manure) and artificial feed as compared to artificial feed alone in semi-intensive system.

POPULATION ESTIMATION OF BROWN TROUT, *SALMO TRUTTA* AND RAINBOW TROUT, *ONCORHYNCHUS MYKISS* IN SWAT RIVERTARIQ KHAN¹, SUMAIRA ABBAS¹, ARSHAD JAVID², MUHAMMAD ASHRAF¹, NOOR KHAN¹, FARZANA ABBAS¹, SABIR KHAN³ AND MUHAMMAD AHMAD¹¹*Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences, Lahore*²*Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Lahore*³*Department of dairy technology, University of Veterinary and Animal Sciences, Lahore*

Total length of Swat river is 250 kilometers from Kalam to river Kabul near Charsadda. The upper part of the river provides habitat to the cold water fish fauna. Two economically important fish species of the area are brown trout and rainbow trout that were introduced in Swat river in 1928. These fish species contribute to the economy of the area by creating employment opportunities and a quality protein diet to local people and the tourists. The present study was planned to find out the population status of brown trout and rainbow trout along a 50 Km long belt of Swat river, from Madayan to Mahoo dand village. Four sampling sites (SSs) namely Madayan (SS₁), Mainkiyal (SS₂), Kalam (SS₃) and Mahoo dand (SS₄) were fixed, visited fortnightly and fish samples were captured through cast nets. Netting index of rainbow trout at SA₁, SA₂, SA₃ and SA₄ was 0.750, 0.082, 0.084 and 0.095, respectively while the netting index of brown trout at SA₁ was 0, at SA₂ 0.670, at SA₃ 0.675 and at SA₄ 0.663. Similarly the percent relative abundance of rainbow trout was 100% at SA₁, 10.86% at SA₂, 11.11% at SA₃ and 12.62% at SA₄ while the same was 0% at SA₁, 89% at SA₂, 88% at SA₃ and 87% at SA₄ for brown trout.

ICHTHYODIVERSITY IN RELATION TO PHYSICO-CHEMICAL PARAMETERS OF RIVER SWAT

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Fish population of any given aquatic habitat can vary significantly from year to year. Consequently, it is necessary to be carried out several studies in the consecutive years in order to get clear picture. The stability of fish is of profound importance due to urgent need of environmental management to know how much fish population naturally change over time. Fish are appropriate indicators of trends in aquatic environment because of the impact they have on the distribution and abundance of other organisms in the water they inhabit. Therefore it is needed to assess the fish distribution and abundance in order to give a good insight into the state of biological production in the stem and the physico- chemical characteristics in evaluating future changes that may occur in response to different anthropogenic activities therefore the study was conducted on the ichthyodiversity of river Swat, Khyber Pakhtunkhwa. The river Swat was divided into four stations namely Kalam, Madyan, Fatehpur and Khwaza Khela. The data was collected month wise from May to December 2012. Twelve fish species belonging to 10 genera, 4 families and 3 orders were recorded during the present study. The family Cyprinidae was dominant with 7 species followed by family Balitoridae with 3 species, Sisoridae and Salmonidae each with 1 specie. Ichthyodiversity was assessed by calculating the various diversity indices such as Shannon-Weiner diversity index, Simpson's index of diversity, Pielou's evenness. Along with the ichthyodiversity, limnological factors were also measured and recorded. Correlation and regression analysis were performed for species abundance and different limnological variables. High values for Shannon-Weiner index, Simpson's diversity index and Pielou's evenness were observed in Khwaza Khela, followed by Fatehpur, Madyan and Kalam. Diversity was higher at Khwaza Khela and lower at Kalam. In the study period high diversity was observed from May to October and low in the months of November and December. The most abundant species was found to be *Schizothorax richardsonii*. The least abundant species was *Salmo trutta fario* among the available species. Significant correlations of fish abundance with Temperature and Dissolved Oxygen were found. The remaining parameters pH, Electrical Conductivity, Chlorides, Salinity and Total Dissolved Solids effects were negligible. On the basis of the current study we conclude that temperature is the deciding factor in dissolved oxygen concentration. These both temperature and dissolved oxygen then decide the diversity of the fish fauna.

BREEDING AND SEED TRANSPORTATION OF *CIRRHINUS MRIGALA*

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Hatchery is main source of fry for fish culture. It is a "place for artificial breeding, hatching and rearing. Hatcheries produce larval and juvenile fish (and shellfish and crustaceans) primarily to

support the aquaculture industry. Carp hatchery includes circular tanks for breeding which provide Riverine conditions for fish breeding and egg development. Rectangular tank was used for holding fish for seed transportation and other purposes. 0.5ml ovaprim was administered using a 5ml syringe. Stripping was accomplished after 8 hours of hormone administration. Fecundity was 88540 eggs/kg female body weight. Different embryonic development stages were observed at different time intervals including fertilized egg, Blastodisc stage, 2 cell stage, 4 cell stage, 8 cell stage, 16 cell stage, 32 cell stage, early morula, late morula, gastrula, yolk absorption stage, optic bud formation, somite stage and hatchling. Fry were transferred into nursery ponds after yolk sac absorption which completed in three days. Seed was transported in polythene bags containing clean, cool and oxygen rich water; bag was tied with plastic string on both sides after stocking fish in it. Optimum stocking density remained 95-143 g/l fingerlings. 1000 to 1500 fingerlings were stocked in a plastic bag of 42 liter volume with maximum mortality rate ranging from 80-100 at the destination of transport. Time duration for transportation with 80% survival rate was observed 5 hours from packing to destination.

EFFECT OF DIFFERENT DIETARY PROTEIN CONCENTRATION ON THE SURVIVAL, GROWTH POTENTIAL AND BODY COMPOSITION OF GMT IN FLOW THROUGH AND SEMI-INTENSIVE CULTURE SYSTEM

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The study was conducted to investigate the effect of artificial feed containing 30%, 35% and 40% crude protein level on the growth, survival and body composition of Genetically Modified Tilapia (GMT) in intensive rearing condition. Mono sex GMT achieved greater individual weight and length than mixed-sex fish. Faster growth of mono sex GMT has been related to the lack of energy expenditure in breeding. The results of fish growth showed significant differences for final body weight, gain in weight, percentage weight gain, specific growth rate and food conservation ratio for all treatment of mix sex culture and mono sex culture. The results of physico-chemical parameters of water quality were within the optimum range for all the treatment of mix sex culture and mono sex culture of GMT. During entire study period no mortality was observed. No significant differences were observed for temperature, pH, salinity, TDS and nitrates while dissolved oxygen at 40% CP level showed significant difference compared to 35% and 30% CP level.

COMPARATIVE EFFECT OF NATURAL AND ARTIFICIAL FEED ON GROWTH, SURVIVAL AND ORGAN DEVELOPMENT IN LARVAL INDIAN MAJOR CARPS

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This study was carried out to determine the effects of live feed (*Brachionus calyciflorus*) and artificial feed (40% CP) on growth and survival of fry of *Cirrhinus mrigala*, *Catla catla* and hybrid

fry (*catla catla* X *Labeo rohita*) at a stocking density of 100 fry per tank. The duration of each trial was 15 days. Fish fed on natural food of rotifers (*Brachionus calyciflorus*) served as control while second group was fed on 40% crude protein containing feed. Among the different feeding trials hybrid larvae fed on artificial feed gained maximum weight (BWG) 52.7mg which was significantly higher than control group. When larvae of different fish species were ranked in decreasing order the following trend emerged hybrid 52.7mg > *Cirrhinus mirigala* 44.6mg > *Catla catla* 32 mg. While larvae fed on control food in all the species grew in the following order hybrid 46.7 mg > *Cirrhinus mirigala* 35.6mg > *Catla catla* 26.4 mg. The hybrid fry nourished on artificial feed displayed maximum survival (71%). Survival in other fish species ranked as hybrid 71% > *Cirrhinus mirigala* 61% > *Catla catla* 52%; live feed: hybrid 64% > *cirrhinus mirigala* 53% > *Catla catla* 44%. Histology of alimentary tract showed considerable muscle deformation in control group but there were slight changes in larvae fed on artificial feed.

FEEDING HABITS OF CYPRINIDAE AND SALMONIDAE IN RIVER SWAT, KP, NORTHERN PAKISTAN

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Aquaculture had gained interest due to the importance of fish as a cheap source of animal protein. Fish require adequate nutrition for proper growth and survival. Analysis of the gut content of fish could provide information about the niche of the particular fish in its ecosystem. Study on food and feeding habits offer information on seasonal changes of fish fauna. The type and significance of available food and season of occurrence of food play important role in the life history of a fish. This information might be beneficial for establishing the inter-specific feeding relationship among different fishes of economic importance. Therefore the current study was conducted on the feeding habits of two fish species (*S. plagiostomus* and *S. esocinus*.) of family Cyprinidae, and one specie (*S. trutta fario*) of family Salmonidae in River Swat, Khyber Pukhtoonkhwa. The River Swat was divided into five stations namely Kalam, Behrain, Madyan, Fatehpur and Khwaza Khela. The fish specimens were collected month wise from May to December 2012. Guts of fish specimens were analyzed and identified. Feeding diversity was assessed by calculating the various diversity indices including Shannon-Weiner diversity index, Simpson's index of diversity, Pielou's evenness. Populations of different orders of insects were compared month wise as well as station wise using student t-test. *S. trutta fario* was found to be the fish with maximum diversity of aquatic and terrestrial insects in its gut content. Among them, the order Diptera was found to be the most abundant (29%), followed by Tricoptera (19%), Plecoptera (18%), Hemiptera (17%), Ephemeroptera (14%), Hymenoptera (3%) and Coleoptera (1%). For *S. plagiostomus* insects belonging to order Diptera (91%) were the main prey whereas the Detritus (9%) were also found in the gut contents of it. For *S. esocinus* was the insects of order Diptera made 75% of the consumed food, whereas the remaining insects from the gut content belonged to order Tricoptera (12%), Detritus (10%), and order Coleopteran (4%). High values for Shannon-Weiner index and Simpson's diversity index were observed in Kalam whereas Pielou's evenness index at Madyan. The most consumed prey was the Diptera. The least consumed prey was the Coleoptera among the available species. This study highlights the role of Diptera as a major food

source for the cold water fishes. On the basis of this we recommend conservation of Dipteran by avoiding water pollution because it may lead to extinction of cold water fish fauna.

GROWTH PERFORMANCE OF CHANNEL CATFISH (*ICTALURUS PUNCTATUS*) IN FERTILIZED PONDS WITH AND WITHOUT ARTIFICIAL FEEDING

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The present study was conducted to find out the growth performance of channel catfish in six fertilized earthen ponds at Aquaculture and Fisheries Facilities, National Agricultural Research Council (NARC), Islamabad. The experiment was carried out with two treatments T₁ (fertilized ponds) and T₂ (fertilized ponds + artificial feed) and each treatment was replicated thrice. All the six ponds (0.04 ha each) were stocked with 120 fingerlings of channel cat fish, *Ictalurus punctatus*. Initial body weight and total length of fingerlings at the time of stocking and thereafter increase in body weight and length was taken on fortnightly basis for a period of four months. No artificial feeding was added in T₁ ponds while the T₂ ponds were provided with pelleted feed (30% CP) twice daily. Physico-chemical variables viz. temperature, dissolved oxygen, alkalinity, total hardness and electrical conductivity of both T₁ and T₂ ponds were monitored on weekly basis. Statistically significant (P<0.05) weight gain was observed in T₂ ponds as compared to the fish from T₁ ponds.

COMBINED EFFECTS OF COW-DUNG AND POULTRY-DROPPINGS ON THE GROWTH PERFORMANCE OF MAJOR CARPS

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The present study was conducted to observe the effect of cow dung and poultry droppings on growth performance of major carps. Experiment was conducted in four earthen ponds of dimensions 25m x 18m x 1.5m (length x width x depth). Ponds were designated as control, T₁, T₂ and T₃. Treated ponds were fertilized: T₁ with cow-dung @ 10 kg/pond/day, T₂ with poultry droppings @ 10 kg/pond/day and T₃ with cow-dung and poultry droppings @ 10 kg/pond/day (5+5 kg) while no organic manure was added in control pond. The fish were weighed and measured at the time of stocking and thereafter increase in weight and length was noted on fortnightly basis for a period of five months. Stocking density in each pond was *Catla catla* 15, *Labeo rohita* 20 and *Cirrhinus mrigala* 15. Statistically significant variations in increase in weight and length were observed between control and treatment ponds in order of T₃>T₂>T₁>control. T₃ showed 2.7 times greater net fish production as compared to control pond similarly T₂ and T₁ showed 2.5 and 2.3 times greater fish production, respectively as compared to control pond. Our study reveals that pond manuring with both cow-dung and poultry-droppings gives better production.

**EFFECT OF VARYING STOCKING DENSITY OF BOTTOM FEEDER FISH
CIRRHINUS MRIGALA AND *CYPRINUS CARPIO* ON GROWTH PERFORMANCE AND
FISH YIELD IN POLYCULTURE SYSTEM**

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The present project was planned to study gradual replacement of *Cirrhinus mrigala* with *Cyprinus carpio* and its impact on pond ecosystem. 900 fish individuals belonging to six species viz. *Catla catla*, *Labeo rohita*, *Cirrhinus mrigala*, *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix* and *Cyprinus carpio* were stocked in four earthen ponds. The dimensions of each pond were 220 X 198 X 7 feet length, breadth and depth. The stocking density in pond 1 (T1) was *C. catla* 150, *L. rohita* 200, *C. mrigala* 200, *C. idella* 150, *H. molitrix* 150 and *C. carpio* 50. The stocking density of *C. mrigala* and *C. carpio* in pond 2 (T2) was 150 and 100, in pond 3 (T3), 100 and 150 and in pond 4 (T4) it was 50 and 200, respectively while the stocking density of all the other four fish species remained constant in all the four ponds. All the fish were fed with a diet of 25.16% crude protein @ 2% body weight. *C. idella* and *C. mrigala* showed maximum growth in T1, *C. catla* and *H. molitrix* in T2, *L. rohita* and *C. carpio* in T3. Maximum growth was observed in T3 followed by T4, T1 and T2. Among fish species *C. idella* and *C. carpio* showed higher growth rates than the rest of fish species. Our results revealed that in polyculture system stocking density of *C. mrigala* and *C. carpio* in a ratio of 1: 1.5 gives better results.

**EFFICACY OF PHYTASE SUPPLEMENTATION ON GROWTH PERFORMANCE AND
MINERAL DIGESTIBILITY FOR *LABEO ROHITA* FINGERLINGS FED ON
COTTONSEED MEAL-BASED DIETS**

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A feeding trial was conducted for 60 days to evaluate the effect of microbial phytase on growth performance and mineral digestibility for *Labeo rohita* fingerlings. The test diet was consisted of 70% reference diet and 30% of test ingredient (cottonseed meal). Seven test diets were prepared by supplementing graded levels (0, 250, 500, 750, 1000, 1250 and 1500 FTU kg⁻¹) of phytase to cottonseed meal-based test diets to assess the optimal dose required for higher fish production, growth and mineral digestibility coefficients. The chromic oxide was incorporated as non-digestible marker in the feed. Phytase supplementation effectively enhanced the growth performance and mineral digestibility of *Labeo rohita* fingerlings at 750 FTU kg⁻¹ level followed by 1000 FTU kg⁻¹ level as compared to reference diet. Relationship between phytase doses and various

growth and digestibility indicators were also established through quadratic regression equations. Our results suggest that phytase supplementation to cottonseed meal-based diets at 750 FTU kg⁻¹ level is adequate to release chelated minerals for optimal growth performance of *Labeo rohita* fingerlings.

POTENTIAL OF PHYTASE AND CITRIC ACID TREATED CANOLA MEAL-BASED DIET TO ENHANCE THE MINERAL BIO-AVAILABILITY IN *LABEO ROHITA*

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Effect of phytase (PHY) and citric acid (CA) supplementation on digestibility of minerals in Rohu, *Labeo rohita* fingerlings fed on canola meal based diet was investigated. Nine experimental diets were prepared by supplementing with CA (%) and PHY (FTU kg⁻¹) at the level of 0,0; 0, 1000; 0, 2000; 1.5, 0; 1.5, 1000; 1.5, 2000; 3, 0; 3, 1000; 3, 2000 respectively. Chromic oxide was used as inert marker in diet to estimate mineral digestibility. Ca, P, Na, K, Mg, Fe, Cu, Mn and Zn showed higher (p<0.05) level of apparent digestibility coefficient (ADC%) in PHY supplemented fish compare to control group. Similarly, CA addition also improved (p<0.05) ADC% of Ca, P, Na, K, Mg, Fe and Cu. However, ADC% of Mn and Zn showed statistically non-significant (p<0.05) but numerically higher values than control group. Also, both the additives (CA and PHY) act synergistically to improve (p<0.05) the minerals digestibility. In conclusion, both the additives (CA and PHY) showed improved digestibility of minerals separately as well as in combination. These results will be helpful to prepare cost effective and environment friendly feed by minimizing the mineral supplementation and their discharge into natural water bodies.

ROLE OF PHYTASE AND CITRIC ACID TO IMPROVE THE NUTRIENT DIGESTIBILITY IN *LABEO ROHITA* FINGERLINGS FED ON CANOLA MEAL-BASED DIET

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A 10 weeks feeding trial was conducted to study the effect of phytase (PHY) and citric acid (CA) supplementation on nutrient digestibility in *Labeo rohita* fingerlings fed on canola meal based diet. Nine experimental diets were prepared by supplementing with CA (%) and PHY (FTU kg⁻¹) at the level of 0,0; 0, 1000; 0, 2000; 1.5, 0; 1.5, 1000; 1.5, 2000; 3, 0; 3, 1000; 3, 2000 respectively. Nutrient digestibility was estimated by using chromic oxide as an inert marker. The higher (p<0.05) digestibility of crude protein, crude fat, and gross energy was observed in fish fed diet supplemented with 1000 FTU kg⁻¹ PHY as compare to control group. Similarly, CA showed improved (p<0.05) digestibility of nutrients at 3% supplementation level. A significant (p<0.05) synergistic effect between CA and PHY was also observed for the digestibility of these nutrients. In

conclusion, both the additives (CA and PHY) showed improved digestibility of nutrients separately as well as in combination. These results showed that CA and PHY have sufficient potential to hydrolyze the phytate to release the bounded nutrients.

GROWTH RESPONSES OF MAJOR CARPS REARED UNDER CHRONIC STRESS OF CHROMIUM

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For the determination of growth responses of major carps viz. *Catal catla*, *Labeo rohita* and *Cirrhina mrigala* under chronic stress of chromium in the laboratory a trail for 12-week was conducted. The fish used in this study were 240-day age group. The growth performance viz. wet weight increase, fork and total length increase, feed intake and feed conversion efficiency were determined. The fish were fed with feed containing a diet 30% DP and 3.14 Kcalg⁻¹ DE to satiation daily. At the termination of growth trial, the fish were dissected and bones, gills, gut, intestine, kidney, liver, scales, skin, muscle and fats isolated for the determination of chromium concentrations. All the three control (un-stressed) fish species exhibited significantly better growth due to significantly higher feed intakes than those grown under sub-lethal exposure of chromium. The exposure of chromium during 12-week growth trials to fish caused significantly variable accumulation in the body organs of three fish species. However, fish liver, kidney and gills were the organs that accumulated significant amounts of heavy metal.

EFFECT OF TEMPERATURE ON THE DEVELOPMENTAL STAGES OF MAJOR CARPS BRED AT RAWAL TOWN FISH HATCHERY, ISLAMABAD

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Studies were made on the developmental stages of major carps hatched in Fish Seed Hatchery and research centre at rawal town, Punjab Fisheries Department, Islamabad. Result revealed that developmental stages of *Cyprinus carpio* in pond and aquarium vary significantly as hatching in pond takes less time than in aquarium. The eggs of *Catla catla* hatch out at 25.5 °C in 18 hours after ovulation (spawning) and yolk absorption took place at 26.5°C in 65 hours after ovulation. It is evident that the diameter of eggs and length of hatchlings both in circular tank indoor and aquarium indoor are almost the same.. The developmental stages in eggs of *Chrinus mrigala* showed that the diameter of eggs increase in size with rise in temperature and eggs hatched out in 25 hour at 28.2 °C after ovulation and yolk absorption took place in 89 hours at 25.2 °C. For *Labeo rohita*, the hatching were observed in 26 hour and yolk sac absorption took place in 72 hours after ovulation both in indoor tub and circulatory tank (indoor) eggs at the same time. The different water quality parameters measured from January to July showed that they changed with the passage

of time and have significant affect on the developmental stages and growth of fishes. This study also provides the base line information on the breeding behavior, survival and development of various fish species in Pothwar region where the temperature is playing a vital role on the developmental stages of carps.

**ASSESSMENT OF GROWTH, MORTALITY AND RECRUITMENT PATTERN OF
TALANG QUEENFISH, *SCOMBEROIDES COMMERSOANNIANUS* (FAM:
CARANGIDAE) IN PAKISTAN**

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Using length frequency data of Talang queenfish, *Scomberoides commersoannianus* landed at Karachi fish Harbour, from July 2012 to Feb 2013, we estimated population parameters of growth, mortality, exploitation and recruitment pattern. The parameters of von Bertalanffy growth function $L_{\infty} = 126$ cm, $k = 0.48$ year⁻¹ and $t_0 = -0.126$ year ($R_n = 0.50$) calculated with ELEFAN 1 programme. The length at first capture $L_c = 32.7$ cm was calculated. The natural (M), fishing (F) and total mortalities (Z) were 0.71 year⁻¹, 0.98 year⁻¹ and 1.69 year⁻¹ (95% CL of Z = 0.55 – 1.69) respectively obtained using length converted catch curve. The annual exploitation rate, U was estimated to be 0.98 year⁻¹. The biomass per recruitment (B'/R) and yield per recruitment (Y'/R) estimated 0.63 and 0.04 respectively. However, annual instantaneous fishing mortality rate of 0.98 year⁻¹ was by far in excess of the precautionary target and biological reference points ($F_{opt} = 0.355$ year⁻¹ and $F_{limit} = 0.437$ year⁻¹) indicating that the resource was over-exploited, it is therefore proposed that reducing fishing pressure would be better for the sustainable exploitation of the resource.

**REPRODUCTIVE BIOLOGY OF AN ECONOMICALLY IMPORTANT MARINE FISH,
EPINEPHELUSFUSCOGUTTATUS ALONG GHARO CREEK “SINDH”**

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Grouper is a popular marine food fish in many parts of the world such as in Kuwait, Indonesia, Malaysia, Thailand, Philippines, Hong Kong, Taiwan, China, Japan and Mexico. Majority of the groupers belong to the genus *Epinephelus*. *Epinephelusfusco guttatus*, is available in limited quantities in the coastal areas of Pakistan. Since this fish can be easily cultured in the brackish water ponds near coastal areas, it can be a good source of protein in the protein-poor high-salinity coastal areas of Sindh and can be a potential export item. At Present, efforts are being made to develop the infrastructure along the coastal areas of Pakistan and introduce techniques utilizing cheap raw material to establish marine fish farms. In one pilot project along Gharo creek, Sindh, *Epinephelusfusco guttatus* was cultured in tidal-fed brackish water ponds and attempts were made to

artificially breed the fish by injecting pituitary extracts from *Tilapia nilotica* and hCG (human chorionic gonadotropin). The *Epinephelus fuscogutatus* is a protogynous hermaphrodite meaning that initially they mature as female but become male as they grow larger and older. However very little or no information is available regarding the sex and maturity of this fish since histological techniques, which is essential for proper identification of male and female, is not available in Pakistan. Therefore, with the newly established histopathological facility in PCMD, we have conducted a study to evaluate the primary sex characters of the fish. As expected, most of the fishes were female showing Oocytes in different stages of development. A few fishes showed testicular tissue characteristic of male while in some fishes both ovary and testis were observed which indicates transitional state. Hormonal injections appeared to fasten the sex change to male. Currently, we are looking at the different stages of the fish in terms of the meat quality using high-resolution immunocytochemical techniques.

**LENGTH-WEIGHT, LENGTH-LENGTH RELATIONSHIPS AND RELATIVE
CONDITION FACTOR (*Kn*) OF TALANG QUEENFISH, *SCOMBEROIDES
COMMERSONNIANUS* (LACEPEDE, 1802) IN PAKISTAN**

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In this study, we present, length-weight (*LW*), length-length (*LL*) relationships and relative condition factor (*Kn*) of the talang queenfish, *Scomberoides commersonnianus* a food fish of the commercial importance. Some 674 individual of *S. commersonnianus* were examined from July 2012 to February 2013. The relationship between length and weight was calculated with an empirical equation $W = aL^b$, parameters *a* and *b* were estimated by linear regression analysis based on the natural logarithm, coefficient of determination R^2 and 95% confidence intervals of *a* and *b* were also estimated.

The length weight relationship equation for the combine sex

$$W = -4.489 L^{2.867} \quad (95\% \text{ CL of } b \text{ } 2.81\text{--}2.92), R^2 = 0.934$$

The estimated length-length relationship equation

$$FL = -0.131 + 0.999 TL \quad (95\% \text{ CL of } b = 0.991 - 1.009) R^2 = 0.986$$

$$SL = -0.06 + 0.995 FL \quad (95\% \text{ CL of } b = 0.987 - 1.007) R^2 = 0.985$$

$$TL = 0.286 + 0.979 SL \quad (95\% \text{ CL of } b = 0.961 - 0.991) R^2 = 0.975$$

Statistical test for isometric growth ($H_0: b = 3$) applied and found slightly negative allometric relationship in length weight relationship. The relationships between *TL*, *FL* and *SL* were highly linear ($R^2 > 9$). The highest *Kn* value was estimated in the month of July (1.26) and lowest in Feb (0.86). This study will help in building stock assessment and better management of an important food fish in future.

**SURVEY OF FISH PARASITE (*LERNAEA* AND ITS SPECIES IDENTIFICATION) AT
DIFFERENT FISH FARMS IN PUNJAB**

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A survey was conducted to study the fish parasites (*Lernaea*) at different fish farms (Fish Nursery Unit Farooqabad, Ganda Singh Nursery Unit Qasoor, Nursery Unit Gujranwala and Fish Seed Hatchery Chhenawan Distt. Gujranwala) in Punjab province. The water temperature of the selected sites on the average was 28.00 ± 1.00 °C. A comprehensive questionnaire was prepared to compile the data on disease status in locally culturable fish species. Five fish species viz. *Catla catla*, *Labeo rohita*, *Cirrhinus mrigala*, *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* were selected with uniform sample size (n = 10 in each). Among all the three indigenous fish species, *Catla catla* were more susceptible to *Lernaea* whereas, both the exotic fish species were more sensitive to this disease as compared to indigenous fish species. *Lernaea cyprinacea* and *Lernaea polymorpha* were the most reported species of *Lernaea* during the whole study period.

**ANTHROPOGENIC EFFECTS ON SPATIAL DISTRIBUTION OF ICHTHYOFAUNA IN
RIVER CHENAB, PAKISTAN**

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The fish fauna of Chenab River is continuously decreasing as a result of human activities viz; industrial, municipal, agricultural and water diversion activities. Present study was conducted to highlight the impact of human activities on the distribution and diversity of fish fauna in River Chenab, Pakistan. A total of 60 fishes species belonging to 12 families were sampled from five different barrages viz; Marala, Khanki, Qadirabad, Trimmu and Punjand. Maximum species richness was recorded from Qadirabad barrage and minimum from Khanki Weir. Cyprinidae was the major represented by 21 species and accounted for 37.69% of the total. Most dominant species viz; *Salmophasia bacaila*, *Puntius sophore*, *Osteobrama cotio*, *Parambasis ranga*, *Cirrhinus reba*, *Puntius ticto* and *Gagata cenia* showed relative abundance greater than 3.00%. These species were least important from economic point of view, whereas, relative abundance of commercially important species were recorded below 3.00% reflecting the intensive human pressure. During this study *Racoma labiata*, *Glyptothorax punjabensis*, *Labeo dero*, *Botia lohachata*, *Clupisoma naziri*, *Bagarius bagarius*, and *Macragnathus pancalus* were least observed species and sensitive species affected due to stress of human activities. Highest values for Shannon (3.50) and Simpson (0.96) indices were recorded from Qadirabad barrage whereas, minimum at Kanki Weir. Fish assemblages are highly influenced by the area of barrage, water flow, bank vegetation, habitat types, substrates and pollution. Highest values of the diversity were measured at microhabitats of each barrage like riffles and pools. A decreasing trend in diversity and abundance as well as in fish catch was observed due to multiple problems originated as a result of human activities. Local and trans-boundary water management along with pollution are putting the aquatic resources under threat

particularly, ichthyofauna of river Chenab. There is an urgent need to address the problems being faced by fishes of River Chenab and present endeavor will be helpful for future management activities at regional level.

ICHTHYOFAUNAL DIVERSITY OF RIVER PANJKORA, DISTRICT DIR LOWER, KHYBER PAKHTUNKHWA

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The present study of river Panjkora District Dir (Lower), K.P., Pakistan was conducted from April 2012 to September 2012 to find out its Ichthyofaunal diversity. Fishes were collected by using different types of hooks and nets of various mesh sizes. During the study period twenty five fish species were recorded from the Panjkora River Family *Cyprinidae* was found to be the richest fish family represented by 13 species viz. *Schizothorax esocinus*, *Schizothorax plagiostomus*, *Racoma labiata*, *Cyprinion watsoni*, *Cyprinus carpio*, *Tor putitora*, *Tor macrolepis*, *Barilius pakistanicus*, *Barilius vagra*, *Barilius modestus*, *Crossocheilus diplocheilus*, *Gara gotyla* and *Ctenoporingodon idella*. Family *Nemacheilidae* was represented by 5 species namely *Schistura alepidota*, *Schistura prashari*, *Schistura macrolepis*, *Triplophysa naziri* and *Triplophysa microps*; family *Sisoridae* included 4 species namely *Glyptothorax punjabensis*, *Glyptothorax stocki*, *Glyptothorax sufii* and *Glyptothorax naziri*; family *Channidae* was represented by 2 species including *Channa punctatus*, *Channa gachua* and family *Mastacembelidae* was represented by a single species, *Mastacembelus armatus*. The study adds 18 new records from River Panjkora and showed its high ichthyic diversity. It is suggested to assess water quality regularly to control anthropogenic activities such as illegal fishing to minimize catch pressure especially during breeding season.

ROLE OF OTHER MEATS IN DECREASING FISH CONSUMPTION IN KARACHI AND REPORTING CONSUMER'S PRACTICES IN KARACHI

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Consumer price index in Pakistan is moving around double digit since couple of years. It is in turn, enforcing consumers to purchase cheaper foods items. Such items will obviously containing low protein level. Continuous consumption of low protein items is opening many social and health problems in the Karachi. Fishes are having reasonable level of zinc that makes marriage life happier and safe people from heart and numbers of other diseases. Therefore, it is become necessary to find the "Role of other meats in decreasing fish consumption in and reporting consumer's practices in Karachi". This study is basically about people satisfaction level by different meat consumption. The authors are identify those factors which really matter to the consumer while consuming meat as well as identify those missing factors which should be included to make people more satisfied and healthier. The authors used convenience sampling technique by

taking the sample of 500 people belong to different income level, and though focus group ask them pre decided set of questions about their income level, their residential areas, their life style, their consumption pattern ,their taste/preferences, etc. While preparing a food on daily basis and occasionally. Through, this study also tried to find out how much people are aware about which meat type is containing more proteins. As we know that there are four categories of meat *i.e.* (beef, mutton, chicken and fish) which are easily available and highly consumed in our society, definitely the level of protein present in each different kinds of meat is different, but factor of awareness about the frequency of consumption certain meat type and information about level of protein present in different type of meat is missing. In addition, through this study it is tried to find out, how fresh and healthy chicken, Mutton, Beef are available to consumer and is it really healthy to eat. The main finding which comes up after pursuing this study is that meat products in Karachi has so much potential in terms of sale as it is available at affordable price, second is accessibility of its meat and meat made products which are available in almost every other corner of the city. Whereas, while talking about taste of its meat made products which is already developed since childhood in our society and preference of chicken and vegetable it is been revealed that mostly people are unaware from the minimum and maximum requirements of daily intake of proteins which are produce from meat made products and unaware from how fresh meat is available in the market. It is concluded that childhood eating practice, price, high exports demand, bones of fish meals, freshness, easily availability, dirtiness and rush of flies at fish shop are main factors that are decreasing fish consumption in our society and increasing role of other meats.

GROWTH PERFORMANCE OF *EPINEPHELUSFUSCOGUTTATUS* (MARINE FISH) IN PONDS ALONGGHARO CREEK

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Coast of Sindh and Balochistan has vast Coast Line ideal for culture of commercialmarine animals but unfortunately marine fish culture has not yet been addressed in Pakistan. Marine fish culture infrastructure along the coastal areas of Pakistan needs to be developed. Present studies are based on the culture of commercially important fish *Epinephelusfuscoguttatus* in ponds. The study will be carried out on ponds owned by farmers situated along Gharo Creek. The site has 18 tidal fed ponds. Only four ponds will be used for carrying out the present M. Phil/PhD studies. The fish seed will be collected from the coastal areas of Pakistan and will be stocked in ponds at Gharo Creek. The studies will be focused mainly on the growth pattern of *Epinephelusfuscoguttatus*. The other aspects of study will included detailed hydrography of ponds such as watertemperature, air temperature, pH, salinity and dissolved oxygen. The study of available natural feed for fish which include plankton in vertebrate (macro and micro) fauna to small fish will also be under taken. This study on culture of marine fish is being planned for first time in Pakistan and will beneficial for the future coastal zone development of Sindh and Balochistan. The study is expected to provide recommendations for establishment of *Epinephelus* culture along the coastal area of Sindh and Balochistan.

AMINO ACID PROFILE IN THE MUSCLES OF *CYPRINUS CARPIO* COLLECTED FROM TARBELA DAM AND RIVER SOAN

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The objective of the present study is to investigate the effect of aquatic environment of different water bodies on the amino acid profile in the muscles of *Cyprinus carpio*. For this purpose, samples were collected from Tarbela dam and River Soan. Total sixteen amino acids were observed though HPLC based amino acid analyzer, out of which ten were essential like arginine, methionine, valine, leucine, phenylalanine, isoleucine, tryptophan, lysine, histidine, threonine and six were non essential such as aspartic acid, glycine, alanine, glutamic acid, proline, and serine. In *Cyprinus carpio* the essential amino acids like threonine, isoleucine, tryptophan, histidine, and lysine and all non essential amino acids except asparagines were significantly higher ($P < 0.05$) in muscle tissues of fish from River Soan compared to fish from Tarbela dam. The significant variation in the contents of amino acids clearly indicated the role of aquatic environment of different water bodies on the amino acid composition of fish.

STUDY OF THE EFFECTS OF DIFFERENT STOCKING RATIOS (*CHANNA MARULIUS*: *OREOCHROMIS MOSSAMBICUS*) ON THE MEAT QUALITY OF *CHANNA MARULIUS* IN FERTILIZED PONDS

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The experiment was designed to study the effects of different stocking ratios of *Channa marulius* with *Oreochromis mossambicus*, fishes were stocked in three earthen ponds designated as T₁, T₂ and T₃ respectively, each measuring 25m x 8.5m x 1.5m (length x width x depth) situated at Fisheries Research Farms, University of Agriculture, Faisalabad. Tube well was used as a supply for H₂O. Ponds were fertilized with organic (cow dung) and inorganic fertilizer (nitrophos) @2gN/100g of a wet fish body weight daily. Ponds were stocked with 1:10, 1:20 and 1:30 ratios of *Channa marulius* with *Oreochromis mossambicus* in T₁, T₂ and T₃ respectively. *Channa marulius* were added @30fish/pond. The culture fish stock was captured randomly and their body weight and total lengths were measured. After obtaining the data, the fish were released back to their respective ponds. Proximate analysis was performed to check the meat quality of *Channa marulius*. Most of the ecological parameters such as dissolved oxygen, water temperature and light penetration were estimated. The maximum weight gain of *Channa marulius* was on account of sufficient supply of tilapia in T₃. It was followed by T₂ in which maximum final average weight was 1025.6 ± 0.56 (predator-prey stocking ratio 1:20). The minimum final average weight was observed in T₁ in which 969.5 ± 0.70 (predator-prey stocking ratio 1:10). The average weight gain was 954.95, 1010.1 and 1117.5g in T₁, T₂ and T₃ respectively. Proximate composition analysis showed that these contents were best in T₃. There was an inverse relationship between water temperature and dissolved oxygen. These two factors cause less increase in weight. These two factors cause less increase in weight in cooler months viz., November during 12th fortnight.

CHRONIC STRESS OF METALS MIXTURE ON THE GROWTH PERFORMANCE OF CHINESE CARPS

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Fish were acclimated to laboratory conditions for one week and were grown under sub-lethal concentrations of 21.48 mg L⁻¹ and 19.55 mg L⁻¹ of metal mixture (Pb+Zn+Mn) for *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix*, respectively for 90 days. As a result, it was observed that lead+nickel+manganese mixture exposed to the two fish species viz. *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* caused significant impact on their wet weights, fork and total lengths, feed intake, feed conversion efficiencies and condition factors. The control fish showed better growth than those stressed with metal mixture. Metal mixture exposure to the fish exerted significant influence on the increments in their wet weights, length-weight relationships, fork and total lengths, feed intake, feed conversion efficiencies and condition factors of the fish. Increase in fish weight was positively and significantly correlated with the water temperature, total ammonia and potassium while it was negatively correlated with the dissolved oxygen contents of the test media. Correlation coefficient among all the physico-chemical variables and increase in the weights of treated *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* were non-significant.

BODY COMPOSITION AND FATTY ACID PROFILE OF CARPS UNDER THE INFLUENCE OF UREA AND FEED SUPPLEMENTATION

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The experiment was planned to observe the influence of inorganic fertilizer (urea) with supplementary feed (rice polish and maize gluten) on the growth performance, body composition and fatty acid profile of carps. Total net fish production of pond 1 and pond 2 was remained 1311.3 and 1518.4 kg/ha/year. The pond 2 treated with urea and maize gluten showed 1.16 times greater growth than the pond treated with urea and rice polish. The physicochemical characteristics of pond water remained within the favorable limits for fish culture. *Hypophthalmichthys molitrix* had highest moisture contents and eicosapentaenoic acid. The maximum value of protein contents, carbohydrates and monosaturated fatty acid was observed for *C. mrigala*. There was inverse relation between moisture contents and crude lipids. PUFA composition of *H. molitrix*, *L. rohita* and *C. mrigala* was 58.89%, 71.89% and 65.32%, respectively. Among the SFAs the most abundant fatty acids were the palmitic acid (16:0) and stearic acid (18:0). In present project, the maximum value of mono unsaturated fatty acid was observed for *C. mrigala* (77.86%). Here palmitoleic and oleic acids were the dominating mono-unsaturated fatty acids. The ratio of PUFA/SFA for above mentioned fish species was 0.98%, 1.2% and 1.13% respectively. This ratio was maximum for *Labeo rohita*.

STUDIES OF THE EFFECT OF ORGANIC MANURE (COWDUNG) ON THE GROWTH PERFORMANCE OF *CYPRINUS CARPIO* V/S MAJOR CARPS (*LABAEO ROHITA*, *CATLA CATLA* AND *CIRRIHINUS MRIGALA*)

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Fish is a very important dietary animal protein source in human nutrition. Fish flesh contains all the essential amino acid and minerals viz., iodine, phosphorus, potassium, iron, copper and vitamin A and D in desirable concentration. It serves a valuable ingredient to a healthy diet because of its low carbohydrate and unsaturated fat contents. In view of the growing demand of proteins in diet there is a need to check the growth of fishes by the treatment of organic fertilizer (cowdung). The experiment was conducted for 6 months on fingerlings of common carp (*Cyprinus carpio*) and major carps in the two earthen ponds to check the better growth of fish. All the ponds were filled upto the level of 1.5m and this water level was maintained throughout the experimental period. In T₁ common carp (*Cyprinus carpio*) was stocked at a rate of 50 fishes. In T₂, the major carp (*Labeo rohita* 20, *Catla catla* 15 and *Cirrihinus mrigala* 15) was stocked. Organic manure (cow dung) was added on weekly basis. The maximum growth was observed in T₁ pond. The physicochemical characteristics of pond water remained within the favorable limits for fish culture. The net fish production /pond/183 days common carps (13.320) and major carps (11.403). The total planktonic biomass increases with the fortnights 133-196, 160-290 in T₁ and T₂ respectively, statistically analysis shows the significant results. The positive correlation between planktonic biomass and physicochemical parameters in T₁ and T₂ ponds. Common carp showed the maximum growth.

FUNGAL INFECTION OF *CATLA CATLA* (HAMILTON) REARED IN EARTHERN PONDS

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Thirty Specimens of *Catla catla* were examined for fungal infection. Fishes were obtained from Punjab University Research Fish Farms Lahore from February to July 2012. The average total length (TL) and body weight (BW) ranged from 15.35 to 28.0cm and 42.45 to 298.3g respectively. The infected fish had typical clinical signs such as: ruptured dorsal fin, lesions on skin and body, caudal peduncle and granuloma on anal fin. The material from various parts of infected, fish such as skin, gills, eyes, head, operculum, buccal cavity and abdomen was inoculated on three different culture media; Malt Extract Agar (MEA), Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA). The agar plates were incubated for seven days at 25-30°C. After 5-7 days, fungal colonies of different colours appeared on agar plates and these were observed with naked eye. MEA showed higher growth (37.93%) SDA and PDA (31.03% each). The control plates showed no growth. Slides were prepared and stained with Trypanblue in lectophenol and photographed. In the anterior part of fish (eyes, head, gills, buccal cavity and operculum) infection was high (81.03%). Skin and abdomen showed 18.97% infection. Colour of the fungal colonies was white, black, grey

and orange. Black colonies were the most prevalent forming 91.77% colonies, followed by orange (32.35%) and grey (25%), white colony appeared the least (1.47%). *Aspergillus* spp., *Blastomyces* sp., *Pencillium* sp. and un-identified hyphae were observed to be present on the fish. The infection of *Aspergillus* sp. was 78.57%, *Blastomyces* sp. 7.1 %, *Pencillium* sp. 3.5% and undidentified hyphae show 14.2% infection. Infection on skin was 46.1 % abdomen. *Blastomyces* sp. was also isolated from 2 fishes from eye and head. *Pencillium* sp. was isolated from one fish 13 from its operculum. Pure culture of fungi was also done with spore suspension method. The pure culture of the fungi was obtained from the preliminary isolated fungi from 6 fish. The pure culture produced the same colour colonies, which were more uniform in outlook and growth. It indicated the establishment of one genus of fungus on pure culture plates. *Aspergillus* sp. is also isolated from pond water which shows that pond water was contaminated with fungal spores. Although these fishes in general, looked healthy but had fungal infection. So great care must be taken in the maintenance of fish farm, regarding feeding, water quality and health management aspect of these fishes.

FEEDING BIOLOGY AND DIET COMPOSITION OF *SARDINELLA GIBBOSA* (PISCES: CLUPEIDAE) IN GWADAR (BALOCHISTAN COAST), PAKISTAN.

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The food habits of planktivorous fish, *Sardinella gibbosa* were investigated in Gwadar, Balochistan coast. A total 351 samples, composed of three size classes were collected seasonally from January 2010 to January 2011. There were analyzed for gut content and zooplankton analyses in the laboratory. Spatial and temporal variations in their food and feeding habits were assessed using the percentage numerical abundance method, percentage frequency of occurrence, stomach fullness indices and T okeshi graphical method. The diet of the fish species showed clear spatial and temporal differences, which were dependent on habit variability. The species belong to the omnivorous trophic category. *Sardinella gibbosa* fed mostly on copepods during the two seasons. Overall, fish species Exhibited generalized and opportunistic feeding habit. Their diet was influenced by changes in the quality and quantity of food in the environment and the fish migratory patterns.

FUNGAL INFECTION IN SILVER CARP, *HYPOPTHALMICHTHYS MOLITRIX* (RICHARDSON) REARED IN EARTHEN PONDS

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An investigation was conducted on fungal infections of freshwater culturable fish silver carp, *Hypophthalmichthys molitrix*. A total of 35 fishes were studied in nine batches. The total length and total body weight of each specimen were measured. Different body parts of fish such as gills, operculum, head, skin, buccal cavity, eyes and all fins were observed. Out of 35 fishes, 13 were infected. The infected fish showed clinical signs such as: rupture skin, eroded fins, cataract in eyes and lesions on skin. Fungi isolated from these tissues of fish were inoculated on three different media, Sabouraud Dextrose agar (SDA), Potato Dextrose agar (PDA) and Malt Dextrose agar

(MEA). A total of 223 agar plates were prepared of which, 71 of MEA, 57 of SDA and 95 of PDA. Isolated fungi from fish in agar plates were incubated for 5-6 days at 25-30°C. Fungal colonies observed on agar plates were black, green, grey, white and orange in colors. Slides were prepared and stained with 0.05% Trypan blue in lactophenol. The results showed isolation of four genera of fungi: *Aspergillus* sp., *Mucor* sp., *Penicillium* sp. and *Rhizopus* sp. Incidence of *Aspergillus* sp. was highest in fish samples. The 17.93% infection was seen from gills, 16.14% from skin, 15.7% from buccal cavity, 15.24% from eyes, 12.10% from operculum, 10.3% from head, 4.93% from caudal fins, 3.14% from pectoral fins, 2.7% from lesions on skin and lowest (1.8%) from dorsal fin. Pure culture was also done to obtain single, bacterial free colony of fish fungi which were useful in identification of fungi.

STOMACH CONTENT ANALYSIS OF SPANISH MACKEREL *SCOMBEROMORUS COMMERSON* (L., 1802) (FAMILY: SCOMBRIDAE) FROM THE KARACHI COAST, PAKISTAN

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Food is a significant factor in the ecology of fishes and required the growth, development, reproduction and extensive migration circuits. Food and feeding habits of fish is a helpful of fishery biology and culture aspects. Analysis of the stomach contents of Spanish mackerel *Scomberomorus commerson* (L., 1802) were collected in Karachi fish harbour during January 2012 to December 2012. After catching the fishes, Total length (cm) and weight (gm) were measured. Stomachs were removed, preserved and taken to the laboratory for analysis of the contents. Prey items in the stomach contents were identified to taxonomic groups. The % frequency of occurrence, % number and % weight of prey types in the stomach contents were evaluated. The percentage of the empty stomach varied seasonally and among size classes. The main diet constituents of *S.commerson* included *Engraulis encrasicolus*, *Stolephorus indicus*, *Duassumeria* spp, *Sardinella* spp squids, shrimps and unidentified foods. Seasonal variations of feeding activity indicated that food consumption was highest in autumn to summer and lowest in winter to spring. Food consumption was related to fish size. Teleost were the most important food items of all size classes.

3. MARINE BIOLOGY

THE ONTOGENIC DIET SHIFTS WITH REFERENCE TO SIZE IN *EPINEPHELUS DIACANTHUS* (VALENCIENNES, 1828) COLLECTED FROM KARACHI FISH HARBOUR

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Present research work is based on 105 specimens of *Epinephelus diacanthus* (Valenciennes 1828) collected from Karachi fish harbor that were studied to find out food contents for the confirmation of ontogenic dietary shifts with reference to size. Fish samples were collected from Karachi fish harbor and for the study of ontogenic diet shifts total length was measured and stomach contents were examined. We have divided available specimens in to three groups *i.e.* small size classes (13.5-19.5cm), medium size classes (19.6-25.5 cm) and large size classes (25.6 - 31.5 cm) Smallest size classes having total length between 13.5 to 19.5 cm fed on penaeid shrimps, small crustaceans and detritus. Medium and large size classes having total length between 19.6 cm to 31.5 cm mainly fed on teleosts fish, benthic crabs small prawns, *Oratosquilla spp* and molluscs. Results show that *Epinephelus diacanthus* (Valenciennes, 1828) changed their diets significantly between 19.5 cm and 25.5 cm total length showing ontogenic diet shift from carnivory to predatory diet mode.

BIODIVERSITY OF MARINE DINOFLAGELLATES ALONG THE PAKISTAN COAST

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Dinoflagellates are micro algae, biflagellated, armored, non-armored. This group is variable in size (1\200th mm -2 mm). Dinoflagellates are the major component of the microbial food web in oceanic ecosystem. They act as biological pump by which the oceanic ecosystems transfer's the energy to the different trophic levels. Dinoflagellates are autotrophic, heterotrophic and symbionts. About 140 genera of dinoflagellates are known in marine environment. Some are toxic and form blooms which can be harmful or toxic to the other living organisms. They possess' great ecological importance. A lot of work has been done on different aspects of dinoflagellates such as their taxonomy, distribution, abundance, mode of nutrition, migration, microbial role, toxicity and there molecular investigations. The sporadic information is available from inshore and offshore waters of Pakistan regarding dinoflagellate and harmful algal blooms (HABs) species how ever no seasonal data is reported along the coast of Pakistan. Few cases of blooms including red tides and green tides are also reported. The present work was proposed to assess the distribution and abundance of this important group of microplankton from different stations along the coast of Pakistan. This is base line data with the reference of Pakistan. The coastal waters along Karachi and off of Karachi were divided into four stations: off of Manora (st.1 inshore and st.4 near shore) and off of Mubarak village (st.2 inshore and st.3 near shore). Samples have been drawn from all stations using 1.7 L Niskin bottle from 1m depth. Total 165 samples have been collected from April 2008-Feb2010 and

each sample was fixed in lugol's solution for total count and identification at genus level. Samples have been analyzed and compared for abundance and seasonal variations. Total chlorophyll, temperature variation, salinity, dissolve oxygen and pH were also recorded. Total count was done using inverted microscope. Highest counts were recorded from st.1, st.3 and st.4 in August (80-140 cell⁻¹). High dinoflagellate diversity was observed in November 2008 and April 2009. A total of 19 genera and 100 species were recorded. Highest diversity was recorded from st.1 (17 genera) follow by st.3 and 4 (14 genera) and st.2 (12 genera). As dinoflagellates occupy an important position in the microbial community and play a vital role in the microbial food chain they are also part of primary producer and have great influence on climate. Therefore it is necessary to understand and conserve the biodiversity at the coast of Pakistan and maintain sustainability.

PHENOMENON OF IMPOSEX AND ROLE OF BIOSENSOR SPECIES IN DETECTION OF ORGANOTIN CONTAMINATION

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This article reviews the part of biosensor marine gastropod species in detection of organotins (OTC) contamination by serving as a cheap and easy bio-tool world over from more than last four decades for screening and reliable testing of presence of OTC in ports, marinas and adjoining natural environment. Review provides the historic stance, general overview of gastropod populations being served as biosensors in different parts of the globe and advances in research methodologies dealing with phenomenon of imposex in marine gastropods.

OBSERVATION ON THE REPRODUCTIVE PATTERN OF THE HERMIT CRAB *DIOGENES CUSTOS* ON THE SANDY SHORE OF CLIFTON, KARACHI

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The hermit crabs in order to protect their delicate abdomen are dependent on empty gastropod shells. A total of 758 hermit crabs were collected from the sandy shore of Clifton, Karachi during the period from December 2009 to February 2011. The population of hermit crabs, *Diogenes custos* was found to occupy 28 different gastropod shells. Out of 758 crabs, 346 (45.6%) were males, 307 (40.5%) were non-ovigerous females and 105 (13.9%) ovigerous females. Cephalothoracic shield length (CL) of *Diogenes custos* varied from 2.0 to 14.5 mm and 2.0 to 12.0 mm, in males and females, respectively. It was observed that the ovigerous females of *Diogenes custos* were found throughout the year which indicated year around reproductive activity with a peak during March to June and in November. The ovigerous females of *Diogenes custos* showed a preference for the *Bullia indusindica*, *B. kurrachensis*, *B. melanoidea*, *Turbo brunneus* and *Surcula javana*.

**ZONAL DIVERSITY AND INVERTEBRATE MACROFAUNAL COMMUNITY
STRUCTURE AT ROCKY INTERTIDAL AREA OF MANORA, KARACHI, PAKISTAN**

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Rocky intertidal zones have been studied for decades in order to identify the several important ecological interactions that cause the diverse assemblages of plants and animals especially characterized by patchiness in the distributions and abundance of species. A survey was conducted in order to recognize the community structure and zonal distribution of macro fauna at Manora rocky shore. The sampling was done at three tidal zone *i.e.* High tide zone, Mid tide zone and Low tide zone and a zonal comparison of fauna was formed to identify the distribution pattern of species. A total 1112 individuals were collected and mainly representatives of phylum mollusca (27 species), crustaceans (12 species), echinoderms (4 species), annelids (2 species), coelenterates (2 species) and porifera (1 species) were identified in the collected samples. The highest faunal diversity ($H' = 1.429$) was observed at low tide zone but the diversity of mollusca was high ($H' = 1.329$) at mid tide zone

**POPULATION STRUCTURE OF *BULLIA* (GASTROPODA: NASSARIADAE) SPECIES
FOUND ON THE SANDY COAST OF CLIFTON, KARACHI**

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A total of 1916 specimens of *Bullia* were collected from the sandy coast of Clifton, Karachi from December 2009 to May 2011. Each month approximately 120 specimens were hand-picked by walking on the lowest tidal level of the coast. The shells were brought to the laboratory and kept in aerated containers. The specimens were identified on their morphological characteristics. The total length (TL) and total breadth (TB) of each shell was taken to the nearest 0.01 mm and total weight (TW) to the nearest 0.1 gm. The animal was removed from the shell to recognize the sex on the coloration of gonads and the size of penis. Hydrographical parameters including dissolved oxygen, pH, salinity, air and surface water temperatures at the collection site was recorded each month. During the period from December 2009 to May 2011 concentration of dissolved oxygen ranged between 3.85 to 5.03 mg L⁻¹ with an average of 4.39 ± 0.45 mg L⁻¹, pH ranged from 7.9 to 8.6 with an average of 8.26 ± 0.17, salinity ranged between 35 to 40‰ while the subsurface seawater temperature ranged between 21–31°C. The population of *Bullia* was found to comprise of three species, that is, *B. melanoides*, *B. indusindica* and *B. kurrachensis*. Among these the most abundant species was *B. melanoides* represented by 73.1% (N = 1401) of the total population. The minimum and maximum total length of *B. melanoides* was 7.5 mm and 33.5 mm respectively. The dominant size-class among the population of *B. melanoides* was 22–24 mm being represented by 40.69% of the total population. The maximum total lengths were 37.0 mm and 52.5 mm for *B. indusindica* and *B. kurrachensis*, respectively.

RELATIVE GROWTH AND MORPHOMETRIC MEASUREMENTS AS AN INDEX FOR ESTIMATING MEAT YIELD OF TWO EDIBLE CRABS *PORTUNUS PELAGICUS* AND *P. SANGUINOLENTUS* FROM THE COASTAL WATERS OF PAKISTAN

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This paper deals with relative growth of long carapace width (LCW) and carapace length (CL) with reference to short carapace width (SCW) in two species of edible crabs, namely *Portunus pelagicus* and *P. sanguinolentus*. The relationship between SCW and CL was found negatively co-related in both the species. Size-weight relationship showed that males are heavier than similar sized females in both species. Meat yield was found higher in *P. pelagicus* than *P. sanguinolentus*. Male *P. pelagicus* had 40.1 to 51.3% (average 44.5% \pm 2.41 SD) meat of the total body weight, whereas female *P. pelagicus* had 35.6 to 48.6% (average 41.9% \pm 2.17 SD) meat. In case of male *P. sanguinolentus* the meat yield varied from 39.0 to 47.7% of the body weight while in female it varied from 32.1 to 43.8% (average 37.7% \pm 2.31 SD). More than half of the total meat was found in the thorax. Female thorax yielded more meat (56 and 55% in *P. pelagicus* and *P. sanguinolentus*, respectively) than the male thorax (53 and 51% respectively). Male chaelae yielded more meat (30 and 32% in *P. pelagicus* and *P. sanguinolentus*) than that of female (27 and 30%, respectively). Peripods yielded 15 to 17% meat only in both species.

POPULATION STRUCTURE OF SEA CUCUMBER FOUND ON TWO ROCKY COASTS OF KARACHI, PAKISTAN

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The economic and nutrition importance of sea cucumber has resulted in an increased fishing of the stocks throughout the world. This over fishing has resulted in the depletion of the stock and it takes decades to restore these stocks, therefore throughout the world the culture of sea cucumber has been established. Sea cucumbers are distributed from shore to the greatest depth. So far in Pakistan no study has been conducted on the population structure and reproductive cycle of this economically important commodity either on the shore or in depths. For the study of population dynamics of sea cucumber, two rocky shores of Karachi coast, Buleji and Manora were selected. A total of 532 and 577 specimens of sea cucumber were collected from Manora and Buleji, respectively during the period from April 2011 to June 2012. The animals were randomly collected in the low tidal zone under boulders and within the crevices at the lowest tide in the month. The specimens were brought to the laboratory in well aerated container and were kept in the aquarium. In the laboratory the numbers of specimens to be studied were narcotized using 2.5% MgCl₂. The species of sea cucumber were identified following the literature of Tahera and Tirmizi (1995) and they were found to belong to a single species, *Holothuria arenicola*. The total length (LT) and total breadth (BT) and total weight (WT) were taken to the nearest 0.1 mm and 0.01 gm. The minimum size of *H. arenicola* recorded was 7.8 mm and 38.0 mm from Manora and Buleji, respectively. The maximum size of sea cucumber *H. arenicola* recorded from Manora was 416.0 mm and that from Buleji was 377.0 mm. The largest sized specimens of this species were recorded

from Manora. The dominant size-class at Manora was 89.0-129.0 mm and at Buleji was 106.0-139.0 mm. Among the larger size-classes very few specimens of *H. arenicola* were found at both sites.

FIRST REPORT OF FRESHWATER CRAB, *POTAMON FLUVIATILIS*, OF PAKISTAN, ITS POPULATION ESTIMATION, DISTRIBUTION AND BEHAVIORAL ECOLOGY

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Due to increase in human population and deforestation, freshwater habitats and inhabitant are under severe threat. Crabs, one of the crustaceans, are member of infraorder brachyuran, having great pharmaceutical and medicinal importance. Approximately 6,793 species of crabs have been identified (Walters, Martin & Johnson, Jinny 2007) out of these 20 per cent of total crab population are freshwater dwellers and are mostly members of super-families: Gecarcinucoidea, Potamoidea, Pseudothelphusoidea, and Trichodactyloidea (Ng et al., 2008). Pakistan, like any other sub-tropical country, is rich in flora and fauna diversity and has approximately 10 different species of fresh water crabs (Cumberlidge et al., 2009). However these species are not yet identified, so a preliminary study was conducted to estimate the population size of fresh water crabs of Potohar region using random sampling methodology using hauling plankton net and recorded some aspects of behavioral ecology of the particular invertebrate. Study areas were ten villages and Rawal Lake in Islamabad, Pakistan. It was found that in 33,600 meter study area out of total estimated population size of 1,183 *Potamon fluviatilis*, 762 were females, 280 males and 141 juveniles. Burrowing habits and sideways movements were seen. These species affords protection by adapting to the occupation of empty cavities and crevices as protective retreats. *Potamon fluviatilis* is a benthotic species living at a preferable depth of 2 feet. Velocity of water flow in surrounding environment was 1.94 m/sec and in habitat of crab it was 0.2m/sec. They are scavengers of variety of zooplanktons, phytoplankton and other microorganisms.

***AREOPAGURISTES PERSPICAX* (NOBILI, 1906) (DECAPODA, ANOMURA, PAGURIDAE), A CASE OF SIMULTANEOUS SACCULIZATION AND BOPYRIZATION FROM PAKISTANI WATERS OF NORTHERN ARABIAN SEA**

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A female diogenid *Areopaguristes perspicax* (Nobili, 1906) was found having an abdominal isopod parasite *Allathelges pakistanensis* Kazmi and Markham, in 1999. The same diogenid host species measuring 3mm in shield length was collected in recent years from Manora Island, Karachi. Interestingly the hermit crab was found to be bearing double parasites. One was a peltogastrid cirriped under its abdomen, the other was a branchial pseudonine bopyrid, and living in the right branchial chamber. Other diogenids in the same lot had either rhizocephalan or bopyrid. The peltogastrid is larger than the bopyrid in size. The adult parasite has lost virtually every crustacean trait, including segmentation, appendages and gut. It consists of an external reproductive body, the externa, connected to a nutrient-absorbing system of rootlets infiltrating the host, the

interna. The peltogastrid is still unnamed and the bopyrid belongs to genus *Parapagurion*. Both of the parasites are new records for the area, both are illustrated and described in this paper. The affects of parasitization are also discussed here.

TWO PHYTAL HARPACTICOID COPEPODS FROM JIWANI, MEKRAN COAST, PAKISTAN

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The Subclass Copepoda traditionally comprises 10 Orders. Most copepods are planktonic, but the order, Harpacticoida is benthic. The order Harpacticoida contains over 3,000 species and subspecies belonging to 463 genera contained in 54 families. Harpacticoid copepod fauna of Pakistan is very much neglected. Very few taxonomic reports are published from here. Effective conservation and management of biodiversity largely depends on the taxonomic determination of species composition. Field observations show that some marine macro-algae harbour large numbers of harpacticoid copepods. In the present report two phytal harpacticoids belonging to the genera *Paradactylopodia* and *Eupelte* (families Thalastridae and Peltidiidae respectively) are described and illustrated for the first time from Pakistan coast.

EXOTIC MARINE CRUSTACEANS OF PAKISTAN - A PRELIMINARY ASSESSMENT

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The significance of introduced species in marine ecosystems worldwide has been highlighted in recent years. An introduced species (also known as exotic, non-indigenous, invasive, alien or non-native species) is any species whose translocation into an environment outside its native geographical habitat, within historical times, has been either man-mediated (either intentionally or accidentally), or has been an action of active dispersal via natural pathways. As marine species know fewer and fewer boundaries, invasive species now constitute one of the four greatest threats to the world's oceans on local, regional and global scales. Several marine alien species have been introduced to the Arabian Sea as coming from the Atlantic to Mediterranean then into the Red Sea, then they geographically spread in the Arabian Sea/other linked seas. They can be termed anti lessepsian migrants -a minor migration in the opposite direction of a Lessepsian or Erythrean migration which started via the Suez Canal opened in 1869. Climatic influence on species introduction and distribution into Pakistani Seas resulting in shift in bio geographical zones and for maritime corridors linking may also be responsible and cannot be ruled out. But net results of oceanographic regimes shifting patterns to alien outcome is such a complex process that is still not fully understood even in high tech developed countries, we as developing country are far behind to explain the presence of these exotic species in our waters. Since these occurrence are of much interest they need to be addressed. A list of exotic taxonomic groups is produced to begin with the *crustaceans*, only for a preliminary assessment. The available

information depends greatly on the taxonomic groups examined by the authors. The number of non indigenous species remains an underestimate, calling for continuous updating and systematic research. The list given below includes amphipods, tanaidaceans, carideans and brachyurans. Amphipoda. *Ampelisca brevicornis* (Costa, 1853), *Ampithoe ramondi* Audouin, 1826, *Ampithoe spuria* Krapp-Schickel, 1978, *Cheiriphotes mediterranea* Myers, 1985; Tanaidacea, *Cristapseudes omercooperi* (Larwood, 1954); Caridea, *Alpheus inopinatus* Holthuis & Gottlieb, 1958; Brachyura *Carcinus maenus* (Linnaeus, 1758), *Planes major* (MacLeay, 1838) The preliminary list can provide the basis for selecting indicator species within the Arabian Sea and thus be the common ground to build cooperation about them within countries in the range. A Marine Invasives Taxonomic Service (MITS) be established to coordinate the distribution, identification and data management for biosecurity.

TO EVALUATE THE ROLE OF TWO OCYPODOID CRABS AS THE BIOINDICATOR OF HEAVY METAL CONTAMINATION

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The concentration of heavy metal in marine environment increases and become a serious global problem due to the industrialization and urbanization. Since in the last few years investigations have focused on searching for bioindicator species that have the ability to accumulate the heavy metals from their surroundings. The aim of this study was to evaluate the role of two crab species as bioindicator for Nickel (Ni), Zinc (Zn), lead (Pb), Copper (Cu) and Cadmium (Cd). Sediment and crab samples (*Uca iranica* and *Macrophthalmus boscii*) were collected from 5 different sites along the coast of Pakistan. Analysis of samples revealed that the both species of crabs were found a good accumulator of Zn, Cu and Cd at all the five studied sites. The mean Cd concentration was high in *Macrophthalmus boscii* $1.01 \pm 0.45 \mu\text{g g}^{-1}$ and *Uca iranica* $1.306 \pm 0.854 \mu\text{g g}^{-1}$ as compare to their respective substrate as was observed $0.82 \pm 0.34 \mu\text{g g}^{-1}$ and $0.75 \pm 0.44 \mu\text{g g}^{-1}$ for *M. boscii* and *U. iranica* respectively. The presence of high metal concentrations in crabs than their surrounding substrate (sediment) explain the fact that these crabs can be good bioindicators and can be include in heavy metal pollution monitoring studies.

SEASONAL VARIATION IN BIOLOGICAL DIVERSITY OF A SANDY BEACH LOCATED AT KUNDMALIR (HINGOL NATIONAL PARK), BALOCHISTAN

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Biodiversity of intertidal area of Hingol National Park studied at exposed beach at Kund Malir, Balochistan. The quantitative assessment of abundance of organisms inhabiting the intertidal area was carried out using quadrat method. Sampling was made during January 2009 to January 2010. A considerable number of animal species belonging to major groups were recorded which include polychaetes, gastropods, bivalves, nematodes, arthropods, isopods and amphipods. Polychaetes were the most abundant group. A total of 2000 individuals of polychaete were collected belonging to different families including Magelonidae, Capitellidae, Eunicidae,

Chaetopteridae and Neridae. High abundance was observed in the winter season. The data obtained improves the knowledge of biodiversity along the coast of Balochistan, particularly with respect to polychaetes.

**SEASONAL VARIATIONS IN TAXONOMIC COMPOSITION OF INTERTIDAL
BENTHO-PELAGIC MARINE ORGANISMS ALONG THE COAST OF MIANI HOR
(SONMIANI BAY), BALOCHISTAN**

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Marine organisms utilize the shallow marine coastal zones and estuaries for various important phases of the life history e.g. nursery and feeding and refuge mostly. The species composition in these areas varies according to season and lunar phase and mainly based on migrations of seasonal visitors and also the result of seasonal settling or consecutive migration waves of young stages. Indeed, most of these species spawn in deeper offshore waters and invade, as late larvae or early juveniles, shallow coastal areas in different times and seasons of the year when such areas are relatively suitable and rich in food. A year long study was conducted along the coast of Miani Hor (Sonmiani Bay), Balochistan (Jan 2003-Dec2003) to identify the seasonal and temporal changes in the composition of some major faunal groups in the semi enclosed lagoon. Two types of fishing gears were used to collect the samples in four seasons (north east monsoon, pre monsoon, south west monsoon and post monsoon). Atleast once, in each month of the season, one set of lunar tides were utilized to account the short term temporal variations in species composition. The distribution and diversity were also affected by physico-chemical factors, observed during the study. Overall 41 species of 29 families of both invertebrates and vertebrates were collected. The highest diversity was seen in pre monsoon (2.74 ± 0.86) whereas south west monsoon showed the lowest diversity (1.84 ± 1.30). The Jaccard's similarity index was used to estimate the assemblage of macrofauna in the intertidal sandy shallow zone and highest estimate was found in pre monsoon (0.75) during spring tide.

**MACROBENTHIC DIVERSITY AND ABUNDANCE DURING SW MONSOON SEASON
AT CLIFTON**

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Sandy beaches occupy three quarters of world's shoreline. Exposed sandy beaches are physically dynamic benthic environment composed of fine grained sediments which have a capacity of retaining water which support the settlement of benthic organisms. The abundance of variety of species on coastal areas influenced by sediment characteristics, physical factors such as sediment texture and by competition among macrobenthic communities depending upon food resources. Medium and fine sands usually have an abundant macrobenthic fauna due to the presence

of more organic matter per unit area. Thus, macrobenthic abundance use to measure environmental disturbances and magnitude of pollution. The study was made at Clifton which is subjected to greater extent of pollution and anthropogenic activities. This study is an approach towards the understanding of sandy beach structure and benthic fauna at studied site (Clifton) which will be helpful in the management of this beach in future. The samples were collected from 2 stations in replicates from quadrates of 0.25 m². The collected sand were immediately sieved. All fauna retained on sieve except polychaetes were preserved in 4% formalin. Polychaete worms were narcotized and preserved separately in alcohol. A total of 16 samples were collected in pre-monsoon & during South West monsoon period. The highest dissolved oxygen (8.11 mgL⁻¹) and Salinity (48‰) was recorded in March. The maximum fraction of sediment texture consist of fine sand. The total macrobenthic abundance at Clifton represented by 5141 organisms comprised of 54 macrobenthic species. The highest species richness throughout the study was exhibited by Molluscs, Polychaetes and Crustaceans respectively. The most abundant organisms were *Donax sp.* and polychaete families *i.e.* family Eunicidae and Glyceridae. The present study shows the variation in macrobenthos abundance. The benthic community structure at Clifton may be governed by the pollution.

GENETIC VARIATION IN MUD CRAB GENUS SCYLLA (FORSKL, 1775) FOUND ALONG COAST OF PAKISTAN

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Management plans and stock assessment of exploited shell fishery should consider the information on the genetic population structure of the species. In this perspective, the genetic diversity was observed between the populations of commercially exploited mud crabs species of genus *Scylla*: *Scylla serrata* and *S. tranquebarica* through allozyme electrophoresis. The genetic variations were estimated through the proportion of polymorphic loci, observed and expected heterozygosity. In the allozyme electrophoresis analysis five enzyme (CD, CAT, GP, G6PDH, ODH) systems were studied. Total 7 loci out of 20 resolved loci were polymorphic. Alleles expressed per polymorphic loci according to the interpretation of the produced banding patterns were 0.26 and 0.28. Mean of alleles per locus (1.35) and (1.39) in *S. serrata* and *S. tranquebarica* respectively. Observed heterozygosity of *S. serrata* and *S. tranquebarica* was (0.35) and (0.388) and expected heterozygosity was (0.34) and (0.39) respectively.

HEAVY METALS CONTAMINATION IN SOME EDIBLE CRAB SPECIES OF FAMILY PORTUNIDAE FOUND ALONG THE COAST OF PAKISTAN

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The presence of metal contaminants in seafood items is a result of human activities and the contaminated fish and shell fish from aquatic environment may become a public health concern. It is important to determine the concentration of heavy metals in commercial and edible fish and shell

fish species in order to evaluate the possible risk of human consumption. A study was conducted to assess the contamination and bioaccumulation of seven heavy metals including Iron (Fe), Zinc (Zn), Cadmium (Cd), Lead (Pb), Copper (Cu), Chromium (Cr) and Cobalt (Co) in edible muscle portion of four commercially important crab species (*Portunus pelagicus*, *Portunus sanguinolentus*, *Charybdis feriatus* and *Scylla serrata*) of family Portunidae. In *Scylla serrata* the concentration of Cr and Cu were highest with the mean value of 36.8 $\mu\text{g g}^{-1}$ and 62.4 $\mu\text{g g}^{-1}$ respectively while in *Portunus pelagicus* the concentration of Cu was highest with the mean value of 70.8 $\mu\text{g g}^{-1}$. The concentration level of all analyzed metals was under permissible range except Cr and Cu, which were higher than the limits of FDA (2001) and FAO (1983). The higher concentration of these metals in food items may pose serious threat to human health through consumption.

MORPHOLOGICAL SEXUAL MATURITY AND RELATIVE GROWTH OF *OPUSIA INDICA* (BRACHYURA: OCYPODOIDEA: CAMPTANDRIDAE) FROM MANGROVE AREAS OF KORANGI CREEK PAKISTAN

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Size at sexual maturity and patterns of somatic growth are important aspects of life history of crab. The main purpose of this study was to provide an estimate for the onset of morphological sexual maturity and relative growth in mangrove crab, *Opusia indica* (Alcock, 1900) from a population located in Korangi creek mangrove area, based on the relative growth. The crabs were monthly collected through quadrat method from March 2001 to February 2002. A total of 1702 crabs were obtained, of which 764 were males and 938 were females. The morphometric measurements of carapace, abdomen, cheliped and males gonopod were related with carapace width which was taken as an independent variable. Based on carapace width (CW) males were significantly larger than female indicating sexual dimorphisms. The size at onset of sexual maturity in males was estimated 5.51 mm CW and 5.3 mm CW in females. Positive allometric growth of female abdominal width is likely related to the incubation process while positive allometric growth of males cheliped and pleopod was related to their reproductive behaviour.

SOME OBSERVATION ON BIOCHEMICAL COMPOSITION OF *CELLANA KARACHIENSIS* (WINKWORTH, 1930) (MOLLUSCA: GASTROPODA) ON DIFFERENT MATURATION STAGES OF GONAD ON THE ROCKY SHORE OF BULEJI, KARACHI

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The true limpets of the Order Patellogastropoda are common inhabitants of the intertidal and subtidal areas of rocky shores extending from the tropics to the polar regions. The species of limpets found on the rocky shore of Buleji, although showed morphological variations, were identified as a single species, *Cellana karachiensis*. The reproductive changes in marine invertebrates are often related to translocation of the biochemical constituents between body tissue and reproductive organs. The paper describes the protein, lipid and carbohydrate concentrations in

the gonads and soft body tissue of females and males limpets during maturation stages, that is, developing, ripe and spawning stages. In the present study protein concentrations increased significantly in gonadal tissue with the advancement of maturation stages in both males and females of *C. karachiensis*. The concentrations of carbohydrate in the developing, ripe and spawning gonads were similar in both sexes. However, the lipid concentration in the gonads of male and female limpets was significantly higher in ripe gonads. The concentrations of protein, lipid and carbohydrate did not vary in the soft body tissue in relation to maturation of male and female *C. karachiensis*.

SOME OBSERVATIONS ON DISTRIBUTION AND ABUNDANCE OF *CLYPEOMORUS BIFASCIATUS PERSICUS* (HOUBRICK, 1985) (MOLLUSCA:GASTROPODA) ON THE ROCKY SHORE OF BULEJI, KARACHI

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Family Cerithidae commonly known as mud creepers, are of common occurrence in shallow waters along the coast of Pakistan. *Clypeomorus bifasciatus persicus* (Houbrick, 1985) was observed to be most dominating species of this family on the rocky shore of Buleji. They live in large colonies feeding decayed algae and are commonly found in mid tidal zones of the rocky and sandy cum muddy shores. The paper describes distribution abundance and other ecological aspects of this species from rocky shores along the coast of Karachi. Because of its resilience to exposure to sun light, comparatively high salinities and common occurrence, this species can be used as an indicator of the health of the rocky beaches.

A MACRO VIEW OF TOP SHELL RESOURCES ALONG THE SINDH COAST

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From the economics point of view Pakistan can generate reasonable volume of income by proper utilization of its top shell resources. But unluckily we are destroying them to the extent that it should not be available for further use of other people as well as for use of coming generations. In this study, sea resources are considered that belongs to invertebrates. The authors are documenting proper and miss use of these marine resources. With respect to mangroves plant the authors are reporting the terrariums that our poor community is doing with mangrove and how it can be avoided, and the magnitude of such loss is also estimated. It is found that the population of top shell is reasonably decline, because the top shell exporters are unable to collect the large size shell. In this connection duo darya and kati bundar area were explored by the researchers, moreover it is also observed by interviewing the exporters that they are having never ending export demand of top shell. The top shells that Pakistan is exporting are locally called marroi, badam, star, and allogula.

DISTRIBUTION AND ABUNDANCE OF *CENTROPAGES* SPECIES (COPEPODA: CALANOIDA) IN THE MANGROVE CREEKS AT SANDSPIT BACKWATERS KARACHI, PAKISTAN

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Zooplanktons are the indicator of fishery potential of the areas and hence information on the interaction with other microplanktonic groups is important in fishery management. Copepods are the most abundant group and have been considered as the major grazer on phytoplankton in the marine ecosystems. The copepods belong to the family *Centropagidae* were reported to dominate the coastal waters. The aim of this study is to evaluate the specie composition and distribution of Genus *Centropages* in the mangrove forests along the coast of Karachi. The samples were collected by Zooplankton net from the creeks of mangroves forests at sandspit backwaters during the SW monsoon season. Four specie *C. dorsipinatus*, *C. orsinii*, *C. furcatus* and *C. karachiensis* were identified in the samples. *C. dorsipinatus* and *C. orsinii* was the most abundant species throughout the SW monsoon season where as the *C. furcatus* and *C. karachiensis* were less abundant.

LABORATORY REARED ZOEAL STAGES OF *ALPHEUS LOBIDENS* DE HAAN, 1850 (CRUSTACEA: DECAPODA: ALPHEIDAE)

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An ovigerous female of *Alpheus lobidens* De Haan, 1850 collected from Bulleji (Long. 66°49'E, Lat. 24°59'N) on January 28, 2006 was kept under the laboratory conditions. On January 29, 2006 larvae were hatched out. Zoeae I- V were obtained within six days at room temperature: 22°C - 24°C, in filtered seawater of a salinity of 35 - 38 ppt and pH 7.9. The larvae are described, illustrated and compared with the earlier studied larvae of *Alpheus lobidens*.

STATISTICAL COMPARISONS OF SOME EXTERNAL MORPHOMETRICAL ASPECTS OF GENUS *SCYLLA* (MUD CRAB) POPULATIONS INHABITING THE KORANGI CREEKS AREA AND SANDSPIT BACK WATERS

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Mud crab samples of the genus *Scylla* were collected from two mangrove areas along the coast of Karachi Pakistan. For discriminating purposes twenty three external morphometric characters and eight morphometric ratio of the genus *Scylla*, were examined and compared between two populations obtained from the Korangi creeks area and Sandspit back waters. A statistical approach, i.e. multivariate morphometric analysis was used to compare external morphometric

characters to determine the most useful characters for discriminating between two populations. The results of Canonical Variate Analysis (CVA) show clear separation of the clusters of some morphometric characters for Korangi creeks area and Sandspit back waters. The results support the proposition that the ratio of the Carapace width (including 9th anterior-lateral tooth) (CW2) and Carapace length (CL) was the main character to distinguish between two studied populations. The results of statistical comparisons were coincident that external morphometric characters of genus *Scylla* between the two populations off the Korangi creeks area and Sandspit back waters are significantly different.

4. PALAEOLOGY

SOME NEW REMAINS OF *HISPANOTHERIUM MATRITENSE* FROM THE MIDDLE MIOCENE OF THE SIWALIK HILLS, PAKISTAN

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New material attributed to *Hispanotherium matritense* from the various localities of 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 sub-hypsodonty. The new material may provide better understanding of the systematics and morphology of the dentition of described species.

SOME NEW REMAINS OF THE PROBOSCIDEANS FROM THE MIDDLE SIWALIKS, NORTHERN PAKISTAN

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New fossils of Proboscideans from the Dhok Pathan Formation of the Chakwal district are described and discussed here. Six specimens are recovered and assigned to the genus *Gomphotherium* of the Order Proboscidea. *Gomphotherium* is an extinct genus of the proboscids which evolved in early Miocene in North America and then emigrated into Asia, Europe and Africa. The specimens are being studied and compared with other *Gomphotherium* specimens studied in Europe, America and Africa till date.

FIRST REPORT OF *MESACERTHERIUM WELCOMMII* FROM THE LOWER SIWALIKS OF LEHRI, DISTRICT JHELUM, NORTHERN PAKISTAN

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Rhinocerotids are particularly abundant in the Neogene deposits of the Potwar Plateau. The rhinocerotids found in the Siwaliks are a least studied group. Only scanty research work has been published, regarding fossil rhinoceroses of the Siwaliks. The present paper describes the dental remains of an extinct rhinoceros identified as *Mesaceratherium welcommi*. These remains were collected from Lower Siwalik Kamlial Formation exposed in the vicinity of Mangla Lake situated in the Lehri Park district Jhelum. This perhaps is the first report of *Mesaceratherium welcommi* in the Siwaliks of Potwar Plateau. In Pakistan previously this species is known only from Neogene sediments of Baluchistan.

**NEW REMAINS OF LISTRODONTINAE (MAMMALIA, ARTIODACTYLA, SUIDAE)
FROM DHOK BUN AMIR KHATOON OF THE LOWER SIWALIKS, NORTHERN
PAKISTAN**

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Early rhinocerotid specimens were collected, described and figured here. The new specimens were collected from the Tertiary hills of the Dhok Bun Amir Khatoon village, Chakwal district, Punjab, Pakistan. The sample belongs to isolated dentition. The remains are identified on the basis of morphological and metrical characters and assigned to *Listriodon pentapotamiae*. The listriodons were present in the Middle Miocene of the Siwaliks with other mammalian groups like proboscideans, ruminants and carnivores. The new sample of *Listriodon pentapotamiae* from Dhok Bun Amir Khatoon contributes to new knowledge about the species.

**SOME NEW FOSSIL REMAINS OF NEOGENE SUIDS FROM LOWER SIWALIKS OF
PAKISTAN**

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Some new fossil remains of suids have been collected from different localities of Lower Siwaliks situated in district Chakwal. These localities include Lawa, Kallar Kahar, Dhok Bun Ameer Khatoon and different areas of Chinji zone. These specimens include isolated teeth and a mandibular fragment identified as belonging to genera *Listriodon*, *Conohyus* and *Hyotherium*. This collection will bring new insight to the understanding of the systematic and evolutionary trends of the family suidae leading to the present-day diversity of this group in Pakistan as well as the adjacent biozones,

**SOME NEW FOSSILS OF GIRAFFOKERYX (MAMMALIA: CETARTIODACTYLA:
GIRAFFIDAE) FROM THE CHINJI FORMATION OF PAKISTAN**

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This paper describes new fossils of *Giraffokeryx* originating from the Chinji Formation of the Lower Siwaliks, northern Pakistan. The fossils comprise upper and lower dentition collected from the middle Miocene outcrops of Chakwal, Punjab, Pakistan. A detailed description of the newly discovered fossils and their taxonomic classification is being provided. The specimens pretty

match with *Giraffokeryx punjabiensis*, a small sized taxon of giraffids, found in the Middle Miocene of the Siwaliks. The Chinji sample adds new information on the morphological variability of *Giraffokeryx* and conforms of an early distribution of the genus in the Middle Miocene of the Siwaliks.

PALEOCOLOGY OF TWO EXTINCT SUINES FROM LATE MIOCENE OF PAKISTANI SIWALIKS

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The Suine remains are known to have broad geographic and long chronologic ranges with diverse dietary and habitat adaptations that provide significant biostratigraphic and paleoecological implications. The paleoecology of *Propotamochoerus hysudricus* and *Hippopotamodon sivalense*, two extinct suine species from Potwar Siwaliks of Pakistan, has been explored applying microwear and hypsodonty methods. The microwear pattern of the extinct taxa is consistent with the fruit dominated browsers. Dietary preference of *Propotamochoerus hysudricus* is indicative of the marked seasonality contrary to *H. sivalense*. The hypsodonty index indicates that the animals fall within the browse dominated omnivorous category. Combining the results from both of the research tools reveal that the extinct suines reflect diverse dietary spectrums ranging predominantly from frugivorous to browsers. The referred species are chronologic indicators of Late Miocene (ca. 10.3-6.5 Ma) of the Siwaliks that corresponds to Vallasian-Turolian of Europe. The ecomorphic information of studied taxa suggest the presence of paleoecological mosaics of woodlands with moist deciduous and dry deciduous forested habitats during the Late Miocene of Pakistani Siwaliks.

FOSSIL ARTIODACTYLA OF DHOK PATHAN DISTRICT CHAKWAL

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The collection of twenty three specimen were studied belonging to 9 Genera, 10 Species and one order i.e Artiodactyla of the Middle Siwalik District Chakwal which comprises Dhok Pathan and Nagri Zone. The collection consists of isolated teeth and madibular fragments. The *Listriodon* have evolved from Bunolistriodon and their first record in the subcontinent is as *Listriodon pentapotamiae* from the upper Kamlial strata on the basis of morphology of premolars and molars from anteriorely protolophid/protoloph posteriorely metalophid/metaloph which form a transverse crest. The species *L. pentapotamiae* ranges from lower to middle Siwalik .It is concluded that the European and African *Listriodon* are descendants of the Siwalik *Listriodon*. The tooth of Genus *Hippohyus* is provided with tubercles which have numerous folds of enamel. In the *Hippohyus lydekkeri* the teeth are sub-hypsodont and multituberculated. The enamel lining of teeth is shiny,thick and corrugated. The Genus *Selenoportax* include species *Selenoportax vexilarius* and *selenoportax lydekkeri*. In *Selenoportax vexilarius* teeth are hypsodont and narrow crowned. The enamel lining is shiny and wrinkled. The *Selenoportax lydekkeri* is characterized by the sub-

hypsodont teeth. Upper molars which are square in shape and having strong median basal pillars. Central cavities are spindle shaped. *Strepsiptax gluten* is characterized by molars are quite hypsodont, slender and are larger. The enamel lining is shiny and rugose. The median basal pillar is large. The Genus *Tragoportax* include *Tragoportax islami*. The upper molars are hypsodont, quadrate and with rugose enamel. The median basal pillars are low in vertical height. The styles are strong and somewhat divergent. *Sivaceros gradiens* generally characterized by lower premolars and molars are narrow crown and Brachyodont. The median ribs on the lingual side is quite thin as compared to labial side of crown. Fourth Premolar of mandibles are extremely simple with one anterior and two posterior wings. *Antelope* Genus is represented by brachyodont tooth. Molars are quadrate and enamel is shiny, thick and rugose. *Proamphibos kashmiricus* is characterized by narrow molars having prominent basal pillars. The enamel lining is thick, rugose with shiny crown surface. Premolars are extremely hypsodont and narrow crown. The species *Gazella lydekkeri* consist of hypsodont molars with thick basal pillars. The enamel lining is smooth and crenulated. The styles are narrow and strong. The central cavities are constricted and crescentic in shape.

NEW FOSSILS OF TRAGULIDS (RUMINANTIA: TRAGULIDAE) FROM THE DHOK PATHAN FORMATION OF HASNOT, PAKISTAN

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Tragulids especially *Dorcatherium* are the best represented ruminants in the Late Miocene Middle Siwaliks of Hasnot, Jhelum, northern Pakistan. The paper deals with the study of the following identified tragulids: *Dorcatherium minus* and *D. majus*. The new remains described here consist of predominantly isolated teeth, maxilla and mandible fragments. The recorded taxa became extinct after the Late Miocene-Pliocene, indicate a dissimilarity of the climatic conditions and landscape in this area in the moment that these sites represent. A strong attachment to wet forested habitats can be assumed for the tragulids where the animals could hide in vegetation or water from predators. However, as it is the case today, these conditions are different in Hasnot in northern Pakistan.

SOME NEW REMAINS OF BOVINES FROM SARDHOK PLEISTOCENE OF PAKISTAN

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Some fossils have been reported from Neogene basin near Sardhok village in district Gujrat, northern Pakistan. The Sardhok bovine sample includes a horn-core, a skull, a hemimandible, a maxillar fragment and isolated dentition. The bovine remains from Sardhok

described and assigned to *Bubalus*, *Hemibos*, *Proamphibos* and *Leptobos*. The new specimen hemimandible provides new information about the complete length of premolar and molar series of *Bubalus bubalis palaeindicus*. These bovines are well known in the Pakistani Siwaliks during Pleistocene. The overall faunal composition seems to suggest affinity with southern Asia rather than northern Eurasia.

**GOMPHOTHERIUM (PROBOSCIDEA, MAMMALIA) FROM THE MIDDLE MIOCENE
LAVA OF THE LOWER SIWALIKS, PAKISTAN**

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Here, a dentary of *Gomphotherium* from the Chinji Formation Lava of northern Pakistan is documented. The material is referred to *Gomphotherium browni*, a rare intermediate species of the genus *Gomphotherium*, only found in the Siwaliks. This is the first mandible of *Gomphotherium browni* from the Chinji Formation of Lava. The fossil record indicates the occurrence of the rare species in the Middle Miocene outcrops of Lava. *Gomphotherium browni* is always found rare in the Lower Siwaliks and lived with other inhabitants of wooded lake margin in the east of Lava during the Middle Miocene.

HYPOPLASIA IN FOSSIL MAMMALS AND ITS PALEOECOLOGICAL IMPORTANCE

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Hypoplasia is underdevelopment or incomplete development of a tissue or organ. These unusual episodes leave unique discernible marking on bones and teeth. Since bones are ever-growing till adult these developmental markings are smudged or even covered up by later growth but teeth are different in this respect as enamel and dentin do not regenerate after they mineralize initially. Enamel hypoplasia is a condition in which the amount of enamel formed is inadequate. This results either in pits and grooves in particular area of the tooth or in widespread absence of enamel leaving behind well-defined linear marking(s). These markings, therefore, act as permanent record of events that occurred during tooth development and, hence, can be tied up with its chronological development. Thus enamel hypoplasia can be a perfect archive for development stress. The fossilized tooth with enamel hypoplasia, thus, also has the potential of providing a unique perspective into environmental conditions present during the growing years of an extinct animal's life, which indirectly reflects the climatic conditions prevailing during that period of time. The analysis of hypoplasia is a useful means for retrospective assessment of the timing and intensity of systematic stress events during the period in which an individual's dentition is formed, and can thereby contribute to the understanding of past ecological and health conditions. It has been

used by biologist, archeologists and anthropologist as an indicator of period of generalized physiological stress during tooth development in hominid and non-hominid primates, domestic pigs and wild boars. The widespread occurrences of Enamel Hypoplasia at some archaeological sites and in a few living human populations have generally been interpreted to be reflective of the physiologic stress that individuals have gone through the natal stages and in the formative (weaning) years. The occurrences of enamel hypoplasia in fossil mammals has recently got the attention of paleontologists; the specific mentions are on Early Miocene Catarrhines monkeys from East Africa, the Miocene Teleoceras rhinoceros from North America and the Early Pliocene Sivathere giraffids from South Africa. The hypoplasia in the first two instances are mostly recorded in deciduous teeth which was interpreted to be the result of physiological stress at or near birth. The Langebaanweg (South Africa) Giraffids has hypoplasia mostly on permanent teeth which is thought to be the result of poor environmental condition, possible seasonal nutritional stresses and in some instances stresses at the weaning stage. The intensive faunal collection from and subsequently their comprehensive systematics and anatomical studies on the Neogene mammalian fauna from various parts of Pakistan in the past three decades have brought a new understanding of not only the evolutionary and migratory pattern of a number on mammalian taxa but also have impact on environmental and climatic changes through in the past 30 million years in South Asia. In this context, a few studies are done on Siwalik bovids, giraffids and Hominoid primates which when tied up with isotopic studies of dental material and of paleosols have led to a better analyses of local environment and regional climatic changes in the Neogene time leading to the present climate regimen of South Asia. The hypoplasia study of the 'Siwalik' rhinocerotids has been initiated with the objective of analyzing this phenomenon through time and with interpolation from other evidences refine the Neogene paleoecologic reconstruction of Pakistan and northern India. The First Author (GR) has recently studied all the notable Siwalik and coeval collections of Pakistan and India residing in selected Museums and Institutions of Pakistan, France, AMNH, Harvard, Yale and the British Museum. Some preliminary results will be shared in the presentation.

NEW REMAINS OF BOVIDAE FROM THE TATROT FORMATION OF THE UPPER SIWALIKS, NORTHERN PAKISTAN

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New Bovidae fossils are collected from the outcrops nearby Tatrot, Jhelum district, northern Pakistan. The Tatrot village is the type locality of the Tatrot Formation belonging to the Upper Siwaliks. The recovered material comprises upper and lower molars of the large sized Siwalik bovids. The recovered specimens provide existing evidence of the large sized bovids in the Tatrot type locality of the Tatrot Formation of the Upper Siwaliks. The diversity of the collected fossilized fauna is helpful in the reconstruction of the paleohabitat of the ancient animals existed in the type locality of the Tatrot Formation of Pakistan.

NEW FOSSILS OF BOVIDS FROM THE CHINJI FORMATION OF DHOK BUN AMIR KHATOON OF THE LOWER SIWALIKS, NORTHERN PAKISTAN

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The new fossil remains of bovids are recovered from the Middle Miocene outcrops of the Dhok Bun Amir Khatoon village of Chakwal district, Potwar Plateau. The sample consists of the upper and the lower dentitions and can be assigned to the medium sized boselaphines. The outcrops belong to the Chinji Formation of the Lower Siwaliks and of late middle Miocene in age (14.2-11.2Ma). The sediments consist of bright red clays with pseudo conglomerates which are silicon in nature. The palaeoclimate of this age can be interpreted warm and humid having extensive forest component and developed grassy sub areas.

NEW REMAINS OF THREE TOED HORSE *SIVALHIPPIUS* FROM THE DHOK PATHAN FORMATION OF THE MIDDLE SIWALIKS, NORTHERN PAKISTAN

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A detailed systematic study of *Sivalhippus* has been worked out in this research project. The three toed horse fossils have been discovered from the Dhok Pathan Formation of the Chakwal district, northern Pakistan. The Dhok Pathan Formation of the Middle Siwalik subgroup is represented by two genera of the Late Miocene horses namely *Hipparion* and *Sivalhippus*. The studied sample is compared well with *Sivalhippus* and consequently speak in favor of the genus *Sivalhippus*.

DESCRIPTION OF LOWER DENTITION OF *HISPANOTHERIUM MATRITENSE* FROM THE LOWER AND MIDDLE SIWALIKS OF CHAKWAL PAKISTAN

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Some new fossil remains of extinct rhinoceros species identified as *Hispanotherium matritense* is presented here in this paper. These fossil remains have been collected from Chinji and Nagri formations of the Siwaliks of Chakwal, Pakistan. The newly discovered material includes isolated lower dentition. The taxonomic identification up to species levels is based on the morphometric measurements and morphological similarities of described specimens with published records of the species both in and outside of Pakistan.

***DORCATHERIUM MINUS* (MAMMALIA: CETARTIODACTYLA: TRAGULIDAE)
FROM DHOK BUN AMIR KHATOON CHINJI FORMATION OF PAKISTAN**

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The *Dorcatherium* assemblage is quite rich in the Chinji Formation of Dhok Bun Amir Khatoon, district Chakwal, northern Pakistan. The specimens comprise isolated teeth and mandible fragments. The newly discovered material can be referred to the tragulid species namely *Dorcatherium minus*. The fossil record of *D. minus* indicates that the sample size increased in the middle Miocene of the Siwaliks. The presence of tragulids in Dhok Bun Amir Khatoon suggests a humid habitat with abundant cover during the Chinji Formation of Dhok Bun Amir Khatoon in northern Pakistan.

**RUMINANT FOSSILS FROM DHOK BUN AMIR KHATOON OF THE LOWER
SIWALIKS, PUNJAB, PAKISTAN**

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The ruminant remains from the Middle Miocene outcrops nearby the Dhok Bun Amir Khatoon (DBAK), district Chakwal, northern Pakistan, have been recovered, described and discussed in this research project. The recorded specimens include horn core, isolated teeth and mandible fragments. Over all 13 specimens are collected representing three ruminant families namely Bovidae, Giraffidae and Tragulidae. The new material from the DBAK reinforces the idea that a variety of ruminants were present in the outcrops of the Lower Siwaliks during the latest Middle Miocene. The Dhok Bun Amir Khatoon ruminant fauna has similarities to the Middle Miocene Eurasian faunas. The analysis of the recovered dental characters was allowed individual variations and contributes knowledge regarding the Siwalik latest Middle Miocene ruminants.

5. POULTRY

GROWTH PERFORMANCE OF DIFFERENT CLOSE BRED FLOCKS OF JAPANESE QUAIL UNDER VARIOUS FEED PROTEIN LEVELS

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Dietary Protein contents play a pivot role in growth and tissues development of a bird. However it effects the cost of production in the farming. Therefore, present study was conducted to optimize the dietary protein levels in quail feed to enhance growth and improve Feed Consumption Ratio (FCR) of Japanese quail. The study was conducted at Avian Research and Training (ART) Centre, Department of Poultry Production, University of Veterinary and Animal Sciences, Lahore, Pakistan. One thousand and eighty, day-old chicks were taken from four different close bred flocks (Local-I Kaleem, Imported-Major, Local-II Saadat, Local-III Zahid) 270 chicks of each flock were randomly divided into 3 sub-groups fed with 3 protein levels (21%, 23%, 25%) up to three weeks. After that both sexes were separated comprising of 15 birds in each replicate. Three experimental rations containing different protein levels i.e., 21 %, 23% and 25% were offered to the chicks till 6th week of age. Different physical parameters were recorded weekly. The results showed that strains did not differ statistically significantly in live body weight, times increase in body weight of both sexes. The weight gain and FCR were also non significant in males. Feed intake (both sexes), weight gain and FCR (female) were effected statistically significantly. Major attained maximum body weight and weight gain while better FCR was showed by saadat. Dietary protein levels affected bird's body weight, feed intake (both sexes), weight gain and FCR (female) of birds significantly ($p < 0.05$). The interactive effects of protein and strains were found statistically significant in weight gain, feed intake and FCR in both sexes. It is concluded that 25% protein level gave higher body weight than birds fed with lower protein (21%) from 1st to 6th week. In first four weeks maximum weight gain, lower feed intake and better FCR was recorded at 25% protein level but in 5th and 6th week 21% protein level showed best results.

STUDY OF SOME EGG QUALITY TRAITS IN BREEDER QUAILS REARED UNDER DIFFERENT REGIMES OF DIETARY LYSINE

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The aim of study was to evaluate egg quality traits of breeder quail reared under different regimes of dietary lysine at Avian Research and Training (ART) Centre, UVAS Lahore, Pakistan. 1440 broiler quail chicks were procured from the hatchery at ART Centre and the birds were reared at different dietary lysine regimes i.e. 1.3% lysine for 28 days (Phase-I), 1.4% lysine for 1st 14 days and 1.2% lysine for last 14 days (Phase-II), 1.5% lysine for 9 days, 1.3% lysine for next 10 days and 1.1% lysine for last 9 days (Phase III). These birds were equally divided into 36 experimental units with four different close-bred stocks, three lysine regimes and every treatment replicated thrice. At the age of 12 weeks 3 eggs from each replicate were picked randomly and

subjected to egg quality analysis. Analysis of data with the help of SAS 9.1 in RCBD factorial arrangements and comparison of means using Duncan's Multiple Range (DMR) test depicted significant differences ($P < 0.05$) in albumen height, albumen weight, albumen pH, yolk weight and Hough Unit value regarding different lysine phases and close-bred stocks. Non-significant differences were observed regarding egg weight, egg shell thickness, egg shell weight, yolk pH, yolk height, yolk width and yolk index.

EFFECT OF COW-DUNG AND POULTRY-DROPPINGS ON FISH GROWTH

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The present study was carried out in four earthen ponds to find out the effect of pond manuring on growth performance of carps. There were two groups, control and treated. Each group had two replicates. The experiment was performed for three months under semi intensive aquaculture system. All the four ponds were stocked with *Catla catla* 300, *Labeo rohita* 300, *Cirrhinus mrigala* 300 *Ctenopharyngodon idella* 300 and *Hypophthalmichthys molitrix* 300. Ponds in treated groups were manured with cow-dung (20kg/acre/day) and poultry-droppings (8kg/acre/day) and supplemented with routine 25% protein diet @ 2% of wet biomass of fish. Control group however received only supplementary feed containing 25%CP @ 2% body weight. Physico-chemical parameters viz. temperature, pH, dissolved oxygen, alkalinity, electrical conductivity and total hardness were recorded on daily basis while nitrates and phosphates were monitored on fortnightly basis. Initial weight and total length of fish was taken at the time of stocking and thereafter weight gain and increase in total length was examined on fortnightly basis. Significant increase in fish growth was observed in manured ponds. Non significant relation in physico-chemical variables in manured and control ponds indicates that cow-dung and poultry-droppings applied in said quantity did not deteriorate water quality.

PRE AND POST-MOULT HATCHING PERFORMANCE INFLUENCED BY AGE IN FOUR VARIETIES OF ASEEL CHICKEN

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The present study was conducted to evaluate pre and post-moulthatching traits in three age groups of four varieties of native Aseel chickens maintained at Indigenous Chicken Genetic Resource Centre (ICGRC), Ravi Campus, University of Veterinary and Animal Sciences, Lahore, Pakistan. For this purpose, a total of 168 eggs from four varieties of Aseel hens (Mushki, Lakha, Mianwali and Peshawari) each having 3 age groups A (50 weeks), B (80 weeks) and C (115 weeks) were collected during pre- and post-moult phases for hatching traits. The data thus collected during

both the phases were analyzed by analysis of variance in Randomized Complete Block Design (RCBD). The results showed non-significant differences in Fertility %, hatchability %, infertile %, dead germ % and cracked eggs % in all four varieties of Aseel during pre and post-moult phase. Dead in shell % was significantly higher in Lakha variety and in pre-moult phase while hatch of fertile % was significantly higher in Mushki variety. Hatchability % and hatch of fertile % was significantly higher in C and A age groups respectively while fertility %, infertile %, dead germ %, dead in shell % and cracked shell % showed non-significant differences in all three age groups.

EFFECT OF FOUR FEED RESTRICTION REGIMES ON THE GIBLET WEIGHT, ABDOMINAL FAT YIELD AND LEG QUARTER YIELD OF BROILERS

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Present study was conducted to investigate the impact of different feed restriction regimes i.e., ad-libitum, 1 hour feeding-3 hour off, 2 hour feeding-2 hour off and 3 hour feeding-1 hour off, on the giblet, abdominal fat and leg quarter yield of broilers. A total of three hundred and sixty day old (Hubbard) broiler chicks were divided into 24 replicates, 6 from each treatment and 15 birds per replicate were maintained under optimum conditions of temperature, humidity and ventilation as per recommendations of Hubbard broiler management guide. Experiment was conducted according to Completely Randomized Design (CRD). Data were analyzed using Analysis of Variance (ANOVA) technique and means were compared using Duncan's Multiple Range (DMR) test. Significantly ($P < 0.05$) higher abdominal fat yield was observed in the birds fed ad-libitum. Giblet weight was not affected by different feed restriction regimes while leg quarter yield was found to be the highest in the 1hr feeding-3 hrs off group.

PHENOTYPIC CHARACTERIZATION OF TWO INDIGENOUS CHICKEN ECOTYPES OF PAKISTAN

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This study documents some morphological characters of Aseel and Naked-neck chicken ecotypes (n=100; 40♂, 60♀ in each) collected from various regions of Pakistan. The characters included head shape; comb type; wattles size; shank color and feathering; types of spurs; number of toes; and colors and patterns of feathers on neck, breast, wing bow, wing bar, wing bay, saddle and tail. It was observed that both ecotypes had non-crested head. Aseel birds had pea comb (100%), absence of wattles (100%), fully feathered necks (100%), predominantly dark yellow shanks (29%), deep dark brown neck feathers (28%), pale-brown breast (32%), wing bow (33%), wing bar (35%), wing bay (35%) and saddle feathers (32%) and black tail feathers (40%). The number of toes in both the ecotypes was four. The Naked-neck birds had single comb (100%), predominantly

medium sized wattles (53%), dark yellow shanks (34%), off-white neck (35%) and breast feathers (36%), deep dark brown wing bow feathers (21%), off-white wing bar (26%) and wing bay (24%), black saddle (22%) and tail feathers (58%). A small proportion (3%) of the single spurs. However, some Aseel hens also possessed spurs. Predominantly plain feather pattern was present in both ecotypes; however, other patterns were also present. It was concluded that both ecotypes differ in their morphology and have rich variation of colors and patterns. Besides morphology, detailed studies on production, husbandry practices and economic importance of indigenous chickens should be conducted to devise breeding strategies and conserve desired characters for extensive production systems.

**CHICK QUALITY OF THREE EGG WEIGHT CATEGORIES THROUGH 4
PRODUCTION PHASES IN THREE DIFFERENT GENETIC GROUPS OF BROILER
BREEDERS**

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The study was conducted at commercial broiler breeder hatchery to compare chick quality from three different egg weight categories (Large, medium and Small) of three genetic groups of broiler breeders (Hubbard, Arbor Acre and Cobb) at four different production phases (Pre-Peak, Peak, Post-Peak and Terminal). At each production phase, 8910 settable eggs categorized in to three egg weight categories from 3 genetic groups replicated 6 times with 165 eggs per replicate were used for studying different parameters. The data were analyzed using ANOVA in Factorial arrangement in Randomized Complete Block Design with the help of SAS 9.1 and the means were compared using Duncan multiple range (DMR) test. The results showed that A grade chick% significantly increased with the advancement in age and egg size. However B and undersize grade chick % significantly decreased with advancement in age and egg size. While C grade chick% significantly increased with advancement in age but not affected by egg size. Different genetic groups showed non-significant differences for A, B and undersized chick percentage whereas C grade chick percentage was found to be highest in Cobb.

**RELATIONSHIP BETWEEN BODY WEIGHT AND EGG QUALITY TRAITS OF FOUR
VARIETIES OF INDIGENOUS ASEEL CHICKEN**

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The aim of present study was to evaluate the relationship of body weight on egg quality characteristics of four varieties (Lakha, Mushki, Peshawari and Mianwali) of native Aseel chicken at Indigenous Chicken Genetic Resource Centre (ICGRC), Ravi Campus, University of Veterinary and Animal Sciences, Lahore. The data of body weight and egg geometry was collected in 2nd, 3rd

and 4th production cycles of post moult phase and was subjected to Analysis of Variance through Complete Randomized Design (CRD) by using statistical package SAS 9.1. Significantly higher body weight was observed in Peshawari variety in all three categories (heavy, medium and light) with average body weights of 9341 ± 512.22 , 8065 ± 83.10 and 6069.33 ± 418.91 g, respectively, as compared to Lakha, Mushki and Mianwali. Higher egg weight (50.50 ± 0.92 g) was observed in heavy body weight category of Lakha, Mushki, Peshawari and Mianwali as compared to medium (46.33 ± 0.49 g) and light (40.00 ± 0.73 g) ones. Significantly average higher egg breadth was observed in heavy, medium and light body weight categories of Mianwali variety 4.20 ± 0.06 , 3.90 ± 0.04 and 3.66 ± 0.03 cm, respectively as compared to Lakha, Mushki and Peshawari. Mianwali variety in case of heavy body weight category has significantly lower egg length (5.08 ± 0.07 cm) while in case of medium and light body weight categories has significantly higher egg lengths 5.16 ± 0.04 and 5.20 ± 0.14 cm respectively as compared to Lakha, Mushki and Peshawari. Significantly average higher shape index of egg was observed in heavy medium and light body weight categories 79.50 ± 0.86 , 80.96 ± 0.63 , and 78.74 ± 1.07 cm respectively of Mushki and Peshawari varieties following by Lakha and Mianwali. Similarly significantly higher egg surface area was observed in heavy medium and light body weight categories 75.38 ± 1.76 , 59.3 ± 0.172 , and 44.67 ± 0.98 respectively of Mushki and Peshawari varieties as compared to Lakha and Mianwali.

HATCHING PERFORMANCE OF EGGS KEPT FOR 3 DIFFERENT STORAGE PERIODS AT 4 PRODUCTION PHASES IN THREE GENETIC GROUPS OF BROILER BREEDER

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The experiment was conducted at a commercial hatchery with the main objective to compare the hatching performance of the eggs stored for three different periods (one day, four day and seven day) from three genetic groups of broiler breeder (Arbor Acre, Hubbard and Cobb) at four production phases (Pre-Peak, Peak, Post-Peak and Terminal). At each production phase, 8910 eggs from 3 genetic groups were stored for 3 different periods with 6 replicates each to study hatching performance. The data were analysed using Factorial ANOVA in Randomized Complete Block Design (RCBD) with the help of SAS 9.1. Comparison of means was worked out using Duncan multiple range (DMR) test. The results showed significantly higher fertility, hatchability and hatch of fertile% at Peak production followed by post peak, terminal and pre-peak phases. Early and late embryonic mortality was found to be increased with advancement in production phase. Whereas non-significant differences in incubation moisture loss and mid embryonic mortality were observed. Significantly lower Fertility and hatchability% were recorded for Arbor Acre. However hatch of fertile and late embryonic mortality remained lowest in Hubbard. Increasing storage period significantly decreased hatchability%, while incubational moisture loss % and early embryonic mortality increased with the storage length. It can be concluded that at Peak production and one day storage hatching performance is better whereas no genetic group can be declared the best regarding hatching performance.

GROWTH PERFORMANCE AND ECONOMICS INFLUENCED BY FOUR FEED RESTRICTION REGIMES AT FOUR INITIAL CHICK WEIGHT CATEGORIES IN BROILERS

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A total of 1440 day-old (Hubbard) broiler chicks were maintained in this experiment. Broiler chicks with 4 initial body weight categories i.e small, medium, A and A⁺ ranging 31-34, 35-38, 39-42 and 43-46 g respectively were subjected to 4 feed restriction regimes i.e ad-libitum, 1, 2, and 3 hour restriction with 3, 2, and 1 hour feeding respectively after observing one week adjustment period. These 16 treatment groups were replicated 6 times making a total of 96 experimental units having 15 chicks each. The broiler chicks under the existing set-up of 4 initial chick weight categories coupled with 4 feed restriction regimes in Completely Randomized Design with two factors were examined for their growth performance and economics. Weekly data recorded on feed intake, body weight gain, FCR, mortality and production efficiency factor (PEF) compared by using Duncan's Multiple Range (DMR) test with the help of SAS 9.1. On the basis of FR regimes birds with 1-hr feeding-3hrs off showed significantly improved FCR by consuming less feed, gaining more weight and overall lower mortality rate. While on the initial chick body weight categories chicks from A⁺ category showed significantly better FCR. PEF was found to be better among the birds from medium weight category in 3hr feeding-1hr off group while economically A⁺ category with 1-hr feeding-3hrs off showed the best results.

PEDIGREE BASED SELECTION FOR HIGHER THREE WEEK BODY WEIGHT IN JAPANESE QUAIL AND ITS EFFECT ON OVERALL GROWTH PERFORMANCE

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The experiment was conducted at Avian Research and Training (ART) Centre, University of veterinary and Animal Sciences Lahore in order to study the possible changes in overall body weight of Japanese quail as a response to fully pedigreed selection for higher three week body weight. A base population of 1080 day-old quail chicks was procured from the hatchery at ART center. These chicks were equally divided into two groups (S and R), each having 60 replicates with 9 birds in each. At the age of 21 days every single bird was weighed and in group S only 20 males and 60 females having the highest body weight were selected to be the parents of next generation, while in group R 20 males and 60 females were randomly picked avoiding any selection and reared further to be the parents of next generation. At the age of 14 weeks the eggs from these birds were set in the hatchery to get next (02) generation chicks. Same selection procedure was adopted in G2 and G3 with a little variation in number of chicks. Statistical analysis of data with the help of SAS 9.1 in RCBD factorial arrangements and the comparison of means using DMR test depicted significant increase in three week body weight of all three subsequent generations of birds selected for higher body weight. Significantly higher body weight was observed for selected (S) than random-bred control (RBC) group. Mortality rate was found to be

significantly lower in each generation for selected group in comparison to the random-bred group.

STUDY OF SOME TRAITS REGARDING EGG GEOMETRY PARAMETERS IN BREEDER QUAILS REARED UNDER DIFFERENT REGIMES OF DIETARY LYSINE

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The aim of study was to evaluate egg quality traits of breeder quail reared under different regimes of dietary lysine at Avian Research and Training (ART) Centre, UVAS Lahore, Pakistan. 1440 broiler quail chicks were procured from the hatchery at ART Centre and the birds were reared at different dietary lysine regimes i.e. 1.3% lysine for 28 days (Phase-I), 1.4% lysine for 1st 14 days and 1.2% lysine for last 14 days (Phase-II), 1.5% lysine for 9 days, 1.3% lysine for next 10 days and 1.1% lysine for last 9 days (Phase III). These birds were equally divided into 36 experimental units with four different close-bred stocks, three lysine regimes and every treatment replicated thrice. At the age of 12 weeks 3 eggs from each replicate were picked randomly and subjected to egg quality analysis. Analysis of data with the help of SAS 9.1 in RCBD factorial arrangements and comparison of means using Duncan's Multiple Range (DMR) test depicted significant differences ($P < 0.05$) in length and egg surface area, while, egg length, egg width, egg shape index and egg volume were not influenced either by Lysine regimes or close-bred flocks

PRE AND POST-MOULT PRODUCTIVE PERFORMANCE OF THREE AGE GROUPS IN FOUR VARIETIES OF ASEEL CHICKEN

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The present study was conducted to evaluate pre and post-moult productive performance of three age groups in four varieties of native Aseel chicken maintained at Indigenous Chicken Genetic Resource Centre (ICGRC), Ravi Campus, University of Veterinary and Animal Sciences, Lahore, Pakistan. For this purpose, a total of 168 hens, 42 from each of four varieties (Mushki, Lakha, Mianwali and Peshawari) divided into 3 age groups A (50 weeks), B (80 weeks) and C (115 weeks) were collected during pre and post-moult phases for production performance parameters. The data collected during both the phases were analyzed by analysis of variance in Randomized Complete Block Design (RCBD). The results showed that overall productive efficiency remained better in post-moult as compared to pre-moult phase. Mushki showed significantly higher egg production, egg mass, egg weight, and better FCR/Dozen eggs. Feed intake was higher in Peshawari variety while body weight and FCR/Kg egg mass showed non-significant differences. First age group showed significantly higher feed intake, egg mass, egg production, and better FCR/Dozen eggs while egg weight and FCR/Kg egg mass were not affected by different age groups. Age group C showed significantly higher body weight.

STUDY OF SOME MORPHOLOGICAL TRAITS AS INFLUENCED BY DIFFERENT COMPOSTED RATIONS IN JAPANESE QUAIL

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Present study was conducted at Avian Research and Training (ART) Center, UVAS, Lahore, to evaluate the effect of composted ration on some phenotypic traits especially body measurements in Japanese quails. For this purpose a total of 1200 day old broiler quails were procured from ART center's hatchery and randomly divided into four experimental groups (A controlled, B 2% compost, C 4% compost & D 6% compost) each having 300 quail chicks. The birds in each group were randomly divided into six replicates. At the age of four weeks 3 birds from each replicate were picked randomly and their body measurements were recorded. Data thus obtained were analyzed through Completely Randomized Design (CRD) using analysis of variance techniques. Means were compared using Duncan's Multiple Range (DMR) test with the help of SAS 9.1 for Windows. Statistical analysis revealed significant differences ($P < 0.05$) among body weight, wing spread, shank circumference, length of keel, shank and drumstick whereas drumstick circumference remained non-significant.

GROWTH PERFORMANCE AND ECONOMICS THROUGH PHASE FEEDING AT DIFFERENT STOCKING DENSITIES IN SEXED BROILERS

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The present study was designed to examine the growth performance and economics of 1440 sexed broilers (720 each sex) subjected to four different levels of phase-feeding at three different stocking densities (0.7, 0.6 0.5 sqft). The group- A was offered diet with 19% crude protein (CP) throughout the grow-out period of 42 days. The group-B was allotted broiler starter diet with 20% CP up to 28 days of age and then broiler finisher diet with 17%CP up to 42 days of age. The diet for birds in group-C was split into 03 phases ie 0-10, 11-26, 27-42 days furnishing with 21, 19 and 18 % CP respectively. The group D had 4 feeding regimes ie. 0-10, 11-20, 21-34, 35-42 days with CP levels of 21, 20, 18 and 17% respectively. At the end of trial all the treatment groups received the same amount of weighed average of 19 % crude protein and 2800 Kcal/kg metabolizable energy (ME).The study was conducted according to Completely Randomized Nested Design. Analysis of data with the help of SAS 9.1 in CRD nested design and comparison of means using DMR test showed that male broilers gained significantly more folds of increase in body weight as compared to female, while, the overall means of all the other parameters differed non-significantly in both sexes. Significant effect of stocking density was recorded on mortality, point spread, production efficiency factor and folds of increase, while, phase feeding had non- significant effect on these parameters. Significantly higher body weight, feed intake, uniformity and better FCR were observed in four phases feeding regimes at 0.5 square ft stocking density, while economically, more profit was observed in four phase feeding program at 0.7 sq. ft. stocking density.

EFFECT OF THREE DIFFERENT ZONES OF THE HOUSE ON THE GROWTH PERFORMANCE, GIBLET WEIGHT AND ABDOMINAL FAT YIELD OF BROILERS

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This study was conducted to explore the effect of three different zones of the house (ventilators, central and variable speed fans) on the growth, giblet weight and abdominal fat yield of broilers. A total of one hundred and eighty day old (Hubbard) broiler chicks were divided into 18 replicates, 6 from each treatment and 10 birds per replicate, were maintained under optimum conditions of temperature, humidity and ventilation as per recommendations of Hubbard broiler management guide. Experiment was conducted according to Completely Randomized Design (CRD) and data regarding growth, giblet (liver, heart, gizzard) weight and abdominal fat yield were recorded and analyzed by using Analysis of Variance (ANOVA) technique and their means were compared using Duncan's Multiple Range (DMR) test. Significantly ($P < 0.05$) higher body weight and better FCR were observed in the ventilator house zone while feed intake and mortality remained non-significant in the all house zones. Abdominal fat yield was significantly higher in the birds which were kept in the fan area, while giblet weight was not affected by the different feed restriction regimes.

A STUDY OF BODY MEASUREMENTS AND SLAUGHTERING PARAMETERS IN FOUR CLOSE-BRED STOCKS OF BREEDER QUAIL REARED UNDER DIFFERENT REGIMES OF DIETARY LYSINE

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The aim of study was to evaluate body measurements and slaughtering parameters of breeder quail reared under different regimes of dietary lysine at Avian Research and Training (ART) Centre, UVAS Lahore, Pakistan. For this purpose 1440 broiler quail chick were procured from ART Centre hatchery, birds were reared at different dietary lysine regimes i.e. 1.3% lysine for 28 days (Phase-I), 1.4% lysine for 1st 14 days and 1.2% lysine for last 14 days (Phase-II), 1.5% lysine for 9 days, 1.3% lysine for next 10 days and 1.1% lysine for last 9 days. A total of 108 birds (3 from each replicate) were slaughtered at the age of 27 weeks and their body measurements and slaughtering parameters were recorded. The data thus obtained were statistically analyzed through Randomized Complete Block Design (RCBD) in factorial arrangement for further interpretations using GLM. The means were separated out through Duncan's Multiple Range (DMR) test using SAS 9.1 for windows. Statistical analysis of data revealed significant differences ($P < 0.05$) among shank length, drumstick length, drumstick circumferences and breast width whereas wing spread, keel length, shank circumference remained non-significant. In slaughtering parameters significant differences were observed in gizzard weight % and intestinal length whereas live body weight (g), dressing %age, heart, liver, giblet and intestinal weight % showed non-significant differences.

IMMUNE RESPONSE AND ENDOCRINE GLANDS WEIGHT INFLUENCED BY FOUR FEED RESTRICTION REGIMES IN BROILERS

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Three hundred and sixty, day old broiler chicks (Hubbard) were used to investigate the effect of four feed restriction (FR) regimes *i.e. ad-libitum*, 1, 2 and 3 hour restriction with 3, 2 and 1 hour feeding respectively, on the immune response and endocrine gland weights. Experiment was conducted according to Completely Randomized Design (CRD) and data regarding pancreas, bursa, thymus, and thyroid weights along with titer of Newcastle disease (ND) and Infectious Bursal disease (IBD) were recorded and their means were compared using Duncan's Multiple Range (DMR) test. On the basis of FR regimes, *ad-libitum* fed birds showed significantly higher bursa and thyroid weight. While the birds kept under feed restriction program were found to have lower immune response when compared with those fed *ad-libitum*.

HATCHING PERFORMANCE AS AFFECTED BY 3 EGG WEIGHT CATEGORIES IN 3 DIFFERENT GENETIC GROUPS OF BROILER BREEDER AT FOUR PRODUCTION PHASES

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The experiment was conducted at a commercial hatchery with the main objective to compare the hatching performance of the eggs stored for three different periods (one day, four day and seven day) from three genetic groups of broiler breeders (Arbor Acre, Hubbard and Cobb) at four production phases (Pre-Peak, Peak, Post-Peak and Terminal). At each production phase, 8910 eggs from 3 different breeder strains were stored for 3 different periods with 6 replicates each to study hatching performance. The data were analysed using Factorial ANOVA in Randomized Complete Block Design (RCBD) with the help of SAS 9.1. Comparison of means was worked out using Duncan multiple range (DMR) test. The results revealed significantly higher Fertility, Hatchability and Hatch of Fertile percentage at Peak Production Phase followed by Post Peak, Terminal, and Pre-Peak phase. While Incubation water loss, early and mid-embryonic mortality % increased with the advancement in production phase. Fertility % was found to be highest in Hubbard while Hatch of fertile % and late embryonic mortality was found to be highest in Cobb whereas non-significant differences were found among genetic groups (strains) for hatchability and incubational weight loss % and early embryonic mortality %. Large egg weight category has more incubational weight loss, fertility % and late embryonic mortality % followed by medium and small. However, Medium sized eggs have highest hatch of fertile % whereas hatchability, early and mid-embryonic mortality % was not affected with egg weight.

EFFECT OF DIFFERENT LEVELS OF LYSINE ON GROWTH PERFORMANCE AND ECONOMICS OF 4-CLOSED BRED STOCK OF JAPANESE QUAIL

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The present study was designed to examine the growth performance and economics on 4 closed bred-stocks (Kaleem, Sadaat, Major, Zahid) of Japenes quail subjected to three different levels of lysine. A total of 1440 commercial day-old quail chicks were used in this experiment. The group- A was offered lysine level of 1.3 percent throughout the grow-out period of 28 days. The group-B was allotted lysine level 1.4% up to 14 days of age and then lysine level with 1.2 up to 28 days of age. The lysine level for birds in group-C was split into 03 phases ie 1.5-1.3-1.1%, for 9-19-28 days. Experiment was designed according to Completely Randomized Design (CRD) with two factor factorial. Analysis of data was done with the help of SAS 9.1 and their means were comparison using Duncan's Multiple Range (DMR) test. The weekly data was collected on feed intake, body weight gain, FCR and mortality. Significantly higher body weight and better FCR were observed in three phases feeding regimes while feed intake was remained non significant in all the 4- closed bred stock. Economically the birds which received three levels of lysine fetched more profit in all closed bred stocks. The findings of the present study suggested that three phase feeding program may improve growth performance, FCR and economics in broiler quails.

PRE AND POST-MOULT EGG GEOMETRY INFLUENCED BY AGE IN FOUR VARIETIES OF ASEEL CHICKEN

ZULFIQAR AHMAD, MUHAMMAD USMAN, MUHAMMAD AKRAM, JIBRAN HUSSAIN, AMJAD IQBAL, MUHAMMAD SHAFIQ, SOHAIL AHMAD, ABD-UR-REHMAN, SANA ASHRAF

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The present study was conducted to evaluate pre and post-moult egg geometry in three age groups of four varieties of native Aseel chickens maintained at Indigenous Chicken Genetic Resource Centre (ICGRC), Ravi Campus, University of Veterinary and Animal Sciences, Lahore, Pakistan. For this purpose, a total of 168 eggs from four varieties of Aseel hens (Mushki, Lakha, Mianwali and Peshawari) each having 3 age groups A (50 weeks), B (80 weeks) and C (115 weeks) were collected during pre- and post-moult phases for egg geometry parameters . The data thus collected during both the phases were analyzed by analysis of variance in Randomized Complete Block Design (RCBD). The results showed that egg breadth and egg shape index were significantly higher in post-moult phase than that of pre-moult one while non-significant differences were observed in egg surface area, egg length and egg volume. Egg length, egg volume and egg surface area were better in Lakha and Mushki while egg shape index was better in Mianwali and Peshawari varieties. Egg breadth was better in Lakha and Mianwali. Non-significant differences were noted in egg volume, egg surface area and egg shape index in different age groups. Egg length was significantly greater in age group C and egg breadth was lowest in age group A.

EFFECT OF DIFFERENT PHASE FEEDING REGIMES ON IMMUNE RESPONSE AND ENDOCRINE GLAND WEIGHTS OF BROILERS

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Present study was conducted to investigate the effect of four different phase feeding programs i.e. single phase (19% crude protein) for 42 days of age, two phase feeding program (17% and 20% CP), for 28 and 14 days, three phase feeding (18%, 19% and 21% CP) for 10,16 and 16 days and four phase feeding (17%, 18%, 20% and 21% CP) for 10,10,14 and 8 days respectively, on immune response and endocrine gland weight of broilers. A total of four hundred and eighty, day-old (Hubbard) broiler chicks were divided into 24 replicates having 20 birds each and each treatment was replicated 06 times. Experiment was conducted according to the Completely Randomized Design (CRD) and data regarding thyroid, thymus, pancreas, bursa weight, Newcastle disease (ND) and Infectious Bursal disease (IBD) titer were recorded. Data thus obtained were statistically analyzed by using analysis of variance (ANOVA) technique and means were compared using Duncan's Multiple Range (DMR) test which revealed that significantly higher thyroid and thymus weights were showed in the birds which were fed according to three phase feeding program. While phase feeding programs showed no effect on the immune response of the broilers.

EFFECT OF DIFFERENT BODY WEIGHT CATEGORIES ON PLASMA MINERAL LEVELS IN FOUR CLOSE-BRED FLOCKS OF ADULT JAPANESE QUAILS (*COTURNIX COTURNIX JAPONICA*)

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The present study was conducted to investigate the macro plasma minerals in adult male and female quails, responded by different body weights in four close-bred flocks of Japanese quails (*Coturnix coturnix japonica*) maintained at Avian Research and Training Centre, University of Veterinary and Animal Sciences, Lahore. A total of 432 adult (12 weeks-old) quails, comprising 108 males and 324 females were randomly picked up from the available stock and were divided into 108 experimental units, comprising three females and one male each. These experimental units were randomly assigned to 12 treatment groups having 4 close-bred flocks x 3 female body weights (heavy 300-350g, medium 250-300g and small 200-250g) with randomized complete block design in factorial arrangements having 9 replicates in each treatment. In the present study, macro minerals data (Ca, P, Na, K and Mg) in blood plasma were studied. The results showed in this study that, the

difference in mean plasma Ca and Na levels in male and female quails of imported and local flocks of Japanese quails was not significant. With respect to body size categories, mean plasma Ca level in both the sexes of quails was not significantly different, however, plasma Na concentration was significantly ($p < 0.05$) different only in female quails. The interaction between flocks and body size for plasma Ca levels was significant ($p < 0.05$) in both the sexes of quails, whereas, for plasma Na it was significant ($p < 0.05$) only in female quails. The mean plasma P and K levels in imported and local flocks of Japanese quails were significantly ($p < 0.05$) different only in female quails, whereas, plasma Mg was significantly ($p < 0.05$) different only in male quails. However, with respect to body size categories, plasma P, K and Mg were significantly ($p < 0.05$) different in female quails only. The interaction between flocks and body size was significant for K and P in female quails only, whereas, it was also significant for plasma Mg levels in both the sexes of quails.

GROWTH PERFORMANCE, IMMUNE RESPONSE AND ECONOMICS INFLUENCED BY DIFFERENT REARING SYSTEMS (CAGE VS FLOOR) IN BROILERS

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The present study was conducted to investigate the effect of different rearing systems involving cages and floor on the growth performance, immune response and economics of broilers. A total of 240 day-old (Hubbard) broiler chicks were used in this experiment to study feed intake, body weight gain, FCR, mortality and production efficiency factor (PEF). Two treatment groups were replicated 12 times making a total of 24 experimental units with 10 chicks in each. At the end of trial (6weeks) two birds from each replicate were picked randomly and slaughtered to observe the antibody titer against New Castle disease. The data thus collect were analyzed using t-test with the help of SAS 9.1. On the basis of analysis of data, birds from cages showed significantly improved FCR and higher PEF by consuming less feed, gaining more body weight and overall less mortality rate, while birds on the floor showed significantly better immune response but remained economically less efficient.

APPLICATION OF MASS SELECTION PROCEDURES FOR HIGHER FOUR WEEK BODY WEIGHT IN JAPANESE QUAIL AND ITS IMPACT ON OVERALL GROWTH PERFORMANCE

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Experiment is in progress at Avian Research and Training (ART) centre, university of veterinary and Animal Sciences, Lahore. The aim is to increase body weight of Japanese quail to 250 grams at the age of 4 weeks. Presently it's the data of four generations of Japanese quail being selected for higher four week body weight through mass selection procedures. 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. Same procedure was repeated for all the four generations. Statistical analysis of data using SAS9.1, with one way ANOVA technique and comparison of means using Duncan's Multiple Range test revealed significant differences/improvement ($P < 0.05$) in body weight of all the four weeks of all generations being selected for 4 week higher body weight.

HISTOPATHOLOGICAL STUDIES ON STUNTING SYNDROME IN COMMERCIAL BROILERS

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Runting stunting syndrome (RSS) is a multifactorial disease with many names and faces that had caused considerable economic losses to poultry through reduced uniformity, reduced livability, decreased body weights, elevated feed conversions and many secondary diseases. The aim of current study was to evaluate the effect of stunting syndrome on body weights, hematology and histopathology in chicks ($n=120$) of different ages collected from nine (9) different farms. Grouping was done on the basis of age (G1=1-10 days, G2=11-20 days, G3=21-30 days, G4=31-40 days) including both stunted and normal chicks. Body weights of stunted chicks were found significantly reduced (32%) as compared to the normal chicks of the same age. Hematological results exhibited lower levels of Hemoglobin (Hb), total erythrocyte count (TEC) while values for total leucocyte count were lower as compared to normal. Absolute lymphocyte count (ALC), absolute monocyte count (AMC) and absolute eosinophil count was also found lower than normal. Histopathological findings were the intestinal lesions (29%), including degeneration of villi, crypts, epithelial cells and lamina propria. Pancreatic histopathological lesions (16.65%) included the fibrosis, vacuolation and degeneration of acinar cells. Degeneration of follicles and epithelial cells of bursa of fabricius (43%) and dilation of glandular cells of proventriculus including lymphocytes infiltration (5.6%) were other histopathological findings. All these changes may interfere with normal digestive processes and normal body functioning resulting in poor weight gain and retarded growth or stunting of chicks.

IMPACT OF GARLIC AND GINGER SUPPLEMENTATION ON MEAT CHARACTERISTICS, BLOOD METABOLITES AND HEMATOLOGICAL PARAMETERS OF BROILERS

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The objective of the present study was to evaluate the effects of garlic and mixture of garlic and ginger powder on the blood cells, hemoglobin concentration (Hb), blood metabolites, meat

characteristics and antibody titer against Newcastle Disease (ND) and Infectious Bursal Disease (IBD) in broilers. One hundred and five day-old Hubbard broiler chicks were randomly distributed into fifteen equal experimental units having seven chicks each. Five commercial experimental diets (A, B, C, D and E) supplemented with 0, 1% garlic, 2% garlic, 1% garlic + 1% ginger and 2% garlic + 1% ginger, respectively were randomly allotted to experimental units such that each diet was fed to three replicates. Results indicated that crude protein (CP) content and pH of meat increased ($P < 0.05$) and fat percentage as well as cooking loss percentage decreased ($P < 0.05$). Total cholesterol, triglycerides, low density lipoprotein (LDL) decreased and high density lipoprotein (HDL) proportion increased ($P < 0.05$). Hematological parameters including RBC, Lymphocytes, Hb and mean corpuscular haemoglobin increased significantly ($P < 0.05$). Heterophils, heterophil/lymphocyte ratio, and mean corpuscular volume decreased ($P < 0.05$) in the blood of birds fed treatment diets. However, WBC, monocytes, eosinophils and mean corpuscular haemoglobin showed non-significant ($P > 0.05$) results among all the experimental groups. Antibody titer against ND and IBD increased significantly ($P < 0.05$). Sensory evaluation score remained unaltered ($P > 0.05$) among all the experimental groups. In conclusion, garlic and ginger powder could be used in broiler diet as antilipidemic agents and enhancer of immunity in broilers.

EFFECT OF DIETARY SUPPLEMENTATION OF MANNAN-OLIGOSACCHARIDES ON VISCERAL ORGAN DEVELOPMENT AND SLAUGHTERING TRAITS IN FOUR CLOSE - BRED STOCKS OF JAPANESE QUAIL BREEDERS

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Use of antibiotics as a growth promoter has been banned in the European Union due to their side-effects like antibiotic resistance and drug residues. Prebiotics are considered as an alternative to antibiotics. Therefore, we investigated the influence of dietary supplementation of mannan-oligosaccharides (MOS) on slaughtering characteristics and relative weights of visceral organs & giblets in four close - bred stocks of Japanese quail breeders. Twelve week old Japanese quail breeders (n=960) of four close - bred stocks were randomly divided into four groups (n = 240), being 12 replicates (n = 20) in each group. Birds were fed a corn-based basal diet or the same basal diet supplemented with 0.25%, 0.5% and 1% MOS for 15 weeks. At the last day of 15th week of trial, three birds (two females and one male) from each replica were slaughtered to determine the relative weights of various visceral organs and various slaughtering characteristics of the birds. Data were analyzed statistically using ANOVA. In the present study, we observed considerable variations in the results among four close - bred stocks of the quail breeders. The imported strain has shown better results than the local strains. Relative weights of some of the visceral organs of the birds were different and slaughtering traits were not significantly different. The birds supplemented with 0.5% MOS were having better results compared to other treatment groups. In conclusion, feeding mannan-oligosaccharides as a replacement for antibiotic growth factor may positively influence production performance and health of quail breeders.

PRE AND POST-MOULT EGG QUALITY PARAMETERS AS INFLUENCED BY AGE IN FOUR VARIETIES OF ASEEL CHICKEN

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The present study was conducted to evaluate pre and post-moult egg quality in three age groups of four varieties of native Aseel chickens maintained at Indigenous Chicken Genetic Resource Centre (ICGRC), Ravi Campus, University of Veterinary and Animal Sciences, Lahore, Pakistan. For this purpose, a total of 168 eggs from four varieties of Aseel hens (Mushki, Lakha, Mianwali and Peshawari) each having 3 age groups A (50 weeks), B (80 weeks) and C (115 weeks) were collected during pre- and post-moult phases for egg quality parameters. The data thus collected during both the phases were analyzed by analysis of variance in Randomized Complete Block Design (RCBD). In the present study, significantly higher Albumen and Yolk pH, yolk index, Albumen percentage, Shell thickness and Haugh unit score was observed in post moult than that of pre moult one while albumen height was non-significantly different between pre and post-moult phases. Albumen percentage was observed to be lowest in Peshawari variety as compared to rest of three. Yolk Index was better in Mushki and Mianwali variety. Highest haugh unit score was observed in Mushki, higher shell thickness in Lakha, higher yolk and albumen pH in Mianwali variety while albumen height was better in both Lakha and Mianwali variety. Statistical analysis revealed that age groups did not influence all these parameters except Haugh unit which was maximum in age group A and Yolk Index Which was higher in age group C.

6. WILDLIFE, DIVERSITY AND CONSERVATION

STUDY ON CAPTIVE BREEDING OF ENDANGERED INDIGENOUS WILD ANIMALS AT NARC ISLAMABAD

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Captive breeding provides a means for conservation of endangered wildlife that may not survive in the wild due to anthropogenic or natural habitat destruction. While captive populations are established for many reasons such as conservation education, recreation. Exhibit of interesting species and research establishment captive population for saving species from extinction is an important task of conservation. The ongoing study is being conducted at Animal Sciences Institute, NARC in research facility of Poultry and Wildlife Program. Five enclosures have constructed and endangered ungulates were kept for captive breeding under observation Hog deer 1 male. 2 female and Black buck 1 male 1 female Hog deer found to be excellent breeder under the captivity that is from three animal we received seven offspring's which implies that even a single oestrous cycle not missed. In case of Black buck we receive three offspring's two from dystocia one survived yet. The hog deer become well acclimatised while Black buck remain shy in comparison. The study recorded offspring's growth rate time, weaning out time, sexual maturity, Area required for captive breeding of wild ungulates, feed needed, animal husbandry techniques and shared it with wildlife farmers, lovers, students Internees. The coordinated management of wild and captive populations as a single entity is desirable and will require unprecedented coordination of in-situ and ex-situ conservation efforts. Although in situ conservation of endangered species is always preferable to ex-situ preservation, there is often no choice. The future of the world's fauna depends upon our willingness and ability to meet the challenges of the task ahead

CHECKLIST OF FIRST RECORDED BAT SPECIES IN PESHAWAR AND ADJACENT AREAS, KHYBER PAKHTUNKHWA, PAKISTAN

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Seven species belonging to 5 genera of 4 families were recorded during May 2011 and September 2012 in Charsadda, Kohat, Mardan, Nowshera and Peshawar, Khyber Pakhtunkhwa, Pakistan. The bat species included: one fruit bat species (*Pteropus giganteus*; Pteropodidae), one fulvus leafnosed bat (*Hipposideros fulvus*; Hipposideridae), one greater mouse-tailed bat (*Rhinopoma microphyllum*; Rhinopomidae), three Pipistrellus bats of Vespertilionidae (Indian Pipistrellus, *Pipistrellus coromandra*; least Pipistrellus, *P. tenuis*; Javan Pipistrellus, *P. javinacum*) and one greater Asiatic yellow house bat (*Scotophilus heathii*; Vespertilionidae). A detailed study is required for further exploration of bat fauna in the study areas.

**POPULATION STATUS, THREATS AND CONSERVATION STRATEGIES FOR
PUNJAB URIAL IN RAKH KHABEKI AND RAKH KHARIOT FOREST SALT RANGE
DISTRICT KHUSHAB**

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Rakh Khariot and Rakh Khabeki 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 Phulai (*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 (Kinhati) and Rakh Khariot 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 akranda Loharan Wala, Churmil 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 the 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.

**DISTRIBUTION OF INDUS RIVER DOLPHIN (*PLATANISTA GANGETICA MINOR*) IN
DERA ISMAIL KHAN RANGE KHYBER PAKHTUNKHWA, PAKISTAN**

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To assess the status of the Indus River dolphin, *Platanista gangetica minor*, and to analyze the threats to their lives, a survey extending upto 103.5 km was conducted in the Khyber Pakhtunkhwa (KPK) of Pakistan in March 2012. It was conducted in the Indus River and its tributaries. The sum of best group size estimates produced an abundance estimate of 35 Dolphins. From Miran to Ramak in the Indus River approximately 48.57% of the dolphin population occurred in 27 km of river length, 0.33% of the dolphin population occurred in 46 km of river length from Dera Ismail Khan Bridge to Miran in the Indus River and 0.1 % of the dolphin population occurred in 30.5 km of river length from Saggu to D.L Khan Bridge. The density of dolphins was highest between Miran and Ramak in the Indus River. Increase in dolphin abundance was observed in a downstream direction. Threats to dolphins include too much vessel traffic, disturbance by the crane and duck hunters, high levels of anthropogenic threat and no effective measures for their conservation. River dolphins are particularly vulnerable to the activities of humans because of their

restricted habitat. Threats vary geographically in their importance, but generally include accidental killing during fishing operations, habitat loss and population fragmentation from water development. Deliberate killing for dolphin products also threatens the animals. Recommendations are given for conservation.

MORPHOLOGY OF GREATER FALSE VAMPIRES BATS *MEGADERMA LYRA* E. GEOFFROY, 1810 OF MALAKAND DIVISION OF PAKISTAN

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Four genera and five species of the false vampire bats exist throughout the world. Of these, the greater false vampire bat *Megaderma lyra* E. Geoffroy, 1810 is the only bat species reported from Pakistan. This bat has not been recorded from Malakand so far. We captured five females from a cave in Tura Gata (N34° 26.762' E71° 49.064') in Heroshah and recorded their body mass, external body and cranial measurements while two bats were also preserved in absolute alcohol in the BatLab, Department of Zoology and Fisheries, University of Agriculture, Faisalabad. This paper describes external body and cranial measurements of the Greater False Vampire bats species recorded from Malakand area. Our findings suggest that more in depth work may reveal some new bat roosts in the same area.

MORPHOLOGY OF GREATER AND LESSER MOUSE TAILED BATS OF MALAKAND DIVISION OF PAKISTAN

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Rhinopomatidae is a monogeneric family with four species worldwide. Three i.e. *Rhinopoma microphyllum*, *Rhinopoma hardwickii* and *Rhinopoma muscatellum* of the four species are represented in Pakistan. We captured eight specimens of both these species from two different caves i.e. *Rhinopoma microphyllum* (2 ♂, 1 ♀) from a cave (N34° 26.783' E71° 49.070'), *Rhinopoma hardwickii* (5 ♂) from another cave (N34° 26.818' E71° 48.973'). Both these caves were situated in Tura Gata in Heroshah of district Malakand. *R. microphyllum* were two male and one was female while all *R. hardwickii* were female. Body mass, external body and cranial measurements of these bats were recorded while two of them was preserved at BatLab, Department of Zoology and Fisheries, University of Agriculture, Faisalabad. This paper describes external body, cranial and bacular measurements of the Greater and lesser Mouse tailed bats species recorded from Malakand area.

STRUCTURAL FEATURES OF THE BACULA OF SOME MICROCHIROPTERAN BATS OF MALAKAND DIVISION OF PAKISTAN

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Baculum is a bone found in the penis 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 12 species of microchiropteran bats, viz., *Rhinopoma microphyllum*, *Rhinolophus ferrumequinum*, *Hipposideros fulvus*, *Scotophilus heathii*, *Scotophilus kuhlii*, *Myotis formosus*, *Scotoecus pallidus*, *Miniopterus schreibersii*, *E.serotinus*, *Rhinolophus lepidus*, *Pipistrellus tenuis* and *Pipistrellus pipistrellus*, taken from Malakand. The nine morphological features of the bacula were Total length of baculum (TLB), Length of shaft (SL), Proximal branch length (PBL), Distal branch length (DBL), Proximal branch width (PBW), Distal branch width (DBW), Height of baculum (BH), Width of proximal extreme (WPE) and Width of distal extreme (WDE).

A PRELIMINARY ASSESSMENT OF THE BAT FAUNA OF HEAD KOPER AND KUZ KOPER, DISTRICT MALAKAND, KHYBER PUKHTUNKHAWA, PAKISTAN

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The bat fauna of the District Malakand with special focus on two villages (Head Koper N34° 24.454' E71° 50.061' & Kuz koper N34° 24.399' E71° 50.171') Khyber Pukhtunkhawa (KP) was explored. The area was divided into three habitat types 1) Old/new buildings, 2) Orchids, and 3) Canal side. Survey was done at dawn and dusk. Bats were captured with the mist net and identified. A total of 34 bats (18♂, 16♀) were captured. These included *Scotophilus heathii* (n=9), *S. kuhlii* (n=1), *Scotecus pallidus* (n=6), *Eptesicus bottae* (n=1), *Eptesicus serotinus* (n=9), *P. dormeri* (n=2) and *P. tenuis* (n=6). Three species of bats; *Scotecus pallidus*, *P. dormeri* and *P. tenuis* were common in the two villages of district Malakand. We calculate netting index and relative abundance for each bats species.

MORPHOMETRY OF THE HORSESHOE BATS OF DISTRICT MALAKAND, KHYBER PUKHTUNKHAWA, PAKISTAN

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The present study had been designed to use external, cranial and bacular morphometry in assessing the variation in Horseshoe bats species and to determine the character of taxonomic

importance. From study area, Malakand, 2 species were captured belonged to two different areas i.e. Loe Agra (N34°34.868' E71°43.114') and University of Malakand (N34°40.054' E72°03.653'). Species named *Rhinolophus ferrumequinum* (Greater Horseshoe Bats) and *Rhinolophus lepidus* (Blyth's Horseshoe Bats). The mean body mass of *Rhinolophus ferrumequinum* captured from Loe Agra was 18.45 g ± 0.35 (SD). The mean head and body length was 60.00 mm ± 1.41 (SD) while the ear was 20.50 mm ± 0.71 (SD) long. Mean thumb and forearm length was 4.00 mm ± 0.00 (SD) and 60.00 mm ± 1.41 (SD) respectively. The mean body mass and forearm length of *Rhinolophus Lepidus* was 3.93 mm ± 0.21 (SD) and 37.67 mm ± 0.58 (SD) respectively. The mean breadths of braincase and zygomatic breadths of *Rhinolophus ferrumequinum* were 9.69 mm ± 0.23 (SD) mm and 12.15 mm ± 0.12 (SD), respectively.

TO-REDFINE GEOSPATIAL DISTRIBUTION OF VARIOUS BATS OF DISTRICT MALAKAND, KHYBER PUKHTUNKHAWA, PAKISTAN

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The present study had been designed to use GPS in assessing the digitized species distribution maps of various bats of Malakand Division. The global position of each species/roost was determined using Garmin Etrax H Global Position System (GPS) to develop digitized species distribution maps. The current distribution patterns of various bat species recorded from the study area were compared with the distribution maps available on the IUCN website (www.iucn.org). Roberts (1997), Bates and Harrison (1997), Srinivasulu *et al.*, 2010 and Mahmood-ul-Hassan *et al.* (2009) were also consulted for obtaining comparable information.

FIELD OBSERVATIONS ON BREEDING BIOLOGY OF SCALY-BELLIED WOODPECKER (*PICUS SQUAMATUS*) IN THE MARGALLA HILLS NATIONAL PARK, ISLAMABAD

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In view of the fact that, no scientific information is available on the breeding biology of scaly-bellied woodpecker (*Picus squamatus*) in the Margalla Hills National Park, Islamabad. Therefore, the current study was designed to study the breeding biology by observing the clutch size, incubation period, dimensions of nests and eggs, nestling and fledgling success of scaly-bellied woodpecker in the study area. A total of twenty eight (28) nests were located in the study area, out of which ten (10) nests were found active. Successful nests (n=10, 100%) were located in tree trunks. The data on the height of the nest from ground shows that the preferred nest height (n=6, 21.43%) by scaly-bellied woodpecker was 1-3 meters as compared to top of the trees (n=5,

17.86%) with height 13.1-15 meters. In the study area, the preferred vegetation for nest building by scaly-bellied woodpecker was *Pinus roxburghii* (n=13, 46%) and *Paper Mulberry* (n=15, 54%). The mean nest height from ground was 6.08 m (range=1.2-14.6 m). The mean cavity-entrance diameter of nest was 5.94 cm (range=5.08-6.5 cm) and the mean cavity depth was about 32.97 cm ranged from 25.4-38.1 cm. The mean clutch size was 5.3 ranged from 4 to 6 (n=10). Four eggs were found in one nest (10%), followed by 5 nests (50%) having five eggs each and 4 nests (40%) having six eggs. The total length of incubation period ranged from 16 to 17 days. Mean length of egg was about 3.29 cm (range=3.02 - 3.43 cm), mean width 2.30 cm (range=2.15 - 2.4 cm) and mean weight was about 14.5 g (range=14 - 15 cm). Eggs found were oval in shape and white in color. The hatching and fledgling success in one nest which had only four egg was 0%, whereas in five nests having five eggs each was 50% and four nests having six eggs each was 40%. The overall percentage of hatching was 90% with same percentage of fledgling. The present data on the breeding biology of scaly-bellied woodpecker needs further research on the parental care, mortality rates, predation, their distribution and vulnerability to climate change in the study area.

THE ILLEGAL WILDLIFE HUNTING AND LAW ENFORCEMENT IN AZAD JAMMU AND KASHMIR: A PIONEER STUDY

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Illegal hunting is one of the common reasons for declining wildlife in Azad Jammu and Kashmir. Effective law enforcement is essential to control this unsustainable exploitation of wildlife. This study investigates the current level of wildlife poaching and effectiveness of law enforcement in Azad Jammu and Kashmir. The study was entirely based on literature review and analysis of the secondary data of wildlife Department. The main findings of this study showed that a total of 202 cases of illegal wildlife hunting were registered in Wildlife and Fisheries Department during 2000-2011. This illegal hunting activities involved 18 wildlife species comprising birds (n=10) and mammals (n=8). Among these, 02 species were Vulnerable, 13 species were Least Concern and 03 species were Near Threatened in the IUCN species status record available during 2011. Koklass pheasant (n=57) was the leading victim of illegal hunting among the birds, while maximum hunting of grey goral (n=24) was recorded among mammalian species. Overall, maximum hunting of bird species was reported in spring (45%) while minimum in summer season (12%). On the other hand, majority (26%) of illegal hunting of mammalian species was reported in winter and minimum (17%) in autumn. About 126 cases of hunting were reported from outside of the protected areas while 76 cases were reported from inside of various protected areas. Among registered cases (n=202), 80% cases were resolved by the Wildlife Department, 10% cases by judicial courts while other 10% cases were still pending. Similarly, among the registered cases, majority of the cases were solved with diminutive actions against the culprit i.e., in term of fine and existing rules and regulations could not actually be implemented by the Department as well as by courts especially in case of protected areas. Due to the deficiency of field staff and required resources, the wildlife poaching cannot be controlled properly as actual rate of illegal wildlife hunting is too high than

being registered or prosecuted by the Wildlife Department. Furthermore, the available field staff also needs proper trainings and awareness to control the illegal hunting effectively. Several factors were found responsible for the illegal hunting of wildlife in AJK; i.e., availability of 12-bore shotguns, poverty, lack of awareness, involvement of politics, lack of alternative entertainment/sports opportunities and poor implementation or ineffectiveness of Wildlife protection laws. To reduce the illegal hunting of wildlife in AJK conservation and management projects with hunting mitigation strategies should be initiated.

OBSERVATION OF WATERFOWLS ON THE COAST OF PASNI BALUCHISTAN

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The coastline of Pakistan extends 1,050 km (650 mi), 250 km falling in Sind province and 800 km in Baluchistan. Baluchistan coastline extends over 750 kms. From Hub near Karachi to the Gwadar Bay on Pakistan-Iran border. The coastal tribes are as colorful as that of central and upper Baluchistan. Their colorful costumes, songs and dances are equally fascinating. The whole area is rich with long unspoiled golden sunny beaches and a variety of sea fish. Because of the importance of this coast, Pasni coast important area of waterfowls. 55 species of water fowl belonging to 12 order and 16 families have been recorded from the area during the period 2010-2012, which included some rare and threatened species. The status of each birds have been recorded

SPATIO-TEMPORAL PATTERNS OF COMMON LEOPARD (*PANTHERA PARDUS*) ILLEGAL HUNTING IN AZAD JAMMU AND KASHMIR (PAKISTAN): A CASE STUDY

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Human-leopard conflict has been alarmingly increased in Azad Jammu and Kashmir (AJK) during the last decade which has resulted in high rate of retaliatory killings of this threatened animal throughout the state. Based on the available records of Wildlife and Fisheries Department Government of AJK (in terms of registered cases of such killings reported by field staff during 1998-2011), an analytical study was conducted to assess the spatio-temporal patterns of illegal leopard killings in AJK during the last decade. The information was collected on the prescribed data sheets from the Directorate of Wildlife and Fisheries Department Muzaffarabad. A sum total of 29 illegal leopard hunting cases were found registered with the Department of Wildlife and Fisheries. Majority of the killings were reported from district Muzaffarabad (55%) followed by Bagh (17%), Hattian (14%), Kotli (10%) and Haveli (4%). Most of the killings (n=10) were reported in the year 2009. During winter season leopard was found to be the most vulnerable to killings (48%) followed by 20% in summer, 17% in spring and 13% in autumn seasons. The main reason for retaliatory killings was livestock depredation (65.51%) by leopard followed by attacks on humans (13.79%), while, rest of the killings (22%) were reported for recreation or unknown

reasons. About (66%) of the killings were reported from inside of the forest area and (34%) in community areas. Department of Wildlife and Fisheries resolved about (62%) of the total registered cases, while (38%) of the registered cases were referred to court for further proceedings. The hunting of leopard is emerging as big issue in AJK; however, the Department of Wildlife and Fisheries has no any formal management plan to tackle these issues.

HUMAN-BLACK BEAR CONFLICTS IN MACHIARA NATIONAL PARK, AZAD JAMMU AND KASHMIR (PAKISTAN): PATTERNS, PERCEPTION AND CAUSES

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Human-black bear (*Ursus thibetanus*) conflict is one of the major conservation and rural livelihood issue in Azad Jammu and Kashmir. The species has been evolved in the crop raiding and livestock depredation and thus persecuted due to elevated conflict levels with communities. To mitigate such conflicts requires a firm understanding of their underlying patterns. To understand these human-black bear conflicts, a study was conducted in Machiara National Park (MNP), Azad Jammu and Kashmir, from April to December, 2011. A sum total of 30 surveys were conducted in three union councils of MNP to collect information about livestock depredation and crop raiding by black bear, local tolerance, perception and knowledge about black bear behavior during the last five years (2006-2011). During these surveys, 108 affectees were interviewed using questionnaire. About 945 acres area with 53.88 metric tons estimated maize crop yield was damaged by black bears during the last five years. Most of the crops raiding incidences were recorded during 2011 on 376 acres and 181.2 metric tons of crop yield loss. The crop raiding season vary between the last weeks of October to November. The financial value of the damaged crop, according to the local market, was estimated as 1.370 million PKR. Similarly, during the study period, about 301 livestock heads (209 goats, 81 sheep, 8 cows, 2 oxes and 1 donkey) were also killed by black bear at different localities in the open forests or forest huts. Majority of livestock (n=122) was killed during 2011. The estimated cost of all these 300 livestock heads was 1.226 million PKR. All the livestock depredation incidences were recorded between June to November with maximum in July (n=62). Five persons were also injured during the last 20 years by black bear attacks at different localities. In response to these incidences, during the last 14 years (1998-2011), 11 black bears were killed by the community. Accordingly, 82% of respondents dislike black bear; while only a small proportion (18%) of the respondents were in favor of bear conservation. About 95% respondents recommended the ex-situ black bear conservation; while only 5% were in favor of black bear conservation in natural habitats. To improve black bear protection and local livelihood prospects around study area, various conflict mitigation strategies (e.g., community loss compensation schemes, livestock and crop insurance schemes, community awareness and improvement in protection strategies) should be initiated.

**STUDY OF SPATIO-TEMPORAL ASPECTS OF HUMAN-WILDLIFE CONFLICTS
AROUND MACHIARA NATIONAL PARK, AZAD JAMMU AND KASHMIR
(PAKISTAN)**

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Today one of the main challenges facing wildlife conservation in Azad Jammu and Kashmir is the increasing negative interaction between people and wildlife resulting human-wildlife conflicts. These conflicts are considered to be a major conservation and rural livelihood issue because both species faces deleterious effects. To mitigate such conflicts, the understanding of their underlying patterns is very crucial. A study was conducted to assess the human-wildlife conflicts in terms of crop and fruit raiding by mammalian pests around Machiara National Park (MNP), Azad Jammu and Kashmir. For this purpose, a total of 25 field surveys were conducted in three union councils comprising 30 villages of MNP during April to November 2011. The data about the crop and fruit raiding by the wild mammals during the last five years (2007-2011), and the tolerance and attitude of community towards crop raiders was collected through structured and semi-structured interviews using questionnaires. Five mammalian wildlife species including black bear, rhesus monkey, Indian porcupine, palm civet, and Kashmir flying squirrel were involved in the crop and fruit raiding activities in MNP. Three crops were found damaged by wildlife species comprising maize, potato and bean. Among total damage of the crops, about 100.60 Metric Ton (mt) of maize crop were damaged sharing about 62% of the total crop loss followed by potato (40.176 mt) and bean (20.206 mt) with 25% and 13% of the total damage, respectively. These damages to crops lead to an estimated amount of 5.738 million PKR (63688.89US\$) to communities during the last five years. Maximum raiding of maize crop was carried out by the black bear (46%) followed by porcupine (36.60%) and rhesus monkey (18.14%); whereas, potato and bean crops were damaged by porcupine solely. However, there was non-significant difference ($p=0.25$) between the percentage share of each raider species. Among fruits, walnut was the most extensively damaged fruit with 39.16 mt loss (34%) causing 6.811 million PKR (75677 US\$). Apple being the second most damaged fruit shared about 27% (30.44 mt) of the loss followed by peach and apricot (16%), date plum (12%) and ficus (11%) with about 17.828 mt, 13.6 mt and 13.08 mt loss, respectively. Accordingly, about 2.568 million PKR (28533US\$), 1.753 million PKR (19477US\$), 1.312 million PKR (14577US\$), 0.478 million PKR (5311US\$) was the estimated financial loss of the community due to loss of apple, ficus, peach and apricot, and date plum, respectively. There was highly significant difference between the quantitative loss ($p=0.0001$) and financial loss ($p=0.025$) of the community due to fruit damaged by different wildlife species during the last five years. The highest intensity of walnut, apple, ficus, date plum, peach and apricot fruit raiding was reported from Thora Baik (a summer pasture), Danna, Kahi, Sarlisacha and Dhair respectively; whereas, the lowest amount of damage of these fruits recorded was Byarr, Chatthiyan, Byarrian, Thora/Bayk and Magra. Majority (83%) of the respondents were of the opinion that wild animals come out of forest because they have no natural food, while remaining (17%) were of the opinion that they prefer crops over natural food resources. The methods used locally by communities for prevention of crop raiding are scare crow, beating drums

and firing crackers. About 92% respondents disliked the crop raiders and 98% people supported the idea of launching a compensation scheme on emergency basis to minimize these human-wildlife conflicts and retrieve community loss.

MORPHOLOGY OF FRUIT BATS OF MALAKAND DIVISION OF PAKISTAN

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Four species of fruit bats are reported from Pakistan. These include *Pteropus giganteus*, *Rousettus leschenaultii*, *R. aegyptiacus* and *Cynopterus sphinx*. Of these three viz., *Pteropus giganteus* (n=3), *Rousettus leschenaultii* (n=41), *Cynopterus sphinx* (n=1) were Malakand division. *Pteropus giganteus* was captured from Jaranda (N34° 24.808' E71° 48.202'), *Rousettus leschenaultii* from Tura Gata (N34° 26.818' E71° 48.973'), Cupni (N34° 27.691' E71° 48.220'), Brah (N34° 29.915' E71° 46.822'), while *Cynopterus sphinx* was recorded from Kashmir Smasta (N34° 25.780' E72° 13.727'). This paper describes external body, cranial and bacular measurements of the three fruit bat species recorded from Malakand area.

CONSERVATION BIOLOGY OF HIMALAYAN GREY GORAL (*NAEMORHEDUS GORAL BEDFORDI* HARDWICKE, 1825) IN MACHIARA NATIONAL PARK, AZAD JAMMU AND KASHMIR (PAKISTAN)

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Study was conducted about the conservation biology (population, habitat utilization and conservation status) of Himalayan Grey goral (*Naemorhedus goral bedfordi*) in Machiara National Park (MNP), Azad Jammu and Kashmir (AJK), from April to December, 2011. For this purpose, direct (physical observation and signs by transect method) and indirect (information through questionnaires) methods were used to collect information. The study area was divided into three main union councils (Machiara, Sarli Sacha and Bheri) and twenty two localities. The study was conducted at dawn and dusk while day time was utilized for taking indirect data. A total estimated population of about 91 animals was estimated at 22 different localities in the national park. Among these localities, the highest population density (2.37 animals/km²) was found in Union council Machiara, followed by Sarli Sacha (1.63 animals/km²) and Bheri (1.56 animals/km²). The overall population density recorded was 1.92 animals/km² with the highest density index recorded at locality Ravri (4 animals/km²) in Union Council Machiara. The habitat of grey goral was quite dense with hilly terrains scrub forests between 1800-3100m elevation above sea level with dominant vegetation as *Pinus wallichiana*, *Quercus incana*, *Asculus indica*, *Berberis aristida*, *Indigofera heterantha*, *Juniperus communis*, *Viburnum nervosum*, *Betula utilis*, *Rumex nepalensis*, *Fragaria nubicola*, *Impatiens edgeworthii* and *Polygonum amplexicaule*. During the last 12 years

(2000-2011) about 24 killings of goral were recorded by the field staffs of Wildlife Department out of which 2 killing were reported from MNP. Out of these 24 killings 9 killings were done in the protected area of AJK, while the remaining 15 were done in the non protected areas of AJK. Although the gorals are protected by laws in AJK, however, habitat destruction, hunting, poaching, over grazing by livestock and disturbance by human activities were the major threats to the conservation of grey goral in the study area.

BAT SURVEY OF MALAKAND DIVISION OF PAKISTAN

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Bats are the least studied group of small mammals in Pakistan. We studied their diversity and distribution in Malakand division. We recorded 315 bats that belonged to twenty two species, fourteen genera and six families. Of these twenty species were recorded Malakand district, eight from Dir district and two from Swat district that was poorly sampled. These included *Pteropus giganteus* (n=3), *Rousettus leschenaultii* (n=41), *Cynopterus sphinx* (n=1), *Megaderma lyra* (n=5), *Rhinopoma microphyllum* (n=3), *R. hardwickii* (n=5), *Hipposideros fulvus* (n=17), *Scotophilus heathii* (n=22), *S. kuhlii* (n=3), *Scotecus pallidus* (n=24), *Eptesicus bottae* (n=2), *Eptesicus serotinus* (n=14), *Rhinolophus ferrumequinum* (n=2), *R. lepidus* (n=3), *Myotis formosus* (n=5), *Miniopterus schreibersii* (n=6), *Pipistrellus coromandra* (n=8), *P. dormeri* (n=16), *P. tenuis* (n=35) and *P. javanicus* (n=1), *P. pipistrells* (n=1) and *Barbastella leucomelas* (n=1). This paper describes the netting and diversity indices of the bats captured from Malakand division.

THE RELATIONSHIP BETWEEN EGG WEIGHT AND GROWTH TRAITS IN RING NECKED PHEASANTS (*PHASINUS COLCHICUS*)

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The present study was conducted to find out the relationship between egg weight and growth traits in ring necked pheasants (*Phasianus colchicus*). Eggs of the ring necked pheasants were weighed and their lengths and breadths were taken. These eggs were divided into three weight groups and were classified as light (23.7 to 28.7g), medium (28.8 to 33.8g) and heavy (33.9 to 38.9g) weight. The eggs were incubated at 37.5 °C and 70 % relative humidity for 21 days during which they were rotated at an angle of 45° in Victoria incubators under standard conditions of incubation. Thereafter the eggs were transferred into hatching machine during the last three days. A temperature of 36.5°C and relative humidity of 85 % was provided to the eggs at hatching period. The chicks were weighed at the time of hatching and wing length and wing span was measured and thereafter the increase in weight and wing length and wing span was noted on weekly basis. The effect of egg weight on chick weight, live weight gain, wing length and wing span was found

significant ($P < 0.05$). Average chick weight at hatching in heavy, medium and light weight egg groups was 25.2, 19.2 and 16.2 g, respectively and the average live weight at three months stage was 564.8, 530 and 492.6g, respectively. Wing span and wing length at three months stage for heavy, medium and light weight egg groups was 77.42, 75.5, 71.52 and 53.8, 47.2 and 35.28 cm, respectively. Wing span and wing length at three months stage for heavy, medium and light weight egg groups was 107.3, 96.7, and 71.5 and 53.8, 47.2 and 35.28 cm, respectively. Our studies reveal that egg weight has pronounced effect on growth traits of ring necked pheasants.

MORPHOLOGICAL DIFFERENCES AMONG FULVOUS FRUIT BAT, *ROUSETTUS LESCHENAULTI* POPULATIONS FROM KHYBER PAKHTUNKHWA AND PUNJAB

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Geographic variations in animal body size have functional significances for a diverse range of life history traits and therefore subjected to an equally diverse array of selection pressures. These variations in size of homeothermic species are typified by pragmatic generalizations as according to most accepted zoological rule, Bergmann's rule, body size is positively related to latitude. The present study was conducted in Malakand district in Khyber Pakhtunkhwa and Lahore district in Punjab. The external body measurements viz. head and body length, ear length, forearm length, claw length, 2nd claw length, thumb length, length of 3rd metacarpal, length of 4th metacarpal, length of 5th metacarpal, length of 1st phalanx on 3rd metacarpal, length of 2nd phalanx on 3rd metacarpal, length of 1st/2nd phalanges on 4th metacarpal, wing span, tibia length, calcar length, hind foot length and tail length were taken and compared to find out morphological differences in *Rousettus leschenaulti* populations of both the areas. Statistically non-significant variations were recorded among all the parameters under study.

THE DIVERSITY AND MORPHOMETRICS OF BAT FAUNA OF CENTRAL PUNJAB

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Bats are considered terrifying and hateful creatures in Pakistan and this mind set makes them one of the least studied groups of small mammals in the country. Present study was conducted to explore the bat fauna of central Punjab. 11 sampling stations were fixed in four districts of central Punjab which included Gujranwala, Lahore, Kasur and Toba Tek Singh and a total of 52 bat samples belonging to two families, three genera and six species were captured. These species included *Rhinolophus blasii* (n = 3; netting index = 0.05), *Scotophilus heathii* (n = 18; netting index = 0.31), *S. kuhlii* (n = 4; netting index = 0.07), *Pipistrellus pipistrellus* (n = 20; netting index = 0.35)

and *P. ceylonicus* (n= 7; netting index = 0.12). The percent relative abundance was highest for *P. pipistrellus* (32.3%) and the lowest for *R. blasii* (4.8%). The external body, cranial and bacular measurements of these bats which are important characteristics for species identification were taken and compared with available literature.

AVIFAUNA DIVERSITY OF CHANGA MANGA FOREST, PUNJAB, PAKISTAN

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Changa Manga is an artificial forest land and wildlife sanctuary in district Kasur. The forest provides roosting place to a variety of birds. The forest area was surveyed from May 2009 to April 2010 to find out the avifauna diversity. The surveys were scheduled during dawn (5:00 a.m. to 8:00 a.m.) and dusk (4 pm to 7 pm). A total of 78 bird species were recorded from the study area, out of which 51 were resident species, 5 were summer breeders, 21 winter visitors and 1 bird species was year round visitor. Seasonal fluctuation in the number of species recorded within forest plantation was attributed to in and out movements of birds from the plantation and seasonal rotation of crops in the surrounding agricultural land 49 bird species were found common, 1 less common, 25 abundant and 3 were frequent species.

DISTRIBUTION OF AND THREATS TO FRESHWATER TURTLES PERCEIVED BY LOCALS IN SELECTED WETLANDS OF CHARSADDA, KP, PAKISTAN

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Turtles and tortoise are among the oldest tetrapod vertebrates having a complex history. Globally 330 species of tortoises and turtles are recognized up till now. Among them 149 are threatened and 98 are either critically endangered or endangered. South East Asia possesses one of the richest varieties of turtles in the world. Pakistan hosts eight species of freshwater turtles. These belong to two families namely *Geoemydidae* and *Trionychidae*. According to IUCN Red list narrow headed softshell turtle (*Chitra indica*) is endangered, ganges softshell turtle (*Nilssonina gangeticus*), peacock softshell turtle (*Nilssonina hurum*), crowned river turtle (*Hardella thurjii*) and spotted pond turtle (*Geoclemys hamiltoni*) are vulnerable, whereas spotted flapshell turtle (*Lissemys punctata*), indian roofed turtle (*Pangshura tecta*) and smiths turtle (*Pangshura smithii*) are at lower risk/not threatened in Pakistan. The survey was conducted with the pre-designed questionnaires from the locals regarding distribution and threats to freshwater turtles at selected wetlands of District Charsadda. It was cleared from the questionnaire analysis that four species of freshwater turtles were present namely *Chitra indica*, *Nilssonina gangeticus*, *Nilssonina hurum* and *Lissemys punctata*. Majority (47.5%) people were of the view that peacock softshell turtle is the most

abundant whereas spotted flapshell turtle was the least abundant (72.5%). The population of turtles species were decreases especially ganges softshell turtle and peacock softshell turtle. The possible reason of their decline is hunting, habitat degradation, adult killing and during fish practice. It was interesting to know that most of the hunters were non local. The respondents also revealed that turtle's species are export to national and international markets. No government level protection was provided for these species. In order to conserve turtles from extinction necessary steps should be taken on emergency bases.

**ROOST CHARACTERISTICS AND FOOD HABITS OF INDIAN FLYING FOX
(*PTEROPUS GIGANTEUS*) FROM LAHORE, PAKISTAN**

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The present work was planned to study roost characteristics and food habits of Indian flying fox (*Pteropus giganteus*) at Jinnah and Lalazar Gardens, Lahore where these bats were roosting. The study sites were visited on monthly basis for a period of one year i.e. from May, 2009 to April, 2010. 44 roosting sites (number of trees) at Jinnah Garden and six at Lalzar Garden were observed and monitored throughout the study period. At Jinnah Garden, the trees where bats were roosting belonged to 18 different species of plants with the population variation of 1100-1508 individuals while six roosting sites at Lalazar garden where population varied from of 90-175 individuals during the whole study period belong to two different species. Out of all 18 species of plants, serving as bat roosts, there were three viz. Wood apple (*Aegle marmelos*), Rayan (*Manalikara hexandra*) and Sabino (*Taxodium mucronatum*) that harboured less than 100 animals throughout the year. The flowers and fruit of Areca palm (*Areca catechu*) served as food for Indian flying fox throughout the year whereas the Papaya (*Carica papaya*), Slow match tree (*Careya arborea*), Sapodilla (*Manilkara zapota*), Mehiva (*Madhuca longifolia*), Mango tree (*Mangifera indica*) were consumed by bats exclusively in spring season. The fruits of Weeping fig (*Ficus benjamina*), Indian banyan (*F. bengalensis*), Cluster fig tree (*F. glomerata*) and Persian lilac (*Melia azedarach*) were used as exclusive food item during summer while the flowers of Sausage tree (*Kigelia pinnata*) and English shisham (*Albizia procera*) were the only food source during autumn.

**AVIFAUNA DIVERSITY CLOSE TO INDIAN BORDER AREA AT RAVI SIPHON,
PAKISTAN**

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This one year study extending from May 2009 to April 2010 was carried out at Ravi Siphon area in Sheikhpura district near Lahore, Punjab, which is an important wetland site along the Indian Border. The observations were taken on monthly basis following total count method and 87

species of birds were recorded from the study area out of which 24 were winter visitors, 54 residents, 6 summer breeders and 3 species were year round visitors. The bird species were designated as very common 1 in number, common 9, fairly common 20, uncommon 34, rare 12 and very rare 11 on the basis of their abundance. The number of birds recorded during May, 2009 was 319, June 354, July 375, August 390, September 316, October 432, November 349, December 395, January, 2010 was 373, February 389, March 401 and in April, 2010 it was 363. Relative abundance, Census Index and Shannon Weiner Diversity Index were also calculated for studied bird data. The most dominant bird of the area was Indian cliff swallow with relative abundance (RA) 5.69. The other dominant avian species were little green bee-eater (RA= 3.4), Large Egret (RA= 3.96), House Sparrow (RA= 2.3), and Bank Myna (RA= 2.6). Census Index was found 1.64 and Shannon Weiner Diversity Index was 5.98.

AVIFAUNA DIVERSITY OF HEAD QADIRABAD, GUJRANWALA

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This one year study extending from May 2009 to April 2010 was conducted to find out the avifauna diversity of Head Qadirabad which is an important wetland, situated at river Chenab. A total of 149 species of birds were recorded from the study area, out of which 76 were winter visitors, 54 were resident, 10 summer breeders and 9 species were year round visitors. Shannon-Weiner Diversity Index (H') was recorded 1.9582 and Census Index / density was 04.37. Threats to birds included habitat loss and degradation, chemical toxins, chemical pollution, avian diseases including avian malaria, humane disturbance of nesting, feeding and roosting areas, shortage of water, degraded quality of foraging habitat by the invasion of introduced species, and public dislike for some species. Many species had become extinct through human activities like excessive hunting, logging, large scale use of insecticides and pesticides in agriculture and industrial pollution.

RECENT RECORDS AND MORPHOMETRICS OF FULVOUS FRUIT BAT, *ROUSETTUS LESCHENAULTII* FROM MALAKAND

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This six month study, extending from October 2011 to March 2012, was conducted in Malakand District in Khyber Pakhtunkhwa to find out the day roosts of *Rousettus leschenaultia*, which was reported from Malakand in 1977. Exploratory visits were made to locate as many bat roosts in the study area as possible. Potential bat roosts such as old and undisturbed buildings, ruins, abandoned wells, farm houses, tree groves and forest plantations were thoroughly searched. Local people were also interviewed for gleaning maximum information about the exact location of various bat roosts. A bat roosts at Malakanad district was located and 9 (6♂, 3♀) specimens were

captured. These specimens were brought to the laboratory and their external body and cranial measurements were taken. Average head and body length of these specimens was 111.89 ± 14.013 , tail length was 10.77 ± 3.308 , average length of forearm was 76.24 ± 3.300 and average wing span was 393.64 ± 34.702 . The average greatest length of skull of 7 *Rousettus leschenaultii* 36 ± 2.160 , condylo-basal and condylo-canine lengths were 33.29 ± 2.059 and 32.57 ± 0.976 , respectively. The external body and cranial measurements of these specimens was compared with available literature.

BOLUS AND GUANO ANALYSIS OF INDIAN FLYING FOX, *PTEROPUS GIGANTEUS* FROM LAHORE

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Mineral composition of bolus and guano of Indian flying fox was analyzed in four seasonal samples i.e. winter spring, summer and autumn throughout the year. The pH ranges of fruit bat bolus were 6.7 and 7.4 whereas that of guano ranged between 7.1 and 7.4. The most abundant elements in bolus and guano are phosphorus and nitrogen whereas potassium is in lesser amounts. On average nitrogen values were higher in bolus (3.36%) than guano (2.72%). The phosphorus content of bolus was higher than that of guano. Ten fungal genera were identified from bolus and eight from guano samples collected during different seasons. *Alternaria*, *Aspergillus*, *Candida*, *Chrysosporium*, *Fusarium*, *Penicillium*, *Debaryomyces*, *Exophiala*, *Saccharomyces* and *Scopulariopsis* were observed from the bolus of the bat. The fungal genera observed in guano included *Alternaria*, *Aspergillus*, *Chrysosporium*, *Cryptococcus*, *Exophiala*, *Histoplasma*, *Scopulariopsis* and *Trichophyton*. Six bacterial genera were identified from bolus and ten from the guano seasonal samples collected from Jinnah Garden. These included *Acaligenes*, *Azotobacter*, *Bacillus*, *Bartonella*, *Corynebacteria*, *Klebsella*, *Listeria*, *Nitrosomonas*, *Nocardia*, *Salmonella* and *Streptomyces*.

A PRELIMINARY BAT SURVEY OF BAJAUR AGENCY AND ADJOINING AREAS OF DIR DISTRICT

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This study was made to collect information on various bat species inhabiting Bajaur Agency (FATA) and adjoining areas of Dir district (KPK). A total of 30 bats were captured and measured for body and cranial parameters. Of these twenty were collected from Bajaur Agency (tehsil Khar =14; tehsil Salarzo = 6). *Scotophilus heathii* (1♀), *S. kuhlii* (2♀), *Pipistrellus ceylonicus* (1♂, 1♀), *P. tenuis* (1♂), *P. cromendra* (1♂, 6♀), *P. javanicus* (1♂) were captured from tehsil Khar while *P. pipistrellus* (2♂), *P. tenuis* (1♂, 1♀) and *P. javanicus* (1♀) and *Rhinolopus hipposiderous* (1♂) were recorded from Teshil Salarzo. Ten bats were captured from Dir (Lower). These included *Megaderma lyra* (1♂, 4♀), *R. ferrumequinum* (1♀) and *R. hipposiderous* (4♀).

**FIELD SURVEY OF FRESHWATER TURTLES IN DISTRICT CHARSADDA, KP,
PAKISTAN**

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Wetlands play an important role in the regulation of river flow. They are vital to the health of worldwide biomes. Turtles and tortoises species are one of the keystone biodiversity components which highly contribute to maintain the integrity of freshwater ecosystem. They play a vital role in the environment, including seed dispersal, control of insects and snail populations and vegetation management. They also keep water clean and aquatic habitats healthy by scavenging dead animals and preying on weak and sick individuals. In order to determine the status, distribution and threats to freshwater turtles a comprehensive survey was conducted at selected wetlands of District Charsadda from June, 2012 to September, 2012. Data were collected from four selected wetlands namely River Jindi, Hisara drain, Kashmaloo drain and Dab drain. Line transect method was applied with fixed length of 1.5 Km on each station. Netted animals were counted, identified and then released back into the same water body. Data were collected from a total of 19 sampling stations, including 6 each from River Jindi and Hisara drain, 4 from Kashmaloo drain and 3 from Dab drain. A total of 618 specimens belonging to four species of freshwater turtles were identified. They all belonged to family *Trionychida*. The species includes ganges softshell turtle (*Aspiderates gangeticus*), peacock softshell turtle (*Aspiderates hurum*), narrow headed softshell turtle (*Chitra indica*) and spotted flapshell turtle (*Lissemys punctata*). Furthermore *Aspiderates hurum* (35.11%) and *Aspiderates gangeticus* (33.33%) had abundant population, whereas *Lissemys punctata* (19.42%) and *Chitra indica* (12.14%) were rare. Only at River Jindi four species namely ganges softshell turtle, peacock softshell turtle, narrow headed softshell turtle and spotted flapshell turtle were observed whereas on rest of the water bodies three species were identified excluding narrow headed softshell turtle. The overall population of ganges softshell turtle and peacock softshell turtle was higher compared to spotted flap shell and narrow headed softshell turtles. It was concluded from the habitat analysis that all turtle's species prefer mostly sandy and muddy bottoms with rich aquatic and riparian vegetation.

**INVESTIGATION ON DIETARY HABITS OF BARKING DEER (*MUNTIACUS
MUNTJAC*) IN MARGALLA HILLS NATIONAL PARK, ISLAMABAD.**

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Present study was conducted to investigate dietary habits of Barking Deer (*Muntiacus muntjac*) in Margalla Hills National Park, Islamabad. A total of 107 faecal pellet groups were collected season wise from eight different locations of the study area. The diet analysis was carried out by microhistological technique following Nagarkoti and Thapa (2005). The estimated mean frequency occurrence (%) of different plant parts in the 34 faecal pellet groups collected during

winter season indicated the presence of eleven plant species including four tree species i.e. *Phyllanthus emblica* (26.2%), *Zizyphus nummularia* (23.6%), *Acacia modesta* (18.7%), *Bauhinia variegata* (16.5%); five shrubs i.e. *Carissa opaca* (22.7%), *Maytenus royleanus* (17.3%), *Grewia optiva* (16.8%), *Myrsine africana* (13.2%), *Justicia adhatoda* (9.5%), and two grasses i.e. *Eleusine indica* (13.8%) and *Themeda anathera* (12.5%). During the spring season 42 faecal pellet groups, we identified 12 plant species including four trees i.e. *Phyllanthus emblica* (29.1%), *Zizyphus nummularia* (25.5%), *Bauhinia variegata* (24.3%), *Acacia modesta* (17.8%); five shrubs i.e. *Justicia adhatoda* (28.6%), *Carissa opaca* (24%), *Myrsine africana* (18.1%), *Maytenus royleanus* (17.5%), *Grewia optiva* (15.5%), *Woodfordia fruticosa* (14.6%) and two grasses i.e. *Eleusine indica* (13.3%) and *Themeda anathera* (9.4%). The summer samples which were 16 faecal pellet groups, showed the presence of 11 plant species, comprising of three trees i.e. *Zizyphus nummularia* (35%), *Bauhinia variegata* (11.3%), *Olea ferigena* (7.9%), six shrubs i.e. *Myrsine africana* (22%), *Justicia adhatoda* (17.4%), *Myrsine africana* (15.9%), *Carissa opaca* (14.5%), *Grewia optiva* (10.5%), *Woodfordia fruticosa* (6.5%) and two grasses i.e. *Eleusine indica* (12.4%) and *Themeda anathera* (7.5%). During monsoon season 15 faecal pellet groups were collected from which 10 plant species were identified as five trees i.e. *Zizyphus nummularia* (34.6%), *Carissa opaca* (25.1%), *Phyllanthus emblica* (10.7%), *Olea ferigena* (9.7%), *Bauhinia variegata* (8.2%); three shrubs i.e. *Justicia adhatoda* (19.3%), *Maytenus royleanus* (10.9%), *Grewia optiva* (9.3%) and two grasses i.e. *Eleusine indica* (13.1%) and *Themeda anathera* (11.3%). The overall results reflected that the most preferred tree species were *Phyllanthus emblica* (Amla) and *Zizyphus nummularia* (Beri), shrub *Carissa opaca* (Grandia), and grass species *Eleusine indica*. The results of this study suggested that there should be proper management and conservation of those species which are include in preferred diet of the deer.

**COMPARATIVE STUDY OF GREY PARTRIDGE, *FRANCOLINUS PONDICERIANUS*
AND BLACK PARTRIDGE *FRANCOLINUS FRANCOLINUS* AT MANG GAME
RESERVE HARIPUR, PAKISTAN**

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The grey partridge (*Francolinus pondicerianus*) and Black partridge (*Francolinus francolinus*) are the prime game birds of Pakistan, however both are endangered at different levels. Therefore, the present study was conducted to estimate the comparative population of these two partridges in Mang reserve Haripur, Khyber Pakhtunkhwa, Pakistan. Direct and indirect approaches were adopted for estimation of population. The game reserve was divided into three sub-habitats namely grassland, hillyland and wetland. The data was collected monthwise from each sub-habitat twice both at early morning and evening. The black partridge (*F. francolinus*) population density was found to be 0.19, 0.16 and 0.25 birds/ hactre in grassland, hillyland and wetland respectively. The estimated population density of grey partridge (*F. pondicerianus*) in grassland, hillyland and wetland was 0.37, 0.30 and 0.25 birds/ hactre respectively. The average population density throughout the game reserve was 0.31 birds/ hactre for grey partridge (*F. pondicerianus*) and 0.18/ hactre for black partridge (*F. francolinus*). The population density of grey partridge (*F. pondicerianus*) was significantly higher ($p > 0.05$) compared to black partridge. The month wise

population of grey (*F. pondicerianus*) and black (*F. francolinus*) partridges shows that, the maximum population for grey partridge (*F. pondicerianus*) were recorded (9.3 ± 3.2), (7.3 ± 1.2) and (7.8 ± 2.1) in grassland, hillyland and wetland respectively, while minimum grey partridge (*F. pondicerianus*) population were recorded (1.5 ± 0.7), (2.0 ± 0.5) and (1.8 ± 1.1) in grassland, hillyland and wetland respectively. Moreover, maximum black partridge (*F. francolinus*) population were estimated (4.0 ± 1.4), (2.5 ± 1.4) and (5.0 ± 2.1) in grassland, hillyland and wetland, while minimum black partridge (*F. francolinus*) population were recorded (1.2 ± 0.3), (1.0 ± 0.7) and (1.3 ± 0.4) in grassland, hillyland and wetland respectively. Our data conclude that grey partridge (*F. pondicerianus*) population were significantly higher in Mang gamr reserve compared to black partridge (*F. francolinus*).

DISTRIBUTION, POPULATION AND HABITAT OF SCALY ANTEATER (*MANIS CRASSICAUDATA*) IN MARGALLA HILLS NATIONAL PARK ISLAMABAD, PAKISTAN

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Scaly anteater (*Manis crassicaudata*), the only member of order Pholidota occurring in Pakistan, is a "Near Threatened" unique mammal species which faces a risk of extinction due to hunting pressure for its scales and trade purposes. The current study investigated its distribution, habitat preference and population in the Margallah Hills National Park Islamabad, from September 2011 to August 2012. The study area, divided into six representative study sites, was thoroughly surveyed for recording occurrence and distribution of the animal species through direct sightings as well as indirect signs like its burrows, faecal matter and information from the local people. The population estimation was carried out by using its active permanent burrows count while habitat was analyzed through quantification of vegetation using "point-centered-quarter" method for trees and quadrat method for shrubs and herbs. Results showed that the Scaly ant-eater had patchy distribution in the park ranging from 462m to 1046m elevation a.s.l. Its occurrence was confirmed at Phalwari, Kalinjar valley, Gandian valley, Rumli, Shahdara, Ratta-Hottar, Bari-Imam, Lake View and Malpur areas of the MHNP. At Sangjani, Talhar, Trail-3 and Trail-5, some of its old burrows revealed its occurrence in the near past. However, no direct or indirect signs of the animal were found at Saidpur village and Daman-e-Koh areas. Population estimates of the animal species revealed a very low average density of 0.0036/ha in the park. Vegetation analysis showed *Dalbergia sissoo* as dominant tree species at site-I (Malpur) and site-II (Phalwari) having IVI=140.45 and 125.2, respectively. At site-III (Gandian), V (Sangjani) and VI (Shah-Allah-Ditta), *Acacia modesta* (IVI =221.24, IVI=113.8, IVI=81.24, respectively) was dominant whereas at site-IV (Talhar), *Pinus roxburgii* (IVI=235.03) was the dominant tree species. The *Dodonea viscosa* was dominant shrub species while *Cynodon dactylon* was most common grass at all sites. The Scaly ant-eater showed special preference for *Lantana capara*, and *Punica granatum* for making its permanent burrows in the soil under and around these plant species.

DISTRIBUTION RANGE AND HABITAT STATUS OF BARKING DEER (*MUNTIACUS MUNTJAK*) IN THE MARGALLA HILLS NATIONAL PARK, ISLAMABADSHAHID MEHMOOD, MAQSOOD ANWAR, TARIQ MAHMOOD, IFTIKHAR HUSSAIN
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Barking Deer or Indian Muntjac (*Muntiacus muntjak*) is a small, solitary deer inhabiting dense tropical and subtropical forests. A study on Barking Deer was conducted to collect data on its distribution range and habitat status in the Margalla Hills National Park, Islamabad during 2011-12. Data on distribution range was collected through direct observations as well as indirect evidences (fecal pellets, foot prints, calls, etc) in its potential habitat. Data on habitat use was collected through vegetation sampling using quadrat method at randomly selected sites within its distribution range. Physical features of Barking Deer habitat such as elevation, aspect, water sources, etc. were also noted. Barking Deer was found in all four ranges of the park i.e Barakau, Saidpur, Nurpur and Golra. It was mostly found on southern slopes of the hills between 500m and 1200m elevation. A total of 52 plant species were identified in Barking Deer habitat in Margalla Hills National Park which included 22 trees, 21 shrubs, 7 grasses and 2 herbs. Individually, number of plant species identified in Barakau, Saidpur, Nurpur and Golra ranges were 28, 27, 39 and 27, respectively. Water sources were found throughout its distribution range in the park. Natural vegetation in four ranges of Barking Deer habitat in the park did not show any significant difference; however, Golra Range had maximum plant cover in their habitat. Major threats to Barking Deer in Margalla Hills National Park included loss and degradation of habitat due to resource use pressure by local human population as a result of livestock grazing, fuelwood removal, grass cutting, encroachment and disturbance by visitors of the park. Habitat protection and development must be done for conservation of Barking Deer in the Margalla Hills National Park.

SOME ASPECTS OF BREEDING BIOLOGY OF PHEASANT TAILED JACANA, *HYDROPHASIANUS CHIRURGUS* IN PUNJAB, PAKISTAN

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Hydrophasianus chiorurgus, of the family Jacanidae is a wader with huge feet and claws which enable them to walk on floating vegetation in shallow lakes, their preferred habitat. The Pheasant-tailed Jacana is capable of swimming, although it usually walks on the vegetation. The females are more colorful than the males and are polyandrous. Some aspects of breeding biology of the bird were observed during the period 2004-2007 at four water bodies, Marala, Qadirabad, Balloki and Sulemanki, in order to facilitate the conservation biologist. The clutch size remains between 1-4 eggs, and most of the fertile females laid 2 consecutive clutches. The field observations suggested that the incubation was totally attended by the males. The incubation periods was found to be 25-29 days. The highest hatchability of the eggs was at Sulemanki (83.7%), followed by Qadirabad (82.7%), and Marala (81.8%), and the minimum hatchability was recorded for Balloki (68.0%). Defensive responses, egg laying and chick care were observed to understand general behaviour of the species.

HABITAT SELECTION AND POPULATION STATUS OF MARCO POLO SHEEP (*OVIS AMMON POLII*) IN KHUNJERAB NATIONAL PARK, GILGIT-BALTISTAN

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Monitoring of animals population is necessary to conserve and manage rare species and to understand their population dynamics. Marco Polo sheep is an inhabitant of high mountainous plateau regions subject to cold winds and arid climate. It is not a permanent resident in Pakistan territory but migrates from Chinese Sinkian to Khunjerab National Park across three passes, Mintaka, Killik, and Khunjerab during summer season. Present study investigated habitat use and population status of Marco Polo Sheep in Khunjerab National Park, Gilgit-Baltistan during 2011-12. Vantage point method was applied to estimate the population Marco Polo Sheep and quadrat method to analyze its habitat. During 2011, 53 Marco Polo Sheep were sighted which were classified as 14 males, 16 females, 12 lambs and 11 yearlings with a density of 0.67 animals/Km². During 2012 a total of 42 individuals were sighted among which 19 were males, 11 females, 07 lambs and 05 yearlings with a density of 0.53 animals /Km². It was distributed in 78.83 km² area of Kerchanai Nallah of the park with mean group size of 47.5±5.5 animals. During vegetation analysis a total of 41 plant species belonging to 21 families were recorded in Marco Polo Sheep habitat. Herbs dominated the flora of the study area (78%), followed by shrubs (14.6%) and grasses (7.31%). Dominant plant species included *Astragalus tibetanus*, *Potentilla pamirica*, *Poa alpine*, *Allium carolianum*, *Artemisia sentolanifolia*, etc. In Khunjerab National Park, Marco Polo Sheep preferred plateau like habitat having boulders that were surrounded by rolling-hills, between 4,300m and 4,900m elevation. Major threats to Marco Polo Sheep are possible fencing from Chinese authorities along the border blocking its migration to Pakistan territory, competition for forage and space with domestic livestock and wild ungulates especially Himalayan Ibex.

BREEDING BIOLOGY OF GREY PARTRIDGE (*FRANCOLINUS PONDICERIANUS*) IN SALT RANGE, PUNJAB, PAKISTAN

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Grey Partridge (*Francolinus pondicerianus*) is a medium-sized game bird which is widely distributed in plains and drier regions of Pakistan including Salt Range in Punjab. Grey Partridge is a favorite game bird of Pakistan and is also considered friend of farmers due to its role as biological control agent of insect pests. Its population in Pakistan has declined during the recent past, mainly due to loss/degradation of habitat, hunting pressure and excessive use of pesticides. A study on breeding biology of Grey Partridge was conducted in Salt Range of Punjab during 2011-2012. Direct field observations were taken in the selected study sites to record data regarding breeding season, nest structure, clutch size, incubation period and hatching success. Results of the study revealed that their breeding season extends from mid March to end of the July. All the nests of grey partridge located during the study were found on the ground in the vegetation which mainly

comprised of plant species including *Desmostachia bipinnata*, *Cynodon dactylon*, and *Zizypus jujuba*. Egg laying occurred during spring and summer seasons mainly in the months of April and May, with mean egg laying span of 11.3 ± 1.68 (range 7-18 days) and a mean clutch size of 6.8 ± 0.78 (range 4-12 eggs). Mean incubation period was 15.7 ± 1.86 (range 13-20 days) and both the sexes took part in incubation. Out of a total number of 68 eggs located in various nests, 53 were hatched (74.80% success) with a mean hatching rate of 5.3 ± 0.85 eggs per clutch. The fledging success was estimated at 4.6 ± 0.81 per clutch (77%). In addition, nest measurements were also taken where mean outer diameter of the nest was 14.82 ± 1.31 (10.16-22.86 cm) while inner diameter was 18.51 ± 1.04 (15.24-22.86 cm). Weight of the egg varied with mean of 11.46 ± 0.93 gm (8-15 gm). Mean length of the egg was 34.24 ± 2.46 mm (25-46 mm) and mean width was 25.62 ± 2.11 mm (16-35 mm). The current study suggested that Gray partridge is successfully breeding in the Salt Range which supports its healthy population, helping in the conservation of this important game species.

ECOLOGICAL IMPACTS ON THE STATUS OF MARSH CROCODILES IN DEH AKRO II WILDLIFE SANCTUARY NAWABSHAH

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An ecological study of impacts on Marsh Crocodiles in Deh Akro II Wildlife Sanctuary Nawabshah was carried during the month of January to December 2008. Marsh Crocodiles are considered endangered around the world due to the vulnerability of marshy habitat due to increase pollution and environmental deterioration. In order to assess, the microbial contamination, detection of pollutant indicator organisms in the water samples, using different physico-chemical parameters were performed. The level of different physico-chemical parameters like as temperature, electrical conductivity, total dissolved solids, calcium, magnesium, bicarbonate, chloride, sodium, potassium, sulphur, carbonate, biological oxygen demand and dissolved oxygen were monitored in water samples collected from Deh Akro II Wildlife Sanctuary to assess the impact of toxic pollutants. Metal concentrations in water samples were estimated by atomic absorption spectrophotometer. Toxic chemical contaminants were estimated below the detection limit, while other several chemicals were found within the range set by WHO. The degree of contamination, proximity to pollution source and the metabolic ability of Marsh Crocodile suggest that the species are at threatened from the environmental contamination by the study of heavy metals.

STUDY OF DIFFERENT CAPTIVE ENVIRONMENT CONDITION ON BEHAVIOR OF AFRICAN LION (*PANTHERA LEO*)

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Humans housed the wild animals in captive environment for the purposes of conservation, public education; research, and recreation. But these wild beasts developed may abnormal behaviors in the captivity because these man made captive facilities are not appropriate for them,

that ultimately leads to compromise their natural or instinctive behaviors. In general Felids, have vast natural home ranges in the wild which lacking at most of zoological gardens. The aim of this study was to assess the behavioral repertoire of African Lions in Lahore Zoological Gardens compared with a Lahore Zoo Safari Park. The main focus of study was to identify whether the enclosure size and environmental conditions have any impact on abnormal and natural behaviors on the behaviors of 10 captive African Lions (one breeding pair, two siblings and one solitary male lion at Lahore Zoological Gardens and one group of breeding pair & two siblings and one solitary male lion at Lahore Zoo Safari Park) in each collection. The total area Lion house at Lahore Zoo is 27400 sq ft (1 acre) while the total area of lion safari at safari park is 653400 sq ft (15 acres). The data was recorded by instantaneous point-sampling technique over 48 hrs in 03 months of period in summers at both captive places to find out the frequency of different behaviors performed by these lions. The both felids were observed for 06 abnormal behaviors like food refusal, pacing, abnormal aggressiveness, auto mutilation, head tossing and Feaces Licking and 12 normal/natural behaviors like Playing, Climbing ,Stalking, Scent Marking, Lying on Back, Sleeping, Roll over/Stretch, Grooming, Digging, Roaring, Use of water pool and Mating. The results of this current study elaborated that lions kept in semi-natural and large enclosures with ample shade, water pool, good vegetation cover, resting places, natural substrate and social groups at Lahore Zoo Safari Park has shown less frequency of abnormal behaviors then lions at zoo at one hand and have high frequency of natural/normal behaviors at other hand. The frequency of abnormal behavior in solitary lion at safari was 0.08 and 0.12 for solitary lion at Zoo, while the same lion at safari has high frequency of 0.25 for normal behavior then 0.18 frequency of zoo lion. The recorded abnormal behavior frequency of breeding pair male lions at safari and zoo was 0.04 and 0.10 respectively. The female of breeding pairs at safari showed less frequency of abnormal behavior i.e. 0.04 then the 0.06 frequency of female at zoo. The normal behavior frequency of breeding pair was same and high i.e. 0.25 then the male and female of zoo which is 0.14 and 0.10 respectively. Both the siblings of zoo and safari shown low frequency of abnormal behaviors and high frequency for normal behavior with little difference then adult lions. The abnormal frequency of behavior for siblings was 0.02 at safari and 0.04 at zoo, while normal behavior frequency of siblings at safari was 0.20 and 0.14 for siblings at zoo. In the light of above findings it is suggested that captive lions should be kept in social groups within big enclosures having natural substrate, good vegetation cover, water bodies, sufficient shade, a multiple resting places, and different enrichment items.

STATUS, DISTRIBUTION AND POPULATION OF HIMALAYAN IBEX IN SHISHPER NULLAH HUNZA, PAKISTAN

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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 km². 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 (≥ 2 years age), yearling (≥ 6 & < 12 months), kid (≤ 6 months) and trophy size males (≥ 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 Shishper 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

DIVERSITY OF INSECTIVOROUS BAT FAUNA OF BAJUR AGENCY, PAKISTAN

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Present study was planned to explore bat fauna of Bajur agency, Pakistan. A total of 30 bat specimens belonging to three families, four genera and ten species were captured from the study area by mist nets and hand net. The netting efforts in all the netting station was same i.e. 3600 m² hrs. Bat species captured from the study area include *Scotophilus heathii*, *S. kuhlii*, *Pipistrellus ceylonicus*, *P. javanicus*, *P. tenuis*, *P. coromandra*, *P. pipistrellus*, *Rhinolophous hipposideos*, *R. ferrumequinum* and *Megaderma lyra*.

POPULATION AND CONSERVATIONAL STATUS OF MARSH CROCODILES, *CROCODYLUS PALUSTRIS* IN HALEJI LAKE WILDLIFE SANCTUARY (THATTA) SINDH, PAKISTAN

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Haleji Lake is the largest bird sanctuary in Asia and also home to Marsh crocodiles (*Crocodylus palustris*) which is located in Thatta, Sindh-Pakistan. Out of 23 species of crocodylian, only one species of Marsh crocodile occurs in Pakistan. The study on the population and conservational status of Marsh crocodiles was carried out from 2006 to 2009. The species have

declined in range and numbers since the turn of the 20th century, mainly through over-hunting for hide and meat in the past, and conversion of their habitat to other land-uses by man at present. Most people think about them as a serious and potentially hazardous pest and so do not be repentant their disappearance from their neighborhood. Marsh crocodiles can be ranched, but the concept of sustainable utilization of wildlife in general is still anathema to a large segment of the population in Pakistan. On the other hand, if crocodiles could benefit people, they are not likely to become extinct.

STUDY OF ECTO- AND ENDO-PARASITES OF DOMESTIC PIGEON (*COLUMBA LIVIA DOMISTICA*) KEPT AT JALLO WILDLIFE PARK, LAHORE

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The present study was conducted to record relative abundance (%) of ecto-parasites (chewing lice) and egg per gram (EPG) of endo-parasites in Domestic Pigeon (*Columba livia domestica*) in captivity at Jallo, Wildlife Park, Lahore. For this purpose 46 (30 male and 16 female) birds were examined. Ecto-parasites were collected, preserved, stained and identified. A total of 1622 lice (705 female, 853 male and 64 nymph) of 19 species were identified. Relative abundance of the total lice examined from 46 adult domestic pigeons was 100 % (female 43.46%, male 52.59%, nymph 3.94%). The dominant lice was *Columbicola columbae* with relative abundance of 80.64% followed by *Physconelloides zenaidurae* (0.31%), *Menacanthus stramineus* (2.71%), *Menacanthus pallidulus* (1.60%), *Menpon gallinae* (2.09%), *Lipeurus caponis* (0.80%), *Lipeurus tropicalis* (0.74%), *Goniodes gigas* (1.79%), *Goniodes dissimilis* (0.49%), *Goniocotes colchichi* (0.68%), *Goniocotes gallinae* (0.49%), *Colpocephalum zebra* (0.37%), *Colpocephalum turbinatum* (0.12%), *Coloceras damicorne fahrenheitzi* (0.12%), *Chelopistes meleagridis* (1.17%), *Cuclutogaster heterographus* (3.27%), *Bonomiella columbae* (0.74%), *Camnulotes bidentatus* (1.11%), *Hohorsteilla lata* (0.06%). During the present study, it was noted on the average, each bird was loaded 14.2 ± 6.3 , on adult female was 19.9 ± 12.1 and on male was 8.5 ± 3.7 . Fresh fecal samples of each pigeon were collected and endo-parasites were identified. A total of 16500 EPG was recorded. All the samples of fecal matter were loaded with the eggs of 7 types of helminthes and 2 types of protozoans. *Eimeria columbae* was dominant species with 5200 EPG and relative abundance (31.52%) followed by *Capillaria columbae* with 4075 EPG and relative abundance (24.70%), *Eimeria labbeana* with 3500 EPG and relative abundance (21.21%), *Capillaria retusa* having 1400 EPG and relative abundance (8.48%), *Capillaria annulata* having 875 EPG and relative abundance (5.30%), *Ascaridia galli* with 825 EPG and relative abundance (5%) , *Capillaria longicollis* with 275 EPG and relative abundance (1.67%), *Heterakis gallinae* with 250 EPG and relative abundance (1.51%) and *Syngamus trachea* with 100 EPG and relative abundance (0.61%). During the present study, it was noted on the average, each bird was loaded 101.9 ± 12.8 , on adult female was 2.1 ± 16.2 and on male was 121.6 ± 19.6 . From this study, it was concluded that it is the need of time for extensive study on ecto- and endo-parasites of domestic pigeon so that, these can be preserved from diseases caused by these agents which ultimately affect the health and reproductive performance of these birds.

**VARIATION IN SOIL MACROINVERTEBRATE COMMUNITIES UNDER
CONVENTIONAL AND ORGANIC FARMING PRACTICE IN WHEAT AND
SUGARCANE**

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The present study was conducted to assess the macro and micronutrients variation and their impact on soil macro invertebrate communities in conventional and organic farms of both wheat and sugarcane fields in Faisalabad district (73°74 E, 30°31.5 N). The soil macro invertebrates were sampled fortnightly from conventionally (HIP) and organically (UP) practiced fields. A total of 3323 individuals of macroinvertebrates belonging to 192 species were recorded from wheat and sugarcane fields. Species richness was recorded higher in wheat (n = 126) than in sugarcane (n = 92) and overall Pulmonates (n = 66) and Coleopterans (n = 55) were the more species rich taxa in both the crops. The organic fields of both wheat and sugarcane were more species rich (n = 102, n = 79 respectively) than conventional fields (n = 62, n = 61 respectively). The macro and micronutrients were recorded significantly effects macro invertebrates assemblages in both wheat and sugarcane crops. It is concluded that low input farming system (rich of organic matter) support the richness, abundance and diversity of macro invertebrate taxa, which in turn play essential role for soil health.

**COMPARISON OF MICROBIAL GROWTH RATE ON WILD AND FARMED *CYPRINUS
CARPIOL CATLA CATLA* FRESHLY HARVEST, ICE STORED AND AFTER
TRANSPORT**

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This study was aimed to estimate the microbial growth and rate of spoilage in freshly harvested fish and its effect after transport. Wild and Farmed *Cyprinus carpio* and *Catla catla* species were used for analysis. Microbial samples were taken from flesh, gills, tail and fins of these fish species that were freshly harvested and even after transport to different fish hatcheries. Microbial growth cfu with variable time periods were determined in laboratory. Results showed that the microbial growth was directly proportional to time. No microbes are present in flesh at the time of harvest, and microbial growth rate was found higher on gills as compare to that of tail and fins. This study realized the fact that fresh fish have lesser number of microbes in body parts and on skin and no in flesh. It showed twelve hour shelf life without storage in ice with lesser microbes depending on temperature. With storage in ice shelf life increases near about four days. Live fishing experiment provides fresh fish till to the reach of consumer without any contamination and live fish showed resistance for the growth of microbes. Fishes stored in ice showed no contamination and high fresh qualities and consumer attraction have fresh qualities and consumer attraction to buy. Live fishing proved to be the best way for the transfer of fishes from fishing point to sale point.

PROSPECT OF UTILIZATION OF ANCHOVIES (FAMILY: ENGRAULIDAE) FOR ALTERNATE INCOME GENERATION FOR FISHERMEN COMMUNITIES ALONG THE COAST OF PAKISTAN

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Anchovies belonging to Family Engraulidae are abundantly found along the coast of Pakistan. Of the ten species of anchovies known from Pakistan, *Stolephorus commersonii* Lacepède, 1803 (Commerson's anchovy) and *Stolephorus indicus* (van Hasselt, 1823) (Indian anchovy) are presently harvested in appreciable quantities using set bag, seine and cast nets in the creek system of the Indus Estuary. Presently these species are sun-dried and used as cheap raw material for poor quality fish meal for the poultry. In a recent study, high valued boiled-dried anchovies were produced. Coastal communities at Keti Bundar were motivated to commercially produce this product from the anchovies harvested from the area and export the product to Southeast Asian countries where it is relished. The paper describes the potential of anchovies processing in Pakistan. It also covers aspects of biology of anchovies found in the Indus Estuary.

AN ACCOUNT OF MOBULID RAYS (FAMILY: MYLIOBATIDAE) FOUND ALONG THE COAST OF PAKISTAN

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Mobulids play an important role in the pelagic ecosystem in coastal and offshore waters of Pakistan. Six species belonging to two genera *Manta* and *Mobula* are reported from Pakistan. Of these *Manta ehrenbergii* (Muller and Henle, 1841), *Mobula eregoodootenkee* (Garman, 1913) and *Mobula japonica* (Muller and Henle, 1841) are abundantly found in the area. Mobulids are caught mainly as bycatch of tuna gillnet fisheryies. A considerable decrease in mobulid landings was observed in Pakistan during last two decades due to increase fishing pressure.

STATUS OF RECENT KNOWLEDGE ON MARINE CETACEAN ABUNDANCE AND DIVERSITY IN PAKISTANI WATERS

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Large vertebrates including whales, dolphins and porpoises constitute marine cetaceans which may inhabit in a particular area and also may perform physiological and behavioral functions in different areas in coastal waters and open oceans overriding geographical limits of countries and continents. Only sparse and discrete information on diversity of marine cetacean is available from

northern Arabian Sea bordering Pakistan. A first scientific survey program was initiated in 2005 under DFID funded Higher Education Linkage and DEFRA's Darwin Initiative programs. The data collected ever since is being presented here. Cetaceans are the top predator and their conservation is important for maintaining balance in the pelagic ecosystem. The information was gathered through boat and beach surveys. Number of cetacean species occurring in Pakistani waters has been confirmed. Two new species (Striped dolphin and Rough toothed dolphin) have recently been identified through skeletal material and sightings. A total of 15 cetacean species including 6 whales, 8 dolphins and 1 porpoise species are now confirmed to inhabit this area. Beach remains (skeletal material) and information on stranded animals appear to be useful in identification and confirmation of new species. Stranding data also provide information on the threats that these animals face in these waters. Further research on abundance and behavior of cetaceans and establishment of regional cooperation are needed to address many unanswered questions about this group of animals and resolve some trans-boundary issues.

**POTENTIAL OF *AEGICERAS CORNICULATA* (L.) BLANCO LEAVES EXTRACTS
AGAINST SOME ATMOSPHERIC FUNGAL ALLERGENS**

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In vitro, antifungal bioassay of *Aegiceras corniculata* (L.) Blanco (Syn. *A. majus* Gaertn.) leaves was carried out. The extracts were prepared in DMSO, DW, chloroform, acetone and ethanol in 2000, 4000 and 6000 ppm 90ses 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 most of the fungi was significant in the case of *S. cerevisiae* where the results were recorded as ineffective. Overall, concentration dependent tendency was observed.

**HABITAT ASSOCIATION OF LITTLE GREBE (*TACHYBAPTUS RUFICOLLIS*) AT
KALLAR KAHAR LAKE, PAKISTAN**

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The data regarding biology and ecology of Little Grebe (*Tachybaptus ruficollis*) are deficient in Pakistan. We studied the association of Little Grebe abundance with habitat parameters at Kallar Kahar Lake, District Chakwal, Punjab, Pakistan, from September 2010 to July 2011. Bird population density data, vegetation and physico- chemical parameters were collected along three strips from different areas of the lake based on habitat variability. The association of Little Grebe abundance with physico- chemical parameters of water was determined using simple linear regression. The physico-chemical parameters differed significantly (P<0.05) among the three strips.

The water temperature and turbidity were negatively related while pH and depth were positively related with grebe population. We concluded that the species more frequently occurred in shallow water habitats with preponderance of reed vegetation, slightly high alkalinity, low water temperature and low turbidity. The reed vegetation such as *Phragmites* and *Typha* provided shelter and nesting sites while open water had abundant planktons and crustaceans, algae such as *Spirogyra* spp. and submerged vegetation such as *Chara* spp. which provided foraging sites. Threats to the species included eutrophication, fluctuation in water level and littering by visitors which need to be addressed through appropriate management interventions.

**FIRST REPORT OF INSECTICIDE RESISTANCE IN HOUSE FLIES (DIPTERA:
MUSCIDAE) FROM PAKISTAN**

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Department of Entomology, Bahauddin Zakariya University, Multan House flies are amongst the major pests of dairy operations with the ability to develop resistance to various insecticides with different modes of action. Adult house fly populations from dairies in Punjab, Pakistan were evaluated for resistance to six insecticides with novel modes of action. The significance level of resistance to most of the insecticides tested was observed in the present study. Low to moderate levels of resistance were found for abamectin, emamectin, fipronil and imidacloprid while very low levels of resistance were observed for spinosad and indoxacarb. Resistance to new chemical insecticides in Pakistani dairy populations of house flies is reported here for the first time. Judicious use of these chemicals is needed to avoid future control failures in the management of house flies.

**INVESTIGATION OF HUMAN-WILDLIFE CONFLICT IN SELECTED VALLEYS OF
CENTRAL KARAKORAM NATIONAL PARK (CKNP)**

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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 determine Human-wildlife conflict in different region of Central Karakoram National Park (CKNP). This study was carried out during the month of December 2011 to March 2012 from different region of CKNP viz Shimshal, Bagrot, Haramosh, Rakaposhi and Hoper/Hisper. The parameters under study included educational back ground of different communities, Agricultural land and livestock they owned and mortality of their livestock due to disease and predation, attitude and perception of local people about carnivorous animals, status of these species and economic loss of local communities due to their predation. Snow Leopard was observed a common species, which followed wolf and then lynx respectively. Brown bear was observed as a rare species in this region except Shimshal. people of these areas have a different perception regarding these carnivore mammals, some wants complete elimination, some said to

reduce their population and few respondents thought their population should be conserved after paying compensation at the expense of livestock. Wolf was considered one of the most dangerous carnivores, followed by snow leopard, lynx and brown bear was ranked as low dangerous carnivore. The highest livestock mortality rate due to disease was observed in Haramosh valley followed by Hoper, Bagrot, Shimshal and Rakaposhi respectively. According to the study goats were important victims in Haramosh valley and sheep and cattle in Hoper/Hisper region. This study reveals the most victimized small ruminant was goats than sheep and cattle. The most predominant carnivore mammal in the study area was observed Snow leopards followed by wolf then lynx. The major cause of economic losses of the farmer in the study area was Predation and diseases of livestock.

SECTION - VI**POSTER SESSION*****ALLIUM CEPA* ROOT TIP ASSAY A EXCELLENT GENETIC MODEL TO MONITOR THE TREATED INDUSTRIAL WASTEWATER BEFORE BEING DISCHARGED INTO WATER BODIES**

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The increasing discharge of hazardous chemicals into the environment has affected the balance of natural ecosystems, which can lead to several health problems and can affect future generations also, since these alterations can be inheritable. Higher plant tests have been employed as genetic models for the screening and monitoring of environmental pollutants. Among the several higher plants, *Allium cepa* (*A. cepa*) has been used as an efficient genetic model for toxicity assessment. In present study, the toxic potential of the raw and treated effluents of three textile industries located in Faisalabad city were investigated using the *A. cepa* root tip test assay. The wastewater from textile industries were subjected to advance oxidation process-AOP,s (UV, UV/H₂O₂, UV/TiO₂, UV/H₂O₂/TiO₂, γ and γ /H₂O₂) and their toxicity reduction were compared. The *A. cepa* roots exposed to untreated textile wastewater for 72 h showed 52, 60 and 77% inhibition in root length, while the reduction in number of root were up to 38, 73 and 84%. The mitotic indices were also found to be lower in treated group of plants. Among all AOP's, UV/H₂O₂/TiO₂, γ /H₂O₂, UV/TiO₂ and γ reduces the cytotoxic effect of textile industries wastewater significantly. However, UV treatment alone did not show promising effect. The abnormalities observed in meristematic cells of *A. cepa* were also correlated linearly with water quality parameters. From results, it is suggested that the AOP,s are efficient for the treatment of textile wastewater toxicity reduction and *A. cepa* test can be used successfully for the monitoring of toxicity reduction of textile wastewater by these methods before being discharged into watersheds.

BIO FUEL PRODUCTION BY ALGAE: PROSPECTS IN PAKISTANTARBIA AAMIR, HINA SHAFIQUE, SAEED WAQAR ALI, MUNEEZA RAZA AND
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The current dilemma of fossil fuel scarcity is on the rise as reliable sources are depleting rapidly with the increased demand. The alternate sources of fossil fuel are insufficient to meet the high demand of the population with a major part of machinery depending upon fossil fuels. The dire need for an alternate source can be satisfied with the emergence of bio-fuel production from algae. Compared to other sources of bio-fuel, algae has been found to have the highest oil content and hence can be utilized as a major source for the production of bio-fuel. Since Pakistan is rich in algal growth, oil extraction from it can be an opportunity for Pakistan to balance its production vs demand graph of fossil fuels. For oil extraction from algal cultures several methods are being used across the globe e.g Solvent/saponification, Bligh and dyer (wet), Bligh and dyer (dry), Wet

milling, French press, Sonnication, Bead-beater, Soxhlet (hexane extraction). Among all these the most inexpensive, easy and suitable method is "Hexane extraction method" also known as conventional method or Soxhelt method. This method has been used for oil extraction in Pakistan at small level on experimental bases. It however, can be used for mass extraction of oil from algal cultures. Being easy and economical its use can lead to the development of a strong Algal Bio-fuel Production Industry in Pakistan. The research done in this field in Pakistan is scarce and theoretical except the recent work published in "Chemistry Central Journal 2012" in which six algal species of Southern Pakistan, both fresh water and marine, were analyzed for their oil content. Though the highest oil content percentage found was 12.6%, yet there are thousands of species available in Pakistan. Hence it is a wide, promising and productive field for researchers to work on. Algal bio-fuel production is prospective for Pakistan considering the climatic conditions which will hence allow algae to thrive. In addition, it will enable utilisation of massive water logged and saline lands of Pakistan creating employment for several people. Being a non competitive group, its growth will have minimum impact on the ecosystem. Algal Bio fuel production requires mostly simple and economical setup which is suitable for Pakistan. The process has least harmful by product, in fact compressed residue left after oil extraction can further be used as animal feed. In addition, it can also help in treatment of sanitary waste as organic matter will be degraded and will eventually provide nutrition for algal growth. To sum up, the use of algae as a source of bioduel is not only cost effective, rather it is an efficient way in contributing to the fuel crisis and more importantly to build a greener environment.

SIMULTENOUS REMOVAL OF SELENATE AND CHROMATE UNDER DENITRIFYING CONDITIONS IN H₂-BASED MBfR

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Simultaneous reduction of multiple oxidized contaminants was challenged in a bench scale H₂-based membrane biofilm reactor (MBfR). Hydrogen gas supplied through hollow membrane fibers provided electron pool to be utilized by the bacterial community growing over fiber for reduction of oxidized contaminants present as electron acceptors. Initially we developed a denitrifying community on Mitsubishi Rayon Fibers using soil inoculum under 5ppm nitrate in a synthetic wastewater as electron acceptor. Later reactor was fed with medium containing two additional oxidized contaminants chromium and selenate as electron acceptors. Reactor was operated for eight different stages under varying hydrogen pressures and contaminant loadings. Complete reduction of nitrate was achieved at a flow rate of 0.2mL/min at 2.5psi at day 13. Addition of selenate and chromium simultaneously put the reactor in competition for electrons and a sudden decrease (70%) in nitrate reduction was observed. Nitrate reduction recovery with increase in hydrogen pressure indicated that reactor was hydrogen limited. In presence of selenate a slight decrease in nitrate removal without hydrogen limitation showed selection of denitrifying community performing selenate reduction as secondary electron acceptor. In presence of all four contaminants oxygen and nitrate were the first to be reduced and utilized maximum hydrogen flux, while chromium and selenate were reduced secondarily. Hydrogen flux distribution among different contaminants was in an order of oxygen < nitrate < selenate < chromium. An interesting

aspect of the study was inhibition of selenate reduction when chromium started reducing while removal of chromium from the media recovered the selenate reduction. This data suggest complex and sequential interactions among different oxidized contaminants and the microbial community. These information are relevant for designing sequential metal bioremediation processes.

PREFERENCE AND SUITABILITY OF ASIAN CITRUS PSYLLID AND CORN LEAF APHID AS PREY FOR THE TWO-SPOTTED LADY BEETLE *ADALIA BIPUNCTATA* (COLEOPTERA: COCCINELLIDAE)

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The two-spotted ladybeetle, *Adalia bipunctata* (Coleoptera: Coccinellidae) is an important bio-control agent, wide spread in crops and forests ecosystem all over the world. Suitability of some Homopterans like aphids and mealy bugs has been examined as a prey for *A. bipunctata*, but there are no reports regarding Asian citrus psyllids *Diaphorina citri* (Homoptera: Psyllidae). We tested predation of *A. bipunctata* for *D. citri* and corn leaf aphid *Ropalosiphum maidis* (Homoptera: Aphididae) and studied life parameters on these two species and frozen eggs of the flour moth *Ephesia kuehniella* (Lepidoptera: Pyralidae). Both larvae and adults of *A. bipunctata* equally prefer *D. citri* and *R. maidis*. All three diets were observed equally suitable for larval development to adults with 93-100% survival rate. All three diets also found suitable for beetle fecundity and fertility with fewer less fertility on diet of *E. kuehniella*. More oviposition events were observed on substrates of citrus jasmine *Murraya paniculata* compare to paper and Petri dish. Field experiments showed 54% reduction of *D. citri* nymphs in colonies with beetles comparative to 4% reduction in control colonies without beetles.

SIMULTANEOUS REMOVAL OF SELENATE AND CHROMATE UNDER DENITRIFYING CONDITIONS IN H₂-BASED MBFR

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Simultaneous reduction of multiple oxidized contaminants was challenged in a bench scale H₂-based membrane biofilm reactor (MBfR). Hydrogen gas supplied through hollow membrane fibers provided electron pool to be utilized by the bacterial community growing over fiber for reduction of oxidized contaminants present as electron acceptors. Initially we developed a denitrifying community on Mitsubishi Rayon Fibers using soil inoculum under 5ppm nitrate in a synthetic wastewater as electron acceptor. Later reactor was fed with medium containing two additional oxidized contaminants chromium and selenate as electron acceptors. Reactor was operated for eight different stages under varying hydrogen pressures and contaminant loadings. Complete reduction of nitrate was achieved at a flow rate of 0.2mL/min at 2.5psi at day 13.

Addition of selenate and chromium simultaneously put the reactor in competition for electrons and a sudden decrease (70%) in nitrate reduction was observed. Nitrate reduction recovery with increase in hydrogen pressure indicated that reactor was hydrogen limited. In presence of selenate a slight decrease in nitrate removal without hydrogen limitation showed selection of denitrifying community performing selenate reduction as secondary electron acceptor. In presence of all four contaminants oxygen and nitrate were the first to be reduced and utilized maximum hydrogen flux, while chromium and selenate were reduced secondarily. Hydrogen flux distribution among different contaminants was in an order of oxygen<nitrate<selenate<chromium. An interesting aspect of the study was complete inhibition of selenate reduction when chromium started reducing while removal of chromium from the media recovered the selenate reduction. This data suggest complex and sequential interactions among different oxidized contaminants and the microbial community. The information are relevant for designing sequential metal bioremediation processes.

BIOCHEMICAL PROFILE OF *CIRRHINUS MRIGALA* FROM DOWNSTREAM LOCATIONS OF RIVER RAVI: EFFECT OF MUNICIPAL AND INDUSTRIAL DISCHARGES

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River Ravi, after receiving municipal and industrial effluents' discharges of the second biggest city of Pakistan the Lahore loses its ecological vigour tremendously. Freshwater fish *Cirrhinus (C) mrigala* were netted from three downstream polluted sites (B, C and D) and compared with a less polluted upstream site A. The fish were sampled during low (winter) and high (post monsoon) flow seasons. The fish muscle from site D showed 28% less total carbohydrates as compared to the value obtained for the fish caught from site A. This parameter showed further decreases at sites B and C and the reductions turned up to 58 and 59% and 77 and 74%, during low and high flows, respectively. Besides the carbohydrates, total lipids, cholesterol and RNA content showed decreases for the muscle of the fish, captured from the downstream locations. However, total and soluble proteins and DNA contents increased in fish muscle progressively downstream during both low and high flow seasons. This trend was especially noticeable for total protein content which showed 97% increase for site C downstream sampling point as compared to the respective value for site A. The levels of biochemical parameters are apparently indicative of the organisms' response to the pollutant stresses which may lead to negative health effects for the fish and influence its dietary importance.

TAXONOMY OF JUMPING SPIDERS, FAMILY SALTICIDAE FROM SOON SAKASER VALLEY

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Family Salticidae, commonly known as jumping spiders were collected by handpicking and sweep net from Soon Sakaser Valley. Specimens were collected from vegetation present in the

surrounding of Uchali, Kabbiki and Jahlar lakes (Collectively known as Uchali Lake Complex, a Ramsar site). After collection specimens were preserved in a mixture of 80% alcohol and 20% glycerine. Specimens were identified by using differ keys (1.Simons key of Salticidae, 2.Keys for Identification of Salticidae Genera of Central Europe , 3.Key to Salticidae Genera of Levant).The data showed that studied specimens belong to 18 genera ; Bianor, Carrhotus, Hasarius, Heliophanus, Harmochirus, Hyllus, Icius, Leptorchestes, Mogrus, Modunda, Myrmerechni, Menemerus, Plexippus, Phintella, Pseudicius, Rhene, Telamonia, Thyene and approximately 30 species.10, 1st time recorded genera from Pakistan are Carrhotus, Heliophanus, Harmochirus, Hyllus, Icius, Leptorchestes, Mogrus, Modunda, Phintella, and Telamonia. We are further identifying the species.

APHIDICIDAL AND CYTOTOXIC POTENTIAL OF *PLEUROTUS* MUSHROOMS

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The current study highlights the insecticidal and cytotoxic potential of organic extract of *Pleurotus* mushrooms. To assess the aphidicidal potential the rose aphids (*Macrosiphum rosae*) were exposed to the different concentrations of organic extract of three different parts (fruiting body, mycelium and fermentation filtrate) of *Pleurotus* mushrooms i.e. *P. ostreatus*, *P. florida* and *P. citrinopileatus*. Similarly the cytotoxicity of these organic extracts were also carried out to relate the cell toxicity, by using crusticians, brine shrimps (*Artemia salina*). The extraction of organic compounds from each part of *Pleurotus* mushrooms were carried out using ethylacetate. The results showed that fruit bodies extract of *Pleurotus ostreatus* exhibit highest cytotoxic potency 61.62 $\mu\text{g mL}^{-1}$ by EtOAc extract of fermentation filtrate of *Pleurotus Citronopileatus* (LC_{50} of 105.9 $\mu\text{g mL}^{-1}$). The mycelial extract of *Pleurotus ostreatus* was 179.79 $\mu\text{g mL}^{-1}$ while *Pleurotus Florida* and *Pleurotus Citronopileatus* did not possess any cytotoxic potential. All the organic extracts showed significant insecticidal and phytotoxic potential except the mycelial extract of *Pleurotus florida*. Maximum insecticidal effect was showed by the fermentation filtrate extract of *Pleurotus citronopileatus* with LC_{50} of 7.67 mg mL^{-1}). Overall it was observed that the fruiting bodies and fermentation filtrate extract of all the three mushrooms used were very potent then the mycelial extract of the same mushrooms. Thus *Pleurotus Ostreatus*, *Pleurotus Florida* and *Pleurotus Citronopileatus* can be used as important secondary metabolites and can be helpful in agriculture and health sector.

DIVERSITY AND RELATIVE ABUNDANCE OF COCCINELLIDS IN THE CROPLAND OF FAISALABAD USING MOLECULAR TOOLS

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The present study was conducted to evaluate diversity and relative abundance of coccinellids (Coleoptera) as well as their genetic diversity in the cropland of Faisalabad district

during 2010 to 2011. A total of 2204 specimens of coccinellids were collected throughout the study period, belonging to 12 species of four sub families including Coccinellinae, Chilocorinae, Epilachninae and Scymninae on five crops viz., wheat, sugarcane, fodder, maize and vegetables and their associated weeds. Out of 2204, 1974 coccinellids were recorded on the major crops (sugarcane and wheat etc) and 230 coccinellids were recorded from their weeds. The species were predatory in nature except *Epilachna indica*. Ample genetic diversity was found among selected species of coccinellids. The technique promises to be a very useful source of markers for maintaining colonies and tracking genes in biological control projects and in identifying species and immature stages of insects.

CELL TOXICITY OF ORGANIC EXTRACT FROM DIFFERENT INDIGENOUS FUNGI

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The present study highlight the cytotoxic effect of the organic extract obtained from *Trichoderma harzianum* and *Rhizopus stolonifer*. Both the extraction were carried out with ethyl acetate. To assess their cytotoxic potential, the brine shrimp larvae were separately exposed to each extract of different concentration 10ppm, 50ppm, 100ppm , 200ppm and one test with negative control was also carried out for each extract. After 48 hours, the mortality rate was observed in both extracts. Both species showed significant cytotoxic effect. *T. harzianum* was more potent as compare to *R. stolonifer*. *T. harzianum* extract indicated 100% mortality at concentration 50-200%. Minimum mortality rate (13%) was shown by 10ppm. For *Rhizopus stolonifer*, the 200 ppm concentration showed maximum mortality (40%) while the lowest lethality value (3.32%) was recorded for the solution having 10 ppm crude concentration. The 50 ppm and 100 ppm concentration exhibited 33.32% and 36.66% lethality of the brine shrimps. No cytotoxic effect was recorded under negative control.

ENHANCED PRODUCTION OF BUBALINE SOMATOTROPIN IN *PICHIA PASTORIS*

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Anterior pituitary gland of most vertebrates secretes a protein of ~22kDa, known as somatotropin (growth hormone), which is capable of enhancing the milk and meat productivity of livestock upto 25%. This protein is amongst one of the most leading recombinant product in biotechnology industry because of its application in live stock industry. In present study, fermentation conditions (both in shake flask and 3.7 liter bioreactor) were optimized to achieve enhanced secretory expression of bubaline somatotropin (BbST) in *P. pastoris*. *P. pastoris* has been proved an efficient expression system for eukaryotic proteins. A *Pichia* transformant carrying multi-copy BbST cDNA integrated into its genome was expression under the regulation of alcohol oxidase promoter and its secretory expression was monitored in culture, BbST expression attained in shake flask culture represented more than 35% of total secretory proteins of *Pichia pastoris*. Different nonionic surfactants were used to facilitate the enhanced expression of BbST. Maximum expression of recombinant BbST was achieved when *Pichia* cells were induced with 0.5% methanol in the presence of Tween 80 for a period of 48 hours. Further a study of medium selection

for bioreactor revealed that modified basal salt medium (M-BSM) is the most suitable medium for the cultivation of *Pichia* transformants. The maximum cell density in MBSM was equivalent to 120g/L of wet cell mass. Further studies related to high cell densities of *Pichia* in cost effective medium with maximum secretory expression of BbST are in progress.

CHARACTERIZATION OF THERMOSTABLE PULLULANASE FROM *THERMOCOCCUS KODAKARAENSIS*

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Amylolytic enzymes are very important in various industries. One of the largest users of enzymes for hydrolysis and modification of material is the starch industry. The thermostable enzymes originating from the hyperthermoacidophiles can play a major role in industrial processes because they are capable of working at higher temperature and lower pH. *Thermococcus kodakaraensis* is a hyperthermophilic archaeon. The thermostable pullulanase from this microorganism has been cloned and expressed in our lab previously. The present study includes the purification of the protein, determination of kinetic parameters of this enzyme using different substrates and site directed mutagenesis of the active site amino acid residues. It was found that maximum velocities (V_{max}) of the enzyme using pullulan, amylopectin, dextrin and γ -cyclodextrin as substrates were 100, 10, 25 and 33.33 U/mg, and the K_m values were 3.03, 10, 0.2 and 1.11 mg/mL, respectively. The enzyme gave highest turnover number (k_{cat}) with pullulan i.e., 141 s⁻¹. Hence, pullulan is the preferred substrate. By using the inverse PCR technique, aspartate at position 503 was changed to asparagine. The mutation resulted in complete loss of enzyme activity proving that Asp-503 is essential for the enzyme activity. Because of its thermoacidophilic nature, this enzyme is potential candidate for its use in starch industry.

HEPATITIS C VIRUS INFECTION AND DIABETES MELLITUS; THE POSSIBLE ROLE OF VIRUS IN THE DEVELOPMENT OF DIABETES

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Total samples from 319 patients (51% Males and 49% Females) were subjected to different biochemical and molecular studies. ELISA positive HCV infected patient's samples were subjected to qualitative detection of HCV by PCR for further confirmation. Four groups were formulated for the categorical analyses of different parameters. Group1; comprised of 123/319 (38%) patients who were HCV positive but without diabetes. Group 2; constituted 66/319 (21%) patients who were positive for both HCV and diabetes, Group3; included 104/319 (33%) patients with diabetes and Group 4; had 26/319 (08%) patients regarded as control group. Different factors like gender, age, socio-economic status, treatment plan and phase were brought under consideration for detailed investigation. The age group ranged from 15-80 years. About 50% patients belonged to lower, 27% belonged to middle and 23% belonged to upper class level. HCV positive and HCV positive

diabetic subjects were prescribed different therapeutic treatments including interferon (INF) (67%), pegylated interferon (PEG-INF) 32% and oral medication (1%). Majority of HCV positive diabetic patients belonged to the age groups of 35 to 60 years, with no previous family history of diabetes. Female and male constituted 59% and 41% of these patients respectively. Stratification of HCV positive diabetic patients in different age groups showed that majority of the subjects lie in the age group of 50-60 years. 15% HCV positive patients developed diabetes before the commencement of the treatment therapy, 45% developed diabetes during the treatment therapy and about 40% HCV positive patients developed diabetes after receiving the therapy. All 66 HCV positive diabetic patients were found to exhibit increased liver transaminase and phosphatase levels in both pre and post treatment phases. Increase in ALT, AST and ALP enzyme levels above the normal reference range proved to be non-significant statistically ($p > 0.05$) at both pre and post treatment phases, when compared with control subjects having normal enzyme level. The increment in total bilirubin level in HCV diabetic subjects showed significant rise ($p < 0.001$, $p < 0.05$) for both males and female patients. RFT analysis showed significant elevation in creatinine level in both male and females ($p < 0.05$). FBG was found to be significantly high ($p > 0.05$) in females whereas RBG level showed no significant increase. Genotype based studies showed that majority of male and female HCV positive diabetic subjects were of the subtype 3a.

**OPTIMIZATION OF SUBMERGED FERMENTATION PARAMETERS FOR
CELLULOSE PRODUCTION FROM PRETREATED LIGNOCELLULOSIC BIOMASS
THROUGH *TRICHODERMA VIRIDE***

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The escalating consumption of energy reserves can be compensated by focusing on the production of bioethanol from the abundant and renewable lignocellulosic biomass. One of the main impediments to the industrialization of lignocellulose conversion processes is the copious amount of cellulase enzymes required for the hydrolysis of cellulose. Cellulase is multicomponent enzyme system and represents the key step for biomass conversion by virtue of synergistic action of exoglucanase, endoglucanase and β -glucosidase. Current research focuses on cellulase production from pretreated lignocellulosic biomass (*Saccharum spontaneum*) by employing fungal strain *Trichoderma viride* under submerged fermentation conditions. Alkaline pretreatment of substrate with 2 % NaOH resulted in enhanced cellulase yield (CMCase 60.39 \pm 0.13 U/ml/min and FPase 30.28 \pm 0.16 U/ml/min) as compared to untreated substrate (30.23 \pm 0.02 U/ml/min and FPase 23.23 \pm 0.05 U/ml/min). Furthermore, the process parameters for submerged fermentation were optimized. Maximum cellulase production (CMCase 64.11 \pm 0.01 U/ml/min and FPase 31.40 \pm 0.01 U/ml/min) was achieved at 6 % substrate at pH 5.0, with 10 % inoculum, incubated at 35°C for 120 hrs of fermentation period. Considerable enzyme yield (CMCase 66.70 \pm 0.2 U/ml/min and FPase 29.78 \pm 0.2 U/ml/min) was recorded with surfactants where 0.15 % Tween 20 gave best results for cellulase yield. The present study has been conducted for proficient cellulase yield through optimization, and by exploiting cheaper substrate thereby governing cost reduction in enzymes to make them commercially viable.

**PROCESS OPTIMIZATION FOR ROBUST BIOETHANOL PRODUCTION FROM
SACCHARIFIED BIOMASS (*SACCHARUM SPONTANEUM*) THROUGH
*SACCHAROMYCES CEREVISIAE***

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Considerable attention has been focused on alternative energy resources as it is an important weapon against rising petroleum costs and exceeding dependence on fossil fuel resources. The production of bioethanol from lignocellulosic biomass is ultimate bioenergy alternative. The main focus of the present study is to obtain the maximum yield of ethanol from pretreated cellulosic biomass employing yeast of *Saccharomyces cerevisiae*. The substrate was pretreated with 2 % NaOH and subjected to saccharification with commercial cellulases. Various process parameters of saccharification i.e. time, temperature, enzyme loading, biomass, pH has been optimized to obtain hydrolysate for ethanol production through aerobic and anaerobic fermentation respectively. To obtain the enhanced ethanol yield different fermentation parameters i.e., hydrolysate size, inoculum size, temperature, reaction time, pH and enhancers were optimized. Saccharification rate of 83 % was recorded with 7 % substrate, 50 FPU/ml, pH 4.8, incubated at 60° C for 5 days. Enhanced bioethanol yield 88.89g/L was achieved with 100 ml hydrolysate, 5ml yeast inoculum, pH 4.8, incubated at temperature of 30°C for 5 days for aerobic and anaerobic fermentation respectively. Results depict that PEG showed positive influence in enhancement of bioethanol yield considerably. The emphasis of present study is to reduce our excessive dependence on energy reserves. Alternative energy sources such as bioethanol can bring down prices at the pumps, increase energy security and can boost our drowning economy.

**GENE CLONING AND EXPRESSION, IN *ESCHERICHIA COLI*, OF TRYPTOPHAN
SYNTHASE A- AND B-SUBUNIT FROM HYPERTHERMOPHILIC ARCHAEON
*PYROBACULUM CALIDIFONTIS***

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Tryptophan synthase belongs to lyase class, which acts on carbon-oxygen bond and releases water, so it is a hydro-lyase that catalyzes the last step of the tryptophan synthesis pathway. The present study covers the gene cloning and expression, in *Escherichia coli*, of Pcal 1211 (tryptophan synthase α subunit) and Pcal 1206 (tryptophan synthase β subunit) from the hyperthermophilic archaeon *Pyrobaculum calidifontis*. Both the genes were amplified by polymerase chain reaction, cloned in PTZ57R/T. Pcal 1211 is a 0.720 kb gene that codes for 240 amino acid protein whereas Pcal 1206 is a 1.239 kb gene that codes for 413 amino acid protein. Both the genes were separately expressed in *E. coli* BL21 CodonPlus (DE3)-RIL under the control of T₇ promoter using pET expression system. Analysis of the recombinant proteins by polyacrylamide gel electrophoresis, revealed that both the recombinant proteins were produced in *E. coli* in insoluble form. Expression was also attempted at lower temperature, 17°C, with and without heatshock at 50°C, but the recombinant proteins were produced in insoluble form.

Furthermore, soluble expression was examined in the presence of pyridoxal phosphate, a cofactor for the enzyme but was unsuccessful. Different strategies were used to solubilize and refold the recombinant proteins.

CHARACTERIZATION OF CATALYTIC DOMAIN OF HUMAN AND CHICK 3-HYDROXY-3-METHYLGLUTARYL-CoA REDUCTASE (HMG-CoA REDUCTASE)

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The enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase is one of few four-electron oxidoreductases. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate, which is known as the rate-limiting step in the biosynthesis of cholesterol and other intermediates of mevalonate pathway. We have designed the primers for the catalytic domain of HMG-CoA reductase and amplified by PCR. The full length amplified DNA product of human catalytic domain is 1329 bp encoding for 437 amino acids. The molecular weight of catalytic domain is 46.7 kDa and isoelectric point is 8.43. The homology modeling was carried out. The ultimate objective of this study is to evaluate the quality of biotechnologically produced statins. For this purpose the inhibition studies of HMG-CoA reductase will be carried out against the statins. To meet this long term objective, characterization of the HMG-CoA reductase was done as an interim objective.

ADVANCED OXIDATION TREATMENT EFFECT ON CYTOTOXICITY REDUCTION OF LEATHER TANNING EFFLUENT

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Present study was conducted to appraise the effect of advanced oxidation process on cytotoxicity reduction of industrial wastewater. *Allium cepa* root analysis was used to evaluate the cytotoxicity of wastewater before and after treatment. The wastewater of leather tanning industry was subjected to various advanced oxidation processes such as UV, UV/H₂O₂, UV/TiO₂, UV/H₂O₂/TiO₂ and γ /H₂O₂. The macroscopic characteristics such as root length and root number were reduced significantly when bulbs were grown in untreated wastewater. The mitotic index of treated group was also found to be lower in response to wastewater exposure. To evaluate the biological efficiency, the *Allium cepa* roots were exposed to treated wastewater and among all the advanced oxidation processes, UV/H₂O₂/TiO₂ showed maximum toxicity reduction efficiency followed by γ /H₂O₂ and UV/TiO₂. After treatment no cytotoxicity was observed for the wastewater treated by UV/H₂O₂/TiO₂, γ /H₂O₂ and UV/TiO₂ as compared to negative control (distilled water). However, UV alone did not show significant effect on toxicity reduction. From results, it is concluded that the advanced oxidation processes are efficient for the cytotoxicity reduction of leather tanning effluents and *Allium cepa* test can efficiently be used for the monitoring of toxicity reduction by these methods.

DETECTION AND SCREENING OF CYTOGENETIC AND MUTAGENIC AGENT IN INDUSTRIAL EFFLUENTS THROUGH *ALLIUM CEPA*-ROOT TIP MICRO-ANALYSIS

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Allium cepa, among higher plants has been recognized as excellent genetic models to detect toxic effects from industrial wastewater. While studying the toxicity of industrial wastewater, contaminated soil, river water and even those systems which are considered non toxic, the *A. cepa* has been used as a test system to detect/evaluate the toxicity load. Regarding toxicity evaluation, the *A. cepa* is distinguished as a low cost test, excellent model (*in vivo* and *in vitro*), sensitive, short term and easily to handle. It offers the detection of dosages in genetic material quantitatively and results can be generalized for diverse animal and plant community. Through comparative studies, various author reported the similarity of *A. cepa* test with other biological assay being in practice for toxicity evaluation. The *A. cepa* test also enables to assess the different macroscopic and microscopic endpoints. Among the endpoints, chromosome aberrations have been used to detect genotoxicity, while cytotoxicity is evaluated on the basis of mitotic index, root length and nuclear abnormalities and micronuclei inductions are correlated with mutagenic action tested agent. From present study, it was concluded that *A. cepa* can be successfully applied for detection and screening of cytogenic and mutagenic agent present in the industrial effluents and results can be used as warning for other biological and ecological systems.

AMPHIBIAN FAUNA AND THEIR ECOLOGY IN DISTRICT LARKANA SINDH, PAKISTAN

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This poster illustrates and describes the amphibian fauna and their ecological conditions to which they interact for their survival in District Larkana being explored for the first time. The studied parameters include population diversity, predators, vegetation and water temperature, pH, conductivity, Total dissolved solids are physico-chemical parameters studied comparatively in four Talukas of district Larkana Sindh explored during April, May and June 2011. The poster describes the diversity of amphibian fauna that district Larkana contains. Population status of amphibians is aimed to compare with population of predators to evaluate their competition and struggle that they make for their survival. The poster describes vegetation for its important role in warming up the water, as those habitats which are more covered by vegetations like trees remain colder than those that have less vegetation cover. Among physico-chemical parameters water temperature is analyzed for its influence on growth and development of amphibians. Water pH analyzed to know acidity of aquatic environment whereas Conductivity and TDS are analyzed as their increased level can be cause of amphibian's mortality. This poster may describe amphibian survival in District Larkana by highlighting their ecological niches that imply the utilization of ecosystem to fulfill varying needs of multiple amphibian species for their development and conservation.

STUDY OF MICROBIAL DIVERSITY IN RUNNING AND STAGNANT WATER OF LAHORE

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The present study was designed to investigate microbial species in water samples collected from different locations of Lahore. 10 samples were collected and examined under microscope. Microscopic study revealed that those samples contained a broad spectrum of microbial life containing micro flora and micro fauna. Microbial count was done. The most abundant species were *Paramecium*, *Euglena*, *Chlamydomonas*, *Amoeba*, *Spirogyra*, *larvae of mosquito* and *crustacean*. The experimental results indicated that the maximum microbial count (2x 10³/ml) was obtained in the stagnant and humidified water.

THE PREVALENCE OF NEUROLOGICAL DISORDERS IN PAKISTAN

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The percentage of death and disability in humans due to Neurological Disorder (ND) is much higher than that of any other disorder. The burden of ND ranges from 4-5% in developing countries as compared to 10-11% in high-income countries. However, the prevalence of ND in Pakistan is higher than expected. The prevalence of ND has been least studied in Pakistan. This study aimed a hospital-based epidemiological investigation of Pakistani patients suffering with ND including Epilepsy, Schizophrenia, Depression, Mental Retardation, Dementia, Parkinson disease, Panic disorder and Bipolar disorder. For the purpose, a scientific survey was conducted to determine the prevalence of ND in Pakistani patients from April 2010 to December 2011 at Jinnah Hospital, Lahore. The data of etiology, pathology and epidemiology of ND in 614 Pakistani patients were collected and analyzed. The occurrences of neurological disorders were found more in males (60.26%) than in females (39.74%). The result of analysis of data revealed the distribution of ND in different age groups as; <5 year (2.61%), 6-15 year (17.26%), 16-25 year (35.83%), 26-35 year (17.26%), 36-45 year (10.75%), 46-55 year (9.12%), 56-65 year (3.91%) and >65 year (3.26%). This study will help geneticist and clinicians to better understand the prevalence and incidence of ND which will lead to formulate objective and credible health policies for the benefit of community.

CLONING AND EXPRESSION OF α -AMYLASE GENE FROM *BACILLUS LICHENIFORMIS*, WITH SIGNAL SEQUENCE, AND CHARACTERIZATION OF THE GENE PRODUCT

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Alpha-amylase from *B. licheniformis* is a highly thermostable starch hydrolyzing enzyme. The present study describes gene cloning, its expression in *E. coli* and characterization of the recombinant protein with and without signal peptide. The protein produced in the insoluble form was solubilized and was found to be highly active. Amounts of extracellular and intracellular enzymes were compared. Molecular mass of the extracellular mature protein was found to be 55411 Da by MALDI-TOFF analysis whereas the calculated molecular mass based on the amino acid sequence was 58492 Da, indicating the removal of the signal peptide. The signal sequence and its cleavage site was determined by N-terminal sequencing and it was found that the *E. coli* signal peptidases recognize and cleave the α -amylase from *B. licheniformis* between amino acids 28 and 29. So the signal peptide for this enzyme was found to be 28 amino acids long contrary to the previous reports where signal peptide was predicted to be 29 amino acids long.

EFFECT OF *NIGELLA SATTA* AND *PLANTAGO OVATA* ON LIPID PROFILE OF EXPERIMENTAL NONALCOHOLIC FATTY LIVER DISEASE *RATTUS NORVEGICUS*

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Nonalcoholic fatty liver disease (NAFLD) is of much focus among all the other liver diseases. Lipid profile can be considered to be one of the markers of NAFLD to predict the severity of disease. The aim behind the present study was to analyze the impact of fat plummeting medicinal herbs on the variant lipid profile in diet induced NAFLD in adult *Rattus norvegicus*. Four groups (n=10) of adult *R. norvegicus* were designated as 0, I, II and III. O group . was given 100% rat chow, while group I was given diet "A" (13% water+ 20% sucrose+ 33% Nestle tea whitener +34% rat chow). Group II and III respectively were provided with the diet "AJJ supplemented with 5% *Nigella sativa* seeds and *Plantago ovata* husks. Animals were kept on feeding for sixteen weeks. The results obtained by the serological analysis revealed that there were highly significant changes in TG (P<0.001) Cholesterol (P<0.01) LDL (P<0.01) and HDL (P<0.05) of all the experimental groups in comparison with 0 group. The variations in Cholesterol! LDL ratios were also observed to be statistically significant (P<0.05). Conclusively, it can be said that among the two fat plummeting agents under study, *N. sativa* seeds are more promising than *P. ovata* husks to reverse the adverse changes in the lipid profile of diet induced NAFLD in rats. Moreover, Cholesterol/LDL are among the markers of NAFLD.

**EXPRESSION OF MODIFIED CATALYTIC DOMAIN OF CELLOBIOHYDROLASE OF
CLOSTRIDIUM THERMOCELLUM IN *ESCHERICHIA COLI***

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Cellobiohydrolase (CBHA) of *Clostridium thermocellum*, which hydrolyzes crystalline cellulose to cellobiose, exhibits a multidomain structure of unusual complexity. Catalytic domain of CBHA, carrying 503 amino acids (molecular mass ~55 kDa) has previously been expressed in *E. coli* in the form of inclusion bodies. In the present study, the native sequence has been modified by deleting stretches of amino acids from the loop regions in an attempt to improve its expression and solubility in *E. coli*. The deletions were made in a manner that didn't affect the overall three-dimensional structure of the catalytic domain. Cloning of the modified nucleotide sequences of catalytic domain in pET22b(+) vector generated two expression plasmids *i.e.*, pCBHA-323 and pCBHA-374. The expression of cloned genes was analyzed in *E. coli* BL21 CodonPlus cells following IPTG induction. Expression levels achieved were quite high and amounted to 40% and 30% of the total *E. coli* cellular proteins, respectively. However, the truncated/modified forms of CBHA catalytic domains still expressed in the form of inclusion bodies. Studies relating to solubilization and refolding of native and modified forms of CBHA are in progress.
