PROCEEDINGS

OF

PAKISTAN CONGRESS OF ZOOLOGY

Volume 31, 2011

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

THIRTY FIRST PAKISTAN CONGRESS OF ZOOLOGY

held under auspices of

THE ZOOLOGICAL SOCIETY OF PAKISTAN

at

UNIVERSITY OF AZAD AND JAMMU KASHMIR

APRIL 19 – 21, 2011

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ACKNOWLEDGMENTS

University of Azad and Jammu Kashmir, Muzafarabad hosted the 31st Pakistan Congress of Zoology (International).

The Zoological Society of Pakistan expresses its deep gratitude to the Vice Chancellor, University of Azad And Jammu Kashmir, Muzafarabad and faculty members and students of the Department of Zoology for extending warm hospitality.

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31st PAKISTAN CONGRESS OF ZOOLOGY (INTERNATIONAL)

UNIVERSITY OF AZAD AND JAMMU KASHMIR, MUZAFARABAD

April 19 – 21, 2011

PROGRAMME

TUESDAY, APRIL 19, 2011

08:30 AM Registration
10:00 AM Inauguration: Recitation from the Holy Quran
10:05 AM Welcome Address
10:15 AM Address by the President, Zoological Society of Pakistan
10:25 AM Distribution of Medals and Awards
10:45 AM Address by the Chief Guest
11:15 AM Vote of Thanks
11:25 AM Refreshment

JOINT SESSION I: Plenary Lectures

Chairperson: Prof. Dr. A.R. Shakoori
Co-chairperson: Prof. Dr. Imtiaz Ahmad

Speakers: 1. **Dr. Muhammad Perwaiz Iqbal**
Professor & Chairman, Department of Biological and Biomedical Sciences, Aga Khan University, Karachi.
Lead Pollution – A grave risk to human health in Pakistan.

2. **Prof. Dr. S.N.H. Naqvi,**
Baqai Medical College, Karachi.
Investigations on the anti ulcer effect of neem leaf extract and *Cedrus deodara* oil.

01:00 PM Lunch and Prayer

HALL – 1

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

SESSION I

	Chairperson:	Dr. Sardar Mohammad
	Co-chairperson:	Dr. Naseem Khan
02:00 AM	Paper reading	
04:30 PM	Tea Time	

SESSION II

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

SESSION III

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

HALL – 2

SECTION II: PEST AND PEST CONTROL

SESSION I

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

SESSION II

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

SESSION III

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

HALL – 3

SECTION IV: PARASITOLOGY

SESSION I

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

SESSION II

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

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

SESSION I

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

WEDNESDAY, APRIL 20, 2011

JOINT SESSION II: (Plenary Lectures)

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

09:00 AM 1. **Prof. Dr. A.R. Shakoori**
Distinguished National Professor & Director, School of Biological Sciences, University of the Punjab, Lahore.
Regulation of copper influx-efflux through plasma membrane in *Klebsiella pneumoniae*

2. **Prof. Dr. M. Suleman**
Department of Microbiology, Hazara University, Mansehra.
Research methodology, sampling methods and sample size.

3. **Dr. Abdul Aleem Chaudhary**
Ex-DG Wildlife
Role of protected areas in the conservation of species and ecosystem.

HALL – 1

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

SESSION IV

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

SESSION V

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

SESSION VI

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

SESSION VII

	Chairperson:	Dr. Shahid Nadeem
	Co-chairperson:	Mr. Ishtiaq Ahmad
06:30 PM	Paper reading	
07:00 PM	Executive Council Meeting	
08:00 PM	Dinner	

HALL – 2

SECTION III: PEST AND PEST CONTROL

SESSION IV

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

SESSION V

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

SESSION VI

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

SECTION III: ENTOMOLOGY

SESSION I

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

HALL – 3

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

SESSION II

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

SESSION III

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

SESSION IV

	Chairperson:	Prof. Dr. F.M. Bilqees
	Co-chairperson:	Prof. Dr. Juma Khan Kakar
20:00 AM	Paper reading	
04:30 PM	Tea Break	

SESSION V

	Chairperson:	Dr. Nusrat Jahan
	Co-chairperson:	Dr. Asmatullah kakar
05:00 PM	Paper reading	
07:00 PM	Executive Council Meeting	
08:00 PM	Dinner	

THURSDAY, APRIL 4, 2011

JOINT SESSION III: (Plenary Lectures)

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

09:00 AM 1. **Dr. Imtiaz Ahmad**
National Distinguished Professor, University of Karachi, Karachi.
Arthropod born haemorrhagic fever and their management strategies in Pakistan

2. **Prof. Dr. Bilqees Mujib**.
Department of Zoology, Jinnah University for Women, Karachi
Histopathology of Fascioliasis in goat and sheep.

3. **Prof. Dr. M. Nasim Siddiqi**
Former Chairman and Professor, Department of Zoology, University of Peshawar, Peshawar
Mangrove – Adeliete Ecosystem, Threats and Opportunities.

HALL – 1

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

SESSION VIII

	Chairperson:	Prof. Dr. Muhammad Ali
	Co-chairperson:	Dr. Khawaja Abdul Majeed
10:30 AM	Paper reading	
11:00 AM	Tea Break	

SESSION IX

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

SESSION X

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

HALL – 2

SECTION III: ENTOMOLOGY

SESSION II

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

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

SESSION XI

	Chairperson:	Prof. Dr. Samina Jalali
	Co-chairperson:	Dr. Basharat Ahmad
02:0 0 AM	Paper reading	

HALL – 3

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

SESSION VI

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

SESSION VII

	Chairperson:	Prof. Dr. Muhammad Javed
	Co-chairperson:	Dr. Zahida Tasawar
11:30 AM	Paper reading	

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

SESSION XII

	Chairperson:	Prof. Dr. Samina Jalali
	Co-chairperson:	Dr. Basharat Ahmad
02:0 0 AM	Paper reading	
04:35 PM	Concluding Ceremony	
	Recitation	
	Congress Report by President ZSP	
	Award Ceremony	
	Concluding Remarks by the Chief Guest	
	Vote of Thanks	
05:30 PM	Refreshments	

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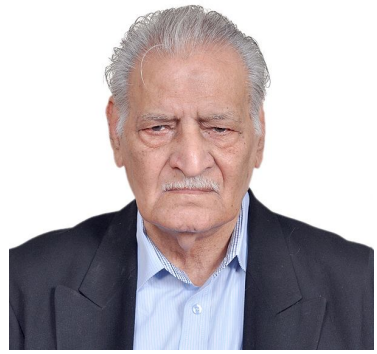


Prof. Dr. Mahmood Hussain Qazi
Vice-Chancellor, The University of Lahore

Dr. M.H.Qazi received his M.Sc. Degree from University of Karachi. Subseqently, selected as Fulbright Scholar, he obtained his Ph.D. degree from Louisiana State University, USA. Over the years he has been a Post Doctoral Fellow at the University of Wisconsin, USA as a Ford Foundation Fellow; and WHO Post Doctoral Fellow at Karolinska Institute, Stockholm, Sweden. He was consultant with WHO at Karolinska Institute. He has been Adviser Women Hospital Munster, Germany. He was Member/Chairman of WHO expanded program in Human Reproduction. He was visiting Professor at Max-Plantk Institute for Reproduction Medicine, Munster, Germany. He has been visiting Professor at State University of New York at Buffalo, USA.

During the past 50 years he bas been intensely involved in teaching and research. He has published about 80 research papers in National and International Journals in the field of Endocrinology focusing on gonadotropic hormones. He worked on purification of hCG and its alpha and beta sub units. His work on development of highly sensitive micro-bioassays has been widely cited.

He held several academic and administrative positions. He has been

Professor of Physiology at University of Karachi, and Professor/Dean of Natural Sciences, Quaid-i-Azam University, Vice Chancellor, Hamdard University, Rector, The University of Lahore, Rector, Dada Bhoy Institute of Higher Education, Adviser and then Member, University Grants Commission, Vice Chancellor, Allama Iqbal Open University and visiting Professor in several International Universities. He has been a member of a large number of several National and International academic and research organizations. He is recipient of Sitar-e-lrntiaz and Tamgha-e-Imtiaz in recognition of his contributions to the Advancement of Science and Technology in Pakistan. He is a founder Member of Zoological Society of Pakistan.

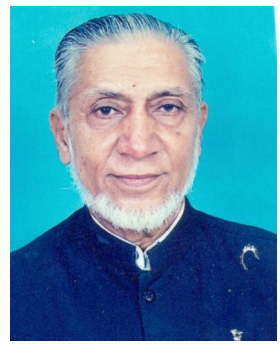
RECIPIENT OF
LIFE TIME ACHIEVEMENT AWARD 2011

Prof. Dr. Mirza Azhar Beg
Department of Zoology, University of Arid Agriculture, Rawalpindi

Prof. Dr. Mirza Azhar Beg obtained his Ph.D. degree In Zoology - Animal Ecology from University of Montana, USA in 1969. He joined University of Agriculture, Faisalabd soon after that, were he was first elevated to Professorship. Later he became Dean, Faculty of Sciences.

Prof. Dr. Mirza Azhar Beg deserves Life Time Achievement award for his significant contributions in the field of ecology, behavior and taxonomy of mammals, birds and spiders.

RECIPIENT OF
LIFE TIME ACHIEVEMENT AWARD 2011



Prof. Dr. Syed Naim-ul-Hassan Naqvi
Professor of Zoology, University of Karachi, Karachi

Prof. Dr. S.N.H. Naqvi was born on October 01, 1936 at Buland Shahr. He did B.Sc. from Muslim University, Aligarh in 1954 and the M.Sc. from Karachi University in 1958. He did Ph.D. in 1966 from Karachi University and was awarded D.Sc. in 1995, on the recommendation of five International experts.

Prof. Naqvi has published 335 research papers in international and national journals and five books. He has partly translated comparative Anatomy of Vertebrates, Weichart and Plant Anatomy by Esau. He has one patent of Phytopesticide (Biosal) with Prof. Salim-uz-Zaman Siddiqui and Dr. B.s. Siddiqui. So far six M.Phil and 25 Ph.D. degrees have been awarded under his supervision and 8 students are working with him at present in addition to 42 M.Sc. project. Prof. Naqvi's is investigation on neem products in collaboration with HEJ Research Institute of Chemistry have been of fundamental importance.

Prof. Dr. S.N.H. Naqvi has been Senior Humboldt Fellow at Philips University, Marburg (1970-72) and University of Hannover (1987-88), visitir.g Professor at University of Illinois, Chicago (1979). He started his carrier as Lecturer at Urdu Science College in 1956 their retired as Professor of Zoology, from University of Karachi in 1996. He has been Chairman,

Pharmacology Department, Baqai Medical University, Karachi (1997-2007). His field is Animal/Human Physiology, Toxicology and Phytopesticides.

He was awarded Writers Guild award in 1969, ABI Gold Medal (1987), Nishan-e- Danish (1995), Pakistan Academy of Science Gold Medal (1988) and Zoologist of Year Award (2009). He has been Vice - President of the Zoological Society of Pakistan.

RECIPIENT OF
ZOOLOGIST OF THE YEAR AWARD 2011*

Dr. Aly Khan
Director, Crop Diseases Research Institute, University of Karachi, Karachi

Dr. Aly Khan did his M.Sc. in first class from Department of Zoology, University of Karachi with specialization in Parasitology in 1974. Later he joined PARC in 1981 as Research Officer at Crop Diseases Research Institute, Karachi. Later he did his post-doctoral studies at the United States Department of Agriculture, Beltsville, Maryland from the Nematology and Microbiology Lab. He has published 12 books and more than 235 research papers in national and International journals. He has received best research publication award from Pakistan Book Foundation and a case prize of *Rs.25,000.* He has received best Scientist award from PARSA; A case prize of *Rs.100,000* and Gold Medal from Pakistan Science Foundation for being most productive principal investigator for a period of 10 years (1999-2009). Received a shield from FAO-NARC on nematode problems and their control and numerous certificates of appreciation from both national and international organizations.

*Other nominee of this award were Dr. Muhammad Ather Rafi, Dr. Khalid Mahmood Prof. Dr. Javed Iqbal Qazi and Prof. Dr. Shahid Mahboob Rana.

RECIPIENT OF
PROF. A.R. SHAKOORI GOLD MEDAL 2011*

Dr. Irfan Zia Qureshi
Associate Professor
Department of Animal Sciences, Quaid-i-Azam Universtiy, Islamabad

Dr. Irfan Qureshi did his Master from University of Agriculture, Faisalabad and obtained M.Phil degree from Quaid-i-Azam University in 1992. He obtained his Ph.D. degree from University of Sheffield, United Kingdom, where he worked on development of neurones. He continued with his research activities as Post-doe at Sheffield and worked on the expression of sulphonylurea (anti-diabetic drug) receptor and showed for the first time that this receptor is also expressed in the kidney and urinary system by using rat as a model. The discovery was seminal with regard to diabetes and helped other scientists in drug development. Recently Dr. Qureshi has shown that kisspeptin, a reproduction-related peptide also acts as an anticoagulant and it degenerates juvenile male gonads. He has coauthor a book on "Thyroid Gland and Chromium Toxicity" in 2009. Dr. Qureshi has produced 48 M.Phil and presently one Ph.D. student is working under his guidance.

*Other applicants for this award were Prof. Dr. Muhammad Jalal Arif, Dr. Muhammad Hamid Bashir, Dr. Abid Farid, Dr. Muhammad Asif Shahzad and Dr. Noor Us Saher.

RECIPIENT OF
PROF. DR. MIRZA AZHAR BEG GOLD MEDAL 2011*

Dr. Muhammad Hamid Bashir
Assistant Professor,
Department of Agriculture Entomology, University of Agriculture, Faisalabad

Dr. Muhammad Hamid Bashir has a first class academic career. He obtained his Ph.D. degree from University of Agriculture, Faisalabad in 2006 and described forty new species of mites. Dr. Bashir has produced 12 M.Sc. (Hons) students and is supervising 10 M.Sc. and 5 Ph.D. students. He has over 60 research publication in journals of national and international repute out of which 18 are in journals of impact factor.

He has successfully completed two research projects and is presently running another on mites.

*Other applicant for this award were Dr. Muhammad Mahmood-ul-Hassan, Dr. Noor Us Saher, Dr. Muhammad Jaffar and Dr. Shaukat Ali

RECIPIENT OF
PROF. DR. NASIMA TIRMIZI GOLD MEDAL 2011*

Prof. Dr. Naureen Aziz Qureshi
Acting Dean,
Faculty of Science and Technology, Government College University, Faisalabad

Prof. Dr. Naureen Aziz Qureshi is presently working as Acting Dean, Faculty of Science and Technology, Government College University, Faisalabad. After passing her M.Sc. examination (Gold Medalist) in 1st Class 1st Position from the Department of Zoology, University of Karachi, in 1978 with specialization in Marine Zoology, she completed her M.Phil in Marine Biology in 1983 from the Centre of Excellence in Marine Biology (CEMB), University of Karachi. She obtained her Ph.D. degree from Louisiana State University, Baton Rouge, Louisiana in May, 1995. Her Post-Doctoral research was at University of Glasgow, as a Commonwealth Fellow during 2002-2003. She has supervised 11 M.Phil and 1 Ph.D. student.

*Other applicant for this award were Dr. Nasira Kazi and Dr. Razia Sultana

RECIPIENTS OF GOLD MEDALS AWARDED BY THE ZOOLOGICAL SOCIETY OF PAKISTAN

1. Muzaffar Ahmad Gold Medal 2011

Fifteenth Muzaffar Ahmad Gold Medal 2011 was received by Ms. Nadia Naeem for obtaining first position in the M.Sc. Zoology examination of the University of the Punjab.

2. Ahmed Mohiuddin Memorial Gold Medal 2011

Eighth Ahmed Mohiuddin Memorial Gold Medal 2011 was given to Miss Tehmina Kaleem, who obtained first position in the M.Sc. Zoology examination of the University of Sindh, Jamshoro.

3. Afsar Mian Gold Medal 2011

Second Afsar Mian Gold Medal 2011 was given to Ms. Ainee Zafar who obtained first position in the M.Sc. Biology/Zoology examination of the Arid Agriculture University, Rawalpindi.

4. Muhammad Afzal Hussain Memorial Gold 2011

Thirteenth Muhammad Afzal Hussain Memorial Gold 2011 was given to Ms. Zainab Najmuddin for obtaining first position in Parasitology for her M.Sc. Zoology examination of the University of Karachi.

5. Mujib Memorial Gold Medal 2011

Fifteenth Mujib Memorial Gold Medal 2011 was given to Miss Asra Batool who obtained first position in the M.Sc. Zoology examination of the University of Sindh, Jamshoro.

6. Prof. Imtiaz Ahmad Gold Medal 2011

Eight Prof. Imtiaz Ahmad Gold Medal 2011 was given to Ms. Safia Razzaq who obtained first position in the M.Sc. Zoology examination in Entomology, University of Karachi, Karachi.

7. Prof. Dr. S.N.H. Naqvi Gold Medal 2011

Seven Prof. Dr. S.N.H. Naqvi Gold Medal 2011 was given to Dr. Sobia Khawaja for his Ph.D. degree in Zoology specializing in the field of Toxicology from University of Karachi, Karachi.

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TWO HUNDRED YEARS OF DARWIN AND RESOLUTION OF THE CONTROVERSY OF ORIGIN OF WHALES

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Abstract.-On February 12, 2009, Darwin turned out to be 200 years old. This gives us an opportunity in the midst of celebration of 200 years of Darwin, to evaluate how much have we advanced in the field of Evolutionary Biology. Not only the theory of immutability of species so much pronounced in Darwin's days by the custodians of the churches has been buried for ever but the truth of natural selection is also clearly dawned upon us through experiments. In the field of paleontology also we have marched a long way after Darwin and recently the controversy of the origin of Whales has also seemed to be resolved.

Key words: Darwin, evolutionary biology, whales, origin of whales.

INTRODUCTION

The year 2009 has marked 200 years after Darwin's birth. It reminds us to evaluate our progress specially in the field of Evolutionary Biology. There is a consensus now at least among Muslim scientists that the theory of immutability of species so strongly held by the custodians of the churches at the time of Darwin has lost the ground for ever, that new species are being discovered almost every day and more profoundly we have been visualizing ourselves the formation of new species in all spheres of biodiversity.

Being Muslims these scientists feel utmost satisfaction that the Almighty in His holy Quran in the 20th chapter prophesized that "He would create an animal in the Arabian desert of Mecca which will speak to the human beings near the end of the world. In the 23rd chapter of the Holy Quran at the end of Sura "Yaseen" our Lord stressed that "Whenever He intendeth to create something He so orders and it is". Dr. Sir Muhmmad Iqbal, the poet of the east in one of his beautiful verse pointed out that the universe is yet incomplete and continuously "let it be" is drummed and new ones are being poured. He in another of his profound verse rejected the theoryof immutability of species forever, "the scenario of fixity in nature is an impossibility and if something appears continuous that is only the scenario of change.

The concept of natural selection and the phenomenon of adaptation and therefore, a continuous change in nature has also been proven beyond all doubt (Ahmad, 2007). Alas! This phenomenon seldom, results in the origin of species. We have learnt after Darwin about other much more pertinent causes of the origin/formation or creation of species *i.e.* the geographical isolation and the gene modification or gene mutation.

In these 200 years the Muslim scientists have also learnt to keep out of their fear raised by Nasr (1964) and Nadvi (1966) that Darwinism "was mostly upheld in Darwin's days by unbelievers and most of the supporters of Darwin such as Huxley (1877) and Weismann (1892,1902) were atheists. They have also recollected in these years that Mivart (1871) and Wallace (1889, 1909) were themselves priests and strong believers of Christianity. They have also realized in these 200 years that evolutionary biology is now based on concrete scientific evidences derived as a result of experiments which could be repeated by any adversary. Unlike today's Christian fundamentalists such as so called creationists or special designers Muslim scientists following their fore fathers i.e. Ibne-Sina, al-Beruni, al-Mastoi, al-Khawarzmi (in Sarton, 1942) learnt to separate Scientific experiments with their faith which they have acquired by logic or philosophy. Like Prof. Behe (2007) who in his famous book "Black Box of Darwin" insists that the personality of the designers should be recognized in every text book of Evolutionary Biology although he has himself realized and has acceded to the truth that the designer could not be placed in a test tube in a scientific experiment and therefore at least for the time being scientific experiments should be carried out keeping it aloof of ones faith. Darwins strongest supporter of his day was the Geologist/ Palaeontologist Charles Lyell and the strongest proof Darwin considered for his origin of species was fossil records, the species now extinct but which paved the way in the evolution of some spectacular modern species. The 1861's fossil discovery of "*Archaeopteryx*" the extinct European Jurassic bird with many reptilian features such as scales on its strong hind legs, claws on its forelimbs and long tail with caudal vertebrae together with beautiful impressions of its avian feathered wings left no doubt in anyone's mind that modem birds evolved from reptilian stock. As it is customarily believed to day that "Dinosaurs" still exist today in the form of "Extant birds" (Ahmad, 2009b).

RESULT AND DISCUSSION

Fossil evidence and revolution of origin of whales

Soon after Darwin, palaeontological evidence kept pouring in leaving no

doubt in any ones mind that amphibians evolved from fresh water fishes and mammals from primitive therapsidian reptiles. The evolutionary history of horses, elephants and camels has been beautifully revealed by their fossil records. Yet some of the enigmatic groups pose definite problems in the minds of evolutionists as well as provide the creationists the last chance to cling on to the end of the rope that Darwin's evolutionary theory was unable to solve the mystery of the origin of such and such group of animals. The modern cetaceans including the extant "Whales", dolphins, porpoises, humpbacks, orcas and other whales proved indeed one of the most enigmatic group. The natural historians even the Greeks like Aristotle has long before recognized that whales are mammals but that was about all, as far as they understood the origin of cetaceans. In his (1859) origin of species Darwin noted a case of a black bear swimming for hours with its mouth a gape catching aquatic insects much as a whale might engage itself in feeding (Monastersky, 1999). Darwin could see no difficulty in a race of bears being rendered by natural selection, more aquatic in structure and habits with larger and larger mouth till a creature was produced as monstrous as whale. The ridicule and attacks engendered by this passage in his origin of species grew to such a pitch that Darwin paired it down and then deleted it altogether in the later editions of his book. Only 24 years after Charles Darwin, in 1883, Flower offered an idea that the apparently legless *1*. Leviatheans had evolved from ungulates, a group of mammals whose best known characteristicis a set of hoofed feet, which claim means that whales are close kins of cows, horses, pigs, goats, buffalos and camels etc.

After the serological tests were evolved, Simpson, a Professor at Harward University suggested that Whales would be ideal for such a test because nothing was known about their origin and about their ancestors. These tests revealed that the whales are closely related to beef, the hoofed artiodactyl mammals of the order Ungulata. The sensitive ring or dilution test using the apparatus Libby's photron reflectometer confirmed this finding (Moody, 1962; Ahmad, 2009a). It was conjectured that both beef and whales probably were evolved from archaic ungulate ancestral group Condylartha.

Sinonyx, a wolf sized (hyena-like, land dwelling mammal with hoofs belonging to archaic/ungulate group Condylartha) from which the artiodactyls evolved have long been supposed as the ancestral group which has already been developing the adaptation un like other mesonychid that later became the basis of the whales specialized way of life. These adaptations include an elongated muzzle, an elongated Jugular foramen a natural opening or perforation through a bone or (a membranous structure), and a short basicranium. They had very

narrow shearing molar teeth, especially in the lower jaw with multiple cusps (Kuhn-Fulton *et al.,* 1996).

The scene moves to Indo-Pakistan Sub-Continent

Thewissen *et al.* (2007) have recently noted that like all fossils and recent cetaceans which differ from most other mammals in the reduction of crushing basins on their teeth there are no trigonid and talonid basins in the lower molars and the trigon basin of the upper molar is very small in Archaeoceti (*i.e.*Pakicetids and Ambulocetids) or absent but unlike these cetaceans in these mesonychids molars have wear facets very unlike that of cetaceans (O'leary and Uhen, 1999). Just before the end of the last century in August 1999, Nikaido *et al.,* from their studies on SINES and LINES of the Junk DNA concluded that whales and hippopotamuses are first cousins. They argue that a part of the animals genetic code that does not specify instructions for any genes, the so called "Junk DNA" is made up of segments that can copy themselves and then splice the copies back into the genetic strands at various points. One set of these moving elements consist of short interspersed materials or SINES, long interspersed elements or LINES. These segments have been insinuating themselves into the genetic code for billions of years (Nikaido *et al.,* 1999). In human SINES constitute more than 10% and LINES more than 15%. They could have played a role in spawning some viruses, *i.e.* "Retrovirus" or the AIDS viruses which could have been generated from LINES. These have unique value in mapping the twists and turns of evolution. By looking for particular examples of these copies in specific sites of genome, researchers could determine when various species split off from related ones. Nikaido *et al.* (1999) found that whales and hippos share four SINES not present in any other artiodactyls tested *i.e.* camels, pigs, deer, giraffes and cows. Nikaido *et al.* (1999) stated that this powerful technique present a really perfect record of evolutionary changes. In 10 years of study these scientists have never noticed that these elements appearing independently in the same spot or once inserted extracting themselves from DNA.

On the other hand the conventional genetic analysis is also not considered foolproof. It goes away when the same mutation happens independently in two different species, it makes two animal sequences with a molecular version of convergent evolution, look similar even though they may have been only distantly related. It could also happen that a mutation in one spot can change again or even correct itself which would make the comparisons of sequences much more difficult.

Other molecular biologists like Gaur of Tel Avive University agree that the SINES and LINES data add weight to close relationship between whales and hippos but many say that the connection was already firmly established by conventional molecular data. As John Gatesy of the University of Wyoming in Laramie has stressed that every gene he ever sequenced suggested this relationship which is generally called "whippo hypothesis". It suggests that some 55 million years ago the artiodactyl ancestors of whales and hippos split of other groups closely related to the camels, pigs, deer and cows. This whippo lineage may have developed an aquatic life style even before the hippos and whales branches diverged. Both groups share particular aquatic adaptation such as the ability to nurse and communicate under water.

Although many people including David M Hillis of the University of Texas at Austin considered the data so compelling that there is not any question about it. The palaeontologists including Maureen 0' Leary at the state university of new York at Stony Brook observed with dimsay that it was just unwise not to consider the fact that the fossil records is in contradiction with molecular data. According to her whales and mesonychids looking like a wolf with hoofs among ungulate seemed to be so unique that they look like carnivorous animals. If whales and mesonychians are the nearest known relatives, whales could not fit near to hippos within the artiodactyls because Mesonychians lack ankle bone "astragalus" with a pulley shaped knob at each end that provides more freedom for flexing the foot up and down and limbs twisting from side to side, in all artiodactyls. Thewissen and Midar (1999) identified the "astragalus" as belonging to a pakicetid whales in Pakistan because no other animal in those deposits could lay claim to a bone of that size in that area. However the "astragalus" was not clearly part of a pakicetid skeleton, therefore many palaeontologists wondered if it probably belonged to an animal other than as ancient as whale. Thewissen however had high hopes that he would find better fossils that would really bear on this question in a near future. The progression from land to sea was indeed problematic because the most ancient whale fossils came from animals already completely at home with in the sea. J.G.M Thewissen of the North eastern Ohio University College of Medicine in Rootstown when examined Gingerich *et al*.'s discovery of 1983 of 50 million years old fossils of earliest whales in Pakistan, *Pakicetus*in the remain of small ephemeral streams only about 1 meter deep he considered that they were doing something different than land mammals do. Thewissen and Hussain (1993) studied the arrangement of ear bones in *Pakicetus* and concluded that they were best adapted to hearing through a medium much denser than air. The ears could have worked well under water or the animals

could listen out of the water by placing their jaws on the ground as some predators do. Thewissen *et al*. (1994) discovery of *Ambulocetus* with a complete skeleton of whale only slightly more recent than *Pakicetus,* had limbs that would have been clumsy on land. Its large feet and supple spine allowed the animal to swim by flexing its hind half up and down in a precursor to the type of motion that modern whales use.

The *Ambulocetus* from the sedimentary deposit, it was found, one could tell that it lived in the ocean close to shore probably at the mouth of a river where it could get fresh water for drinking. It was suggested by the studies of the oxygen isotopes of its bones that it had not fully developed the ability to use sea water as today's whales consume.

Recently Thewissen *et al.* (2007) demonstrated that whales originated from aquatic artiodactyls in the Eocene epoch of India. The first ten million years of whale's evolution as noted above are remarkably documented by a series of fossil skeletons, the link to the ancestors of cetaceans were missing. In this discovery Thewissen and his group showed that the Eocene south Asian raoellid artiodactyls are the sister group to whales. The raoellid *Indohyus* is similar to whales and unlike other artiodactyls in the structure of its ears and premolars, in the diversity of its limb bones and in the stable oxygen isotope composition of its teeth. They have fully demonstrated that a major dietary change occurred during the transition from artiodactyls to whales and the raoellid were aquatic waders. This meant that the aquatic life in this lineage occurred before the origin of the order Cetacea.

Thewissen*et al.* (2007) mainly argued against whippo hypothesis on the ground that cetaceans originated about 50 million year (MYr) back in south Asia where as the family Hippopotamidae is only 15 MYr old and the first hippopotamids recorded from Asia is only 6 MYr old. On the other hand the middle Eocene artiodactyl family Raoellidae is considered coeval with the earliest cetaceans and both are endemic to south Asia. In the past also some raoellid genera were added to some phylogenetic analysis (Thewissen *et al*., 2007. Geisler and Uhen, 2005) but no close relation to whales was found because raoellid fossils were limited to dental material alone. Thewissen *et al.* (2007) claimed to have studied new dental cranial and post cranial material for *Indohyus* middle Eocene raoellid artiodactyls from Kalakot region of occupied Kashmir. Thewissen *et al.* (op. cit.) found in *Indohyus* thickened medial lip of its adulatory bulla, the *involucrum* a feature previously thought to be present exclusively in cetaceans. The anterior posterior arrangement of incisors in the jaw and the high

crowns in the posterior premolars were the derived similarities shared by *lndohyus* and cetaceans. Earlier it was thought that the involucrum was the only character occurring in an extinct and extant cetaceans but in no other mammals, the finding of involucrum in *lndohyus* either necessitate the inclusion of *lndohyus* in cetaceans or the involucrum ceased to characterize cetaceans. Thewissen *et al* (cop. cit.) argued that cetaceans should remain a stable monophyletic group to include Pakitcetidae, Ambulocetidae, Reminglonocetidae, Protocetidae, Basilosauridae, Mysticeti and Odontocetidae (including *Pakicetus, Ambulocetus, Rodhocetus* and *Artiocetus* including ancient and recent cetaceans) but excluding Artiodactyla (including Raoellidae but excluding Cetacea) which remained a paraphyletlic group. They did not however prefer the alternative scheme to include *lndohyus* in Cetacea with both cetaceans and remaining artiodactyls monophyletic because it causes instability by significantly altering the traditional content of both Artiodactyla and Cetacea.

As noted above reduced crushing basins (with no trigonid and talonid basins in the lower and the trigon basins of the upper molar is very small as in pakicetids and ambulocetids) or absent in all fossils and recent cetaceans which differ from most of the mammals and also from roellids including that of *lndohyus* but very similar to mesonychids (but in them the wear facets are very unlike those of cetaceans). All cetaceans also have a particular shape of post orbital and temporal region of the skull. In early cetaceans this region.is long and narrow. The change in the later cetaceans must have had effects on the sense organs and also on oral function. Thewissen *et al.* (op. cit) argued that changing diet led to a change in food processing organs in whales.

The controversy of origin of whale appears to be at least for the time being resolved and Darwin was at least right to this extent that a black bear (not really but an even toed ungulate artiodactyl) swimming for hours with its mouth agape catching aquatic insects turned out to be a monstrous whale through evolution/natural selection. It is certainly satisfying that both palaeontologists and molecular biologists have reached to a consensus that ancestors of whales were even toed ungulate artiodactyl land mammals. It also defeated Creationists the so called Christian fundamentalists and other antievolutionists who boasted that connecting links between whales and their so called terrestrial ancestors are missing and therefore these were separately and specially created. It is also satisfying to note that we are marching in the right direction as Darwin claimed that different species share a common ancestry.

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ROLE OF PROTECTED AREAS IN THE CONSERVATION OF SPECIES AND ECOSYSTEMS

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Abstract. Wildlife, their habitats and ecosystems in Pakistan have been undergoing changes for the worst. Main factors affecting this change are mainly anthropogenic including habitat deterioration and indiscriminate hunting of wildlife species. According to latest data on the status of faunal species there are 23 mammals, 11 birds and 13 reptiles which are threatened with extinction; 30 mammals, 78 birds and 14 reptiles are likely to become extinct if immediate measures are not taken to help improve their populations; populations of 20 mammals, two birds and 12 reptiles are being regulated. Various measures are taken to check the deterioration or loss of habitats and illegal hunting of wild animals. Some of the measures taken to address the threats include: *Strengthening the enforcement of legislation, stakeholders participation, awareness campaigns and collaboration among the Government/s, NGOs and donors*. Control and enforcement mechanisms adopted include: *good governance, legislation enforcement also with the help of communities, human resource development and through outreach programs*. Historically, establishment of Protected Areas (PAs) is seen as a sufficient measure to conserve species, habitats and ecosystems. *Pakistan has designated 227 Protected Areas: 26 national parks, 97 game sanctuaries and 104 game reserves covering* 10,060,850 *hectares of land area (Table I, Fig. 1) with the following management objectives: (i) Scientific Research; (ii) Preservation of species and genetic diversity; (iii) Maintenance of environmental services; (iv) Protection of specific natural/cultural features; (v) Tourism and recreation; (vi) Education and (vii) Sustainable use of resources from natural ecosystems; (viii) Maintenance of cultural/traditional attributes.* The article describes the rationale for the establishment of Protected Areas and their establishment and management in the context of Pakistan.

Key words: Protected areas, conservation, species, habitats, ecosystem, legislation, communities, awareness

INTRODUCTION

Protected Areas are established with the specific objective of '*conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings'* Pakistan has a great variety of ecosystems and habitats rich in flora and fauna. Roberts (1986, 1991, 1992, 1995, 1997), Scott (1989), IUCN (2000a) and Chaudhry (2011a) have amply described the faunal and floral richness and the ecosystems

supporting them. Anthropogenic pressures including population pressure, change in land use practices, forest clearing, over-exploitation of rangelands, illegal hunting and poaching, lack of capacity, mass awareness, and resources required to manage and enforce legislation and non-cooperation on the part of communities has greatly pressurized these ecosystems. The establishment and proper management of Protected Areas is perhaps the single most important step taken by the Governments to conserve the natural resources.

Pakistan (Provincial Governments as the land managers) has designated 227 Protected Areas: 24 national parks, 97 wildlife sanctuaries and 104 game reserves covering 10,060,850 hectares of land area (Table I, Fig. 1) with the management objectives: (i) Scientific Research; (ii) Preservation of species and genetic diversity; (iii) Maintenance of environmental services; (iv) Protection of specific natural/cultural features; (v) Tourism and recreation; (vi) Education; (vii) Sustainable use of resources from natural ecosystems; (viii) Maintenance of cultural/traditional attributes.

TABLE I.- PROTECTED AREAS EXTENT BY CATEGORY IN PROVINCES.

Province	**National Park**		**Wildlife Sanctuary**		**Game Reserve**		**Total**	
	No	**Area (ha)**	**No**	**Area (ha)**	**No**	**Area (ha)**	**No**	**Area (ha)**
AJ&K	7	99,191	---		12	14,164	**19**	**113,355**
Balochistan	2	634,598	16	786,539	6	232,673	**24**	**1,653,810**
Islamabad	1	17,386	1	7,000	1	69,800	**3**	**94,186**
Gilgit - Baltistan	5	1,751,132	5	185,444	9	238,544	**19**	**2,175,120**
Khyber Pakhtunkhwa	6	134,351	3	34,212	38	387,719	**47**	**556,282**
Punjab	4	108,873	37	198,682	24	3,853,615	**65**	**4,161,170**
Sindh	1	308,733	35	758,547	14	239,647	**50**	**1,306,927**
TOTAL	**26**	**3,054,264**	**97**	**1,970,424**	**104**	**5,036,162**	**227**	**10,060,850**

The rationale for the establishment of Protected Areas and their management has been described in the context of Pakistan.

Protected areas defined

International Union for the Conservation of Nature and Natural Resources (IUCN) defines Protected Areas as “specifically delineated areas designated and managed to achieve the conservation of nature and the maintenance of associated ecosystem services and cultural values through legal or other effective means” (Dudley, 2008).

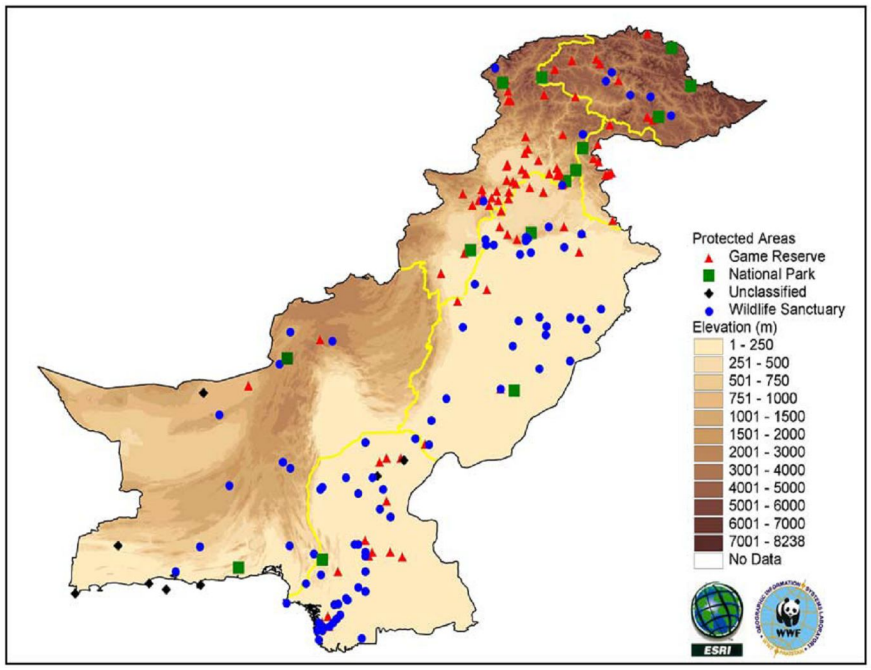

Fig. 1. Protected Areas of Pakistan.

The Convention on Biological Diversity (CBD) (Glowka, 1994) defines Protected Areas as "geographically defined areas, which are designated or regulated and managed to achieve specific conservation objectives".

Establishment and management of protected areas together with conservation, sustainable use and restoration initiatives in the adjacent land and seascape are central to Article 8 on "In-situ Conservation" of CBD. "*In-situ* Conservation" is defined by the CBD as “the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings, and in the case of domesticated or cultivated species, in the surroundings where they have developed their distinctive properties”.

Article 8 of the CBD makes it mandatory for each Contracting Party (Pakistan is a Contracting Party) that as far as possible and as appropriate: (i) Establish a system of protected area or areas where special measures need to be

taken to conserve biological diversity; (ii) Develop, where necessary, guidelines for the selection, establishment and management of protected area or areas where special measures need to be taken to conserve biological diversity; (iii) Regulate or manage biological resources important for the conservation of biological diversity whether within or outside protected areas, with a view to ensuring their conservation and sustainable use; (iv) Promote the protection of ecosystems, natural habitats and the maintenance of viable populations of species in natural surroundings; (v) Promote environmentally sound and sustainable development in areas adjacent to protected areas with a view to furthering protection of these areas; (vi) Rehabilitate and restore degraded ecosystems and promote the recovery of threatened species, inter alia, through the development and implementation of plans or other management strategies (IUCN, 2000b).

Articles 8(a) and 8(b) of the CBD state that a system of protected areas forms a central element of any national strategy to conserve biological diversity. The word "system" in Article 8(a) implies that the protected areas of a country or region may be designated and designed to form a network, in which the various components may conserve different portions of biological diversity, often using a variety of approaches to management. In addition, Article 8(c) calls for the regulation and management of protected areas, while Article 8(d) aims to "Promote the protection of ecosystems, natural habitats and the maintenance of viable populations of species in natural surroundings".

Protected areas are a central part of the Convention in that the Parties themselves have consistently identified that their efforts to develop and maintain their national protected area system are the central element of their strategy to implement the Convention. A well-designed and managed system of protected areas can form the pinnacle of nation's efforts to protect biological diversity. Such a system compliments other measures taken to conserve biological diversity outside protected areas.

The efforts for *in-situ* conservation are augmented through parallel measures in the form of *ex-situ* conservation to ensure survival and thriving of endangered species for their reintroduction and rehabilitation in their native areas. Wildlife parks, zoological gardens, botanical gardens, refuges, pheasantries, aquaria etc. are therefore, established in public and private sectors to achieve the desired objectives.

Protected Areas are far more than just places set aside for wild plants and animals. They provide: (i) environmental services, such as soil protection and

drinking water supplies, and secure homes for vulnerable human communities including many indigenous peoples; (ii) they protect places of cultural and spiritual significance; (iii) supply economic benefits, for instance through tourism, and (iv) give us all space for recreation and renewal.

It follows that the term 'protected area' does not describe a single entity or management regime and that an enormously wide range of different approaches to protection can be found around the world (Bishop *et al*., 2004).

Objectives for the establishment of protected areas

All protected areas are aimed to: (i) conserve the composition, structure, function and evolutionary potential of biodiversity; significant landscape features, geomorphology and geology; conserve significant landscape features, geomorphology and geology; (ii) contribute to regional conservation strategies (as core reserves, buffer zones, corridors, stepping stones for migratory species etc.); (iii) maintain diversity of landscape or habitat and of associated species and ecosystems; be of sufficient size to ensure the integrity and long term maintenance of the specified conservation targets or be capable of being increased to achieve this end; (iv) maintain the values for which it was assigned in perpetuity; (v) be operating under the guidance of a management plan, and a monitoring and evaluation programme that supports adaptive management; (vi) possess a clear and equitable governance system; (vii) Provide regulatory ecosystem services, including buffering against the impacts of climate change; (viii) conserve natural and scenic areas of national and international significance for cultural, spiritual and scientific purposes; (ix) deliver benefits to resident and local communities consistent with the other objectives of management; (x) deliver recreational benefits consistent with the other objectives of management; facilitate low-impact scientific research activities and ecological monitoring related to and consistent with the values of the protected area; (xi) use adaptive management strategies to improve management effectiveness and governance quality over time; (xii) help to provide educational opportunities (including about management approaches); (xiii) help to develop public support for protection.

Protected areas categories (IUCN)

In an attempt to bring some order into this rapidly developing use of land and water, IUCN (Dudley, 2008) has developed a system of categories for protected areas recognizing the following six categories: (i) Strict Nature Reserve/Wilderness area; *Ia*. Strict Nature Reserve (managed mainly for science

or wilderness protection); *Ib*. Wilderness Area (managed mainly for wilderness protection) (ii) National Park (managed mainly for ecosystem protection and tourism); (iii) Natural Monument (managed mainly for conservation of specific natural features); (iv) Habitat / Species Management Area (conservation through management intervention); (v) Protected Landscape/Seascape (landscape/ seascape conservation and recreation); (vi) Managed Resource Protected Area (sustainable use of natural ecosystems)..

Category Ia are strictly protected areas set aside to protect biodiversity and also possibly geological/geomorphological features, where human visitation, use and impacts are strictly controlled and limited to ensure protection of the conservation values. Such protected areas can serve as indispensable reference areas for scientific research and monitoring. The *Primary objective* is to conserve regionally, nationally or globally outstanding ecosystems, species (occurrences or aggregations) and/or geo-diversity features: these attributes will have been formed mostly or entirely by non-human forces and will be degraded or destroyed when subjected to all but very light human impact.

Category Ib protected areas are usually large unmodified or slightly modified areas, retaining their natural character and influence, without permanent or significant human habitation, which are protected and managed so as to preserve their natural condition. The *Primary objective is* to protect the long-term ecological integrity of natural areas that are undisturbed by significant human activity, free of modern infrastructure and where natural forces and processes predominate, so that current and future generations have the opportunity to experience such areas.

Category II protected areas are large natural or near natural areas set aside to protect large-scale ecological processes, along with the complement of species and ecosystems characteristic of the area, which also provide a foundation for environmentally and culturally compatible spiritual, scientific, educational, recreational and visitor opportunities. The *Primary objective* is to protect natural biodiversity along with its underlying ecological structure and supporting environmental processes, and to promote education and recreation.

Category III protected areas are set aside to protect a specific natural monument, which can be a landform, sea mount, submarine cavern, geological feature such as a cave or even a living feature such as an ancient grove. They are generally quite small protected areas and often have high visitor value. The *Primary objective* is to protect specific outstanding natural features and their associated biodiversity and habitats.

Category IV protected areas aim to protect particular species or habitats and management reflects this priority. Many category IV protected areas will need regular, active interventions to address the requirements of particular species or to maintain habitats, but this is not a requirement of the category. The *Primary objective* is to maintain, conserve and restore species and habitats.

Category V Protected landscape/seascape is a protected area where the interaction of people and nature over time has produced an area of distinct character with significant ecological, biological, cultural and scenic value: and where safeguarding the integrity of this interaction is vital to protecting and sustaining the area and its associated nature conservation and other values. The *Primary objective* is to protect and sustain important landscapes/seascapes and the associated nature conservation and other values created by interactions with humans through traditional management practices.

Category VI protected areas conserve ecosystems and habitats, together with associated cultural values and traditional natural resource management systems. They are generally large, with most of the area in a natural condition, where a proportion is under sustainable natural resource management and where low-level non-industrial use of natural resources compatible with nature conservation is seen as one of the main aims of the area. The *Primary objective* is to protect natural ecosystems and use natural resources sustainably, when conservation and sustainable use can be mutually beneficial.

Application of categories

In applying the categories system, the first step is to determine whether or not the site meets this definition and the second step is to decide on the most suitable category (Dudley 2008).

Applicability of these categories in Pakistan is however, restricted. Wildlife Sanctuary in Pakistan corresponds to IUCN category IV (Habitat/ Species Management Area: conservation through management intervention); National Parks correspond to IUCN category II (managed mainly for ecosystem protection and tourism); Game Reserve however does not correspond to any IUCN category even though it is claimed to correspond to category VI where the PA is managed for sustainable use of natural ecosystems whereas the Game Reserve is managed only for specialized hunting and all the other aspects of ecosystem are ignored.

Protected areas are also allowed to be established in the Private Sector as (Shafique, 2002) Community Hunting Reserves where the owner/s of the Community Hunting Reserves can issue hunting permits in collaboration with the Government Wildlife Departments. The proceeds may be shared between the owners and the Government in a ratio of 80 and 20, the Government getting the share for managing the resources.

Corresponding to IUCN category VI Conservancies have been established in Khyber Pakhtunkhwa (KPK) and Gilgit-Baltistan (GB) where the ecosystems are being managed for sustainable use of natural resources with the help of local communities (Chaudhry, 2011b). The initiative was taken with the help of United Nations Development Programme (UNDP) and Global Environmental Facility (GEF) established by the World Bank.

Beside the Protected Areas declared by the Governments (227), a range of global or regional efforts / initiatives exists to address conservation in areas of land and water, including:

(i) UNESCO World Heritage – natural and mixed natural and cultural sites agreed by the World Heritage Committee to be of "outstanding universal value"; River Indus-the habitat of Indus blind dolphin (*Platanista gangetica minor*), Palas valley in Kohistan- Khyber Pakhtunkhwa, and Mangla Dam have been proposed to be declared as UNESCO World Heritage sites.

(ii) UNESCO Man and the Biosphere (MAB) – biosphere reserves are sites where conservation is integrated with sustainable use; Lal Suhanra National Park-Bahawalpur is the only Biosphere Reserve declared in Pakistan. No area has been declared as UNESCO Man and the Biosphere (MAB) in the northern mountainous region of Pakistan but the conservancy areas in Khyber Pakhtunkhwa and Gigit-Baltistan qualify to be declared as MAB areas by UNESCO.

(iii) Ramsar sites (Scott, 1989) – important freshwater and tidal waters listed by the Ramsar Convention as Wetlands of International Importance; Nineteen (19) wetlands in Pakistan have been declared as Ramsar sites. There are however a number of potential Ramsar wetland sites in the northern mountainous region. Government is trying to get the potential sites declared as Ramsar sites.

(iv) IUCN category VI areas – Village Conservation Areas, Valley Conservation Areas and Conservancies established on private/public participation basis; Village Conservation Areas, Valley Conservation Areas and Conservancies have been established in Khyber Pakhtunkhwa and Gigit-Baltistan (Chaudhry, 2011b).

(v) Traditional protected areas – practiced under the customary laws also provide for protection of certain areas like graveyards, tombs etc. where all sorts of grazing and cuttings are strictly prohibited;

(vi) Nagha system: Certain areas are also seasonally protected through NAGHA to ensure regeneration of plants and rehabilitation of degraded landscape especially in rangeland areas in Balochistan and Pakhtunkhwa where seasonal protection improves grazing.

(vii) Trans-boundary Protected Areas: Preliminary discussions have been held between Sinkiang Academy of Sciences and Worldwide Fund for Nature-Pakistan for the establishment of a trans-boundary protected area in Northern Pakistan, and adjoining areas of China and the neighbouring Central Asian states.

(viii) Regional Corridors: The potential for establishment of regional corridors for migratory birds under the Convention on Migratory Species (Bonn Convention) has not been utilised so far. Furthermore, there are no plans under consideration for action in the near term.

National system of protected areas

To conserve the Biodiversity of Pakistan a Protected Areas (PAs) network has been established. PAs have been established under the Provincial Wildlife Acts, provinces being the custodians of land, in three categories viz., Wildlife Sanctuaries (WS), National Parks (NP) and Game Reserves (GR). All sorts of hunting and habitat disturbances are prohibited in WSs and NPs whereas hunting on special permits/licenses is permitted in Game Reserves. Besides PAs officially designated, Community Hunting Areas/Private Game Reserves have also been established in provinces under the relevant legislation.

A protected area system review was carried out in 2000 and an action plan prepared (IUCN, 2000a). The Plan included elements for filling ecological gaps, securing financial resources, and capacity-building, and addressed policy,

legislative and institutional barriers. The overall goal for Pakistan's Protected Area System was developed as 'To protect representative samples of Pakistan's full range of biodiversity, and help maintain ecosystems, and associated cultural heritage, for the sustainable benefit of present and future generations.'

Listed PAs were re-classified according to international standards and IUCN categories assigned to them. Of the 227 PAs listed at the time 58 were considered to be satisfying the IUCN criteria whereas 169 PAs were considered to have been established mainly to control hunting. The management of these areas is required to be upgraded to conform to the principles of conservation. Simultaneously Environmentally Significant Areas (ESAs) covering all the ecological zones that required immediate attention for conservation were also identified (IUCN, 2000a).

The review observed that management of the protected areas was required to be upgraded to conform to the principles of conservation. The follow up on this Action Plan has rather been insignificant as for as the re-classification of PAs, up gradation of PAs, and establishment of ESAs is concerned. Progress has however been made in achieving partial targets such as filling ecological gaps, capacity-building, addressing policy, legislative and institutional barriers and to some extent in securing financial resources.

Management of at least six national parks is being integrated into the broader landscape and local communities are actively involved in the planning processes. Site based management planning with key stakeholders is a standard procedure. The protected areas are established under the wildlife laws by the provinces. Some provinces have revised their laws to bring them in conformity with the convention and present day management needs. There has been no assessment of the protected areas so far to determine the status of biodiversity and effectiveness of the management.

There has been a significant increase in the extent of PAs since the first PA was established in 1972. The number of Protected Areas since 1998 has almost remained the same however the area protected and its status of protection has been upgraded. Coverage of ecological regions however has not been equitable. Except for marine ecosystems, most other major ecosystems are covered in the protected area system of Pakistan. However, because of the great diversity in habitats, some habitats are not covered by the PAs. The status and extent of *ESAs* not included in the protected area system remain to be determined. In general, the representation of coastal areas is rather low and the dry and sub-humid lands are over represented in the PA system.

A list of National Parks representing the eco-systems and the flagship species contained in them is given in the following Table II.

The important biodiversity areas are covered under the protected area system. A few such areas that were not covered in the PA system or were not being effectively protected are now receiving attention. The prominent examples are:

Conservation of endemic reptiles

Chagai desert is home to six endemic species of reptiles. These were being unsustainably harvested for export as pets and other uses. A GEF/UNDP medium scale project has initiated program to conserve and develop protocol for a sustainable collection of these reptiles for marketing by the local communities.

Conservation of brown and black bear

Himalayan Wildlife Foundation has been actively involved in the conservation of Himalayan Brown Bear (*Ursus arctos*) in alpine ecosystem of Deosai Plateau. Through their efforts a new national park has been established in Azad Jammu and Kashmir which will not only extend the home range of brown bear but also bring to fore the conservation of Musk Deer (*Moschus chrysogaster*) as the National Park has been named after musk deer. Sustainable Use Specialist group of IUCN for Central Asia is actively taking actions to conserve a remnant population of a sub species of Asiatic Black Bear (*Ursus thibetanus gedrosiana*) in Balochistan.

Trophy hunting

Through effective management of Community Conservation Areas for the purpose of trophy hunting (The communities get 80% share of the total Trophy hunting fees whereas 20% is retained by the Governments for the management that again is spent on conservation activities), there are now healthy populations of wild ungulates – Markhor (*Capra falconeri*), Blue sheep (*Pseudois nayaur*), Himalayan Ibex (*Capra ibex himalayensis*), Sindh wild goat (*Capra aegagrus*), and Urial (*Ovis vignei*), in many parts of the country. In addition, the decline of reptiles in Chagai desert has been reduced through the work of GEF MSP for conservation of species and habitats.

TABLE II.- NATIONAL PARKS OF PAKISTAN, THEIR EXTENT, ECO-SYSTEM AND WILDLIFE SPECIES THEY CONTAIN.

Sr. No.	National Park	Territory / Province	Eco-system	Area (ha)	Significance of the Park
1	Margallah Hills	Islamabad	Tropical dry mixed deciduous forests	15,883	Rawal Lake, Barking Deer, Leopard, Rhesus monkey, White crested kaleej pheasant
2	Hazarganji Chiltan	Balochistan	Balochistan Hills, and Pistacia and Juniper forests	15,555	Chiltan Markhor
3	Hingol	Balochistan	Tropical thorn forest, Riverine ecosystem, marine and coastal areas	619,043	Geological formations, Mud volcanoes, Marsh crocodile, Wild goat, Urial, Chinkara, Biodiversity
4	Khirthar	Sindh	Tropical thorn forest	308,733	Wild goat, Urial, Chinkara, Common leopard, historic graveyards and Rannikot fort
5	Ayubia	Khyber Pakhtunkhwa (KP)	Himalayan moist temperate forests, Tropical thorn forests	3,122	Common leopard, Rhesus monkey, Goral, pheasants,
6	Chitral Gol	KP	Himalayan dry coniferous forests, Dry alpine zone	7,750	Flare-horned markhor, Snow leopard
7	Sheikh Badin	KP	Dry Sub-tropical Semi-evergreen Scrub forests	15,540	Straight-horned markhor
8	Saiful Maluk	KP	Cold desert and dry alpine zone, valley bottoms and stream beds	12,026	Lake Saif-ul-Maluk, Snow leopard, Himalayan Ibex, Brown & Black bear.
9	Lulusar Dodipat	KP	Cold desert and dry alpine zone, valley bottoms and stream beds	75,058	Lakes, glaciers, snow bound peaks, Snow leopard, Lynx, Black & Brown bear, Snow partridge, Ram chakor
10	Broghil	KP	Cold desert and dry alpine zone, valley bottoms and stream beds	134,744	Lakes, glaciers, snow bound peaks, Snow leopard, Lynx, Himalayan Ibex, Snow partridge, Ram chakor
11	Khunjerab	Gilgit-Baltistan	Cold desert and dry alpine zone	227,143	Marco Polo's sheep, Snow leopard, Marmot, Blue sheep, Himalayan ibex, Wild ass,
12	Hunderab Shandoor	Gilgit-Baltistan	Cold desert and dry alpine zone, alpine wetlands	165,000	Himalayan Ibex, Snow Leopard, trout
13	Deosai plains	Gilgit-Baltistan	High altitude wetlands, Alpine plateau meadows	363,600	Brown bear, Snow leopard, Himalayan ibex, waterfowl

14	Central Karakoram	Gilgit-Baltistan	Himalayan dry coniferous forests, Cold deserts and dry alpine zone	973,845	Glaciers and Peaks like K-2, Gashabrum, Snow leopard, Black bear, Himalayan Ibex, Musk deer, Ladakh urial, Snow partridge, Ram chakor
15	Karamber	Gilgit-Baltistan	Cold deserts and dry alpine zone	74,100	Snow leopard, Himalayan Ibex, Musk deer, Himalayan pheasants, Snow partridge, Ram chakor
16	Machiara	AJ&K	Himalayan moist alpine zone, Sub alpine scrub, Himalayan moist temperate forests	13,532	Snow leopard, Black bear, Himalayan Ibex, Musk deer, Himalayan pheasants, Himalayan langur
17	Ghamot	AJ&K	Himalayan dry coniferous forests, Dry alpine zone	27,271	Snow Leopard, Himalayan Ibex, Musk Deer, Black Bear, Brown Bear, pheasants, Himalayan Griffon Vulture, *Yew* and *Kuth*
18	Pir Lasoorha	AJ&K	Sub-tropical pine forests, tropical thorn forests	1,580	Common Leopard, Rhesus Monkey, Palm Civet, Kaleej and Koklass Pheasant, chukar, partridges (black and grey), quail
19	Toli Pir	AJ&K	Himalayan moist temperate forests	1,000	Common Leopard, Rhesus monkey, Black bear, Palm civet, Pheasants (Koklass and Kaleej), chukar partridge
20	Musk Deer Gurez	AJ&K	Himalayan moist temperate forests, Himalayan dry coniferous forests, dry alpine zone	52,815	Common leopard, Musk deer, Black bear, Brown bear, Yellow-throated martin, wolf, Pheasants (Koklass, Monal), snow cock and Himalayan Griffon Vulture, Golden eagle, *yew*
21	Deva Vatala	AJ&K	Dry Sub-tropical Semi-evergreen Scrub forest, Sub-tropical pine forests	2,993	Nilgai, barking deer, hare and grey partridge, black partridge, red jungle fowl, Indian peacock; waterfowl, and Indian python
22	Punch River	AJ&K	Riverine areas, and the river stretch in Moist temperate areas		Snow trout, Mahseer, indigenous fish species, introduced trout
23	Lal Suhanra	Punjab	Tropical thorn forests, Sand dune desert	51,368	Blackbuck, Chinkara, Nilgai,Houbara bustard, Snake bird
24	Chinji	Punjab	Sub-tropical thorn forests	6,097	Punjab Urial, Fossils dating back to Tethys sea
25	Kala Chitta	Punjab	Dry Sub-tropical Semi-evergreen Scrub forest		Punjab Urial, Chinkara, Chakor, partridges
26	Kotli Sattian-Kahuta- Murree	Punjab	Sub-tropical thorn forest, Sub-tropical pine forest, Himalayan moist temperate forest		Barking deer, Common leopard, Leopard cat, Himalayan palm civet, Koklas pheasant, White-crested Kaleej pheasant

Convention on International Trade of Endangered Species of Flora and Fauna

There are 23 mammals, 11 birds, and 13 reptiles which are threatened with extinction and are included in Convention on International Trade in Endangered Species of Flora and Fauna (CITES) Appendix I. There are 30 mammals, 78 birds and 14 reptiles on CITES Appendix II, that is the species that are likely to become extinct unless trade is closely controlled. Twenty mammals, two birds and 12 reptiles are included in Appendix III – species of which trade is already regulated. There have been no surveys to assess the present status of these species and these continue to be in threatened status.

Prevention and mitigation of the negative impacts of key threats

The key threats to PAs include: (i) illegal use of natural resources; (ii) habitat degradation and in some cases even the habitat loss; (iii) lack of stakeholders' participation; (iv) week enforcement of legislation; (v) lack of capacity; (vi) lack of awareness; (vii) poverty and environment nexus, and (viii) financial constraints.

Some of the measures taken to address the threats include: Strengthening the enforcement of legislation, stakeholders participation, awareness campaigns and collaboration among the Government/s, NGOs and donors.

Control and enforcement mechanisms adopted include: good governance, legislation enforcement with the help of communities, human resource development and through outreach programs.

A number of awareness raising events have been organized at places in and around biodiversity hotspots. No key threat has been mitigated completely, partial successes have however been achieved to mitigate all the threats. This varies from site to site and threat to threat. Generalization in this case is rather difficult.

Historically, establishment of protected areas was seen as a sufficient measure to conserve species, habitats and ecosystems. The canvass of biodiversity conservation has now been expanded to include the land and sea-scape.

The high yielding varieties of crops, fruit trees, poultry and livestock were seen as the only way forward to meet the growing demand for food. The importance of the need to conserve the genetic diversity is now being increasingly realized and measures being taken for its conservation.

Exotic species of flora and fauna were being indiscriminately introduced in the natural habitats. The convention has played an important role in raising awareness about the threats of invasive alien species and introduction of alien species is now being strictly regulated.

Financial resource allocations for biodiversity, both national and international, and international cooperation has increased after the adoption of the convention.

The protected area system needs to be strengthened ensuring that at least 10% area of all major ecosystems, habitats, and ecologically sensitive areas are effectively conserved.

The conservation status of species of flora and fauna threatened with extinction or endangered by trade, hunting or loss of habitat needs to be improved.

Conservation of landscapes should be the basis to maintain capacity of ecosystems to deliver goods and services and support livelihoods.

Mitigation measures need to be developed in selected ecosystems to adapt to climate change, and enhance resilience of biodiversity.

Integration of protected areas into broader land seascapes

There has been significant progress to integrate the protected areas into the broader landscape. Most of the protected areas are surrounded by agro-pastoral communities who have been historically dependent on the natural habitats for some of their subsistence needs such as of fuel wood, forage, and grazing animals. One mangrove ecosystem has seen reduction in biotic pressure due to changes in the life styles of the local communities and similar changes are taking place around other PAs as well. Where the communities still rely heavily on the natural habitats surrounding the PAs, collaborative natural resource management practices are being promoted to achieve the objectives of connectivity through integration of PAs into broader landscape. Serious efforts to reduce anthropogenic pressure on the natural habitats in and around the PAs have been made in at least seven of the 26 PAs that are being integrated into the broader landscape.

Site-based protected area planning and management

Protected area planning and management has until recently been the job of professionals alone. However, the site based consultations involving protected area functionaries, local stakeholders, and researchers is now being increasingly adopted. Eleven National Parks out of 26 have management plans in place. Most of these management plans were prepared in the recent past and employed participatory approach for planning. In general most of the PAs so far declared have partially achieved the conservation objectives. A comprehensive review has however not been undertaken and thus it is difficult to report on their effectiveness.

Involvement of indigenous and local communities and relevant stakeholders

The local communities and relevant stakeholders, until recently neither consulted nor involved in planning and management of the PAs are now being increasingly involved. This is true of almost all of the national parks. The initial consultative meetings are held in the villages all around the protected areas and villagers are invited to nominate representatives who would work closely with the functionaries during the planning process and report back to them on any issues of concern to them. During the planning process, the functionaries and the local communities agree on the structure and functions of the joint committees for management.

The collaborative approaches for planning and management of the protected areas have not yet been integrated into the formal policy and legal instruments, provisions have however been made in the drafts under revision. There has been no formal assessment of the effectiveness of participatory or collaborative management. However, informal evidence suggests that these approaches are working well for now.

There is no protected area that is being managed by indigenous or local communities. However, more than 30 areas across the country are now being managed as community conservation areas for the main objective of trophy hunting.

Use of Geographic Information System (GIS) and Geographic Positioning System (GPS) is now widely used to collect the base data on natural resources, land use classification, Cadastral data, animal populations, evaluation of the

habitat and habitat use. Modern scientific tools are also being used to record data on animal populations and the habitat use.

One important use of GIS is the Boundary Demarcation and subsequently re-notification of PAs. Almost all the PAs have been notified based on empirical description of the boundaries that led to disputes among different agencies and private individuals. GIS is being effectively used to remedy the embarrassing situation/s.

At least 10 PAs, the plans of which have been written have benefited from the technological innovation; 15 PAs (including Wildlife Sanctuaries National Parks and Game Reserves) have been taken up as Pilot Areas for boundary notification and re-notification by WWF Pakistan.

Financial sustainability of protected areas

Financial sustainability in terms of ecological and financial costs and benefits of PAs has not been determined for any of the PAs so far. A trust fund has been established and capitalized under the Protected Areas Management Project. The fund is not yet in operation, and it is expected that in future this fund will provide funds for better management of the PAs. Other than this Fund, There are no other mechanisms in place to secure the cost on management and offset the ecological costs.

The national funding for the management of PAs is mainly in the form of staff salaries and other operational costs. The funding for conservation planning, and other capital expenses is rare. Major funding for the three protected areas came from a GEF project. Another GEF project catalyzed the establishment of community conservation areas. Some help has also been provided by the Asian Development Bank and European Commission for management planning and enabling activities.

Minimum standards and best practices

Site management

The three protected areas included in the Protected Areas Management Project have strong elements of community participation. Conservation and Enterprise Committees (CEC) were formed by the project through the intermediation of locally-active NGOs. Where Village Organizations already

existed, the CECs can be subgroups of these organizations. CECs consist of representatives from local communities and enter into formal agreement with the government. The CEC provide a decision-making mechanism for the participatory planning and implementation of management and development interventions under the project.

Governance

At each PA included in the GEF project, a Local Advisory Committee (LAC) was formed during project preparation and maintained through the project implementation period of 5 years and beyond. At implementation, each LAC had representatives of the project management, the local communities, local government, NGOs and related rural support agencies. The LAC's role is to ensure the integration of project activities in the overall strategies and activities of development in the three project areas. It will liaise with other governmental agencies and other agencies active in the area to coordinate project implementation.

Although local communities have or no direct role in the governance of the protected areas, their participation in planning and management has raised their awareness of the rights and obligations. Further it has broken the communication barriers between them and functionaries. The increased awareness and improved interaction among stakeholders has improved the governance of the PAs included in the project.

Change in area

In 1997, the protected areas covered 9.01 million hectares while present there are 10.061 million hectares under protected areas. National Parks is the most important and prominent category of the PAs. In 1997, there were 12 national parks while today there number stands at 26. Except for the addition of Central Karakorum National Park, the increase is mainly due to change in the status of existing PAs or changes in their boundaries.

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MODERN SCIENTIFIC LIVING EXAMPLES FROM THE PLANTS AND ANIMALS: SUPPORTS THEORY OF AADAM HYPOTHESIS FOR SECOND HUMAN ORIGIN TO MAINTAIN THE PAIR LEVEL OF HUMAN BEING

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Abstract.- Human is the most dominant species in the living creature of the world, but they are believed the descendents of the animals (apes). Due to the same reason human is called the social animal, but in the present work, two examples from the animals *i.e. Metridium senile* Linnaeus and *Hydra vulgaris* Pallas the most primitive multicellular animal from phylum coelenterata which also give rise to another individual/clone by Natural Method of Propagation (NMP) through budding process asexually. The other two examples from succulent plants *Kalanchoe daigremontiana* and *Bryophyllum daigremontianum* have also been studied for the same purpose and these plants also give rise to a complete plantlet/miniature/clone with complete root and shoot system by the same NMP, through budding process, asexually. These examples may be practiced and experienced on scientific grounds to understand the possible method/process/ phenomenon for evolving Hazrat Bibi Hawwa (Radiyallaho Taala Anha) from Hazrat Aadam (Alaihis Salam). The examples from the animals *i.e*. *Metridium* and *Hydra* and the plants *i.e*., *K. daigremontiana* and *B. daigremontianum* have been presented by describing their reproduction through Natural Method of Propagation (NMP) by means of budding, asexually. In these four examples only a pre-existing single individual (animal/plant) is required to reproduce the other individual/ plantlet/clone. This NMP by means of budding in spite of the meiosis cells (in reproductive organs only) uses the mitotic/somatic cells *i.e*. body cells. This type of reproduction, from the somatic cells is now referred as the natural cloning in the modern scientific term. This method may reflect the idea for Hazrat Bibi Hawwa (Eve) Radiyaullaho Taala Anha who evolved/originated from the body of pre-existing Hazrat Aadam Alaihis Salam (♂) as the second human (♀) through natural budding / natural cloning, to make a pair level of human by nature for the first time to start the sexual reproduction and then countless men and women were spread through this pair as stated in Holy Qur'an, Hadeeth of Mishkat and Bukhari Shareef. It has also been proved by Science, DNA evidence suggested that " all modern races are closely related and shared a common ancestor no more than 200,000 years ago" it is the 1st point of "Out of Africa Theory" and the single origin model of Human Evolution, that "all existing races of *Homo sapiens* arose from a single ancestor". Therefore"The single origin model of human Evolution has been confirmed and multiregional model for modern Human origin has been rejected"as proved in the present findings through the Theory of Aadam Hypothesis.

Key words: Origin of woman from a man, the Aadam, Qur'an and Science Hadeeth and Science.

INTRODUCTION

Human is a par excellence creature on the surface of earth. He has

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dominated all the rest creatures present on this planet. Since the 1st day of human existence the population of human beings is continuously increasing. Human beings are inventing a new invention, in every new coming day. The approach of human being is spreading and increasing from earth to other planets. But Allahs! Some of the human being wants to insult and degrade the status of human beings by enforcing misconceptions, misguidance, mis-experimental data, wrong ideas, telling lie, diverting researcher in negative direction by misusing the name of science, whereas the science is a source, truly based on real experimental and practical data, providing true and authentic information for the betterness of humanity. It is the duty of an individual/scientist that how he/she make the use of its knowledge/invention. Such as pistol has been invented for the security of human being, but if the same pistol, some one uses for the killing of another innocent person, then it is not the fault of pistol but the fault of user and the method of use. Similarly the science is being used for the betterness for human being so much so that it has become the backbone of human beings. On the other hand the same science is also being used by someone for cheating, misguiding, devaluing and degrading the status and dignity of human beings. Those all scientist, researcher and workers who are involved in this scientific lie, are not human beings in reality.

In this connection one example for instance may be presented here is the case of "Human Evolution", a lot of papers are present on different websites in different scientific journals and books *e.g.,* Kimball (1977), Mader (1987). The general extract of these publications, journals and books is "The human evolved from the apes", but Yahya (2001) and Behe (2006) have strongly not only criticized this concept but also they have proved this concept, invalid and unscientific, in their books separately. The other common concept is "The Eve hypothesis", means all humanity evolved from Eve. Now the question, arise about the origin of Eve? Because the modern age is the age of science and the genetics is that branch of science which can solve this problem on scientific grounds with scientific modern evidences.

Therefore in the present work, the same problem is discussed in the light of genetics, saluting Sir Gregor Mendel (1822-1884), the father of genetics. The asexual (Budding) reproduction of *M. senile* & *H. vulgaris* and *K. daigremontiana* & *B. daigremontianum* have been taken as the example, on which the base line of the idea has been constructed and explained. These are the examples of living multi-cellular animal and angiospermic plants which also reproduce by an asexual method of budding. The same kind of asexual reproduction might have happened for the 2nd human (♀), Hazrat Bibi Hawwa

(Radiyallaho Taala Anha) from the pre-existing 1st human (♂), Hazrat Aadam (Alaihis Salam). Under consideration Theory of Aadam Hypothesis for Human Evolution is not only the reflection of pondering upon three verses of the Holy Qur'an which is the most reliable book in the world (Bucaille, 2001), but also a Hadeeth of the Holy Prophet Sallallaho Alai-Hay-Wa-Aalayhi-Wasallam that Indeed the woman was grown (evolved) from the rib of Hazrat Aadam (All agreed), Narrated by Hazrat Abu Huraira Radiyallah ho Taala Anho, cited in Saheeh Bukhari Shareef & Saheeh Mishkat Shareef.

Therefore keeping in mind the Qur'anic verse "And Allah hath caused you (Human being) to grow as a growth of plants from the earth" (Al-Nooh), both animal (*Metridium* and *Hydra*) and both plants (*K. daigremontiana* & *B. daigremontianum*) have been taken into consideration for the same purpose that How the Eve might had grown/evolved/originated/created from the body of Hazrat Aadam (Alaihis Salam) by NMP, through budding like phenomenon asexually. In this process hard part of the body, the skeleton (soft cartilaginous floating rib) took part in the growth of Hazrat Bibi Hawwa (Radiyallaho Taala Anha) from Hazrat Aadam (Alaihis Salam), as xylem, phloem & veins in plants and ecto, meso and endoderm in *Hydra* and *Metridium*. They give rise to new individuals by somatic cells as well.This may also be considered true for origination/creation/evolution of Hazrat Bibi Hawwa (Radiyallaho Taala Anha) from the body of Hazrat Aadam (Alaihis Salam) asexually in the beginning before pairation of these two humans, because most of the livings have more than one method for their propagation, for example sexual and asexual method of propagation for any plant or animal, such as date palm tree is grown from seed sexually but may be grown from suckers/babies asexually and human being reproduce from zygote sexually but may be grown asexually i.e. birth of Hazrat Issa Alaihis Salam from Hazrat Bibi Marium Radiyallaho Taala Anha and from the somatic cells i.e. cloning of human being as the Dolly was cloned from somatic cells in 1996.

MATERIALS AND METHODS

Four specimens were selected for the study of Natural Method of Propagation (NMP) Dutta (1972). The most primitive multicellular animal *M. senile* Linnaeus commonly called as sea anemone and *H. vulgaris* Pallas (Annandale 1911) from the phylum coelenterata reproduce by NMP through budding process asexually from the pre-existing animal. In this process only one individual of *Metridium* and *Hydra* was required to reproduce the other member of its species, the *Metridium* and *Hydra*. The speed of increasing population of

Metridium and *Hydra* was increasing with the passage of time. The different NMP stages were also studied directly on Karachi coast in case of *M. senile*, where thousands of sea anemone of different colours, stages and species were found there. They were brought to BRC laboratory and were studied in the direction of interest, and *H. vulgaris* (Vidyarthi, 1979) from a fresh water pond grown on water plant. Secondly, the two examples from plants from Cactus (succulents) the *Kalanchoe daigremontiana* Brickell (1989), commonly known as back bone plant and *B. daigremontianum* (Sarwar 2002; Riha and Subik 1981). These plants increase their population by developing miniatures/plantlets/ clones on the margin of their leaf. The miniatures/plantlets have complete root and shoot system. They grow by NMP through budding asexually directly from leaf of pre-existing plant leaf. The newly evolved plant has usually 4-8 leaves and has complete root system.

The two examples each from animals and plants were chosen due to the importance of development of new individual directly from the body of pre-existing individual asexually and the most primitive type of Natural Method of Propagation (NMP). The other reason for selecting these specimens was to avoid the change in number of chromosomes, which might change during sexual reproduction but in NMP it was quite clear that the individual evolving/ developing/originating from the 1st pre-existing animal/plant will have automatically the same number of chromosomes as have the 1st pre-existing animal/plant. Furthermore, these *Metridium* and *Hydra* and *Kalanchoe* and *Bryophyllum* are living specimens they may be studied not only in laboratory, but also in field by any one, any where and any time on modern scientific grounds.

RESULTS

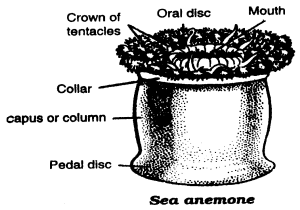
The practical study in laboratory and field revealed that, the sea anemone *Metridium senile* and *Hydra vulgaris* reproduced by NMP through budding process. The bud starts from the body of pre-existing animal as a small out growth, containing the body layers and an extension of the gastro vascular cavity. The second evolving animal was nourished by the 1st pre-existing animal. The second evolving individual was the same species. After detaching from the 1st pre-existing individual, the second evolving individual/clone started passing its life independently, as shown in (Fig. 1A-D). In the case of *K. daigremontiana* & *B. daigremontianum* as well the 1st pre-existing plant gave rise to a new plantlet by NMP through foliar budding at the margin of the leaf directly and asexually. The new plantlet had 4-8 leaves and complete root and shoot system. In one leaf of 1st pre-existing plant there were 60-70 miniatures/plantlets in case of

K. daigremontiana and 25-30 in case of *Bryophyllum daigremontianum*. Each miniature/clone in the beginning was nourished by the 1st pre-existing plant leaf. After its maturity, it was detached from the pre-existing plant leaf body. The detached miniature/plantlet due to complete organs/parts (stem, leaves and roots) soon started passing its life independently as shown in (Fig. 1E-H).

What is important about Natural Method of Propagation here is to note that (i) The most primitive type of propagation and found in both the plants and the animals (ii) Found not only in Monera but also in protoctista and is an asexual type of propagation STBJ, XI. (2005) (iii) Only a single pre-existing individual was needed to give rise to the second individual/clone. STBJ, XII, (2005) (iv) No any reproductive organs were needed used in this NMP through budding/natural cloning (v) The bud (second new individual) may evolve from any vegetative/somatic part of the body of pre-existing individual (vi) The second/next individual is nourished by the 1st pre-existing individual till the new one individual matures physically (vii) After detachment the second one pass its life freely and independently in all respects as the 1st one (vii) The second new individual will have the same number of chromosomes as the pre-existing 1st individual have due to asexual reproduction. Alberts *et al*. (2010) (viii) This type of propagation in animals is found in both Monera and Protoctista *e.g. Hydra* and *Metridium* (ix) This type of propagation in plants is found in both Monera and Protoctista (Uni. and Multicellular) *e.g. Bryophyllum pinnatum, B. daigremontianum, Kalanchae tubiflora* (Syn. *K. verticillata*), *K. daigremontiana*, spider plant Riley (2010) (x) Natural Method of Propagation (NMP) takes place naturally by means of budding/cloning like process in favourable conditions of healthy environment, frequent food supply and for healthy individual (xi) In term of plants and animals it is an asexual method of propagation, for increasing the population of an individual/organism, where there is no population *i.e.* single individual/plant (xii) NMP checks/stops/opposes the process (Phenomenon) of evolution (xiii) The above all presented examples are modern and living examples (xiv) These examples may be seen, observed, experimented, practical and studied by any one, anywhere, any time on scientific grounds to understand the NMP through budding asexually.

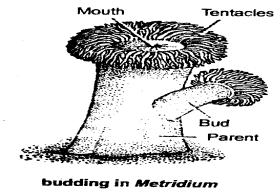
The above all points favour the evolvement / origin of second human, Hazrat Bibi Hawwa Radiyallaho Taala Anha (Identical Female) by NMP through budding like process from the 1st pre-existing human, Hazrat Aadam Alaihis Salam (Identical Male).

A



Pre-existing *M. senile*

B



M. senile with growing bud

C

H. vulgaris before budding

D

H. vulgaris with growing bud

E

K. daigremontiana full plant

F

K. daigremontiana with plantlets

G

B. daigremontianum full plant

H

B. daigremontianum with plantlets

I

Carica papaya male with fruits

J

Male fruit with seeds un ripen

DISCUSSION

Commonly a simple question, arise in the minds of people that How the 1st individual of each animal and each plant came into existence? This question can not be answered by anyone because there are so many species of animals and plants. How the example and evidence you will provide for each and every animal and plant on earth planet for its evolution or its beginning/origin i.e. the 1st individual of each animal and each plant species. It seems to be if not impossible, but very difficult, such as How the 1st plant of Banana was originated? Because it has no seeds and reproduces only asexually. Only a few examples are presented theoretically e.g. of horse evolution. Some people have used the gestures, rough estimates or assumptions which may or may not be correct. But if hinds are taken from the Holy Qur'an which is a Devine, unchanged, unchallenged, un-refutable book and is the most modern book from Almighty Allah (Bucaille, 2001). Then the question may be answered on scientific grounds such as in Surah Al-Nisa # 4: Yaaa - 'ayyuhan-naasut-taquu Rabbakumullazii khalaqakum-min-nafsinw-waahida-tinw-wa khalaqa minhaa zawjahaa (Al-Nisaaa) Translation: "O mankind! Be afraid of your Lord who created you from a single man and from him created his mate" Here man means Aadam (Alaihis Salam) and mate means H.B. Hawwa (Eve) Radiyallaho Taala Anha which means that the second human (Eve) was evolved/originated/ created/grown from the first human (Aadam) present previously (pre-existing), having the same shape, species and number of chromosomes (2N + 1 = 46 + 1 = 47), as we observed in the case of *M. senile* & *H. vulgaris*. Therefore from a single individual (Aadam), the second individual (H.B. Hawwa) was produced by NMP process through budding like phenomenon in the beginning of their life to make up a pair level and then the sexual reproduction started to increase their population. The same concept of human evolution has been described in Surah Al-Hujrat # 49 (Al-Qur'an). Similarly the example may be taken from the plants as well as it has been signaled in the Holy Qur'an in the verse of Surah Al-Nooh # 71: *Wallaahu 'amhatakum-minal-'ardi nabaata* (Al-Qur'an). Translation: "And Allah hath caused you (Human) to grow as a growth of plants from the earth".

Fig. 1. Evidence of budding phenomenon in animals and plants where new animals plants are borne after budding from the parent animal/plant.
A,B, *M. senile,* Pre-existing (A) and with growth bud (B)
C,D, *H. Vulgaris,* before budding (C) and with growing bud (D)
E,F, *K. daigremontiana,* full plant (E) and with plantlets (F)
G,H, *B. daigremontianum,* full plant (G) and with plantlets (H)
I,J, *Carica papaya,* male with fruits (I) and male fruit with seeds un ripen (J)

Bryophyllum) have also been taken into consideration to understand the same process in animals and plants simultaneously. Now this verse also invites us to ponder upon the plants and their budding system in plants, to understand the budding growth in animals. These plants and animals click/switch the idea in our minds to think upon the evolution/origin/creation of female human from a male human by NMP process.

Now to understand these verses of Holy Qur'an, the Hadeeth (Sayings) of the Holy prophet (Sallallaho-Alaihay-Wa-Aalayhi-Wasallum) serves as reference or guide. Therefore to understand that, How a female Human can take creation from a male Human? A Hadeeth is presented. Hazrat Abu Hurairah may God be pleased with him narrated that the Holy prophet (Sallallaho-Alaihay-Wa-Aalayhi-Wasallum) said "Indeed the woman was created from rib" All agreed (Al-Hadeeth). The presented Hadeeth provides clue about the importance of skeleton. In the process of NMP, the skeleton provides the main support to the evolving individual, therefore the Hazrat Bibi Hawwa (Radiyallaho Taala Anha) was evolved/created/developed/originated from the (floating) rib of Aadam (Alaihis Salam). As we observe in the *Metridium* and *Hydra* that all two layers are involved during budding process. Similarly in the case of plants *K. daigremontiana* and *B. daigremontianumthe* the miniature arises from the leaf at the margin of the leaf blade, where the veinlet is ending. This veinlet arise from the mid rib and the mid rib has main supporting cells, the xylem and phloem, providing complete root and shoot system to the plantlet/miniature which after detaching from the pre-existing plant, pass an independent life, as the previous one. Hence similar idea clicks here for Eve (Radiyallaho Taala Anha) from the floating rib of Aadam (Alaihis Salam). So it becomes clear that the Holy Qur'an provides the main idea and the Hadeeth verifies the happening/process/ method/probability of the idea as we noted here. This means that the human beings were evolved from a man and a woman. Note here that the first human (Aadam) was created by Almighty Allah and then from this human the other human (H.B. Hawwa) was created/originated/evolved and from these two (Hazrat Aadam and H.B. Hawwa) all the *Homo sapiens* were evolved, and the population is increasing day by day up till now.

Now the question arises in the minds of common people that how the male human can give rise to a female human? This question may be answered from the findings of a Japanese scientist Fujisawa who reported the experimental work on male liverwort.

Fujisawa *et al.* (2001) reported that only from XY, the female (XX) and male (XY) may be obtained genetically *e.g.* the liverwort *Marchantia polymorpha* has X and Y chromosomes in the respective female and male haploids. They reported the successful explanation of representational different analyses to isolate DNA markers for the sex chromosomes. Two female-specific and six male-specific DNA fragments were genetically confirmed to originate from X and Y chromosomes respectively. In this report which is a scientific attempt proves that the liverwort with XY chromosome, can give rise XX and XY genetically. This example may be accepted for the above question i.e. the 1st plant/individual will produce the same chromosome number in the plant/individual grown from the pre-existing organism and the sex may be the same or different and it was also proven that a male can give rise to a female genetically, which means that it was possible scientifically according'to genetics that from the body of Hazrat Aadam (Alaihis Salam) who was an identical male, having 2N+1=46+1=47 chromosomes, a female Hazrat Bibi Hawwa (Radiyallaho Taala Anha) could be evolved, as we noted in Fujisawa report. It also means that, this scientific statement that male can give rise to female organism is accurately in line with the statement of Holy Qur'an and Holy Hadeeth. And the present work provides the idea about the possible process/phenomenon taken place for reproduction of H.B. Hawwa (Radiyallaho Taala Anha) from the body of H. Baba Aadam (Alaihis Salam) by presenting the examples of *Metridium* and *Hydra* and *Kalanchoe* and *Bryophyllum* reproduction asexually by means of NMP through budding and a complete animal/plantlet from an animal/a plant by the same phenomenon.

The other example may be given from the findings of Storey (1976) who described the papaya as hermaphrodite for the first time having xY sex chromosomes. According to him the male (Hermaphrodite) papaya tree, which is identical to the pure male tree bears the fruit like the female papaya plant. In the same way if Aadam (Alaihis Salam) is supposed to be hermaphrodite having (xxY) chromosomes. Then it becomes very easy to understand because, hermaphrodite is capable of producing both the male and the female. Furthermore hermaphrodite is an identical to a pure (xY) male, as we still note in this modem age that there are xxY male in Human beings which are normal males, and don't have Klinefelter Syndrome as reported online, "I never refer to new born babies as having Klinefelter because they don't have a syndrome". Said 'Arthur Robinson MD, a Pediatrician at the University of Colorado Medical School in Denver and the Director of the National Institute of Child Health and Human Development (NICHD) checked the chromosomes of more than 40,000

infants. The ratio was one infant with xxY in 500-1000 males. Sponsored this study of xxY males "Presumably, some of them will grow up to develop the syndrome Dr. Klinefelter described, but a lot of them won't". For this reason the term "Klinefelter syndrome" has fallen out of favour with medical research. Most prefer to men and boys having the extra chromosome as xxY males. Therefore, researchers identified the males having extra sex-chromosome in new born babies, one of the largest studies in 1970's.

Lieu *et al.* (2004) reported that the papaya sex chromosomes have virtually all of the features that the human sex chromosomes have. Male and hermaphrodite papaya plant having xY and xxY = mMh and xx = mm chromosomes are identical to each other. Here hermaphrodite papaya has the ability for producing fruits like the female papaya plant. This practical scientific evidence also supports that when male (Hermaphrodite) papaya plant can bear the fruits like, the female papaya plants, then how it is not possible for *Homo sapiens* to give rise, a female through NMP like process. Because we know that in this modern age, the male persons having xxY chromosomes are still taking birth, and not only they are normal males but also they are exactly identicals to the pure male (xY) persons (online information), which is a clear proof that Aadam was xxY due to which the evolved progeny still shows this in this modem age as well, which is also a scientific proof.

Alberts *et al*. (2010) write in their book “Many plants also reproduce asexually, forming multicellular offshoots that later detach from the parent to make new independent plants. Even in the animal kingdom there are species that can reproduce by budding, and some worms can be split into two halves, each of which regenerates its missing half. And the females of some species of insects, lizards, and even birds, can produce eggs that develop *parthenogenetically* – that is, without any need for males, sperm, or fertilization – into healthy daughters that can then reproduce in the same way. But while such asexual reproduction is simple and direct, it gives rise to offspring that are genetically identical to the parent organism”. The example has been furnished by them is of *Hydra*, which is a simple multicellular animal and reproduces by forming bud that is genetically identical to the parent. Eventually this bud will detach from its parent and live independently. The same result has been concluded in the present work through the observation of *Metridium senile* and *K. daigremontiana* budding.

Riley (2010) writes in his book “A clone is an organism that is an exact copy of its parent. Clones can occur naturally when some organisms reproduce.

Amoeba, a Protoctist, produces a clone by simply dividing in two. *Hydra*, a small animal related to sea anemones, which lives in ponds and ditches, grows a bud which detaches itself and becomes a copy of its parent. Some plants can also form clones. The spider plant is a familiar houseplant which grows small plants on side shoots. The small plants can become detached and live on their own". In the present work the same idea has been supported by the natural cloning of sea anemone and *K. daigremontiana.*

Tariq (2011) reported that male *Carica papaya* plant having not only male flowers but also bisexual flowers containing Androecium + Gynoecium bear the fruits (As shown in Fig. 1I,J) like a female papaya plant, which has only female flowers containing ovary. This is possible only due to the XXY sex chromosomes in identical male plant. This characteristic of the male papaya plant provides a strong support to the idea and statement described by the Holy Qur'an and Hadeeth. Hence the other evolutionary theories and hypothesis other than Aadam Hypothesis are not valid/satisfactory and reliable as they were constructed on assumptions, whereas Aadam Hypothesis has been proved not only from Qur'an and Hadeeth, but also from the modern scientific, practical evidences from both the plants and animals.

Therefore the *Homo sapiens* should not be mixed with the animals, due to having no practical data and evidences. Instead of this *Homo sapiens* may be classified independent of the other primates because of independent creation of man, as suggested by Farooq and Shakoori (2002) and they have also proposed evolutionary Tree of Man as well beside the describing 16 differences between Human and other primates.

CONCLUSIONS

Human species evolved from the 1st pre-existing human species "Aadam". As according to Modern Cell Theory "a live cell cannot exist until unless the pre-existing cell" Similarly a species cannot exist until unless the pre-existing species. Therefore according to Theory of Aadam Hypothesis "Eve" evolved from the pre-existing Human species Aadam, asexually by means of natural budding / natural cloning to maintain the pair level of Humans for the sake of sexual reproduction" According to Cann *et al*.(1987) DNA evidence suggested that " all modern races are closely related and shared a common ancestor no more than 200,000 years ago" it is the 1st point of "Out of Africa Theory" According to Stringer and Andrews, (1988); Cavalli - Sforza and Feldman, (2003). They

strongly support to the single origin model of Human Evolution, that "all existing races of *Homo sapiens* arose from a single ancestor". According to Benton, (2005), "The single origin model of human Evolution has been confirmed again and again and multiregional model for modern Human origin has to be rejected".

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DORCATHERIUM FROM HASNOT (MIDDLE SIWALIKS), NORTHERN PAKISTAN

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Abstract.– The late Miocene-early Pliocene Hasnot, northern Pakistan, has yielded remains of the Siwalik tragulid *Dorcatherium.* The same unit has also yielded *Selenoportax*, *Pachyportax*, *Gazella* and *Bramatherium*. The known tragulid teeth are well worn. In this paper two new specimens of *Dorcatherium* have been identified, described and discussed in detail. This discovery adds weight to the suggestion that the late Miocene tragulids inhabited well wooded to forested regions rather than open country.

Key Words: Tragulidae, *Dorcatherium*, Siwaliks, Late Miocene, Hasnot.

INTRODUCTION

Palaeontological and geological research began in the 1830s in India, including the region that is now Pakistan, continuing through much of the past century (Fatmi, 1973; Barry *et al.*, 2002). The Potwar Plateau of northern Pakistan is a highly fossilized area of the Siwaliks comprises ca. 30,000 km^2 with thickness of over 4,000 m. The exposure area and fossiliferous nature of the Siwalik sediments of the Potwar Plateau make it the best terrestrial Neogene record of sediments and vertebrate fossils in all of South Asia (Badgley *et al*., 2008).

The Siwalik deposits of the Potwar Plateau have produced a wealth of both macro- and micro-vertebrate fossils, most of which are mammals but also including abundant reptiles and fish as well as occasional birds (Barndt, 1978; Badgley and Behrensmeyer, 1980; Badgley and Tauxe, 1990; Barry *et al*., 1985, 1995, 2002; Khan, 2007, 2008; Khan *et al*., 2008, 2009, 2010, 2011). Most of the larger vertebrates (*i.e.*, >1 kg original body weight) are represented by localized concentrations of fragmentary surface remains, although a number of excavations in particularly rich patches have produced more complete material. Microsites

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are recognized initially by a concentration of small bones on the outcrop surface, followed by excavation, screen-washing of sediment, and picking of the concentrate. Some Siwalik localities have both macro- and micro-remains, but more typically they occur in different places, indicating that different sets of sedimentologic and taphonomic processes were responsible for concentrations of large vs. small animals (Badgley *et al.*, 1998).

The Hasnot area exposes the most complete sequence of the Siwalik Group and yields a diversified sequence of the Middle Siwalik Formation (Fig. 1). They have been referred recently to the late Miocene to the early Pliocene age and belong to fluvial deposits (Pilbeam *et al.*, 1977; Khan *et al.*, 2009). The Hasnot fossil record (Table I) is very rich (Bhatti, 2004; Farooq *et al.*, 2007a-d, 2008; Khan, 2007, 2008; Khan *et al.*, 2007, 2008, 2009, 2010) and provides the opportunity for high resolution of sampling and reconstruction of palaeoenvironments. Palaeoenvironmentally, the fauna suggests a mosaic of landscapes among which humid environments, grasslands and forests, are predominant. The purpose of this paper is to document the tragulid *Dorcatherium* of the Siwaliks from the locality of Hasnot, northern Pakistan (Fig. 1).

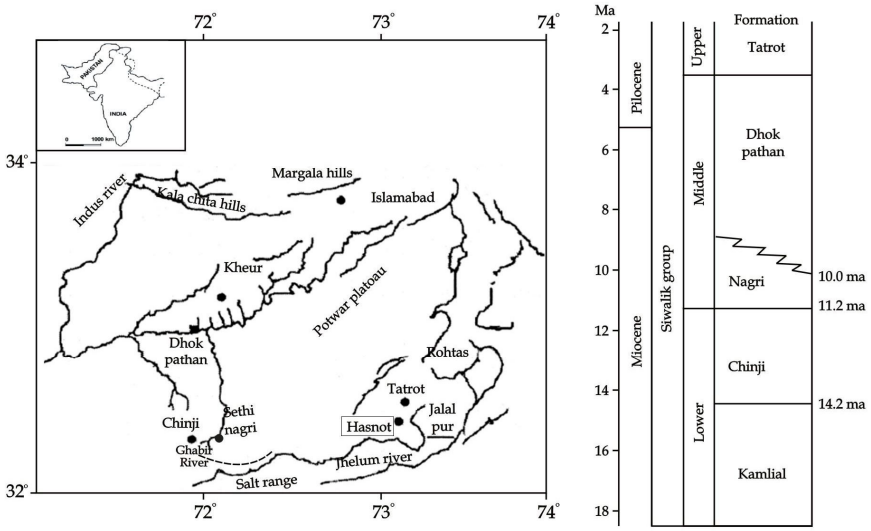

Fig. 1. Location of the Potwar Plateau in northern Pakistan; the studied area is encircled (map is modified from Behrensmeyer and Barry, 2005 and the boundary dates are from Dennell, 2008 and Nanda, 2008).

TABLE I.- MAMMALIAN FAUNA FROM HASNOT AREA (REFERRED DATA ARE TAKEN FROM COLBERT, 1935; PILGRIM, 1937, 1939; HUSSAIN, 1971; SARWAR, 1977; BERNOR AND HUSSAIN, 1985; AKHTAR, 1992; FAROOQ *ET AL*., 2007A-D; KHAN *ET AL*., 2009; KHAN A. M., 2010; GHAFFAR *ET AL*., 2010).

Cercopithecidae	
Cercopithecus hasnoti	*Macacus sivalensis*
Rodentia	
Rhizomys sivalensis	*Rhizomys* sp.
Hystrix sivalensis	
Carnivora	
Amphicyon lydekkeri	*Indarctos punjabiensis*
Promellivora punjabiensis	*Enhydriodon falconeri*
Sivaonyx bathygnathus	*Vishnuictis salmontanus*
Ictitherium sivalense	*Hyaenictitherium indicum*
Lycyaena macrostoma	*Lycyaena macrostoma-cinayaki*
Precrocuta carnifex	*Precrocuta gigantea*
Precrocuta gigantean-latro	*Adcrocuta eximia*
Mellivorodon palaeindicus	*Acluropsis annectans*
Paramachairodus orientalis	*Felis* sp.
Propontosmilus sivalensis	
Proboscidea	
Dinotherium indicum	*Paratetralophodon hasnotensis*
Tetralophodon falconeri	*Tetralophodon punjabiensis*
Zygolophodon chinjiensis	*Choerolophodon corrugatus*
Anancus perimensis	*Stegolophodon latidens*
Stegolophodon cautleyi	*Stegodon bombifrons*
Stegodon cliftii	*Stegodon elephantoides*
Equidae	
Cormohipparion antelopinum	*Cormohipparion theobaldi*
Sivalhippus perimense	*Hipparion* sp.
Rhinocerotidae	
Chilotherium blanfordi	*Chilotherium intermedium*
Subchilotherium intermedium	*Alicornops* sp.
Brachypotherium perimense	
Suidae	
Tetraconodon magnus	*Propotamochoerus ingens* (?)
Listriodon pentapotamiae	*Propotamochoerus hysudricus*
Hippopotamodon sivalense	*Hippopotamodon vagus*
Sivahyus punjabiensis	*Hippohyus lydekkeri*
Anthracotheriidae	
Microbunodon silistrensis	*Merycopotamus dissimilis*
Tragulidae	
Dorcabune anthracotherioides	*Dorcabune nagrii*
Dorcatherium majus	*Dorcatherium minus*
Dorcatherium minimus	

Cervidae

Rucervus simplicidens	*Cervus triplidens*
Cervus sivalensis	*Cervus punjabiensis*
Cervus rewati	

Giraffidae

Bramatherium megacephalum	*Bramatherium perimense*
Giraffa punjabiensis	

Bovidae

Taurotragus latidens	*Tragoportax salmontanus*
Tragoportax punjabicus	*Tragoportax browni*
Proleptobos birmanicus	*Selenoportax vexillarius*
Selenoportax lydekkeri	*Pachyportax latidens*
Pachyportax giganteus	*Gazella lydekkeri*
Gazella padriensis	*Elaschistoceras khauristanensis*
Eotragus sp.	?*Tragoportax curvicornis*

In the present study two specimens of the tragulid from Hasnot is described and housed in the Palaeontology laboratory of the Zoology Department, University of the Punjab, Pakistan (PUPC – institutional abbreviation) and the Zoology Department, Government College University Faisalabad, Punjab, Pakistan (PC-GCUF – institutional abbreviation). The catalogue number of the specimens consists of series i.e., yearly catalogued number and serial catalogued number, so figures of the specimen represent the collection year (numerator) and serial number (denominator) of that year (e.g. 04/21). All measurements are expressed in mm. The upper and lower teeth positions are given using upper and lower case letters respectively (e.g. M1 and m1).

SYSTEMATIC PALAEONTOLOGY

Suborder Ruminantia Scopoli, 1777
Infraorder Tragulina Flower, 1883
Family Tragulidae Milne-Edwards, 1864
Dorcatherium Kaup and Scholl, 1834

Type species

Dorcatherium naui Kaup and Scholl, 1834.

Dorcatherium cf. *majus* Lydekker, 1876

Studied specimen

A fragment of left mandible with posterior lobe of m2 and the posteriorly broken m3 – PUPC 04/21.

Description and comparison

Sample PUPC 04/21 comprises a mandible fragment with broken molars (Fig. 2(1). The length of the preserved mandible is 46.0 mm and its maximum preserved depth below the m3 is 31.3 mm. The specimen is in an excellent state of preservation, rugose and in a late stage of wear. It is fairly preserved and no cement deposition is found all around the specimen. The m2 is broken anteriorly and only the hypoconid and the entoconid are preserved. The ectostylid is present in the transverse valley and it is practically unworn. The principal preserved conids are not so high and the tooth seems to be brachydont. The posterior central cavity is absent, owing to the late stage of wear, and dentine is present all over the crown surface. The dentine island shows that the animal must have died in an old age. The enamel is rugose and the rugosity is more prominent and evident on the buccal side of the tooth. The m3 is a well-preserved tooth except from the hypoconulid, which is missing. The ectostylid is present in the

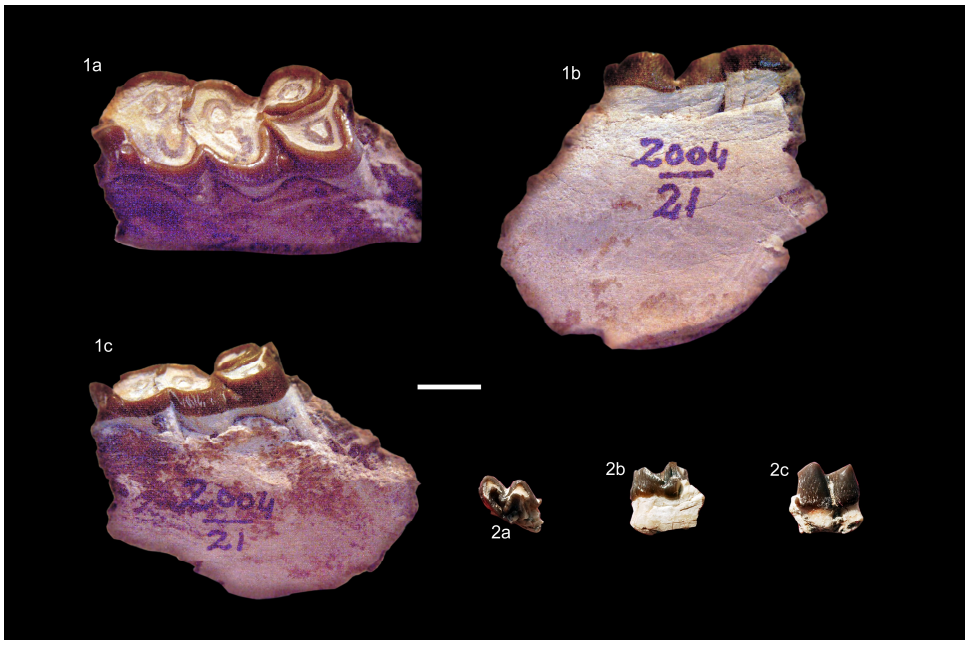

Fig. 2. *Dorcatherium* cf. *majus*: 1, PUPC 04/21 – A fragment of left mandible with posterior lobe of m2 and the posteriorly broken m3. *Dorcatherium* cf. *minus*: 2, PC-GCUF 10/96 – A partial third molar broken anteriorly and preserved hypoconulid and posterior lobe. a = occlusal view; b = lingual view; c = labial view. Scale bar equals 10 mm total.

transverse valley and it is unworn. The anterior fossette is absent and its marks can be seen in the exposing dentine. The posterior fossette is present but not as crescentic as observed in the other molars. The posterior fossette is closed anteriorly and opened posteriorly to form the entostylid, prior to the broken hypoconulid on the lingual side. The median ribs and stylids are strong. The entostylid looks stronger than the other ones. The protoconid and the metaconid are more worn than the hypoconid and the entoconid. So, the posterior conids are higher than the anterior ones. The hypoconid is more crescentic than the other cusps. The praehypocristid is connected to the postprotocristid and longer than the posthypocristid. The hypoconid is projected backwardly and presents a late wear stage. The comparative measurements of the studied teeth specimens are provided in Table II.

TABLE II.- COMPARATIVE MEASUREMENTS OF THE CHEEK TEETH OF THE HASNOT *DORCATHERIUM* IN MM. * STUDIED SPECIMENS. REFERRED DATA ARE TAKEN FROM COLBERT (1935), FAROOQ *ET AL*. (2007A-B, 2008).

Number	Description	Length	Width	W/L ratio
D. majus				
PUPC 04/21*	m3	ca.19.0	11.6	0.61
PUPC 86/3	m3	25.0	11.4	0.45
PUPC 86/152	m3	23.0	11.0	0.47
PUPC 96/64	m3	22.0	11.0	0.50
PUPC 98/61	m3	16.0	11.0	0.68
AMNH 19939	m3	25.5	12.0	0.47
GSI B593	m3	25.0	11.4	0.45
D. minus				
PC-GCUF 10/96*	m3	ca.14	ca.7.0	
PUPC 68/311	m3	14.8	7.80	0.53
PUPC 68/313	m3	15.6	7.40	0.47
PUPC 83/626	m3	12.5	8.00	0.64
PUPC 85/35	m3	15.0	7.00	0.64
PUPC 85/59	m3	14.2	7.00	0.49
PUPC 86/266	m3	14.5	6.40	0.44
PUPC 96/66	m3	13.0	6.30	0.48
AMNH 19366	m3	16.0	8.00	0.50
GSI B594	m3	16.7	8.30	0.49

The sample was erroneously assigned to *Cervus sivalensis* in 2007 by Akbar (Khan, 2007). Later on, the keen reinvestigation of the sample indicates that the specimen shows typical tragulid features: the bunoselenodonty, isolated strong styles, rugose, vestigial ectostylid and basal cingulids. However,

Dorcatherium-fold is absent due to the late wear. The morphology of the molars completely reflects the pattern of *Dorcatherium* and metrically, the molars fall in the range of *Dorcatherium majus* which is the large Siwalik species (Lydekker, 1876; Colbert, 1935; Farooq *et al.*, 2007a-b, 2008; Rössner, 2010). However, the sample is too complete to determine the specific identification and *D.* cf. *majus* can be assigned to the sample.

Dorcatherium minus Lydekker, 1876

Studied specimen

A partial third molar broken anteriorly and preserved hypoconulid and posterior lobe – PC-GCUF 10/96.

Description and comparison

The hypoconulid and hypoconid are preserved in this molar, and they are separated by a vertical groove labially (Fig. 2(2)). The hypoconid displays a deep incisure on its posterior part. The hypoconulid is situated lingually in the main axis of the molar. The antero-labial crest of the hypoconulid connects with the posthypocristid posteriorly. The antero-lingual crest of the hypoconulid forms the postero-lingual edge of the m3 and not to be connected to the post-entocristid leaving the open post-fossette posteriorly which is broken. The basal lingual cingulid is present lingually.

The sample is characterized by small size, brachydont, wrinkled enamel, basal cingulid, open lingual valley and bunoselenodonty. These are the features represented by the small size Siwalik *Dorcatherium.* The small size Siwalik *Dorcatherium* include *D. minimus, D. nagrii* and *D. minus* (Colbert, 1935; West 1980; Farooq *et al*. 2007a-b, 2008; Iqbal *et al*. 2011; Khan and Akhtar, 2011). *Dorcatherium minimus* and *D. nagrii* are too small to include the studied sample. Morphometrically, the sample is associated with *D. minus* and can be named *D. minus*. The material is insufficient and it may be assigned *D.* cf. *minus* from Hasnot.

DISCUSSION AND CONCLUSION

Dorcatherium coexist with *Selenoportax, Pachyportax, Tragoportax, Gazella, Elaschistoceras, Eotragus, Dorcabune, Bramatherium* and cervids (Table I). The bovids are more often associated with this tragulid, they do co-

exist, implying that they probably exploited different feeding resources within a same environment or these could have been an obligate drinker. Large size, long horns and sexual dimorphism of *Selenoprtax* and *Pachypotax* are perhaps slightly in favor of a more open habitat (Khan *et al*., 2009). Therefore, dominance of open woodlands in Hasnot had been suggested by faunal analysis (Table I). *Selenoprtax* and *Pachypotax* had relative lengths of long bones similar to those of the nilgai (*Boselaphus tragocamelus*) which could suggest an intermediate habitat showing the development of herbaceous, shrubby and bushy vegetation.

The ecology of the living tragulids would rather suggest the abundance of bushes and shrubs with mosaic of wet lands in the Hasnot surroundings (Meijaard *et al*., 2010; Geraads, 2010). However, the mixed feeders (*Gazella*, *Selenoportax* and *Pachyportax*) demonstrate the presence of an abundant herbaceous vegetation, which excluded a dense tree cover. This suggests herbaceous, grassy and bushy vegetation in the Hasnot area. The data demonstrates the presence of a rich herbaceous layer with undoubtedly dense forested habitats in Hasnot.

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ISOLATION AND CHARACTERIZATION OF COAL DESULFURIZING BACTERIA

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Abstract.- Coal is considered to be one of the major energy options for next few hundred years. However, the biggest hurdle in this way is air pollution originating through sulfur oxide emission from burning of coal. The present study was undertaken to isolate bacteria from local environment capable of transforming sulfur compounds found in some indigenous coal samples into separable forms. Soils sampled from different localities of Lahore were processed for isolation of sulfur oxidizing bacteria on the medium, EM-I. Nine bacteria were isolated and based upon sulfate deduction potential from coal, the bacterial isolates, MHA-1, MHA-3, MHA-8 and MHA-9 were selected for further studies. On the basis of different phenotypic characteristics, the isolates designated as MHA-8 and MHA-9 were identified as *Bacillus circulans* and *Sulfobacillus thermosulfidoxidans*, respectively. Maximum level of sulfate yield was obtained with 15% inoculum size and 50°C incubation temperature for all the select isolates. Optimum pH for MHA-3 was found to be 4.5 and 5.5 for the isolates, MHA-8 and MHA-9. Deduction of sulfate was investigated in monocultures of the isolates as well as employing a co-culture of MHA-8 and MHA-9. The co-culture was found to remove sulfate significantly on 4^{th} and 8^{th} days of incubation. On these observational points sulfate contents of the co-cultured fluid appeared as to be 2766.4 and 1698.2 µg/ml with corresponding CFU/ml values of 2.07 $x10^7$ and 2.41 $x10^7$, respectively for Lakhara coal sample under the optimized conditions at 100rpm shaking. In case of monocultures the isolates, MHA-3, MHA-8 and MHA-9 yielded, 1192.8, 1339.8 and 1320.2 µg/ml of sulfate, respectively for Khushab coal at 6^{th} day of incubation. Bacterially treated Khushab coal was found to attain higher gross calorific value as compared to the control. Pilot scale studies are required to further define the coal desulfurizartion potential of these bacterial isolates.

Key words: *Bacillus circulans*, *Sulfobacillus thermosulfidoxidans*, sulfate from coal.

INTRODUCTION

A secure energy supply is one of the basic pre-requisites for a sound economic development, sustained standard and quality of life and eventually for social well being of each individual. Much of the world's population has too little

energy to meet basic human needs. Monetary costs of energy are rising nearly everywhere and the environmental impacts of energy supply have been growing and have passed through air, water and oceanic pollutions to global climatic changes. (khalid and Aleem, 1989; Holdren, 2005).

Fossil fuel constitutes an important and extensively exploited natural fuel resource. Coal is one the most important energy sources of fossil origin. It comprises about 75% total world resources of fossil fuels. Many countries are reliant on coal for their energy demands including U.S., India and China. At present 68% of the primary energy needs of Pakistan are met by commercial (oils, gas, coal, hydro and nuclear) and 32% by the traditional fuels (wood, crop residues and animal wastes). In India 73% of coal production is used for power generation. About 93% of U.S. energy comes from combustion of coal. In fact, coal is the fuel of choice for most intensive industrial productions (Reddy and Venkataraman, 2002; Acharya *et al.,* 2005).

Burning of coal is always accompanied with generation of atmospheric pollutants. Its oxidation yields, dust, soot, smoke and other suspended matter, respiratory irritants and various gases such as sulfur dioxide (SO_2), nitrogen oxides (NO_x) and volatile organic compounds. Magnitude of these pollutants depend upon the nature and quality of coal and nature of burning processes itself. Of the various pollutants that are outcomes of coal utilization, the sulfur oxides have specifically been emphasized for their deteriorative properties ranging from lessening the working life of boilers to damaging human tissues such as lungs and skin. (Monticello and Finnerty, 1985; UK Clean Coal Technology, 1998; Boshu and Chen, 2002; Gupta *et al*., 2005).

Of the two forms inorganic sulfur is represented by disulfides and sulfates in coal. Disulfides contents are commonly referred as pyritic sulfur, which appears in two crystalline forms, pyrite and marcasite (FeS_2). Pyrite sulfur may be present as large particles or as microscopic crystals in coal matrix. It is generally the preponderant inorganic sulfur in coal. Organic sulfur is spread throughout the hydrocarbon matrix of coal and is found in the forms of thiophanes, arylsulfides, cyclic sulfides, aliphatic sulfides and aryl and aliphatic thiols (Wise, 1981; Calkins, 1994; Klein *et al.*, 1994).

Desulfurizing high sulfur coal has become a prerequisite for exploiting this resource in present environment and public health perspectives. Accordingly,

numerous methods for coal desulfurizing have been attempted. These include physical separation techniques, chemical treatments and biological means. Although the physical methods do separate, much of the mineral material, their yield decreases considerably when mineral matter in carbon matrix is more disperse. Whilst, depending on the type of coal, a considerable amount of finely distributed pyrite as well as organic sulfur can remain in and attach to the coal particles (Rossi, 1990; Klein, 1998). The inability of physical methods to completely remove even the inorganic sulfur has led to the development of many chemical desulfurization processes, which mainly rely on carbonization in different atmospheres; ethanol solutions, oxidizing agent, alkaline/ acidic solutions and employing variety of solvents *viz.* quinoline, toluene, petroleum ether and house hold bleach. These solvents separate the mineral matter from coal. Besides appealing yields, part of combustible matter of coal is lost in such treatments. This has rendered many of the chemical desulfurization processes to be economically and technically inacceptable at industrial scale. In addition, the processes do not work well for organosulfur, particularly the polyaromatic heterocyclic sulfur(Yaman *et al.*, 1995; Monticello, 1998; Mukherjee and Borthakur, 2001; Pyshyev *et al.*, 2004).

In the above context, microbial processing of coal purification (desulfurization) appeared more attractive to many researchers and the liquefaction, gasification and desulphurization gained massive attention. Application of new developments in biotechnology provides an innovative approach in coal desulphurization. In general, biodesulfurization of coal seems feasible employing less energy intensive operations, low capital and operating costs (Wise,1990; Acharya *et al*., 2001). Another additional advantage of microbial desulfurization is the decrease of trace element concentration as a result of their biologic and chemical leaching during treatment process (Moran *et al*., 2002).For a progressive country like Pakistan, it is obligatory that all energy options must be pursued vigorously. Coal utilization, which being relatively the large resources available, is considered to be one of the major options for next few hundred years (khalid and Aleem,1989). Some workers have reported the bioprocessing of different varieties of Lakhara and khushab coal, in this country (Farhat, 1986; Izhar, 1986). The present study was aimed to isolate and characterize the bacteria capable of transforming sulfur compounds into easily separable forms found in some indigenous coal samples. An attempt has also been made to augment activities of the coal desulfurizing bacterial isolates with low-cost organic additives like bread crumbs and molasses.

MATERIALS AND METHODS

Sample collection

Samples for bacterial isolation were collected from soils rich in decomposing plant residues/humus from different localities of Lahore in sterilized glass bottles. While the two coal samples used as a substrate for bacterial enrichment were from Centre of Coal Technology, Quaid-e-Azam Campus, University of Punjab, Lahore. Referring to their deposits' localities they were named Lakhara and khushab. The coal samples were processed according to the methods of Gupta *et al*. (1990) and Crawford and Gupta (1991). Accordingly, approximately 200g of a sample was treated with 20% HNO_3 for 6h at room temperature. The acid was employed in an amount that soaked the coal completely. The treated sample was then washed, dried at 105°C, powdered, and dissolved in equal weight of 1-*N* NaOH. The solution was centrifuged to remove any undissolved particles, and the supernatant was neutralized (pH 7.0) with dilute HCl. The coal dissolved at pH 7.0 was used as a substrate in the subsequent experiments.

Preparation of enrichment and isolation medium

A modified enrichment medium (EM-I), used for the isolation of bacteria comprised gl^{-1} of $(NH_4)_2SO_4$, 2.0; KH_2PO_4, 2.0; $MgSO_4$, 0.5; and $CaCl_2$, 0.1, yeast extract 0.1, Agar agar, 15 and substrate coal 2.0, derived from Lakhara and khushab deposits. pH of the mineral salts solution was adjusted to 5.5. Following mixing with other ingredients the medium was autoclaved at 121°C for 15 minutes, poured into sterilized Petri plates and allowed to solidify (Crawford and Gupta, 1991).

Each soil sample, 0.1g was suspended in 100ml of autoclaved water for about one hour. Then 100 µl of suspension was evenly spreaded over the plated medium with the help of a glass spreader. The inoculated plates were then incubated at 37°C and growth checked after every 24h up to 120h. Isolated colonies were processed for pure culturing by streaking twice on the isolating medium and on nutrient agar plates. Working stock culture of the pure cultures thus obtained were maintained on M-I agar slants. While the isolates MHA-1, MHA-3, MHA-8 and MHA-9 were also preserved at -20°C in M-I and nutrient broth glycerol stocks.

Growth of bacterial isolates on different media

Besides EM-I, bacterial isolates were cultivated in eight other media (Table I). pH of all the media was adjusted to 5.5 before autoclaving. Bacterial isolates were inoculated on agar media and incubated at 37°C. The media; Muddy-IV (K), Muddy-IV (L), Muddy-V (K) and Muddy-V (L) were selected for further study as they supported best growth of bacterial isolates within 24-36h.

Sulfate estimation

Procedure of Vogel (1978) was used for the photometric estimation of sulfate. Stock solution 0.1% of Na_2SO_4 was diluted to make eight concentrations ranging from 32.4 µg to 972 µg/3ml of deionized water to prepare standard curve. Sulfate contents were calibrated by mixing of 0.5 ml of a dilution with 2.5 ml of water followed by the addition of 0.09gm of $BaCl_2$ and 0.15ml of propanol. After thorough shaking absorbance was read at 520nm aganist distilled water, as blank. Standard curve was then made by applying regression analysis 1.0 (O.D) correspond to 420µg/ ml of Na_2SO_4/ml of the solution.

Selection of bacterial isolates

For the selection of fast growing and maximum sulfate recovering bacteria, the isolates were inoculated in 50ml of Muddy-IV (K), Muddy-IV (L), Muddy-V (K) and Muddy-V (L) taken in culture bottles of 125ml capacity and incubated at 37ºC and 100rpm. Culture samples (3ml) were taken after every 2^{nd} day upto 8^{th} day and analysed for bacterial growth and sulfate estimation. For sulfate estimation given bacterial culture, was centrifuged at 5000rpm for 10 min. Supernatant, 0.1ml was diluted with 2.9 ml water and processed for sulfate estimation as described above. Bacterial growth for these experiments was assessed by determining their C.F.U./ml. Considering maximum sulfate removal at all observational days, medium Muddy-V (K) and the bacterial isolates MHA-1, MHA-3 ,MHA-8 and MHA-9 were selected for further experiments.

Optimization of select bacterial isolates for removal of sulfate and growth

The select bacterial isolates, MHA-1, MHA-3, MHA-8 and MHA-9, were optimized for temperature (37 and 50), pH (4.5, 5.5, 6.5), and inoculum size (1, 5, 10, 15, 20%) in the medium Muddy-V (K). One day old cultures were used as inocula and after 48h of incubation at 100rpm sulfate released in the medium and the bacterial growths were determined.

TABLE I.- COMPOSITION (G/L) OF VARIOUS MEDIA[a] USED IN THIS STUDY.

Medium	Ingredient								
	$NaNo_3$	KH_2PO_4	$MgSO_4$	$MgCl_2$	$CaCl_2$	Yeast Extract	Coal	Molasses	Bread Crumb
Muddy-II(K)	2.0	2.0	0.5	-	0.1	0.1	10.0(k)	-	-
Muddy-II(L)	2.0	2.0	0.5	-	0.1	0.1	10.0(L)	-	-
Muddy-III(K)	2.0	2.0	-	0.5	0.1	0.1	10.0(k)	-	-
Muddy-III(L)	2.0	2.0	-	0.5	0.1	0.1	10.0(L)	-	-
Muddy-IV(K)	-	-	-	-	-	-	10.0(K)	-	5.0
Muddy-IV(L)	-	-	-	-	-	-	10.0(L)	-	5.0
Muddy-V(K)	-	-	-	-	-	-	10.0(k)	5.0	-
Muddy-V(L)	-	-	-	-	-	-	10.0(L)	5.0	-

- = The ingredient was not employed in the respective medium.

[a]: All the media contained 15(g/l) of agar and had initial pH 5.5

Cultivation of bacteria at their growth conditions optima.

Bacterial isolates, MHA-1, MHA-3, MHA-8 and MHA-9 were cultivated in Muddy-V (K) and Muddy-V (L) media and incubated at their corresponding optimum growth conditions. Culture fluids were taken after every 2nd day upto day 8th and processed for determination of colony forming units(C.F.U) /ml and sulfate contents.

Characterization of the bacterial isolates

For determination of morphological and biochemical characteristics, the bacteria were processed for routine Gram's and endospore stainings, motility, oxidase and catalase tests as described by Benson (1994). Lecithinase, nitrate and sulfide tests were performed according to Collins *et al*. (1995). While flagellar staining was done by modifying Rye's method as described by Collins (1995). Isolates were further processed accordingly (Merck, 1996-1997) for citrate utilization. Voges- Proskaur, Carbohydrate utilization, acid production and glucose utilization tests. Based upon the above mentioned characterization the bacterial isolates were identified after Holt *et al*. (2000).

RESULTS

Eight, bacterial isolates capable of oxidizing sulfur from the coal were isolated on, the selective medium EM-I. Colony morphological and biochemical characteristics of the isolates are shown in Table II. Scanty growth appeared when the isolates were grown in Muddy-II (K) and Muddy-II (L); Muddy-III(K) and Muddy-III(L) media, containing mineral salts. While they showed a broad range of growth (O.D) and sulfate removal potential when cultured in media designated as Muddy-IV (K), Muddy-V (K), Muddy-IV (L) and Muddy-V (L).The bacterial isolates designated as MHA-1, MHA-3, MHA-8 and MHA-9 yielded 1178,1500,1594 and 1225 µg/ml of sulfate at first sampling point compared to the inoculated (control) value of 230 µg/ml when grown in Muddy-V (K).Further incubations decreased the sulfate contents drastically so that the corresponding values for the bacterial isolates described above appeared as to be 498,996,1035 and 758 µg/ml at 4th day of incubation which did not alter, in general at the last study period (Fig.1a). When the bacteria were cultured in Muddy-IV(K) a steady decrease of sulfate contents appeared as compared to

TABLE II.- COLONIAL AND BIOCHEMICAL CHARACTERISTICS[a] OF THE BACTERIAL ISOLATES GROWN ON NUTRIENT AGAR AND (EM-I) MEDIA AFTER 24 HOURS OF INCUBATION AT 37°C

Bacterial isolate code	Colonial Characteristics					
	Shape	**Color**	**Margin**	**Elevation**	**Opacity**	**Consistency**
MHA-1	Round (Round)	Creamy (Off white)	Smooth (Smooth)	Raised (Flat)	Opaque (Opaque)	Soft (Buttery)
MHA-2	Round (Round)	Off white (Off white)	Smooth (Smooth)	Flat (Flat)	Opaque (Opaque)	Soft (Buttery)
MHA-3	Round (Round)	Creamy (Off white)	Smooth (Smooth)	Raised (Flat)	Opaque (Opaque)	Buttery (Soft)
MHA-4	L-form (Filamentous)	Creamy (Greenish)	Hairy (Irregular)	Flat (Flat)	Opaque (Translucent)	Matt (Matt)
MHA-5	Round (Round)	Off white (Dusty green)	Hairy (Smooth)	Flat (Flat)	Opaque (Opaque)	Matt (Matt)
MHA-7	Round (Round)	Creamy (Off white)	Smooth (Smooth)	Raised (Flat)	Opaque (Opaque)	Buttery (Buttery)
MHA-8	L-form (Filamentous)	Greenish (Dusty green)	Smooth (Wavy	Raised (Flat)	Opaque (Opaque)	Matt (Matt)
MHA-9	L-form (L- form)	White (Dusty green)	Smooth (Wavy)	Raised (Flat)	Opaque (Opaque)	Matt (Smooth)

[a]: The isolates, MHA-1, MHA-3 were found gram –ve, non endospore former, catalase +ve non-capsular oxidase –ve and expressed diplococci cell arrangement. The isolates MHA-8 and MHA-9 were gram +ve, endospore former, catalse +ve, non capsular and oxidase +ve. MHA-8 and MHA-9 expressed diplobacilli and coccobacilli cell morphologies, respectively.

Muddy-V (K) for all the sampling points. The corresponding values for the above mentioned isolates were 17.64, 50.4, 43.68 and 57.96, for the day 2 and 7.56, 14.28, 39.48 and 10.08 µg/ml, respectively at 4th day of incubation. A slight increase in the sulfate contents was observed at the last sampling period (Fig.1b). In Muddy-V (L), 1374.24, 1345.68, 746.76 and 1456.92 µg/ml of sulfates appeared by the bacterial isolates MHA-1, MHA-3, MHA-8 and MHA-9, respectively at 2nd day with a control value of 1122.4. Further incubation caused a drastic decrease in the sulfate contents and the corresponding values dropped to 962.64, 1179.56, 367.92 and 1006.32 µg/ml at 4th day while 866.56, 1005.48,

289.8 and 898.8 µg/ml on the 8th day of incubation (Fig.1c). When the bacteria were cultured in Muddy-IV (L) decreases in deduction of sulfate were noticeable compared to the control (uninoculated) all days of incubation (Fig 1d).

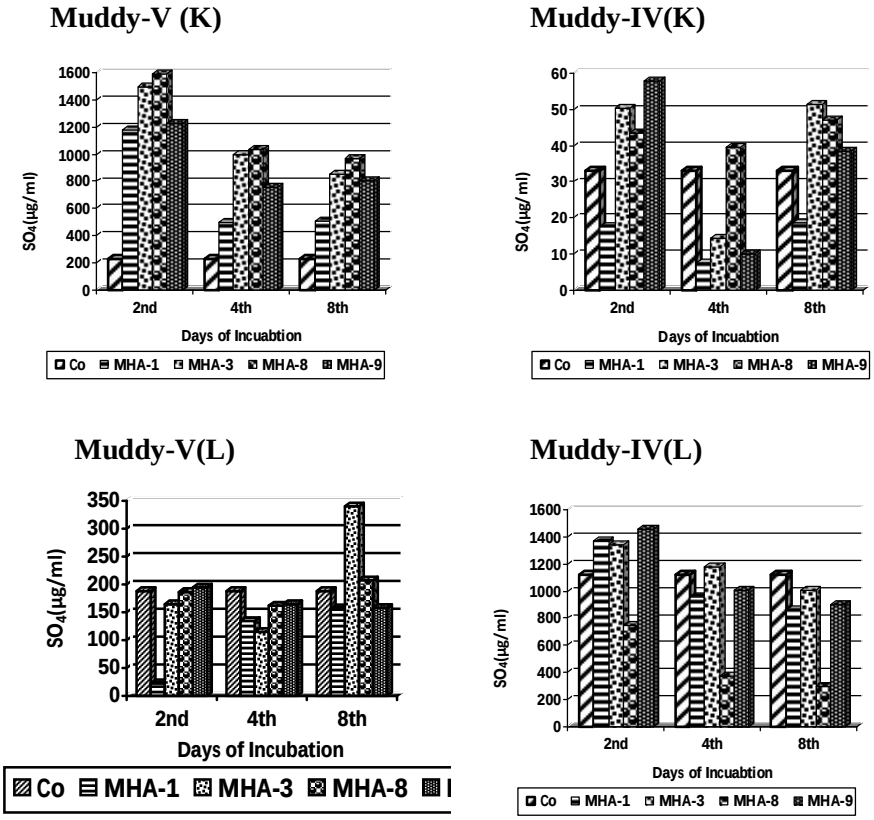

Fig. 1 Deduction of sulfate in control (Co) and the bacterially treated Khushab and Lakhara coal samples in the media. Muddy-V (K), Muddy-IV(K), Muddy-V(L) and Muddy-IV(L), respectively.

Based upon higher amount of sulfate contents in the culture fluids, the isolates MHA-1, MHA-3, MHA-8 and MHA-9 were selected for further experiments. The isolates MHA-1, MHA-3, MHA-8 and MHA-9 were optimized for their growth and sulfate yielding potential at different pH, temperature and inocula size at 100 rpm. The isolates MHA-1, MHA-3 grew best at 50°C, 4.5 pH and 5% inoculum, while MHA-8, and MHA-9, showed optimum growth with

15% inoculum and at 50°C incubation temperature with 5.5 initial pH at 100 rpm after 2 days of inoculation.

For the isolate MHA-1 bacterial growth was found to be maximum with a C.F.U/ml of 2.13 x10^7 for Khushab coal on day 2nd and 2.49 x10^7 C.F.U/ml for Lakhara coal on 4th day. The isolate expressed 2.20 x10^7 and 2.31 x10^7 C.F.U/ml for Khushab, while 2.34 x10^7 and 2.226 x10^7 C.F.U/ml for Lakhara coal samples on 6th and 8th days of incubations, respectively. Sulfate contents were found to be maximum on day 2nd for Khushab and 4th day for Lakhara coal samples with respective values of 1264.2 and 1072.4 µg/ml (Fig 2a).

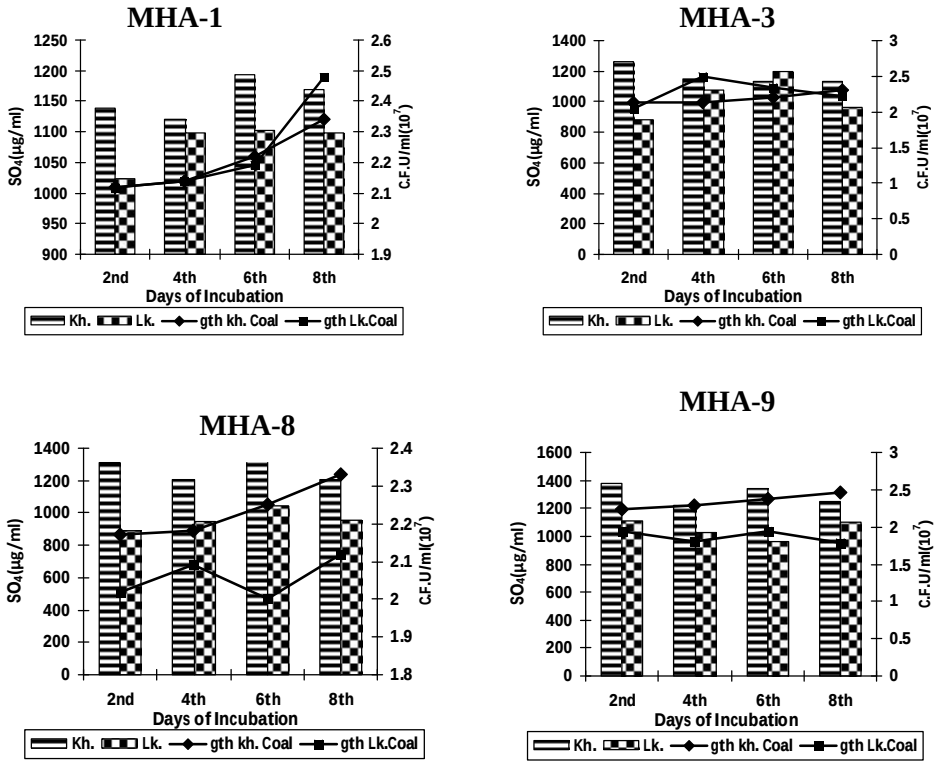

Fig. 2. Growth (C.F.U/ml) and the corresponding sulfate contents for Khushab and Lakhara coal samples (µg/ml) in the culture fluid of MHA-1, MHA-3, MHA-8 and MHA-9 at various days post incubation.

The bacterial isolate MHA-3 showed progressive growth for the post incubation days with maximum C.F.U/ml of 2.34 x10^7 for Khushab coal on 8th

day. For the Lakhra coal the parameter ranged from 2.12 $x10^7$ to 2.48 $x10^7$ C.F.U/ml during the observational period. Sulfate contents were found to be maximum on day 4^{th} for Lakhara coal with a value of 1099 µg/ml. No significant difference in the sulfate contents was observed for Khushab coal in all days of incubation (Fig 2b). Maximum C.F.U/ml of bacterium MHA-8 (2.47 $x10^7$) appeared for Khushab on day 8^{th} day. The isolate showed maximum sulfate contents up to 1339.8 and 1030.4µg/ml on 6th day for Khushab and on day 4 for Lakhara coal samples, respectively (Fig 2c). For the isolate MHA-9, growth ranged from 2.02 $x10^7$ to 2.12 $x10^7$ and 2.17 $x10^7$ to 2.33 $x10^7$ C.F.U/ml for Lakhara and Khushab coal sample, respectively from day 2 to 8^{th} day post inoculation. Maximum sulfate contents were found on day 4^{th} and 6^{th} with values 950.6 and 1320.2 µg/ml, for the respective coal samples (Fig 2d).

The consortium (MHA 8 and MHA 9) was found to remove sulfate significantly on 4^{th} and 8^{th} days post incubation. On these observational points sulfate contents of the co-cultured fluid appeared as to be 2766.4 and 1698.2 as compared to the control value of 715.4 and 847µg/ml, respectively. The corresponding values of C.F.U/ml were 2.07 $x10^7$ and 2.41 $x10^7$, respectively, for the Lakhara coal sample.

TABLE III.- PROXIMATE ANALYSIS OF INTACT, CONTROL AND THE BACTERIALLY TREATED KHUSHAB AND (LAKHARA) COAL SAMPLES

Sample	Moisture (%)	Volatile Matter (%)	Ash contents (%)	Fixed carbon (%)	Total sulfur (%)	Gross caloric value (Kcal/Kg)
Intact	2.81 (7.07)	35.80 (47.91)	24.73 (7.45)	36.66 (40.56)	12.70 (3.58)	5661 (6058)
Control	4.87 (4.93)	43.50 (44.89)	7.52 (8.41)	44.19 (41.77)	10.92 (2.53)	5828 (5534)
MHA-1	4.33 (3.61)	37.11 (49.73)	17.12 (4.16)	41.44 (42.50)	8.68 (2.51)	6241 (5939)
MHA-3	6.44 (4.78)	40.26 (50.42)	11.65 (3.80)	41.65 (40.99)	8.84 (2.54)	5843 (5969)
MHA-8	6.17 (4.74)	39.48 (51.42)	12.93 (5.47)	41.42 (37.82)	8.54 (2.60)	6024 (5928)
MHA-9	3.70 (4.85)	43.52 (50.40)	9.32 (4.74)	43.45 (40.01)	10.38 (2.44)	6181 (6052)

The proximate analysis of khushab coal sample treated with MHA-1, MHA-3, MHA-8 and MHA-9 showed 17.12%, 11.65%, 12.93% and 9.32% ash

contents respectively. While the parameter was found 24.27% and 7.52% for the crude and control coal samples, respectively. The bacterial isolates MHA-1, MHA-3, MHA-8 and MHA-9 also caused a general decrease in total sulfur compared to control value of the parameter. The bacterial inoculation elevated gross caloric value of the treated coal was found so that for Khushab sample the increases were as 7.00, 26.00, 3.6 and 6% for the isolates MHA-1, MHA-3, MHA-8 and MHA-9, respectively as compared to the corresponding control values of the sample (Table III). For the Lakhara sample, the bacterial treatment caused, in general, decrease in ash contents while other parameters remained more or less indifferent to the bacterial attack, except for MHA-9 treated substrate which showed 9.36% higher gross caloric value than the respective control (Table III).

DISCUSSION

In the present study the two coal samples Khushab and Lakhara were treated with bacterial isolates, MHA-1, MHA-3, MHA-8 and MHA-9. The isolates, MHA-8 and MHA-9, when co-cultured yielded the highest removal of sulfur. This consortium removed a maximum amount of sulfur from coal on 4^{th} day, with a value of 2766.4μg/ml, as compared to the uninoculated control value of 715.4μg/ml. The same trend was observed on 8^{th} day with corresponding values of 1698.2 and 847 μg/ml. Fossil fuel's consumption tied pollutants, the-sulfur and nitrogen oxides and their involvement in the formation of acid rain and deleterious effects cannot be ever emphasized. The known harmful effects of acid rain are numerous and include the destruction of aquatic life and forestry, the destruction of buildings by the dissolution of calcium carbonate, and the effect on public health through increased toxic metal concentration in domestic wastes supplies, causing osteomalacia and related bone diseases (Wang *et al.*,2000). Thus it imperative to reduce its sulfur content before combustion. Biodesulfurization is being considered to offer an environmental friendly solution of the problem.

Chemical oxidation of coal sulfur contents may explain appearance of sulfate contents in the uninoculated, but otherwise treated similarly coal samples. For instance, in Lakhara untreated (uninoculated) coal sample sulfate appeared as 1076.6μg/ml on 2^{nd} day, while for MHA-1, MHA-3 and MHA-9, the values could approached upto 882, 1023.4 and 886.2 μg/ml, respectively. Conversely, other bacterially treated samples always desulfurized/yielded sulfate contents more than the corresponding control values. This trend was observed for MHA-8

on 2nd, 4th and 8th day for Lakhara and in all days for Khushab coal samples. So, in all experiments, it appeared that simple soaking of coal in water fortified with molasses activated chemical desulfurization upon the provision of proper physicochemical environment and time frame. It is important to note here that in the present study sulfur deduction was assayed through determination of sulfate contents, which might had transformed after formation to other sulfur compounds not detectable by the method employed. This possibility explains reduction of sulfate contents at increasing incubations, as observed at same study points. When temperature was set at 37°C, the bacterial isolates showed a minimal amount of sulfate deduction, whilst at 50°C, 300 folds increase in sulfate was observed for the bacterial isolate MHA-8. Other isolates, showed a similar trend too.

The volatile matter in general increased after the bacterial treatment in coal samples. This increase may be attributed to formation of jarosite, which decompose rapidly when exposed to heat. The fixed carbon of the treated samples of Khushab coal was found to be higher than in the untreated sample. In short, the bacterially treated coals were found to attain some desirable features such as deduction of sulfur, lowering in the ash contents and higher gross caloric values as compared to the respective control figures. However, searching suitable microorganisms from the local habitats for rendering the sulfur rich coals a suitable fuel remains an attractive field of work in this country.

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SOCIO-ECONOMIC IMPACT OF TROPHY HUNTING ON COMMUNITIES IN GILGIT BALTISTAN

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Abstract.- The study was conducted to find out the socio economic impact of trophy hunting on communities of the Northern Area from November 2008 to February 2009. The main objective of the study was to find out the benefits, socio economic conditions of the community and impact on ecosystem and conservation of endangered species. For that purpose the data was collected from ten communities randomly from the entire trophy hunting area. Interviews were conducted on the basis of specially designed questionnaires. The questions were asked and filled in by an interviewer in face to face interview with the respondent. Almost all respondent had same perception about general effect of the conservation of wildlife and trophy hunting projects upon the area. Majority (90%) of the local community member were of the view that the project had brought positive change in the income and technical skills of the people living in the project area. Only (10%) people opined that the project changes were not visible. The study concludes that through the conservation of wildlife and trophy hunting projects, now the people are well aware about the wildlife and environment. The people want to protect the wild life for recreational value and preservation of natural environment and their sustainability as well.

Key words: Trophy hunting, Gilgit Baltistan, Community, Conservation, Socio economic impact

INTRODUCTION

Trophy hunting is a very demanding venture and due to the ruggedness of the terrain and remoteness of the region hunters must be in top form. However in the event of an accident or illness helicopters can be acquired for evacuation of causality. CTHPs were developed by IUCN between 1995 and 1999 under the UNDP-funded Pre-Investment Feasibility project for -Maintaining Biodiversity in Pakistan with Rural Community Development (PRIF). A major objective of PRIF entailed the promotion and sustainable use of wild resources by encouraging conservation of wild species and contributing to local community

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development. A CTHP involving two species of *Caprinae* (wild goat and sheep), markhor and *Asiatic ibex*, was developed to provide an economic incentive for communities to conserve biological diversity including habitat for wildlife (Shackleton, 2001). In the most comprehensive scientific assessment of the world's animal species to date, IUCN-The World Conservation Union reported in October 1996 that fully one-fourth of all known mammal species are threatened with extinction, along with 20% of reptiles, 25% of amphibians, and 34% of fish (IUCN-The World Conservation Union, 1996). Human population increases and economic development, habitat fragmentation and degradation, the introduction of nonnative species, and commercialization of wildlife have all played a part in the decline and disappearance of species. In the face of such threats, conservationists now recognize that the preservation of many species depends on establishing their economic value and providing incentives for sustainable use, with the increasing involvement of local communities as wildlife custodians (Cohn, 1994; Butler, 1995; Kelso, 1993; Rihoy, 1995). Both former and enduring communal property arrangements are being examined, and new resource-management concepts developed, tested, and refined, according to local circumstances and the unique attributes of individual species. Many of these conservation programmers and projects are viewed as experiments in community-based conservation, which recognizes that the people who coexist with wildlife are in the best position to oversee its use. Especially in developing countries, central agencies responsible for wildlife management are understaffed, underfunded, ill-trained, and ill-equipped to conserve habitats and species (Kothari, Anuradha & Pathak, 1998). As a result, many conservationists argue that the long-term survival of many species can be assured only by involving local people in wildlife management and allowing communities to derive economic benefits from their wildlife resources (Baskin, 1994; Steiner and Rihoy, 1995; Ecotourism — ethical profits, 1995). The objective of present investigation was to find out the socio-economic impact of trophy hunting on communities of the area and future prospective of the trophy hunting.

MATERIALS AND METHODS

The study was carried out in the trophy hunting areas of Gilgit Baltistan which comprised of 970,347 persons (Source 1998 population censes data).

Sample size

The study was conducted through random sampling of communities and owners with the help of list prepared from voters list in order to have reliable data.

Sample intensity

Ten communities were randomly selected to collect the data from the entire area. Interviews were conducted on the basis of specially designed questionnaires and schedule. The questions were asked and filled in by an interviewer in a face to face interview with the respondent.

Data collection

The primary data was collected with the help of questionnaires. The data collection was composed of two parts, one leading to the general information and the other related to activities of the trophy hunting .In order to check the validity of the questionnaires it was pretested in the field and was corrected accordingly. Access, identification, sampled communities and respondents was made with help of Khunjerab National Park Department. The data was collected from November 2008 to February 2009.

RESULTS AND DISCUSSION

After data collection, the data was transferred on the tally sheets, which facilitated the analysis of data. Basic statistical information, were gathered by computing mean, percentage and preparing graphs, and finally results were used for the interpretation and discussion of the data. The data was analyzed by using MS-Excel computer program. The majority of the respondent from local community (53%) were falling in the age group of 41-60years.A small (20%) of the respondents belonged to age group of up to 40 years, while 26% of the local community members of more than 60 years The mean age of the sample local community members was about 52 years. It appears that elders have main role in making decision for the welfare of the concerned communities. That is why we find very few young person's as local community members. The data analysis regarding age composition of respondent as shown in Table I.

Approximately half of the respondents of local community member were found to be illiterates. However the level of education among literate members of the communities is increasing (Table II).

The data analysis depicts that majority of the household earned their live hood from physical labour, agriculture, livestock horticulture and business. Through Business and livestock about 29% earned money. The short falls in

income of the house holding augmented by raring livestock, labor and services in income from their livestock.

Majority (66%)of the respondent were found owing land .However ,the remaining population (17%) cultivating land as tenants while others are landless and not involved in cultivation activities for sharing crops (Table III).

TABLE I.- AGE COMPOSITION

Age(year)	**Northern Areas Number of Respondents.**	**Percentage**
Up to 40	15	60%
41-60	40	53.33%
Above 60	20	26.66%

TABLE II.- EDUCATIONAL LEVELS

Educational Level.	**Northern Areas Number of Respondents.**	**Percentage**
Illiterate	45	60%
Primary	9	12%
Middle	10	13.3%
High & above	11	14.6%

TABLE III.- SOURCE OF INCOME

Profession	**Frequency**	**Percentage**
Agricalture + Forest Earning + Livestock	3	4%
Agricalture + Labour + Livestock	5	6.66%
Agricalture + Labour	7	9.33%
Labour + Livestock	10	13.33%
Agricalture + Livestock.	13	17.33%
Govt Servent + Agricalture + Livestock.	15	20%
Business + Livestock.	22	29.33%

The data analysis indicates that about 73% of the local community members planted seedlings on their lands, before Trophy hunting project; only 50% had undertaken this activity at their own. This shows that to some extent the remaining had been motivated to plant their areas under project activities.

Majority of respondents guide have been exposed to trainings for wildlife conservation and acting as hunting guides.

The analysis reveals that people take interest in training regarding conservation activities and earns live hood by acting as hunting. The project activities also generated employment for some of the local community members in the study areas. About 11% of respondents worked as guide the hunting areas and participated in activities leading to protection of wildlife. This can also be considered as one of the incentives to keep their interest alive in the Trophy hunting activities.

Reason for participation

The member gives many reasons for their participation in the project program. Majority of them 70% stated that they protect the endangered species and earn money through trophy hunting. About 13% said that they wanted to protect and add to the forest resources to promote aesthetic value of their areas in addition to tangible benefits. Similarly 11% stated that they can get more fodder income whereas 6% were of the views of increasing. Animals play a vital role in the survival of rural communities. Livestock are primarily kept for milk and dung cake/manure production and reared for ploughing and transportation .The farmers generally reared the highest numbers of livestock. Milk and sale of animals is their major source of income. There is a shortage of fodder in the grazing area due to numbers greater then grazing capacity therefore stall-feeding is required. The rural population meets its fuel demand from the adjoining hillsides and agricultural and horticular fields. Fuel wood is comparatively a cheaper source of energy in most of the rural areas .At present 57% of the people consumed their own trees to meet fuel wood meet as against 39%before the initiatives of wildlife projects. There is no change in the use of fuel wood sources like forestland and hillsides. However, less than 36% member now relied on purchased wood for fuel as compared to 54% before the projects. In addition to fuel wood, dung cakes also an important fuel source .The utilization of commercial fuels such as gas etc was higher in villages where local sources of wood were source. Low income, high cost of alternative sources and easy access to resources are the major reasons behind the high rate of fuel wood use.

The average household consumption per month indicates that fuel wood remains the major sources of energy. There is no apparent difference in the opinion of respondents. Almost all respondents have same perception about

general effect of the conservation of wildlife and trophy hunting projects upon the area. Majority (90%) of the local community members were of the view that the project had brought positive changes in the income, vegetation cover and technical skills of the people living in the project area. Only 10% opined that so far the projects changes were not visible. About 48% of the respondents gained extra income through labor, guide shrub and grass selling etc whereas 52% concerned that no income has increased .However majority (73%) observed that more fodder was now available for animals Regarding production of fuel wood from farmlands and forests majority (89%) said that more fuel was available. Majority (81%) also felt that their technical skills were upgraded and people's awareness about environment had increased .Everybody unanimously agreed about improvement of vegetation cover on hillsides through trophy hunting and wildlife conservation initiatives. Small population (17%) of the tenants wished not to continue this activity after the phasing out the projects, as it was not bringing any return to them. other (56%) wished to continue conservation of wildlife in the area as they could foresee future benefits . Out of total respondents majority (80%) expected extra income from the scale of local handicraft to the foreigners. Majority (68%) wanted to have fuel wood source. In Gilgit Baltistan the NGOs have taken primary role in Community based Trophy Hunting programs whereas in NWFP the wildlife Department has been much more actively involved, in some cases in co-operation with NGOs. In Gilgit Baltistan NGOs have organized the communities, trained the VWGs and helped to organize and market the hunts, working with outfitters or hunters. NGOs have an understandable propensity to continuously developed new programs because they are often necessary to attract donors. This can be detrimental to community development because the life of NGO conservation programs is short, whereas communities are conservative and resist change. It usually takes much effort on the part of NGO present personnel to convince communities to buy into projects. When a NGOs involvement ends and where a community is expected to continue the

Programs, the NGO must ensure sufficient community capacity end/or ongoing support. This might be achieved by involvement and training government staff, as well as building the capacity of community to work independently. During survey, I come across many examples where communities had been left without adequate capacity and/or support from NGOs/Government to continue CTHPs effectively. It is recommended that every effort should be made to consider that program can be directly linked to existing or previous programs. This will ensure a sense of continuity and a level of trust will develop

within the affected communities. Through the study, we can conclude that these types of project are very beneficial for the local communities and for the improvement of environment. Through the conservation of wildlife and trophy hunting projects the people are well aware about the wildlife and environment. The people want to protect the animals for the recreational value. The local person are getting employment and training through the Trophy hunting. Through the conservation of animals the availability of fuel and fodder for their daily use is increasing.

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PREVALENCE OF *PSEUDOMONAS* AND LACTIC ACID BACTERIA IN RAW MILK AND YOGURT SAMPLED FROM LAHORE

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Abstract.- Microbial contamination is considered, in general, characteristics of foods derived from / and / or prepared in underdeveloped countries. Poor sanitary conditions, less public health awareness and absence of strict regulations by public health authorities contribute for this situation. This survey reports presence of lactic acid and Pseudomonas bacteria in the whole lot of raw milk and yogurt sampled from different locations of the study. Varied nature of the bacterial contaminations of frequently consumed food commodities in this area are indicative of poor food quality control if any. This situation leads to unpredicted effects on the health of consumers. Some suffer from food born illnesses following consumption of the contaminated foods. While depending upon the nature of microbial content other may get benefits in term of ingesting beneficial bacterial species such as LAB. Gastrointestinal disturbances and enteric diseases are not uncommon in this country. Microbially contaminated foods are responsible, to a greater extent, for such disease conditions.

Key words: Lactic Acid Bacteria, Bacterial Contamination of Milk, Bacterial Content of Yogurt.

INTRODUCTION

Milk is an excellent medium for growth of diverse kinds of bacteria. Microbial content of milk is kept in safe limits and controlled by the processes of pasteurization and sterilization, respectively. Presently many developing countries are passing through a process of transformation from traditional ways of living to the scientific based norms. Consequently, both categories of life styles are going hand in hand. Rather, majority of the public in this country still insist for raw milk supplies and even its consumption to a considerable extent without boiling or any other microbial controlling treatments. This latter aspect is reflection of a myth that raw milk is more nutritive and imparts special vigour to the consumers. While owing to geometric population growth and consequently rapidly growing towns and cities alongwith some municipal legislation, animal farming has been shifted hundreds of miles away from the bulk of consumers. Raw milk is transported in traditional containers which themselves are /may be highly contaminated with microbes of the environment. High ambient

temperature in most part of year together with vigorous shaking during the transport provide conditions favourable for the growth of the milk contaminants. Raw milk samples and uncooked foods prepared from yogurt have been reported to be highly contaminated with diverse kinds of microorganisms, including coliform bacteria in this country (Qazi and Qureshi, 2002; Naureen 2003; Butt, 2008).

Yogurt made with traditional practices employing open containers for the fermentation and without a concept of quality assurance still make the greater part of the industry than the quality controlled provision of the produce in Pakistan and many other comparable countries. Consumption of such yogurts and uncooked foods prepared from it such as "Dahi Bally" and Fruit cocktail locally called as "fruit charts" are heavily loaded with microbial contaminations (Qazi and Qureshi, 2002; Rukh, 2008). Owing to diverse kinds of contaminations' sources it is not surprising to find diverse kinds of microbes in samples of raw milk, yogurt and ready to eat foods, referred above, prepared from such commodities. Lactic acid bacteria (LAB) can frequently be expressed on the selective growth media from such foods and yogurt samples Aslam and Qazi (2010). Health promoting and probiotics' role of LAB are well known. While members of *Pseudomonas* sp. are involved in spoilage of milk and milk products. *Pseudomonas* sp. are aerobic, gram-negative bacteria that are recognized for their chees and milk spoilage potential (Brown and Luke, 2010; Arslan *et al*., 2011). Further *P. fragi*, *P. lundensis* and members of *P. fluorescens* group may spoil ultra high temperature treated milk and dairy products due to the production of heat-stable proteases in the cold chain of raw milk. It has been established that *apr* X gene in such bacteria codes for heat resistant proteases (Marchand, *et al*., 2009). Consumption of locally prepared yogurt, full of diverse kinds of microbes including LAB, is generally considered in this country a remedy for controlling different forms of diarrhoea. The remedial effect may be considered a function of bacterial antibiosis by LAB which resist varying levels of pH and thus pass through stomach and colonize /compete with pathogenic bacterial content within intestine. The present study aimed at looking with this preview, the raw milk and yogurt samples collected from Lahore. A part of this reports is concerned with the presence of *Pseudomonas* spp. in these samples.

MATERIALS AND METHODS

Collection of samples and isolation of the bacteria

Raw milk and yogurt samples were obtained from different sale points

within the city Lahore, in sterilized containers. All the samples were serially diluted in sterilized water and spread on respective selective media for isolation of lactic acid bacteria (LAB) and *Pseudomonas* sp.

Isolation of LAB

Lactic acid bacteria were isolated on MRS Medium (Deman *et al*., 1960). pH of the medium was adjusted at 5.7±0.2. The prepared plates appeared brownish. The processed samples were spread on the plates and incubated at 37^{o}C in CO_2 enriched atmosphere in an anaerobic jar up to 5 days. Bacterial isolates were considered different on the basis of differences in their colonies' morphologies. A representative colony of each category was processed for pure culturing on nutrient agar.

Isolation of Pseudomonas *sp.*

Pseudomonas F base agar (Merck, 1996) was employed for isolation of *Pseudomonas* spp. pH of the medium was adjusted at 7.00-7.2. The plates appeared light yellow in color. Samples were spread on the plates which were incubated at 37^{o}C and the colonies processed for pure culturing as described above.

Following the establishment of pure culturing, the selected bacterial isolates were streaked on their respective selective media. Fresh cultures were then mixed with sterilized glycerol (10%). and the glycerol stocks stored at -20^{o}C.

Characterization of the bacterial isolates

The bacterial isolates were grown on nutrient agar plates for 24 hours and color, configuration, margins, elevation, size and optical features of their colonies were recorded. The isolates were processed for Gram and endospore (Benson, 1994) and flagella stainings (Collins *et al.*, 1995). Overnight grown cultures were further processed for catalase, oxidase and motility tests (Benson, 1994). The bacterial isolates were also cultivated on MacConkey, cetrimide and Simmon's citrate media as described by Wayant *et al*. (1996). Based upon the morphological and physiobiochemical characteristics the bacterial isolates were identified according to Holt *et al*. (2000).

RESULTS AND DISCUSSION

From different raw milk and household yogurt samples, collected from different areas of Lahore, a total of 32 bacterial isolates on the two selective media i.e., Pseudomonas F base (for *Pseudomonas*) and MRS (for lactic acid bacterial) were isolated and characterized. Tables 1 shows sources, number of isolates and their colonies. Bacterial isolates were designated with prefixes M/Y and A B----G. The source prefixes show either milk (M) or yogurt (Y) sampled from the localities called Samanabad (A), Chouburgi (B), Sunderestate (C), Township (D), Railway station (E), Walton (F) and Muslim Town (G) while the sufficies L and P indicate *Pseudomonas* and LAB, respectively.

Eight bacterial isolates were isolated on *Pseudomonas* F base from the raw milk samples while six from yogurt samples (Table I). Eighteen bacterial isolates were selected on MRS medium under anaerobic conditions. However, they were cultured on nutrient agar under aerobic conditions, to select facultative anaerobic bacteria. Twelve LAB were isolated from milk samples (Table I).

Characterization and identification of the bacterial isolates

Various characteristics of the bacterial isolates are given in Tables I-III. The isolates varied in colonial characteristics such as colour transparency, and optical features etc. Colonies of most of the isolates were found opaque. Different isolates yielded colonies having butyrious, viscous semi viscous, dry and gummy consistencies. Size of the isolates' colonies varied from <1 to 7 mm. Likewise the isolates varied in configuration, margins and elevations as shown in Table I.

The bacterial isolates showed diverse patterns of biochemical characteristics as shown in Tables II and III. Based upon the colonial, morphological and biochemical characteristics such as Gram staining, endospore and flagellar stainings, catalase, oxidase, motility and other tests (Tables I, II) the organisms were identified upto genus/species levels (Weyant *et al*., 1996; Holt *et al*., 2000). LAB were represented by various genera (Table I). Amongst the *Pseudomonas* species from the different samples all the isolates should growth on Mackonkey agar. All the *Pseudomonas* isolates, expect the MA–P1, were found motile and possessed unipolar flagella. Several other characteristics of the *Pseudomonas* bacteria isolated in this study are given in Table III.

TABLE I.- COLONIAL CHARACTERISTICS* OF LACTIC ACID (L1 – L18) AND *PSEUDOMONAS* (P1 – P16) BACTERIAL ISOLATES.

Isolate No. (C.F.U./ml)	Color	Configuration RF/FF/RRM/IS/ LF/ LIM	Margins	Elevation	Consist.
MA-L1(30)	Off white	RF	Entire	Raised	Semi-viscous
MA-L2(10)	White	FF	Undulate	Umbonate	Butyreus
YA-L3(100)	White	FF	Thread like	Umbonate	Butyreus
MB-L4(40)	Off white	RRM	Undulate	Raised	Butyreus
MB-L5(30)	Off white	LF	Entire	Raised	Butyreus
MB-L6(50)	White	IS	Lobate	Flat	Butyreus
MC-L7(10)	Dirty yellow	RF	Entire	Convex	Semi-viscous
MC-L8(30)	Off white	LF	Undulate	Raised	Gummy
YC-L9(30)	Yellow	IS	Wavy	Raised	Semi-viscous
MD-L10(100)	Off white	LF	Undulate	Raised	Butyreus
YD-L11(80)	Light yellow	RF	Entire	Convex	Butyreus
ME-L12(20)	Yellow	RF	Entire	Raised	Butyreus
MF-L13(30)	Off white	LIM	Wavy	Raised	Butyreus
YF-L14(20)	Creamy	RRM	Entire	Flat	Butyreus
YF-L15(30)	Off white	LF	Irregular	Umbonate	Butyreus
MG-L16(40)	Creamy white	LF	Wavy	Convex	Butyreus
MG-L17(70)	Creamy white	LF	Wavy	Convex	Semi-viscous
YG-L18(40)	Off white	LF	Irregular	Raised	Butyreus
MA-P1(tmtc)	Creamy white	RF	Entire	Convex	Butyreus
MA-P2(150)	Off white	RRM	Wavy	Convex	Butyreus
YA-P3(tmtc)	Creamy white	IR	Wavy	Raised	Butyreus
YB-P4(tmtc)	Creamy white	RRM	Undulate	Raised	Butyreus
MB-P5(tmtc)	Creamy white	FF	Wavy	Umbonate	Semi-viscous
MB-P6(200)	Creamy	RF	Entire	Convex	Butyreus
YC-P7(300)	Transparent	RF	Entire	Convex	Semi-viscous
MC-P8(300)	Yellow	IS	Irregular Branching	Flat	Dry
YD-P9(tmtc)	Off white	LF	Undulate	Convex	Dry
MD-P10(tmtc)	Creamy	RF	Entire	Convex	Butyreus
ME-P11(tmtc)	Off white	LIM	Wavy	Umbonate	Semi-viscous
YE-P12(tmtc)	Off white	LF	Undulate	Raised	Gummy
MF-L13(tmtc)	Off white	LF	Lobate	Flat	Semi-viscous
YF-L14(tmtc)	Off white	LF	Undulate	Raised	Butyreus
MG-L15(tmtc)	Creamy	RF	Entire	Convex	Butyreus
YG-L16(tmtc)	Off white	LF	Wavy	Raised	Butyreus

[a] Prefixes M and Y for each isolate indicate milk and yogurt nature of the samples, respectively. While A – G represent the sampling localities.

Conf. configuration of bacterial colony

Consist. Consistency of bacterial isolates.

*All the colonies of lactic acid bacteria appeared opaque. In case of *Pseudomonas*, the isolates colonies also appeared opaque, except the YC-P7 and MD-P10 and MG-15 whose colonies transparent and shiny, respectively.

TABLE II.- BIOCHEMICAL CHARACTERIZATION AND IDENTIFICATION OF LAB FROM DIFFERENT RAW MILK AND YOGURT SAMPLES.

Isolate code	**Gram's reaction (Cell size µm)**	**Cell morphology**	**Endospores Shape (Size) Location**	**Catalase (Oxidase)**	**Motility (O_2 demand)**	**Identified Genus**
MA-L1	+ve (2 x 1)	Round ended rods	Oval (1 x 0.5) (Central)	-ve (+ve)	+ve (FA)	*Clostridium*
MA-L2	+ve (3.5x~1.1)	Ended round rods	-ve	-ve (+ve)	+ve (NR)	*Lactobacillus*
YA-L3	+ve (3 x 1.2)	Ended round rods	-ve	-ve (+ve)	+ve (FA)	*Lactobacillus*
MB-L4	-ve (1.5 x 0.8)	Round ended rods	Oval (1 x 0.5) (Sub-terminal)	+ve (-ve)	(FA)	*Enterobacter*
MB-L5	+ve/G var (1.5 x 0.8)	Rods ends tapering	Oval (1.1 x 0.8) (Central)	+ve (-ve)	(FA)	*Bacillus*
MB-L6	+ve (2 x ≤ 1)	Round ended rods	Elliptical (1.5 x 1) (Terminal)	-ve (+ve)	+ve (FA)	*SporoLacto bacillus*
MC-L7	+ve (1.5 x 1)	Chains of Cocco-bacillus	-ve	+ve (-ve)	(NR)	*Styphylo-coccus*
MC-L8	-ve (1.5-2 x 0.8)	Round ended rods	Oval(1 x 0.5) (Sub terminal)	(+ve) (-ve)	(FA)	*Enterobacter*
YC-L9	+ve (1.5 x 0.8)	Round ended rods	-ve	+ve (+ve)	(FA)	*Kurthia*
MD-L10	-ve (2 x 1.1)	Round ended rods	-ve	(+ve) (+ve)	(NR)	*Pseudomonas*
YD-L11	+ve (3.5 x 1.1)	Rods with one ovoid end	Oval (1.5 x 1) (Terminal)	-ve (+ve)	(NR)	*Sporolactobacillus*
ME-L12	+ve (1 x 1)	Cluster of cocci	-ve	+ve (-ve)	(A)	*Marinococcus*
MF-L13	+ve (3 x 1.2)	Streptobacili	Oval(2 x 1) (Subterminal)	+ve (+ve)	(NR)	*Bacillus*
YF-L14	-ve (1.5 x 1)	Round ended rods	Oval(1.2 x 1) (Sub terminal)	+ve (+ve)	A	*Aeromonas*
YF-L15	+ve (3 x 1)	Streptobacilli	Oval(2 x 1) (Subterminal)	-ve (+ve)	FA	*Sporolacto bacillus*
MG-L16	-ve (2 x 0.8)	Diplobacilli	Elliptical (1.5 x 1) (Central)	-ve (-ve)	+ve (NR)	*Streptobacillus*
MG-L17	-ve (1.5 x 1)	Round ended rods	Oval(1 x 0.8) (Central)	+ve (+ve)	FA	*Enterobacter*
YG-L18	+ve (3 x 1.2)	Streptobacilli	Elliptical (1.2 x 1) (Terminal)	-ve (+ve)	NR	*SporoLacto bacillus*

TABLE III.- BIOCHEMICAL CHARACTERISTICS* AND IDENTIFICATION OF THE *PSEUDOMONAS* ISOLATES FROM THE DIFFERENT RAW MILK AND YOGURT SAMPLES.

Isolate code	Gram's reaction (Cell size µm)	Cell morphology	Growth on Cetrimide (Simmon citrate) agar	Urease activity	Catalase (Oxidase)	Motility (O_2 Req.)	Identified as
MA-P1	-ve (1.5 x 1)	Pointed rods	-ve (+ve)	-ve	+ve (+ve)	-ve (NR)	*P. mallei*
MA-P2	-ve (1.5 x 0.8)	Round ended rods	-ve (+ve)	-ve	+ve (+ve)	+ve (A)	*P. diminuta*
YA-P3	-ve (1.5 x 1)	Diplobacilli	+ve (-ve)	-ve	+ve (+ve)	+ve (FA)	*P. alcaligenes*
YB-P4	-ve (2 x 1)	Round ended rods	+ve (+ve)	-ve	+ve (+ve)	+ve (A)	*P. pseudomallei*
MB-P5	-ve (2 x 1)	Streptobacilli	+ve (-ve)	-ve	+ve (+ve)	+ve (A)	*P. alcaligenes*
MC-P6	-ve (1.5 x 1)	Round ended rods	-ve (-ve)	-ve	+ve (-ve)	+ve (FA)	*Edwardsiella hoshinae*
YD-P7	-ve (2 x 1.2)	Round ended rods	-ve (+ve)	+ve	+ve (+ve)	+ve (A)	*P. pseudomallei*
MD-P8	-ve (1.2 x 1)	Plympy rods	+ve (+ve)	-ve	+ve (-ve)	+ve (NR)	*P. gladioli*
ME-P9	-ve (2 x 1)	Streptobacilli	+ve (-ve)	-ve	+ve (+ve)	+ve (NR)	*P. pseudomallei*
YE-P10	-ve (2 x 1)	Round ended rods	+ve (+ve)	+ve	+ve (+ve)	+ve (NR)	*P. stutzeri*
MF-P11	-ve (2 x 1.2)	Streptobacilli	+ve (+ve)	+ve	+ve (+ve)	+ve (NR)	*P. putida*
YF-P12	-ve (2 x 1)	Pointed rods	+ve (+ve)	+ve	+ve (+ve)	+ve (A)	*P. pseudomallei*
MG-P13	-ve (1.5 x 1)	Round ended rods	+ve (+ve)	-ve	+ve (+ve)	+ve (FA)	*P.aeroginosa*
YG-P14	-ve (3 x 1.5)	Round ended rods	+ve (-ve)	-ve	+ve (+ve)	+ve (FA)	*P. aeroginosa*

[a]. Prefixes M and Y for each isolate indicate milk and yogurt nature the samples, respectively. While A ----- G represent the sampling localities.

[b]. Number of + sign(S) indicate intensity of the reaction.

NR= Test was not required.

A = Aerobic; FA = Facultative anaerobic.

* The isolated bacteria were found positive for Voges Proskouer test. Whereas the isolates, P4, P11, P13 and P14 showed negative results for methyl red test. All the bacterial isolates, except MA-P1, had unipolar flagella. All the isolates showed growth in the absence and presence of 6% NaCl. Likewise all the isolates expressed growth at 25°C, 37°C and 45°C.

Many earlier reports for the milk and foods prepared from milk and yogurt from the study area have elaborated presence of coliform content in the food commodities (Qazi and Quereshi, 2002; Naureen 2003; Butt, 2008). Such reports warrants for quick public health measures to be taken for protecting the health of consumers, especially children. Majority of the families of this region cannot afford quality milk / food product for feeding their infants. And the practice is that raw milk or dilution of yogurt locally called "Lassi" is offered to infants as well as adults. This practice spreads contamination and food born illness. While a local house hold remedy for controlling several types of diarrhoea is ingestion of yogurt. It is pertinent here to mention that Aslam and Qazi (2010) reported isolation of four species of lactic acid bacteria viz. *L. bulgaricus, L. casei, L. acidophilus* and *L. salivarius* from a yogurt sample collected from Lahore. The bacterium *L. salivarius* showed maximum growth at 37^{o}C but with initial pH 4. The bacterium *L. casei* showed maximum growth at 25^{o}C at pH 5. All the above four bacteria formed smooth textured and white yogurt at 2% inoculum. *L. acidophilus* tolerated acid upto 1 pH. However, *L. bulgaricus* and *L. salivarius* showed more resistance to acid when cultured in yogurt as compared to their cultivations in the selective medium. These authors have demonstrated antimicrobial activity of the lactic acid bacteria against different isolates of *E. coli*, *Staphylococcus* sp. *Salmonella* sp. and yeasts. The *L. acidophilus* was found to be the most potent antagonistic microbe against *E. coli*. While the isolate *L. bulgaricus* expressed vivid antifungal activity. Acid tolerance, yogurt fermentation and bacterial and fungal antagonistic characteristics of these bacterial isolates have been considered to render them good candidates for their consideration as probiotics. However, the yogurt made available to the masses in this country is generally not a quality controlled product of modern fermentation technology. Rather it is raised by inoculating a previous lot to boiled milk often in containers subject of various sorts of contaminations such as from handling of the illiterate persons during preparation, dust from the roads and use of non-disinfectant containers etc. Consequently, such foods (milk and milk products) do contain large number of diverse kinds of bacteria. And it is just a matter of change for a healthy consumers to remain healthy or follow a food borne illness. While an ill person consuming such a product may recover following admitting beneficial bacterial flora to his/her gastrointestinal tract which may exert antibiosis effect to the earlierly colonized pathogenic bacteria. Or he/she may suffer still from another kind of food born infection. This kind of highly unpredictable outcome of nutritional and remedial uptake of milk and its product necessitates prompt and desired level of attention of public health regulating authorities in this country for provision of quality insured food commodities.

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REDUCTION IN IODINE DEFICIENCY DISORDERS: A PROSPECTIVE STUDY OF 2550 SCHOOL CHILDREN IN DISTRICT GILGIT

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Abstract.- To asses iodine deficiency disorders in 14 villages of district Gilgit, a survey was conducted in children aged 6-12 years.2550 school going children were surveyed for the purpose. Of 2550 children 07 male and 05 females were found to be suffering from goiter showing over all goiter rate (TGR) of 0.47%.This shows that prevalence of goiter among males and females children is nearly same. Estimation of urinary iodine analysis revealed that 37 (8.22%), 24 (5.33%), 49 (10.88%), and 340 (75.55%) children (male and female) were suffering from severe, moderate, mild and no iodine deficiency respectively. On the basis of our studies we can conclude that iodine deficiency disorders in school going children has been reduced in the Northern areas presumably due to the utilization of iodized salt provided by Aga Khan Health services and Government of Pakistan.

Key words: Iodine deficiency, children, goiter rate, TGR, Iodized salt.

INTRODUCTION

Iodine deficiency is the major preventable cause of irreversible mental retardation, affecting 1 billion people worldwide (Maberly *et al*., 1994). Nutritionally, iodine is incorporated in thyroid hormones, which in turn play a major role in the growth and development of both humans and animals. The effects of iodine deficiency, often called iodine-deficiency disorders (IDDs), include goiter and an increased incidence of stillbirths, abortions, and congenital abnormalities, including endemic cretinism (Maberly *et al*., 1994). The effect of IDD on apparently "normal" populations in iodine deficient areas have also been studied Because micronutrient malnutrition, including IDD, is an important barrier to socioeconomic development and improved quality of life (Boyages *et al*., 1989).

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Because more than ninety percent of ingested iodine is excreted in urine, urinary iodine determination is an accurate means of assessing iodine status (Dunn *et al*., 1993). Consequently urinary iodine excretion from a population can provide a relatively accurate estimate of the dietary iodine intake of that population. Measurement of urinary iodine is technically simple and cheap. Thus, it remains the method of choice and the most widely used biochemical marker of iodine deficiency (Knudsen *et al*., 2000). The present goiter survey was done **i**n District Gilgit o investigate the present situation of IDD in school going children aged 6-12 years; to determine the iodine intake by measuring urinary iodine concentration; to show that how many children are facing severe, moderate, mild and no deficiency; and to provide the guide lines for the future planners.

MATERIALS AND METHODOLOGY

This Study was conducted on 2550 (1240 male and 1310 female) students in age group of 6-12 years. Students were selected from 30 schools of 14 villages from 03 tehsils of district Gilgit-Baltistan were selected out of which 12 schools from Hunza I, 09 schools from Hunza II and 09 schools from Gilgit. From each school 85 (total 2550) students were selected randomly for clinical check up of goiter of which urine samples of 15 (total 450) were obtained for further analysis. These 15 students were selected on the basis of 02 students from age 6 to 11 and 03 from 12 years age.

Assessment of goiter

Every child in target group was assessed for the thyroid size, using the WHO method of palpation. Goiter size was graded according to the criteria provided by WHO/SEARO.

Urinary iodine analysis

After each child was palpated, 15 out of 85 students from each school were asked to bring their urine (5ml) in the sterilized tubes provided. Analysis was performed according to the recommended method of WHO (1985). Color absorption was read in Spectrophotometer

RESULTS AND DISCUSSION

A total number of 2550 students aged 6-12 years were included in this study. This population includes 1240 males and 1310 females. Of total 343 males

and 390 females children were in the age of 6-7 years, 546 male and 566 female were in the in the age of 8-10 years while 351 male and 354 females children were in the age of 11-12 years as shown in the Table I.

Assessment of goiter

Thyroid size was assessed as recommended by (WHO/UNICEF/ICCIDD, 1994). A total of 07 (0.56%) male and 5 (0.38%) female children were suffering from grade II and grade III goiter. This shows that there is no major difference between male and female children regarding goiter as shown in Table I.

TABLE I- OVERALL PREVALENCE OF GOITER AMONG 2550 CHILDREN

Sex	**Total No.**	**Goiter Grade**	**Total Goiter rate**
Male	07	IA	0.56%
Female	05	IA	0.38%
Total	12	IA	0.47%

TABLE II.- OVERALL PREVALENCE OF GOITER AMONG 2550 CHILDREN ACCORDING TO THEIR AGE GROUP.

Age group	**Sex**	**Total No.**	**Goiter Grade**		**Total goiter rate**
			0	**IA**	
6-7	Male	343	341 (99.42%)	2(0.58%)	2(0.585)
	Female	390	387 (99.23%)	3(0.77%)	3(0.77%)
8-10	Male	546	543 (99.45%)	3(0.55%)	3(0.55%)
	Female	566	565 (99.83%)	1(0.17%)	1(0.17%)
11-12	Male	351	349 (99.43%)	2(0.57%)	2(0.57%)
	Female	354	353 (99.72%)	1(0.28%)	1(0.28%)

Table II shows that there is no major difference between different age groups as well as male and female children regarding the goiter rate. It is most presumably due to usage of iodized salt in the region for the last many years. Last evidence revealed that wide range of disorders resulting from severe iodine deficiency affected more than 400 million people in Asia alone (Stanbury and Hetzel , 1980). There is a census that 800 million people are at the risk from living in iodine deficient environment, 190 million suffer from goiter, more than 3 million are cretins and millions more are suffering from intellectual deficit (SEARO/WHO, 1985).Total number of people at the risk of ID is 1570 million

i.e. 295 of world's population (Pandav *et al.*, 1997).In contrast to the present findings, IDD had been a major problem in the area previously. In 1908 McCarrison estimated that 80% of the population in Gilgit and Chitral had visible goiter, while cretinism was also found to be an endemic problem (McCarrison, 1908).A survey in 1960 indicates 76% goiter prevalence rate in school children in village near Gilgit. Similarly in another survey conducted in 1972 showed 82% goiter prevalence in the aged 10-20 years (cited in Dunn *et al*, 1986). Recently Ali *et al*., 1992 reported less than 5% goiter prevalence in population of all ages and sexes in Gilgit and Ghizar District. The TGR 0.47% recorded in the present study suggests that the prevalence of iodine deficiency in school going children in Gilgit area is very low. The TGR reported here is very encouraging as compared to last surveys in other areas of Swat which was having severe iodine deficiency (Bano, 1998).The probable reason for this low prevalence are: 99% of the school going children were using iodized salt and It may due to the diet changes occurred because of Karakoram Highway construction in last seventies

Urinary iodine excretion

Urine samples were collected from 450 children (17.6% out of the total students surveyed for goiter) for urinary iodine. Table 3 shows that out of total 450 samples 37 (8.22%) have urinary iodine level of 2µg/dL, 24 (5.33%) have urinary iodine in the range of 2.0-4.9µg/dL, 49(10.88%) have urinary iodine in the range of 5.0-9.9µg/dL, while remaining 340 (75.55%) have urinary iodine level greater than 10 µg/dL, indicating severe, moderate, mild and no deficiency according to the WHO criteria (WHO/UNICEF/ICCIDD/ 1994).

TABLE III.- URINAY IODINE EXCRETION LEVELS AMONG 450 CHILDREN ACCORDING TO WHO CRITERIA (1994)

Severity of IDD	**UI Level**	**Total Number**	**Males**	**Females**
Severe	<2	37(8.22%)	18(48.68%)	19(51.35%)
Moderate	2.0-4.9	24(5.33%)	13(54.16%)	11(45.83%)
Mild	5.0-9.9	49(10.88%)	22(44.89%)	27(55.10%)
No IDD	>10	340(75.55%)	172(50.58%)	168(49.41%)
Total		110 (24.44%)	53(48.18%)	57(51.81%)

On the whole 24.44% (110) out of 450 students are suffering from one or other (mild, moderate, severe) form of iodine deficiency. Normal levels of iodine ranges from 67% in Gilgit female and 74% in Gilgit male as compare to only 30% in 1989 survey where urinary iodine excretion was used as indicator. This

shows significance progress in ID from the high IDD prevalence rates of earlier years. severe and moderate levels combine were 33% in 1989 survey while in 1994 it ranges from 15% (Ghizar male) to 26% (Gilgit female).However severe iodine deficiency by itself was only 3% in 1989 survey while in 1994 it was ranges from 5% to 16% in Gilgit males(AKHSP and NIHI, 1994). Similarly in our studies there is no major difference between male and female goiter rate and urinary iodine excretion of the three District of Gilgit Baltistan. On the basis of goiter survey some areas of Swat still are facing severe iodine deficiency as the TGR> 30 which is criteria for severe iodine deficiency (Bano, 1998). Zargar *et al*., 1995 declared Kashmir valley as moderate iodine deficient on the basis of these criteria. On the basis of iodine excretion in urine the prevalence of severe, moderate and mild was 8.22%, 5.33% and 10.88% respectively. The overall urinary excretion was 24.44% which is very less as compared to WHO criteria which is >30% for the whole population (WHO/UNICEF/ICCIDD, 1994).So this area has very low iodine deficiency on the basis of results we found.

CONCLUSIONS

On the basis of this study we can conclude that there has been a significant reduction in the prevalence of Iodine deficiency Disorders since the last 1970s when targeted control efforts were begun and due to establishment of Karakoram high way created much access to the Gilgit Baltistan which results in to increased amount of food being imported from the down country leading to change in diet. According to our study it may be concluded that Gilgit Baltistan has very low Iodine deficiency in school going children between age group of 6-12 years. Further study of those who use iodized salt as compared to those who do not use might be useful in helping to assess the impact of this control measure.

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THREE YEARS' SOIL-BURIED EXPOSURE OF MILD STEEL TO CORROSION INFLUENCING BACTERIA

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Abstract.- Corrosion is a consequent of destructive effects of environment on metals and their alloys. Microbiologically influenced corrosion (MIC) causes heavy losses to metallic installments. The present study describes fate of long term soil buried mild steel coupons (MSCs) exposed to two species of bacteria in nutrient added and without added soils for a period of 36 months. Control MSCs without added nutrients showed the values of corrosion rate and APWL as 19.36×10^{-3} $mgdm^{-2}d^{-1}$ and 44.55%, respectively in the presence of 15.8% moisture and 5.93×10^{-3}% organic contents. While the coupons from soils with added nutrients had corrosion rate and APWL as 11.59×10^{-3} $mgdm^{-2}d^{-1}$ and 32.56%, respectively in the presence of 19.05% moisture and 5.13×10^{-3}% organic contents. Corrosion rate and APWL due to MIC in soil inoculated with bacterial isolate *Penibacillus dendritiformis* -MB14 resulted in 1.41% weight loss with a corrosion rate of -3.5×10^{-3} $mgdm^{-2}d^{-1}$ in the presence of 15.47% moisture and 7.17×10^{-3}% organic contents. In case of nutrient added soil, inoculated with the same bacterium, APWL was 17.68% with a corrosion rate of 5.9×10^{-3} $mgdm^{-2}d^{-1}$ in the presence of 21.1% moisture and 4.7×10^{-3}% organic contents. The corrosion rate and APWL of MSCs due to MIC in soil without added nutrients and inoculated with *Bacillus subtilis* -MB6 were -1.9×10^{-3} $mgdm^{-2}d^{-1}$ and 3.39%, respectively in the presence of 17.85% moisture and 5.57×10^{-3}% organic contents. Whereas in case of nutrient added soil inoculated with this bacterium, the corrosion rate and APWL due to MIC were 3.8×10^{-3} $mgdm^{-2}d^{-1}$ and 14.99%, respectively in the presence of 20.4% moisture and 3.12×10^{-3}% organic contents. The two bacterial species' interaction involved antibiosis effect of *P. dendritiformis* -MB14 against the *B. subtilis* -MB6. The corrosion rate and APWL of MSCs due to MIC in soil without added nutrients and inoculated with both bacterial species were -6.3×10^{-3} $mgdm^{-2}d^{-1}$ and -10.48%, respectively in the presence of 17.1% moisture and 3.7×10^{-3}% organic contents. Whereas in case of nutrient added co-cultured soil, the corrosion rate and APWL due to MIC were 0.2×10^{-3} $mgdm^{-2}d^{-1}$ and 0.683%, respectively in the presence of 19.17% moisture and 2.8×10^{-3}% organic contents. Drastic decrease in APWL of MSCs exposed to the co-culture as compared to those treated with monocultures of *Penibacillus dendritiformis* -MB14 *and Bacillus subtilis* -MB6 demonstrated importance of structure of bacterial community in rendering a given environment suitable for man made structures and their installments.

Keywords: Mild steel corrosion, Microbiologically influenced corrosion, *Bacillus subtilis*, *Penibacillus dendritiformis*,

INTRODUCTION

Humans depend upon the utilization of metals in various ways. Structures made from metals are applied for their various stable features. However, metals and their alloys are influenced negatively by the environment. Corrosion is a well known physiochemical interaction between a metal and its environmental factors and may often lead to impairment of its functions and causes dramatic economic losses. It can be very serious if deterioration of critical components is involved. Serious corrosion problems, such as the pitting of condenser tubes in heat exchangers, degradation of electronic components in aircrafts and corrosion fatigue of propellers can lead to catastrophic failures (Ahmad, 2006).

Corrosion is an electrochemical process involving the transfer of electrons from zero valent metal to an external electron accepter causing release of the metal ions into the surrounding medium. This process ultimately results into deterioration of the metals (Beech and Sunner, 2004). It consists of an anodic reaction involving the ionization (oxidation) of the metal (the corrosion reaction), and a cathodic reaction based on the reduction of a chemical species (Beech and Gaylarde, 1999; Zuo, 2007). Rusting of iron is a familiar example of corrosion in the presence of water and oxygen (Lee and Newman, 2003). When metals such as iron or mild steel are exposed to oxygen in aqueous environment, corrosion or rusting takes place. The deterioration is characterized with the formation of iron oxide/hydroxide as corrosion products. Following reaction describe corrosion of iron in aqueous solution in the presence of oxygen.

$Fe^0 \rightarrow Fe^{2+} + 2e^-$	(Anodic reaction)
$Fe^{2+} + 2H_2O \rightarrow Fe(OH)_2 + 2H^+$	(Iron hydrolysis)
$2Fe(OH)_2 + 1/2O_2 \rightarrow Fe_2O_3 + 2H_2O$	(Further oxidation)
$H_2O + 2e^- + 1/2O_2 \rightarrow 2OH^-$	(Cathodic reaction)

In principle, any set of conditions that promote iron oxidation will accelerate corrosion and any that retard iron oxidation will inhibit corrosion (Lee and Newman, 2003).

Metallic structures are influenced with their immediate environmental factors especially those which influence the process of corrosion. Such factors act differently in the atmosphere, water and soil. Soil is a heterogenous complex,

porous and discontinuous environment constituted by mineral and/or organic solid phase, water liquid phase and gas phase including air. Corrosion of metal in soil environments depends on various soil parameters such as the moisture contents, degree of aeration, pH, ionic species, electrical resistivity and microbial activities etc. (Beech, 2004; Kholondenko *et al*., 2000; Li *et al*., 2007). The latter cause of corrosion is called microbiologically influenced corrosion (MIC). MIC or biocorrosion, refers to deterioration of metals owing to the presence of biofilms on their surfaces (Beech, 2004). MIC does not invoke any new electrochemical mechanism of corrosion; rather it just promotes physiochemically reactions not normally favoured under otherwise similar conditions (Beech and Gaylarde, 1999). MIC problems have been widely documented for storage tanks, piping systems, aquatic structures and cooling towers. About 50% - 70% of corrosion of underground pipes is due to MIC (Zhao *et al.,* 2007). Due to their low cost mild steels are widely used in many applications. For corrosion protection mild steels are normally coated, while cathodic protection may also be used for select applications. However, biofilms tend to form at flaws in the coating surfaces. Delamination of the coating, in turn, creates an ideal environment for further microbial growth (Lane, 2005). Prevention of MIC mostly includes the use of various biocides, which are problematic as the chemicals not only cause environmental problems but also induce resistance in bacteria.

Biocontrol of MIC needs delineating microbial ecological interactions with metals and among themselves. The bacteria exerting antibiosis for corrosion causing microbes can be exploited under select situations to keep a check on the deteriorative organisms. It is well documented that antibiosis as well as applications of bacterial exoproducts may control/inhibit processes of biocorrosion (Jayaraman *et al*., 1999; Korenblum *et al*., 2005). The formation of biofilm by microbes inhibitory to the MIC process on metal surface has been reported by many workers to protect the metal from corrosion (Little and Ray, 2002; Ornek *et al.,* 2002; Zuo *et al*., 2005). Such reports declare the existence of likelihood that promising bacterial exoproducts may in future found application for protecting the metal structure from MIC. However, stability and retention of functional attributes of such biological products under different sets of physicochemical conditions, mimicking the field environment where metallic structures are installed will decide their potential of application. The present study describes influences of corrosion escalating and inhibiting bacteria on the process of soil buried mild steel following i.e. 3 years of soil burial, under differing physico-chemical environments.

MATERIALS AND METHODS

The present work is a continuity of a previous study in which Bano (2008) isolated several bacterial species from soils sampled from surroundings of microbiologically influenced corroded buried Sui Northern gas pipelines. The soil was collected from Sheikhupura, Province Punjab. Many of the bacterial isolates were found corrosion enhancing and several as inhibitory to the process of corrosion of mild steel coupons (MSCs). The MSCs were exposed to different experimental conditions and for different time periods. This study reports effects of two bacterial species on MSCs, buried in wet and nutrient added soils up to 36 months.

Pre- harvest preparation of MSCs

As described by Bano (2008), MSCs (2.5×2.5×0.1cm) were degreased with analytical grade acetone (Merck). The degreased coupons were gently polished with 240 grit polishing paper and rinsed with distilled water. After the cleaning they were washed with ethanol followed by rinsing with distilled water and wiped immediately with paper towel and dried in an oven at 80ºC for 10 minutes and then cooled to measure the weight/coupon (gm) on an electric balance (Zuo and Wood, 2004). The MSCs were then autoclaved and buried in soil within glass containers.

Soil sample, for simulation of field conditions in laboratory was collected from about 2 meter depth in vicinity of Quaid-e-Azam campus, University of the Punjab, Lahore. Soil was brought to laboratory, air dried, thoroughly mixed several times and sieved through 20 mesh sieve. For the estimation of MSCs corrosion in soil, 300gm of the prepared soil was added in transparent glass jam bottles having plastic screw caps. The bottles (uncovered) were then placed in oven for 2 days at 105°C for removal of moisture contents. They were then capped and sterilized at 121°C for 30 minutes in autoclave.

Inoculum preparation

Freshly grown bacterial cultures were spun at 5000rpm for 10 minutes. The pellet was washed twice with sterile distilled water. The cells suspensions in water/nutrient broth were then prepared to achieve 0.80 O.D. at 600nm for harvesting the bacterial in soil conditions.

Experimental procedure

For control containers the sterilized soil was moistened with 80ml of sterilized distilled water/nutrient broth to provide 25% moisture contents. For experimental containers, the amount of water/broth introduced was adjusted for respective inocula. MSCs of known weight were inserted in the moist soils. Bacterial suspensions prepared in water/nutrient broth were inoculated in the soil according to predetermined optimum inoculum size for a given bacterium (Bano, 2008). The glass containers were capped, air tightened and sealed with thick black tape. The inoculated bottles were then placed in inverted position to minimize the interference of air with the bacterial growth conditions (Bano, 2008).

For one set of experiments, bacteria suspended in water were inoulated in soils around the coupons. For this set sterile water was used for control coupons. In the second set of experiments, bacterial cells were suspended in nutrient broth. And sterile nutrient broth was provided to the control containers. One set of experimental containers was inoculated with *Penibacillus dendritiformis* MB-14. Another set of soil buried MSCs was inoculated with *Bacillus subtilis* MB-6. In the last set, the coupons were exposed to inocula of the both bacteria.

Post-harvest preparation of MSCs

Following 3 years post-exposure, 1.00 g of soil was saved aseptically for viable counting of bacteria. The MSCs were then recovered and corrosion products' impregnated soil was removed from the coupons' surfaces with the help of fine needles and scalpels and saved for XRD. Finally the MSCs were made free from corrosion products by the method of Angeles-Chavez *et al.* (2001). Accordingly, the coupons were dipped in the Clark's solution comprising of 20g Sb_2O_3 and 50g $SnCl_2$ in 774ml of HCl, for 5-15 minutes to remove corrosion product. The coupons were then washed with distilled water, dried at 80ºC for 20 minutes and weighed to determine the weight loss. Weight loss of each coupon was calibrated as percent loss from its initial mass. The weight loss of control coupons was subtracted from the experimental MSCs to calculate their percent-corrected weight loss. Corrosion rate ($mgdm^{-2}d^{-1}$) was then calculated according to the following formula as described by Majumdar *et al.* (1999).

$$C=W1-W2/AT$$

where C is the corrosion rate ($mgdm^{-2}d^{-1}$), W1 and W2 are weights of MSCs

before and after the harvesting, A is the surface area of coupons and T represents experimental periods in days. Photographs of the representative control as well as experimental coupons were taken with the help of a digital camera.

Viable counting of bacteria

Before removal of coupons, 1.00g of the sampled soil was mixed with 10ml of sterilized distilled water. Further dilution of the sample was made by mixing 0.1ml of the suspension within 9.9ml of distilled water. Then 0.1ml of a given dilution was spread on nutrient agar plates and bacterial colonies counted after incubation at 37°C for 24 hrs. Plates containing 30-300 C.F.U were taken into account only for determining the parameter/g of soil.

Analyses of experimental soils

Fifty grams of a soil sample was put in a pre-weighted container and placed in oven at 105 ºC till constant weight to determine percent moisture contents (Gupta, 2000) method. Organic contents of dried the soils were measured by heating 5-10gm. For this purpose, (105^{o}C) ground soil taken in tarred crucibles was heated at 360^{o}C for two hours. Percent organic contents then were measured according to the following formula:

Percent organic contents= Weight at 105^{o}C – Weight at 360^{o}C/ Weight at 105^{o}C

For determination of soil pH, 5g of a given sample was mixed with 5ml of distilled water in a beaker, stirred vigorously for 5 seconds and then allowed to stand for 10 to 30 minutes. Electrode of pH meter was placed in the slurry, swirled carefully and pH recorded immediately. During the process it was ensured that the electrode tip should remain immersed in the swirled slurry (Watson and Brown, 1998). Soil salinity/ electrical conductivity was estimated by mixing 20g of a soil sample with 20ml of distilled water. The suspension was mixed periodically and allowed to equilibrate for 15 to 20 minutes and finally the conductivity was measured by inserting the conductivity cell into the suspension in millivolts (Whitney, 1998).

X-rays diffraction of corrosion product

Dried corrosion product(s) were pulverized into a fine powder by using pestle and mortor X-ray diffraction (XRD) analysis of the prepared samples was done to determine the nature of oxides present in the corrosion product. A semi

computer controlled XRD (RIGAKU Model DMAX/ ΠA) between 10^{o} and 85^{o}-2Θ with copper Kα radiation at a rating of 35KV, 25mA was employed for this purpose.

RESULTS

Corrosion rate (CR) and average percent weight loss (APWL)

After three years of soil burial under different experimental conditions, MSCs looked badly damaged. The MSCs were completely and heavily laden with corrosion products and the soil. After removal of corrosion products, metal losses varied for the different treatments (Table.1). The MSCs treated with *Penibacillus dendritiformis* MB-14 lost the metal considerably but patches remained intact and retained the metallic luster. After 36 months of soil burial, corrosion rates of MSCs due to microbiologically influenced corrosion (MIC) by the *Penibacillus dendritiformis* MB-14, *Bacillus subtilis* MB-6 and their co-culture in the presence of 15.47, 17.85 and 17.1% moisture contents were found -3.5×10^{-3}, -1.9×10^{-3} and -6.3×10^{-3} $mgdm^{-2}d^{-1}$, respectively. Average percent weight losses (APWL) of these MSCs turned out to be 1.41, 3.39 and -10.48% respectively (Table I, Fig, 1).

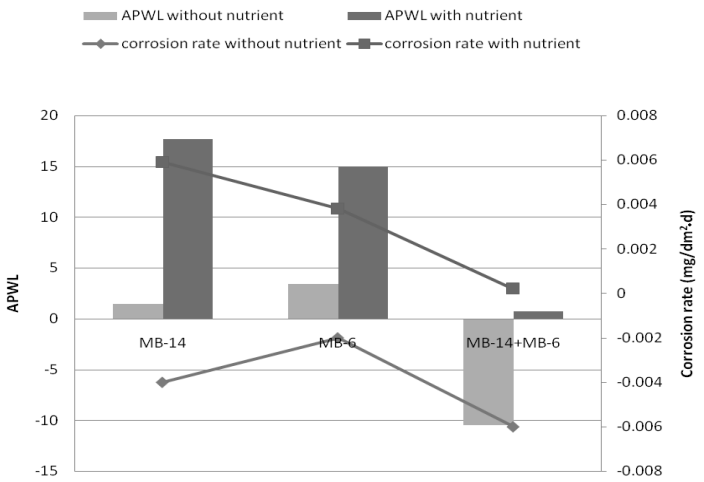

Fig 1. Effect of MIC on corrosion rate and average percent weight loss (APWL) exposed to the bacterial isolates in soils with and without added nutrients for 3 years.

TABLE I.- ESTIMATION OF CORROSION OF MILD STEEL COUPONS AND ACCOMPANYING LEVELS OF MOISTURE AND ORGANIC CONTENTS BACTERIAL C.F.U., PH AND CONDUCTIVITY LEVELS OF CONTROL AND SOILS FOLLOWING INOCULATION WITH, *PENIBACILLUS DENDRITIFORMIS* MB-14, *BACILLUS SUBTILIS* MB-6 AND THEIR CO-CULTURE WITHOUT ADDED NUTRIENTS (A) AND WITH ADDED NUTRIENTS (B) AT AN INCUBATION PERIOD OF 36 MONTHS AT 10-30°C.

Experiment	**C.R ($mgdm^{-2}d^{-1}$)**		**APWL**		**Moisture (%)**		**Organic contents (%)**		**C.F.U./g**		**pH**		**Conductivity (mV)**	
	A	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**
Control	19.36 ±0.44 $\times10^{-3}$	11.59 ±0.60 $\times10^{-3}$	44.55 ±6.73	32.56 ±11.34	15.80 ±1.10	19.05 ±1.13	5.93 ±0.39 $\times 10^{-3}$	5.13 ±1.79 $\times10^{-3}$	----	----	8.54 ±0.019	8.67 ±0.02	77.43 ±3.63	109 ±4.52
Paenibacillus dendritiformis MB-14	15.86 ±0.57 $\times10^{-3}$	17.58 ±0.60 $\times10^{-3}$	45.96 ±11.34	50.24 ±11.6	15.47 ±1.04	21.1 ±0.05	7.17 ±0.92 $\times10^{-3}$	4.70 ±1.30 $\times10^{-3}$	19.87 ±19.43 $\times10^{7}$	94.25 ±17.50 $\times 10^{7}$	8.65 ±0.10	8.81 ±0.08	85.33 ±9.54	111.13 ±4.55
Bacillus subtilis MB-6	17.10 ±0.59 $\times10^{-3}$	15.43 ±0.32 $\times10^{-3}$	47.94 ±11.28	47.55 ±9.23	17.85 ±1.18	20.4 ±0.69	5.57 ±0.56 $\times10^{-3}$	3.12 ±1.92 $\times10^{-3}$	88.80 ±12.00 $\times 10^{7}$	224.24 ±6.75 $\times 10^{7}$	8.61 ±0.07	8.80 ±0.09	85.20 ±8.99	116.90 ±4.08
Paenibacillus dendritiformis MB-14+ *Bacillus subtilis* MB-6	12.99 ±0.14 $\times10^{-3}$	11.85 ±0.43 $\times10^{-3}$	34.07 ±3.08	33.24 ±8.07	17.1 ±0.06	19.17 ±2.84	3.70 ±0.2 $\times 10^{-3}$	2.80 ±0.55 $\times10^{-3}$	42.92 ±31.95 $\times 10^{7}$	214.34 ±33.50 $\times 10^{7}$	8.52 ±0.05	8.70 ±0.16	96.87 ±2.72	119.67 ±9.38

C.R. = Corrosion Rate ($mgdm^{-2}d^{-1}$); APWL= Average percent weight loss; Values are means of six replicates ±S.E.M.

For nutrient added soils, corrosion rates of MSCs exposed to *P. dentritiformis* MB-14, *B. subtilis* MB-6 and their co-culture were found 5.9×10^{-3}, 3.8×10^{-3} and 0.2×10^{-3} $mgdm^{-2}d^{-1}$ in the presence of 21.1, 20.4 and 19.17% moisture contents, respectively. And APWL of MSCs as a result of these treatments appeared as 17.68, 14.99 and 0.683% respectively (Table I, Fig. 1). Corrosion rates as well as APWL due to MIC of MSCs exposed to soils without added nutrients were significantly lower than those exposed to the nutrient added soils in the presence of similar conditions, thus nutrient deficient microbe added soils showed protective role against corrosion. It must be noted here that these figures of MIC reflected corrosion rates as well as APWL after subtracting the values of control coupons.

Analyses of the soils and corrosion products

Bacterial C.F.U./g of soil for without added nutrients soils inoculated with *Penibacillus dendritiformis* MB-14, *Bacillus subtilis* MB-6 and their co-culture were $19.87\pm19.43\times10^{7}$, $88.80\pm12.00\times10^{7}$ and $42.92\pm31.95\times10^{7}$, respectively (Table I). In case of co-culture no discrimination could be made for the C.F.U. of individual bacterial species. For nutrient added soils the respective figures emerged as $94.25\pm17.50\times10^{7}$, $224.24\pm6.75\times10^{7}$ and $214.34\pm33.50\times10^{7}$, respectively (Table I).

Moisture contents of soils without added of nutrients, inoculated by *Penibacillus dendritiformis* MB-14, *Bacillus subtilis* MB-6 and their co-culture were found to be 15.47±1.04, 17.85±1.18 and 17.10±0.06% respectively (Table I). Whereas the moisture contents of nutrient added soils containing the above isolates were found to be 21.1±0.05, 20.40±0.69 and 19.17±2.84%, respectively (Table I).

Organic contents of soil inoculated with *Penibacillus dendritiformis* MB-14, *Bacillus subtilis* MB-6 and their co-culture were found to be $7.17\pm0.92\times10^{-3}$, $5.57\pm0.56\times10^{-3}$ and $3.70\pm0.20\times10^{-3}$%, respectively (Table I). Respective values for the nutrient added soils were $4.7\pm1.30\times10^{-3}$, $3.12\pm1.92\times10^{-3}$ and $2.80\pm0.55\times10^{-3}$%, (Table I). pH of without added nutrients and inoculated with *Penibacillus dendritiformis* MB-14, *Bacillus subtilis* MB-6 and their co-culture soil were found to be 8.65±0.10, 8.61±0.07 and 8.52±0.05, respectively (Table1). Corresponding values for the nutrient added soils were 8.81±0.08, 8.80±0.09 and 8.70±0.16, respectively (Table I). Soil salinity/ electrical conductivity of without added nutrients and inoculated with *Penibacillus dendritiformis* MB-14, *Bacillus subtilis* MB-6 and their co-culture were found to be 85.33±9.54, 85.20±8.99 and

96.87±2.72 mV, respectively (Table I). Whereas the electrical conductivity of nutrient added soils containing the above isolates were found to be 111.13±4.55, 116.90±4.08 and 119.67±9.38 mV, respectively (Table I).

XRD data of the corrosion products yielded peaks of higher intensities of $Fe(OH)_3$, β-$Fe_2O_3.3H_2O$ and β-FeOOH. These products demonstrate the chemical basis of corrosion of MSCs in the present experimental models.

DISCUSSION

The present study indicated that 3 years exposure of mild steel coupons to sterilized soil in the presence of 15.80% moisture contents, resulted into 44.55% weight loss. While this loss reduced to 32.56% in the presence of nutrient added sterilized soil with moisture contents terminal of 19.05%. These vivid differences explain importance of chemical environment having strong influence on the process of abiotic corrosion.

The coupons of mild steel lost 45.96% weight following burial in *Penibacillus dendritiformis* MB-14 inoculated soils in the presence of 15.47% moisture contents. While this loss increased to 50.24% in the presence of nutrients provided in the form of nutrient broth. Coupons of mild steel lost about 47.94% weight following burial in *Bacillus subtilis* MB-6 inoculated soils in the presence of 17.85% moisture contents and the loss was not changed in the presence of 20.4% moisture contents in nutrient added soil. The coupons of mild steel exposed to the co-culture indicated weight loss about 34.07% in soil (without added nutrients) in the presence of 17.1% moisture contents. While this loss reduced to 33.24% in the presence of nutrient added soil of 19.17% moisture contents.

Bacterially influenced corrosion rates of *Penibacillus dendritiformis* MB-14 was -3.5×10^{-3} $mgdm^{-2}d^{-1}$ and for *Bacillus subtilis* MB-6 it was -1.9×10^{-3} $mgdm^{-2}d^{-1}$. These two bacterial species have been reported to cause MIC and to inhibit the corrosion, respectively following soil buried upto 150 days (Bano, 2008). The corrosion rate as well as APWL of coupons that were exposed to the co-culture, turned out significantly lesser from the values observed for each monoculture. This is attributable to antibiosis effect of *Bacillus subtilis* MB-6 against *Penibacillus dendritiformis* MB-14. Thus for soil buried mild steel interplay of microbial agents, nature and amounts of organic and inorganic contents become decisive in determining the corrosion caused structural

deterioration. Several categories of soil buried metallic pipelines meet different supplies and within towns. Soils of different localities do vary in terms of their physiochemical and biotic characteristics. Several of such features have a bearing on the process of metal corrosion. The process is initiated or maintained in the presence of moisture and oxygen. Besides such abiotic corrosion causing or enhancing factors different biotic components of environments have been reported to influence the process of corrosion. In this regard several writers have described bacteria which accelerate the process of corrosion (Beech 2004; Bano and Qazi, 2010). While relatively recently microorganisms and their activities have also been reported which retard and even show shielding effects for metals against corrosion (Jayaraman *et al*., 1999; Korenblum *et al*., 2005; Bano and Qazi, 2010). Such category of bacteria, infact exert an antagonistic effect on the corrosion escalating microbes or communities. Further their exopolymeric secretions have also a protective role for the metal surfaces against abiotic as well as biotic agents of corrosion.

Conclusively while planning for controlling corrosion of soil buried metallic structure in addition to consideration of well known physicochemical parameters influencing the deteriorative processes, the microbial and nutritive nature of the environment should also be taken into account for predictable corrosion control measures.

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MEAN HAEMOGLOBIN LEVEL ASSOCIATED WITH DIFFERENT TYPES OF ANEMIA DURING PREGNANCY IN THE HUMAN POPULATION OF GILGIT BALTISTAN

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Abstract,- Anemia is regarded as a major risk factor for unfavorable pregnancy outcomes, but there have been no previous studies describing the pattern of hemoglobin concentration during pregnancy in Gilgit-Baltistan and the relationship between altitude and Hemoglobin concentration in the pregnant women living in the different areas of Gilgit- Baltistan. The main objective of this study was to study the hemoglobin levels and prevalence of anemia in the human population of Gilgit-Baltistan and to evaluate potential associations of hemoglobin and anemia with women's characteristics. Out of 361 blood specimens 167 (46.26%) were found anemic. Out of 167(46.26%), 97(58.08%) were anemic with Vitamin B12, 39 (23.35%) were anemic with Iron deficiency and 31(18.56%) were found anemic with Megaloblastic anemia. In age wise investigation during pregnancy, out of 361 blood specimen, 213 (59.00%) cases were clinically investigated in the age group of (15- 25years), 120 (33.24%) in the age group (26-35), 18 (4.98%) were in the age group of (36-45years), 10 (2.77%) in the age group of (46-55). The anemic cases were found highest in the age group of 15-25years i.e. 96 (45.07%), 53 (44.16%) in the age group of (26-35years), 13 (72.22%) in the age group of (36-45years) and 05 (50%) in the age group of (46-55).In area-wise distribution out of 361 the highest cases were clinically investigated in District Gilgit *i.e.* 231 (63.98%) of which 100 (43.29%) were anemic, 66 (18.28%) cases were recorded in District Ghizer out of which 24 (36.36%) cases were anemic, 36 (9.97%)cases were recorded in District Diamer out of which 28 (77.77%) were anemic and only 28 (7.75%) cases were recorded in district Hunza Nagar out of which 15 (53.57%) cases were anemic. The highest anemic cases were found in District Gilgit and District Ghizer respectively. In month-wise distribution the highest cases were clinically investigated in the month of July i.e. 106 (29.36%) and lowest cases were investigated in June which were only 30 (8.31%). In July out of 106 clinical investigations 48 (45.28%) were found anemic and in June out of 30 clinical investigations 18 (60%) were found anemic.

Key words: Prevalence of anemia, mean hemoglobin, pregnancy, Vitamin B12 and megaloblastic anemia

INTRODUCTION

In mammals the protein makes up about 97% of the red blood cells' dry

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content, and around 35% of the total content (including water). Hemoglobin has an oxygen binding capacity of 1.34 ml O_2 per gram of hemoglobin (Dominguez, 1981) which increases the total blood oxygen capacity seventy fold compared to dissolve oxygen in blood. The mammalian hemoglobin molecule can bind (carry) up to four oxygen molecules (Costanzo and Linda, 2007). There are marked physiological changes in the composition of the blood in healthy pregnancy, mainly to combat the risk of haemorrhage at delivery. Plasma volume and red-cell mass increase by 50% and 18-25% respectively, resulting in dilutional decrease in Hb concentration called the physiological anemia of pregnancy, maximum at 32 weeks of gestation (Jetsky, 1998). WHO has recommended a cut-off value of 11.0 gm/dl to define anemia at any time during pregnancy (Breymann, 2001). Pathological anemia of pregnancy is mainly due to iron deficiency (Beard, 2000). Folate deficiency is a minor component contributing to anemia. Folate deficiency may be masked by co-existing iron deficiency. Vitamin-B12 deficiency and thalasemia major are usually associated with infertility. Deleterious effects occur in mother, as well as baby, as a result of anemia, which is multifactorial in a community like ours. Multiparity, poor socio-economical and educational status is the principal reasons for a h Normal values of hemoglobin in 1st and 3rd trimester of pregnant women must be at least 11g/dl and at least 10.5g/dl during 2nd trimester (Murray *et al*., 2006). Hemoglobin levels change in pregnancy with a normal reduction at the beginning of pregnancy and a slight rise towards the end of pregnancy (Reveiz *et al*., 2007).

Anemia is a common problem of underdeveloped countries. Patients of anemia usually have various symptoms especially generalized weakness, easy fatigability and poor concentration etc. If anemia is marked and prolonged it may precipitate cardiac problems. Anemia in pregnancy is a special issue. Here anemia has deleterious effects both on mother as well as on growing fetus. (Rizwan *et al*., 2010).

The process of transporting Vitamin B 12 to fetus is responsible for the progressive decline of maternal levels that occurs during pregnancy **(**Edelstein and Metz, 1969). Fetal demands for the vitamin have been estimated to be approximately 0.3 g/day (0.2 nml/day) (Herbert, 1987). Vitamin B12 deficiency in the lactating mother may cause severe consequences in the nursing infant. Several reports have described megaloblastic anemia in infants exclusively breast-fed by vitamin B12deficient mothers (Lampkin *et al*., 1966).The main objectives of the present study are; To find out the mean hemoglobin level during pregnancy in the human population of district Gilgit; to know the prevalence,

types and causes of anemia during pregnancy in the human population of district Gilgit; and to see frequency of anemia and its related risk factors during pregnancy in the human population of District Gilgit.

MATERIALS AND METHODS

An observational, prospective study was conducted at the family health Hospital, Gilgit. This hospital provides medical facilities to a population of about 2, 00000 individuals, which also includes antenatal care to at least 10,000-12,000 women per year. All the pregnant women, irrespective of age, duration of pregnancy or any concurrent illness, presenting in the said hospital, were evaluated according to the following protocol *i.e.* clinical evaluation was used to know the cause of anemia, *i.e.*, H/O blood loss, fetal loss, lactation, multiple pregnancy, worm infestation, use of NSAIDS (non steroidal anti inflammatory drugs), multiparity and blood transfusion, socio-economic status was assessed from the monthly income of the head of the family and dietary habits were rated as good, average and poor. Hb values and blood counts were obtained in all the women at presentation using Reflotron photometer, Roche diagnostics. A cut off value of < 11.0 g/dl of haemoglobin, irrespective of the duration of pregnancy, was used to define anemia[4].

Collection of blood samples

Blood of suspected anemic (hemoglobin deficiency) patients referred by gynecologist of family health Hospital, Gilgit. Blood samples for clinical investigation were collected with the help of sterilize syringe and poured blood in to fresh K3-EDTA– anti coagulated hemoglobin. All blood samples were analyzed within 4 hours of phlebotomy. Patient blood samples were from individuals with hemoglobin/RBC disorders such as iron deficiency, Folic acid, Vitamin B12 thalassemia, and sickle cell disease during pregnancy. The duration of blood sample collection was from June, 5th 2009 to November 2009. The bio-data (name, age, sex and address) of the patients was also noted.

Blood sample analysis

All the collected samples were analyzed for hemoglobin deficiency with the help of Microlab 300, Sahli's hemoglobin meter method and Microscopy.

Red blood cells morphology

After the determination of hemoglobin of all pregnant women's referred by gynecologist, anemic (abnormal Hb%) cases was further studied to investigate the type of anemia (Iron deficiency, Vitamin b12, megloblastic anemia) through microscopic study of red blood. There are two standard methods which are commonly used to morphological study of red blood cell that is field stain method and Leishmen stain method.

RESULTS AND DISCUSSION

Anemia in pregnant women constitutes a real concern all over the world more so in developing countries. Various studies have reported about the variable prevalence rates of anemia during pregnancy and it varies from 33% to 75% (Kiwanuka *et al* 2000; Dim, 2007). In present study frequency of anemia in pregnant ladies is 46.27% while UNO has reported 56% anemia in pregnant ladies from low-income group (4th report on World Nutrition situation, 2000). However a study from Lahore, Pakistan has reported the prevalence 11 of anemia in pregnant ladies as 66% (Sohail *et al*., 2004). Our findings are close to the figures reported by UNO and a small variation from other studies may be due to the selection of study population. In present study mostly pregnant ladies were not anemic 53.73% while 46.27% were moderately and mild anemic (Table I).

TABLE I.- TOTAL SUSPECTED ANEMIC PATIENTS REFERRED FOR LABORATORY INVESTIGATION OF HAEMOGLOBIN LEVEL DURING PREGNANCY.

No. of cases studies	No. of anemic cases	Types of anemia investigated		
		Iron deficiency anemia	Megaloblastic anemia	Vitamin B12
361	167 (46.26%)	39 (23.35%)	31 (18.56%)	97 (58.80%)

A study from India has reported moderate anemia in pregnant women's which is close to our findings (Toteja, 2006). In present study 17.4% of the anemic pregnant ladies were in first trimester, 29.2% were in 2nd and 53.4% in 3rd trimester. A study from Pakistan has reported the prevalence of anemia to be 54% in 3rd trimester, this study also supports our findings. High frequency of anemic patients in 3rd trimester may be due to increased demand of micronutrients during this period (Rizwan *et al.*, 2010) in the background of poor dietary habits. The dietary habits also affect the hemoglobin level in pregnant

women. In our study, 46.27% of anemic women were having poor dietary habits. The same has been (Salick *et al.,* 2004) supported by other studies.

TABLE II.- DIFFERENT TYPES OF ANEMIA FOUND IN DISTRICTS OF GILGIT BALTISTAN.

Name of districts	No. of investigations	No. of anemic patients	Types of anemia		
			Iron deficiency anemia	Vitamin B12	Megaloblastic
Gilgit	231(63.98%)	100(43.29%)	11.59G/100ML	62	62
Hunza-nagar	28(7.75%)	15(53.57%)	11.7G/100ML	08	08
Ghizer	66(18.28%)	24(36.36%)	12.9G/100ML	11	11
Diamer	36(9.97%)	28(77.77%)	12.0G/100ML	16	16

In our present study out of 167(46.26%), 97(58.08%) were anemic with Vitamin B12, 39 (23.35%) were anemic with Iron deficiency and 31(18.56%) were found anemic with Megaloblastic anemia (Table II). Different studies have reported iron deficiency as an important cause of anemia in pregnancy (Awan *et al.,* 2004).Similar findings were observed in our study and anemia was seen less common in ladies taking oral iron supplements. It was 53.74% in ladies who were on iron supplements while it was 46.26% in ladies who were not on iron supplements. Increased parity and gravidity have also been associated with more anemias, underlying (because being depleted iron stores and our study results also favors these findings (Sohail *et al*., 2004). Out of 361 specimens 213 (59%), were investigated in the age group of 15-25years, followed by 120 (33.24%), were investigated in the age group of 26-35years and 18 (4.98%), were investigated in the age group of 36-45years and 10(2.77%) were investigated in the age group of (46-55) (Table III). The highest anemic cases were found in the age group of 15-25years *i.e.* 96 (45.07%), followed by 53 (44.16%) in the age group of 26-35years, 13 (72.22%) in the age group of 36-45 and 05 (50.0%) in the age group of (46-55).

In district wise mean haemoglobin level, 231(63.98%) cases were recorded from District Gilgit out of which 100(43.29%) were found anemic and mean hemoglobin was 11.59G/100ML while in District Hunza-Nagar out of 28(7.75%), 15(53.57%) were found anemic and average mean of hemoglobin was 11.7g/100ml. In District Ghizer out of 66(18.28%) , 24(36.36%)women were found anemic and average mean of hemoglobin was 12.9g/100ml while in District Diamer out of 36(9.97%), 28(77.77% were found anemic and average mean of hemoglobin was 12.0g/100ml (Table II). On the basis of our studies we

can conclude that in Gilgit region most of anemic cases are found in age group 15-25 and this is most probably due to the unawareness of Nutritional values during the pregnancy, gestational age, ethnicity, residence and income significantly associated with the hemoglobin concentration and prevalence of anemia in the study population (Xing *et al*., 2009); less intake of Iron and folate (Theresa, 2008). The results of present study showed high frequency of anemia in the targeted population. Anemia was related with poor dietary habits especially poor iron intake. By correction of anemia in pregnant ladies at initial stage we can ameliorate maternal and fetal complications related to this correctable cause and ladies should be motivated for early antenatal check up. It will also ensure safe motherhood and safe baby. Awareness programs (Family), balance diet, regular checkup during and before pregnancy and Regular doses/injections.

TABLE III.- AGE WISE DISTRIBUTION 0F TOTAL SUSPECTED ANEMIC PATIENTS REFERRED FOR HAEMOGLOBIN INVESTIGATION DURING PREGNANCY

Age in years	**No. of specimen investigated**	**No. of anemic patients**	**Percentage anemia**
15-25 years	213 (59%)	96	45.07%
26-35years	120 (33.24%)	53	44.16%
36-45 years	18 (4.98%)	13	72.22%
46-55years	10 (2.77%)	05	50%

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REVASCULARIZATION AND REINNERVATION OF FREELY GRAFTED EXTENSOR DIGITORUM LONGUS MUSCLES IN RATS RESPOND POSITIVELY TO TESTOSTERONE PROPIONATE

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Abstract.- Extensor digitorum longus (EDL) muscles were autotransplanted in gonadectomized, testosterone propionate replaced, GTP-I (0.2mg/100bw/day) and GTP-II (2mg/100bw/day) rats. The muscle grafts were recovered at weekly intervals upto one-month post grafting period. They were processed for routine histological analysis following haemotoxylin and eosin as well as nerve stainings. Diameters of the arteries (8.00µm) were found higher in GTP-I than orchidectonized control as well as GTP-II muscle grafts following one month post transplantation. This indicated positive role of appropriate amount of the hormone on the process of revascularization following intact muscle orthotransplantation. Thickness of the arterial wall (2.08µm) in the GTP-I grafts increased significantly at 4th week stage than the control. Likewise, diameter of regenerated nerves (6.93µm) and thickness of myelinated sheath (1.17µm) increased in GTP-I grafts at 4th week postgrafting both than the control as well as GTP-II grafts. It is concluded that the anabolic hormone enhanced the process of skeletal muscle fiber regeneration by promoting the processes of revascularization and reinnervation following Arthotopic transplantation of EDL muscles.

Key words: Muscular transplantation; muscle regeneration and androgen, hormone and muscle grafting, angiogenesis and androgen, innervation and androgen.

INTRODUCTION

A skeletal muscle is integrated with the body through its blood supply, innervation and tendons. The tissue has a remarkable ability of regeneration following various kinds of injuries. In fact every skeletal muscle fiber is endowed with a reserve set of cells; the cells which are located outside sarcolemma and beneath the basal membrane. These cells survive accidental conditions of ischaemia and denervation etc. Following degeneration of sacroplasm of injured/ damaged muscle fibers, the satellite cells get activated for their divisions and form myotubes which may further develop into mature muscle fibers (Hansen-Smith *et al.*, 1980; Carlson, 1996; Qazi and Mufti, 2001). The process of regeneration is, however, dependent upon the blood supply which provides

macrophages (for removal of redundant sarcoplasm) and space and nutrients for myosetallite cells to populate and form myotubes. In case of freely grafted muscle tissues, it is the revascularization of the transplants which provides the conditions for the degeneration to proceed the regeneration of muscle fibers. For freely grafted Extensor Digitorum Longus (EDL) muscle grafts in rats, the entire graft is revascularized by the end of first week and ultimately a fairly normal relationship is established. The situation is followed with progress so that within weeks the grafts attain regenerated muscle fiber with about 50% structural and functional recoveries. (Hansen-Smith *et al*., 1980). Stability of the regenerated muscle tissue is mainly dependent upon its reinnervation. Revascularization and reinnervation are accomplished through spontaneous outgrowth and regeneration (Rossi, *et al*., 1987; Carlson, 1996). Incomplete innervation has been described among the factors responsible for failure of grafts to retain the mass of control muscles (Carlson, *et al.,* 1979).

Owing to the importance of clinical and cosmetic transplantations, many attempts have been made to improve structural and functional recoveries of skeletal muscles grafts. In this regard besides many other post-operative managements, anabolic effects of insulin, thyroid hormones and androgens have been well documented: Qazi (1996), Qazi and Mufti (1989, 1990, 1998, 2001, 2003) and Qazi and Riaz (2005) have reported a series of studies mentioning effects of insulin, testosterone and thyroid hormones on the process of skeletal muscle fibre regeneration following grafting.

Regarding the effects of androgen on the process of muscle regeneration Qazi and Mufti (2001) reported that presence of testosterone propionate reversed the atrophy and degeneration of regenerated muscle fibers of freely EDL muscle grafts, observed in orchidectomized rats. The hormone replacement rather promoted the development process so that from 2nd week onward (up to 4th week), average cross-sectional area of regenerated muscle fibers turned significantly higher than the hormone deprived grafts. Revascularization of skeletal muscle grafts is pivotal for the removal of degenerated mass and development of regenerating muscle fibres. In as much as the effects of androgen on axonal outgrowth, is concerned, Kokoris *et al*. (1987) demonstrated that gonadotropin-releasing hormone neurons transplanted into the lateral ventricle survived and extended processes into the host brain, often projecting to site of normal gonadotropin-relasing hormone innervation. Their success in contacting these sites suggests that gonadotropin-releasing hormone fiber outgrowth may be influenced by regionally specified trophic and/or guidance factors. Whereas,

Wan-hua (1982) while studying the effect on testosterone on regeneration of the hypoglossal nerve in rats demonstrated that testosterone propionate(TP) treatment promoted axonal outgrowth of the transected hypoglossal nerve as indicated by a larger proportion of hypoglossal neurons labeled by retrograde transport after injection of horseradish peroxidase (HRP) into the tongue 2 weeks after the lesion. It was postulated that TP exerted its effect on the metabolic activity of the axotomized neurons and influenced the healing process of the nerve. The HRP axonal transport method further revealed that regeneration of the severed nerve resulted in an alteration of the somatotopic organization of the hypoglossal nucleus and a random growth of the axons into branches of the nerve in all the rats irrespective of the treatment. Purpose of the present study was to viznalize fate of the processes of revascularization and innervation of freely grated EDL muscles in orchidectomised and testosterone propionate replaced rats. It was found that supply of testosterone propionate supported better revascularization and innervation in the transplants so that the regenerated muscle fibers not only attained maximum recovery, but also retained it during the study period.

MATERIALS AND METHODS

Fifty two male rats (*Rattus norvegicus*) weighing between 100 to 300g were employed in this study. They were kept in standard animal room facilities with roughly 12 hours dark/light cycle. The rats were fed commercially prepared food and given a constant supply of drinking water.

Experimental design

EDL orthotransplants in one group of gonadectomized (GVC) rats served as control. These animals were injected with 0.2ml of corn oil/100 g body wt/day. While two other groups of orchidectomized rats were administered with 0.2mg (GTP-I) and 2.0 mg (GTP-II) of testosterone propionate (anhydrous, Sigma) / 100 g body wt/day intraperitoneally following EDL muscles transplantations.

Surgical procedure

Details of the procedure have been described elsewhere (Qazi and Mufti, 2001). Briefly described the rats were anaesthetized with ether and gonadectomized 4-days prior to the muscle transplantation. Two cut ends of the skin following castration were sutured with 4-O silk. The extensor digitorum

longus (EDL) muscle was orthotopically transplanted, in all the three groups of animals. For this purpose the muscle was taken out and then grafted back in its original bed in proper orientation by suturing both proximal and distal tendons with respective stumps with 6-O silk. After suturing the fascia, the two cut ends of the skin were sutured with 4-O silk. The operated animals were given 0.06% terramycin in drinking water for 3-4 days postoperative.

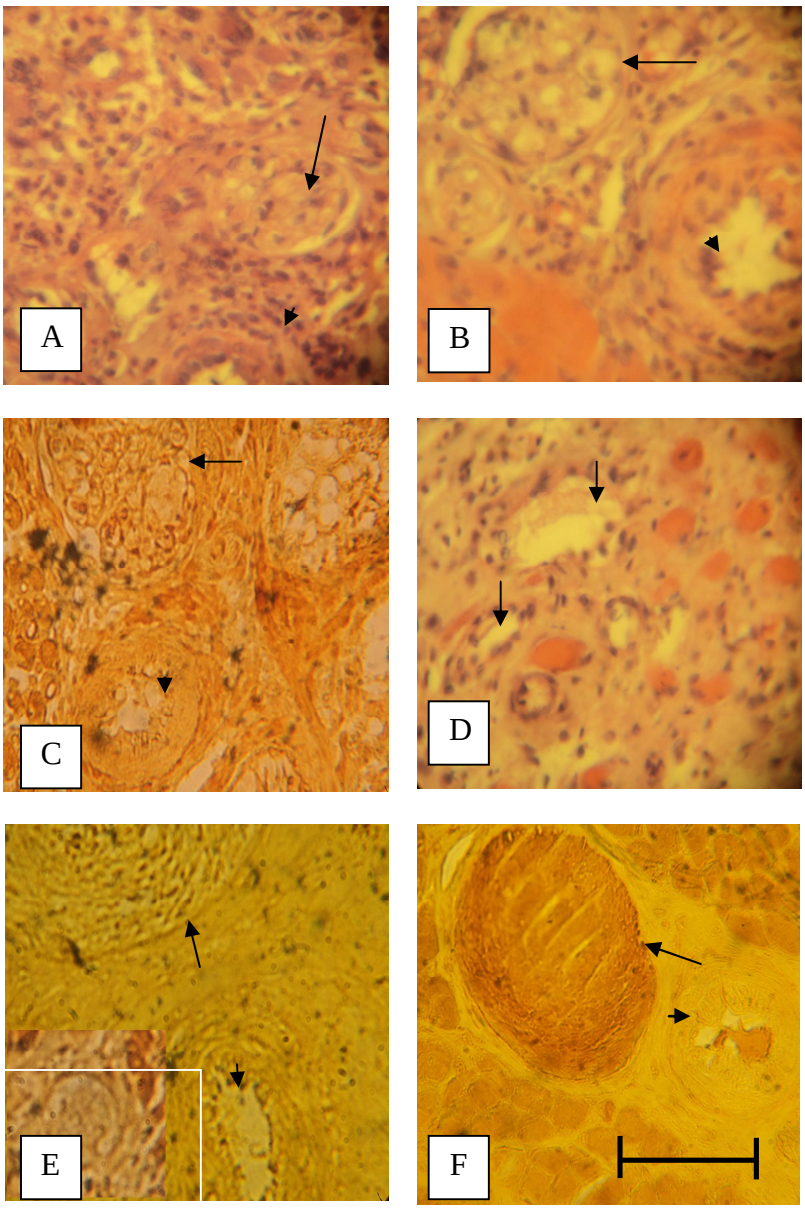
Processing of the grafts for histological observation

The muscle grafts were recovered at each week upto one month. and fixed in Bouin's fluid. The fixed tissues were processed for routine paraffin embedding and sectioning at 8µm with the help of a Cambridge Rotary Microtome. The sections were stained with Hematoxylin Eosin (H&E) and silver impregnation for nerve fibres (Goshgarian, 1977). Representative cross-sections of the EDL muscle regenerates of the three categories of experimental groups were studied microscopically for the levels of revascularization and reinnervation by measuring diameters of regenerated nerves and blood vessels. Wall thickness of blood vessels as well as of myelin sheaths was also recorded. The prominent nerves as well as vasculature within a given cross-sectional were focused. Diameters of arteries, veins and nerves were measured from their outermost boundaries. In case of recording thickness of walls of blood vessels and nerves three reading of each unit structure were taken from different locations and then their average values represented a given animal. These morphometric parameters were measured with the help of an ocular micrometer fitted microscope. The ocular micrometer was calibrated at 40X objective as to represent nearest µm for a given reading. The values were averaged for a given control group and compared with the respective experimental ones. For statistical comparison single factor analysis of variance was applied (Campbell, 1989). Sizes (diameters) of the regenerated nerves as well as the arteries and veins were then discussed in light of the information about average-cross sectional areas of the regenerated muscle fibers in the three series of the experiments as reported by Qazi and Mufti (2001). Photomicrographs of representative sections were taken with the help of a camera-fitted microscope.

RESULTS AND DISCUSSION

At end of first week period post-transplantation regenerated parts of the three categories of the grafts could be seen characterized with sprouted blood vessels and regenerated nerves (Fig. 1A, B, C). Diameters of arteries in such grafts ranged from 4.49 to 6.01µm with wall thickness ranging from 0.88 to 1.88

μm. The smallest diameter was recorded in the grafts treated with 2 mg of the hormone / 100 b.w. / day. But the differences were statistically non-significant (Table I). The arteries' diameters reduced about 50% at 2nd week post grafting in GVC muscle transplants. While a more vigorous decrease in the parameter was noticed for both categories of the hormone replaced grafts. At third week post grafting the situation recovered and became comparable to arteries found within one-week old transplants of respective category. It was interesting to note that arteries of 4-week old EDL muscle regenerates treated with 0.2 mg of TP had diameters of 8.26 μm and the value was 43 and 123 % higher than the corresponding values of GVC and GTP grafts, respectively. However, the differences appeared statistically non-significant (Table I). Histologically, the revascularization of the hormone treated EDL muscle grafts also had a much healthier look than the respective GVC transplants (Fig. 1D,E). This observation verifies the importance of proper dosage of the anabolic hormone for development of regenerated muscle mass. The better revascularization of the hormone replaced GTP-I and GTP-II grafts at the end of first month postgrafting (Fig. 1E, F) was in concordance with significantly higher average cross-sectional areas of regenerated muscle fibres in such grafts upto 73 and 82%, respectively as compared to the values of the parameter in the respective GVC grafts (Qazi and Mufti, 2001). Regarding the diameter of veins the parameter ranged from 2.72 to 6.54, μm within first couple of weeks post grafting in all categories of the grafts. It was again interesting to note that at the end of one-month period, the GTP-I transplants had 7.8 μm diameter of the veins which was 27 and 66 % higher than the corresponding values of the GVC, and GTP-II categories of the transplants, respectively (Table I). Revascularization of freely grafted skeletal muscles has been reported to occur quite earlier during the process of regeneration. For instance, Hansen-Smith *et al*. (1980) while describing revascularization of the freely grafted extensor digitorum longus muscle in rat, elaborated that the original blood vessels of the graft undergo a sequence of intrinsic and cell-mediated destruction. Scattered sinusoidal vessels begin to grow into the graft starting on the second day, and ingrowing blood vessels progressively invade the deeper tissues of the graft. Most new vessels form in the connective tissues, but some vessels, especially larger ones, grow into persisting basal laminae from preexisting vessels and, occasionally, even into basal laminae from degenerated muscle fibres and nerves. And that by the end of the first week, the entire graft is revascularized, and ultimately a fairly normal relationship between new capillaries and regenerating muscle fibers is established. In mature grafts, however, irregularities are sometimes found in the organization of smooth muscle cells associated with larger vessels. The present results indicate



See caption on next page

importance of anabolic factors for the process of revascularization in regenerating skeletal muscles in general and particularly in free small intact muscle transplants. Such information might be of value in escalating the process of revascularization in regenerating muscles in select clinical situation.

Reinnervation of freely grafted muscles is the final factor which determines the viability and further maturation of regenerated muscle fibers within the regenerates. Diameters of regenerated nerves in control i-e, gonadectomized rats appeared upto 6.3µm at 1 week stage. Then the parameter followed a retrogressive trend with values of 6.09, 5.7 and 4.9 µm at 2nd, 3rd and 4th week, respectively. From these results it appears that process of nerve regeneration in initial phases of muscle regeneration may be independent to presence of the anabolic hormone. However, for retaining the regenerated nerves, proper supply of the hormone is imperative. This might further explain secondary degeneration of regenerated muscle fibres in the hormone deprived grafts as compared to the hormones supplied EDL muscle transplants (Qazi and Mufti, 2001). The regenerated nerves' diameter ranged from 4.33 to 6.93 µm for the GTP-I grafts, while the parameter ranged from 4.6 to 7.2 µm for the second series of the hormone replaced EDL muscle transplants. The latter value is of regenerated nerves from 3-week old muscle transplants. Whereas diameters of regenerated nerves in 4-week old transplants could measure upto 4.7 µm. While in case of GTP-I category of the muscle transplants highest value of the

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Fig. 1. A, A portion of cross-section (C.S) one week old control (GCV) EDL muscle regenerate. A regenerated nerve (Arrow) and blood vessels (arrow head) are seen, H&E; **B,** A portion of C.S. of one-week old EDL muscle regenerate from a GTP-I (Supplied with 0.2 mg of testosterone propionate/100g b.w./day) rat. Note prominent size of regenerated nerve (arrow) and well developed (thick walled) artery (arrow head) as compared to the previous photomicrograph H & E., **C,** A portion of C.S. of one-week old EDL muscle regenerate from a GTP-II (supplied with 0.2 mg of testosterone propionate / 100g b.w./day) rat. The regenerated nerve (arrow) and blood vessel (arrow head) are of lesser sizes as compared to the respective regenerated structures observed in the previous photomicrograph; Nerve staining. **D;** A portion of C.S. of 4-week old GVC graft showing regenerated blood vessels (arrows) H & E. **E;** A portion of C.S. of 4-week old EDL muscle regenerate from a GTP-I rat showing a portion of well developed regenerated nerve (arrow) and a prominent artery (arrow head). Inset indicates area occupied by a regenerated nerve (arrow) surrounded by regenerated muscle fibres (arrow heads), nerve staining. **F;** A portion of 4-week EDL muscle regenerate from a GTP-II rat showing a prominent regenerated nerve (arrow) and an artery (arrow head). Well developed regenerated muscle fibres are visible at above right and below left corners of the photomicrograph; Nerve staining. Bar = 50µm. All photomicrographs were taken at the same magnification.

TABLE I.- DIAMETER AND (THICKNESS) OF WALLS OF ARTERIES (A) , DIAMETER AND (THICKNESS) OF WALLS OF VEINS (B) AND DIAMETER OF REGENERATED NERVES AND (THICKNESS) OF MYELIN SHEATH (C) OF EDL MUSCLE GRAFTS OF DIFFERENT AGES IN GONADECTOMIZED (GVC), GONADECTOMIZED AND SUPPLIED WITH TESTOSTERONE PROPIONATE GTP-I AND GTP-II RATS.

Type of graft	Stage of Regeneration		
	A	**B**	**C**
1st week			
GVC	6.01±4.2 (1.88±0.6) n= 4	6.54±8.27 (0.69±0.1) n= 4	6.3±4.9 (0.79±0.214) n=4
GTPI	6.86±9.6 (1.5±2.85) n= 4	4.45±2.9 (0.56±0.07) N= 4	4.33±0.94***** (0.75±0.15) n= 4
GTPII	4.49±3.15 (0.88±0.19) n= 6	4.42±10.9 (0.5±0.05) n= 6	4.6±7.3***** (1.2±1.63) n= 6
2nd week			
GVC	3±0.12 (0.88±0.03) n= 3	2.72±1.81 (0.5±0.08) n= 3	6.09±0.32 (1.00±0.2) n= 3
GTPI	1.5±4.43 (1.9±0.16) n= 4	6.18±37.4 (0.44±0.02) n= 4	5.5±9.7 (0.8±0.2) n=4
GTPII	0.88±6.12 (1.44±0.55) n= 4	6.33±3.98 (0.38±0.03) n= 4	6.07±1.3 (1.06±0.09) n= 4
3rd week			
GVC	6.013±6.25 (1.7±1.02) n= 4	4.26±6.25 (0.56±0.15) n= 4	5.7±0.69 (1.02±0.08) n= 4
GTPI	5.07±0.2 (1.19±0.54) n= 4	4.13±2.9 (0.63±0.54) n= 4	6.11±4.03 (0.8±0.04) n= 4
GTPII	4.53±0.8 (1.15±0.33) n= 5	4.21±3.2 (0.55±0.19) n= 5	7.21±53.9 (0.93±2.14) n= 4
4th week			
GVC	5.76±12 (1.06±0.21) n= 4	6.13±18 (1.06±0.21) n= 4	4.9±9.4 (0.52±0.08) n= 4
GTPI	8.26 (2.08±5.5) n= 6	7.8±25.21 (0.6±0.19) n= 6	6.93±38.6 (1.17±3.95) n= 6
GTPII	3.7±2.13 (1.13±0.53) ♣ n= 4	4.7±9.84 (0.5±0.4) n= 4	4.7±1.7 (0.85±0.09) n= 4

Values are means ± S.E.M. Those with asterisks are significantly different from the respective control values. ♣ = significant difference between the values of GTP-I and GTP-II grafts of respective stage. Single factor analysis of variance $P \leq 0.05$.

GTPI rats were gonadectomized and received 0.2 mg of testosterone propionate / 100g b.w. daily. While the GTPII rats were administered with 2.00 mg dose of the hormone.

parameter *i.e,* 6.93 µm was recorded for the one month old muscle regenerates (Table I). In this regard it is pertinent to mention that proper innervation has been well documented for retention of regeneration of skeletal muscle fibres. For example, Carlson (1995) discussed that if the regenerating muscle fibers are non-innervated, regeneration will not be complete; instead the regenerating muscle fibers will atrophy. Concerning the role of anabolic harmones on nerve regeneration, Kujawa *et al.*, (1991) described that testosterone differentially regulates the regenerative properties of injured hamster facial motoneurons. They suggested mechanism of gonadal steroids action on injured nervous system as partly through the differential regulation of the regenerative properties of the injured cell, presumably via hormone receptor-mediated action at the level of the neuronal genome. Likewise, Tanzer and Jones (2004) identified effective temporal window of TP exposure sufficient to enhance regenerative properties of injured facial motoneurons and functional recovery from facial paralysis induced by facial nerve injury. They pointed to a critical 6-h interval immediately after injury when TP enhances nerve outgrowth distances and augments behavioral recovery. It can be speculated that higher amount of the hormone i.e., 2mg of testosterone propionate / 100g b.w. /d escalated the process of nerve regeneration within the muscle transplants upto 3rd week. While later on the higher amount of the hormone could not support the retention of diameter of the regenerated nerves as the value (4.7µm) equaled to the respective control value. While low dose of the hormone supply (0.2mg/ g b.w./ d) caused a progressive increase in diameter of the regenerated nerves, as values of the parameter measured upto 4.33, 5.5, 6.11 and 6.93µm at one through the 4th week following the free muscle transplantations, respectively. Thus determination of proper dosage of the anabolic hormone is very important for getting optimum effect of an application on a desired biological phenomenon.

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EFFICACY OF A PARASITOID, *DIRHINUS GIFFARDII,* ON BIOLOGICAL CONTROL OF FRUIT FLY UNDER LABORATORY CONDITIONS

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Abstract.- Studies were carried out to determine the efficacy of *Dirhinus giffardii* on biological control of fruit flies under laboratory conditions. Pupae of fruit flies were used as a host for *Dirhinus giffardii*. There were four replications, each contained four treatments. Fifteen pupae were provided to one pair of *Dirhinus giffardii*. To find percent parasitism, adult emergence and longevity, four treatments *e.g.*, 1, 2, 3, 4 and 1 pair of *Dirhinus giffardii* was compared with 15 pupae of fruit flies. Percent parasitism was highest (28.3%) at 15 pupae with one pair of *Dirhinus giffardii* in R_2, whereas percent emergence was highest (13.3) in R_2.The longevity was highest in R_2 that is 40 days, so 15 pupae with one pair of *Dirhinus giffardii* were suggested for rearing under laboratory conditions.

Key Words: *Dirhinus giffardii*, biological control, parasitism, parasitoid, parasitoid, fruit fly.

INTRODUCTION

Dirhinus giffardii is collected as a model system because of several special life history and behavioral traits ideal for qualifying the fitness consequences of body size dependent host species selection. Firstly, *D. giffardii* is a general parasitoid because most its hosts are Polyphagus tephritids species that vary significantly in size, host species selection, behavior by *D. giffardii* may have important sequence for the size fitness relationship of its offspring. .Secondly, like other ectoparasitoids (Godfray, 1994; Quicke, 1997), female *D. giffardii* produce only a few large eggs at the time and have relatively low Egg limitation is likely to occur in this parasitoid. Thirdly, there may be high searching cost for this parasitoid fecundity (Podoler, 1954; Mackauer *et al*., 1993). An ectoparasitoids of *tephritids* fruit fly pupae, female *Dirhinus giffardii* pierce the puparial wall and ovipositor onto the host puparium (Silvestri, 1914). The host continues to develop until the parasitoid larvae hatch, at that time the host is paralyzed and consumed by the larvae. Wang and Messing (2004) provide

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evidence that in laboratory settings *D. giffardii* females prefer to attack larger host species when given a choice, resulting in larger parasitoid offspring. The fitness gained by the offspring in foraging efficiency due to larger size did not appear to come at the cost of development time or survival. *Dirhinus giffardii* has also been reported to attack other parasitoids, including *Fopius vandenboschi* (Dresner 1954) creating a concern about the potential impact of introduced *D. giffardii* on no target species. In additional laboratory studies, Wang and Messing (2004), studied the ability of *Dirhinus giffardii* to attack parasitoids used for biological control in Hawaii, including *Fopius arisanus* (Sonan), *Diachasmimorpha longicaudata* (Ashmead), *Diachasmimorpha tryoni* (Cameron), and *Psyttalia incisi* (Silvestri). They found that *D. giffardii* readily hyper parasitizes *Ceratitis capitata* previously parasitized by any of the 4 parasitoid species. However, when given a choice *D. giffardii* females preferred to oviposit in unparasitized fly pupae due to greater overall fitness experienced by the parasitoid offspring reared in those conditions.

Silvestri (1914) brought *Dirhinus giffardii* from Africa to Hawaii, where it was *Dirhinus giffardii* also reported to attack other parasitoids, including *Fopius vandenboschi* (Dresner, 1954) creating a concern about the potential impact of introduced *D. giffardii* on non target species. In additional laboratory studies, Wang and Messing (2004) studied the ability of *Dirhinus giffardii* to attack parasitoids used for biological control in Hawaii, including *Fopius arisanus* (Sonan), *Diachasmimorpha longicaudata* (Ashmead), *Diachasmimorpha tryoni* (Cameron), and *Psyttalia incisi* (Silvestri). They found that *D. giffardii* readily hyper parasitizes *Ceratitis capitata* previously parasitized by any of the 4 parasitoid species. However, when given a choice *D. giffardii* females preferred to oviposit in unparasitized fly pupae due to greater overall fitness experienced by the parasitoid offspring reared in those conditions. The objective of the present study is to; to study the efficacy of *Dirhinus giffardii* on biological control of fruit fly in laboratory condition and; to study about the effects on population of fruit fly in laboratory condition.

MATERIALS AND METHODOLOGY

To study the efficacy of *Dirhinus giffardii* on biological control of fruit fly, an experiment was conducted in completely randomized design with treatments repeated two times in each biological laboratory, IPDM Northern Areas Gilgit during November to December 2008. *Dirhinus giffardii* which is biological agent were procured from NARC for rearing in the insect ecology Lab department of Agriculture Northern Areas. The following research trails were

conducted to achieve the main objectives of study: T1, host rearing for obtaining pupae and T2, percentage parasitization of *Dirhinus giffardii*

Host rearing for obtaining pupae

Fruit flies were reared on apple in wooden cage .Room temperature was maintained at 25°C to 30°C and humidity was 20% to 30%.Adult male mate frequently at four to five days intervals**.** Male will mate more often when provided with sexually mature females. As single mating ensure production of fertile eggs for life but frequent mating appears to be required to sustain maximum fertility (Christen and Foote, 1960). The female make efforts to displace other flies of the same or different species attempting to enrich upon ovipositional sites they are selected .Several females may use the same ovipunture made by other species .Female deposits 4-13 eggs in a single puncture ,about 100mm below the skin of the fruit. Incubation period is 2-4 days in summer, 5-10 days in autumn and early winter .Egg was white cylindrical, slightly curved on one side, measuring 0.1-0.2nm.Full grow larvae are light yellow, pointed at the head and have 12 segments. Enriching bands of spiracles on segment 1 and 3 of larvae frequently incospious on dorsum .Distal margin of spiracle with 11-13 digits in a single slightly concave line, spiracular chamber about 4 times wider then long pupa barrel shaped and was light orange when fresh formed but light ochrous at maturity. Pupal life varies between 12-30 days in summer and 29-60 days in winter. It is active between March to November and passes through 7 generations in a year. Its female reaches sexual maturity earlier mate sooner and lay eggs earlier when kept in bright light rather dim light (Bateman, 1972). Body color of the adult was reddish brown with 2 longitudinal yellow stripes at thorax the ovipositor of the female is short and brown (Hashmi, 1994). When fruit flies lay eggs on fruit they were shifted into soil in other jars and jars were observed daily with the help of artificial diet prepared in the laboratory.

Percentage parasitization of Dirhinus giffardii

Pair of *D. giffardii* was applied on newly emerged fruit flies pupae. There were four replications. In each treatment 15 pupae were placed in each glass jars with 1 pair of *D. giffardii* .Diet was prepared by mixing water with honey and pasted on the upper side of the glass jars on daily basis, only 2-4 lines were pasted with the help of camel brush to determine the efficacy of *D. giffardii* on larva of fruit flies. After parasitization these pupae were transferred to other jars which were covered by cotton cloths and jars were observed on daily basis. The

data were collected for the parameters like percent parasitism**,** percent emergence**,** adult longitivity and adult Mortality.

These parameters were determined as follows:

For determination of percent parasitism, the number of parasitized (Blackish) and unparasitized (light brown) were counted and added to get total number and percent parasitism was calculated simply as

Percent Emergence =Emergence/Total parasitism x 100

For determination of percent emergence the total number of adult of D.giffardii and fruit flies emerged in a glass vial was counted .The number of parasitized egg were already known .So percentage emergence was calculated:

Percent =Emergence/Total parasitism x 100

For determination of adult longitivity, the Pupae of *Dirhinus giffardii* was taken and kept in transparent glass jar .Each trail was observed daily ,date of emergence and date of death ,each individual was noted and thus longevity (in days) was determined.

For determination of adult mortality, the different number of *Dirhinus giffardii* were emerged in different replications and rate of adult mortality was different in each replication depending on temperature and humidity of room. Date of death and individual was noted and mortality was determined in days.

RESULTS AND DISCUSSION

Effect of parasitoid on host

A pair of *Dirhinus giffardii* was compared against 15 pupae of fruit fly with treatments of T_1, T_2, T_3 and T_4, respectively. There were four replications and each replication contained the following number of parasitized pupae (Fig.1).

Dirhinus giffardii have efficacy to parasitized pupae within 11-12 days. Statistical analysis shows that replication two had high parasitism of 28.3%, followed by replication one 26.3%, replication three 25% and replication four 23.3% parasitism. The results of emergence by one pair of *Dirhinus giffardii* compared against the 15 pupae of fruit fly. Table I shows percent parasitism, percent emergence and adult longitivity.

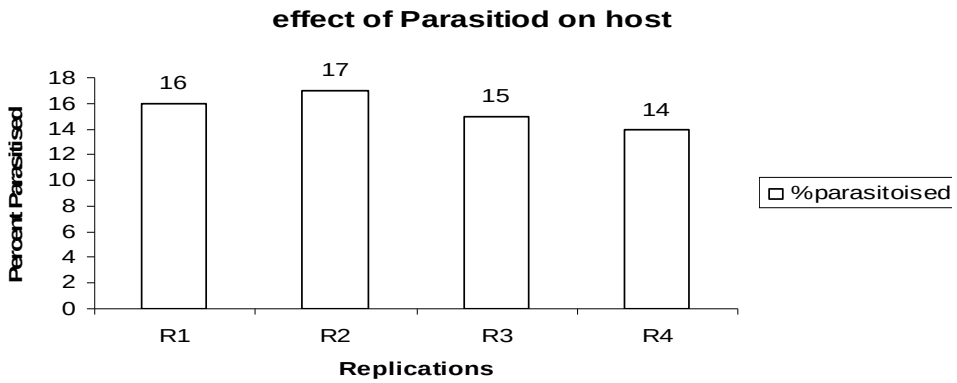

Fig. 1. Effect of parasitoid (*Dirhinuss giffardii*) on host(fruit fly)

Statistical analysis were not significant but in R_3, 13.3% emergence of adult was recorded, 12.5% emergence in R_1, 11.76% in R_2 and emergence in R_4, 7.1% as shown in Table I.

TABLE I.- PERCENT PARASITISM, PERCENT EMERGENCE, ADULT LONGITIVITY OF FRUIT FLY AFTER EXPOSURE TO DIFFERENT DENSITIES OF PARASITOID.

Parasite densities	**Percent parasitism**	**Percent emergence**	**Adult longitivity**	**Adult mortality**
R1	26.65	12.5%	40 days	20 days
R2	28.3%	5.85%	35 days	30 days
R3	25%	13.13%	30 days	35 days
R4	23.3%	7.1%	20 days	40 days

Dirhinus giffardii was solitary parasitoids attacking puparia of many fruit flies. They were not typical ectoparasitoids, as they feed on host pupae within puparia that develop from the exoskeleton of host larvae. *Dirhinus giffardii* did not kill its host until the parasitoid egg developed into a larva but *D. giffardii*, although suffering higher mortality than in older host puparia, still showed a level of successful development. In a choice experiment, both parasitoids preferred to attack 2- to 3-day-old puparia in which the host pupae had fully formed, rather than 1-day-old host puparia. *Dirhinus giffardii* preferred to attack unparasitized hosts rather than hosts previously parasitized by *P. vindemmiae*. The average emergence of *Dirhinus giffardii* was seven in different replications.

There longitivity depend on the temperature. The longitivity of *Dirhinus giffardii* which were emerged in R_1 was 40 days; the adults in R_2 had longitivity 35 days, in R_3 30 days, R_4 20 days respectively. The *Dirhinus giffardii* which were arise in R_1 had high longitivity as compared to R_4 this is due to fluctuations in temperature and humidity (Fig.2).

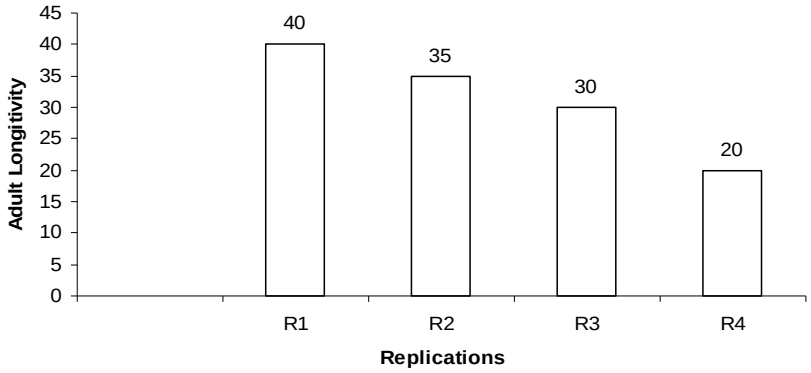

Fig. 2 .Adult longitivity of *Dirhinus giffardii* on 04 replicates

The average mortality of *Dirhinus giffardii* was different in each replication. The mortality of *Dirhinus giffardii which* were emerged in R_1 was 20 days; the adult in R_2 had mortality 30 days; in R_3 35 days; and in R_4 40 days respectively. The graphical representation showed that the *D. giffardii* which was emerged in R_4 had high mortality as compared to R_1.The mortality was high in R_4 because of decrease in temperature and humidity (Fig. 3).

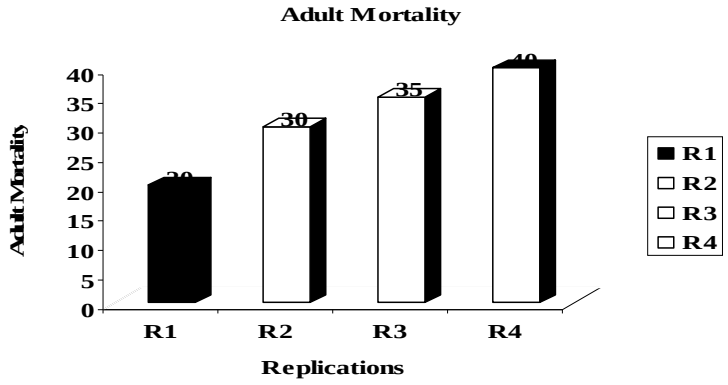

Fig. 3. Adult mortality of *Dirhinus giffardii* in its 04 replicates

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WANG, X.G., MESSING. AND RUSSELL, H., 2004. Department of Plant and Environmental Protection Sciences, University of Hawaii, 7370 Kuamoo Road, Kapaa, HI 96822, USA. xingeng@hawaii.edu) or http://lib.bioinfo.pl/meid:247259/pmid.

Some Abstracts

SECTION - I

CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY, TOXICOLOGY

TO INVESTIGATE THE MECHANISM INVOLVED IN THE PROGRESSION OF PROSTATE CANCER AND IDENTIFICATION OF POSSIBLE DIAGNOSTIC MARKERS FOR DIFFERENT STAGES OF PROSTATE CANCER

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Prostate cancer is a form of cancer that develops in the prostate, a gland in the male reproductive system. The cancer cells may metastasize (spread) from the prostate to other parts of the body, particularly the bones and lymph nodes. Many factors, including genetics and diet, have been implicated in the development of prostate cancer. Tight junctions are the closely associated areas of two cells whose membranes join together forming a virtually impermeable barrier to fluid. The major types of tight junction protein are ZO-1 (Zona occuludin-1 ot *TJP1),* claudins (1 and 7) and occludins. Current study aims to investigate the mechanism involved in the progression of prostate cancer and identification of possible diagnostic markers for different stages of prostate cancer. It will be relevant for both the molecular diagnosis of cancer and for the patient prognosis. For this purpose, PNT2 (normal prostate epithelium), prostate cancer cell lines LNCaP (established from a human lymph node metastatic lesion of prostatic adenocarcinoma), PC-3 (established from human prostatic adenocarcinoma metastatic to bone), and DU-145 (established from human prostatic adenocarcinoma metastatic to brain) have been selected to investigate the mechanism involved in the progression of prostate cancer by analyzing the expression of these protein by Real-time and western blotting.

EFFECT OF FEEDING BRODIFACOM AND FOOD ENERGY INHIBITOR ON HEART AND LIVER ENZYMES OF FIELD RATS (*RATTUS RATTUS*)

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A study was conducted in field rats to see the comparison effect of brodifacom and food energy inhibitor as rodenticide on heart and liver enzymes. For this purpose 42

rats were kept in department of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan. The rats were divided into 3 equal groups. One group served as control while the other were marked as brodifacom fed and food energy inhibitor (FBI). Feed and water were available to the rats *ed libitum.* Blood sampling was done on 14 days post-treatment. It was revealed from the result that creatinin kinase (CK-MB) was significantly increased in brodifacom fed group rats. Alanine Transaminase (ALT) and Aspartate Transaminase (AST) were significantly higher in brodifacom fed rats. Lactate dehydrogenase (LDH) was significantly high in FBI group compared to the other groups. It was revealed from these results that while brodifacom acting through affecting CK-MB, AST and ALT, the FBI act mainly through influencing LDH.

LEAD POLLUTION – A GRAVE RISK TO HUMAN HEALTH IN PAKISTAN

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Lead (Pb) pollution is a global problem; however, developing countries in South Asia appear to be more affected due to improper regulatory measures. Pb is not known to support any biological function therefore its inhalation or ingestion leads to accumulation in the body which may result into harmful effects including cardiovascular disease (CVD), anemia and cognitive dysfunction. These disorders, unfortunately, are highly prevalent in South Asia. Exposure to Pb comes mainly from polluted air, smoke, contaminated water, drinks and food, cosmetics, Pb-based paints, batteries, etc. Effects of Pb pollution on human health can be assessed by determining the levels of blood Pb. In 1991, Centers for Disease Control and Prevention (CDC) indicated that blood Pb levels ≥ 10 mcg/dl should be considered as high blood Pb levels. Research during the past 15 years has indicated that developed Asian countries with strong economies such as, Japan, Korea, Singapore and Taiwan, have mean blood Pb levels well below this threshold level, while developing countries in South Asia (Iran, India, Pakistan and Bangladesh) have mean blood Pb levels well above 10 mcg/dl despite introducing unleaded gasoline for use in the automobiles. "Phasing out lead from gasoline" has been the most significant measure in bringing down Pb pollution. However, the mean levels of blood Pb in most of the South Asian countries have remained well above 10 mcg/dl. For example, in Pakistan mean blood Pb levels have come down from 38 mcg/dl in 1989 to 15.6 mcg/dl in 2002. It is noteworthy that some of the other developing countries in Asia (Indonesia, Thailand, Srilanka, Philippines, and Malaysia) have succeeded in bringing down the mean blood Pb levels in the range 2.4 mcg/dl – 6.9 mcg/dl. There could be several reasons for failure of some of the Asian countries in bringing down levels of blood Pb: While developed countries such as USA, have strictly enforced that new paints should not have concentration of Pb more than 90 ppm, several South Asian developing countries such as, India and Srilanka have 31% -45% of paint samples with Pb concentrations far in excess of 90 ppm. There is high prevalence of iron and folate deficiencies in populations of

South Asia. Studies have shown that iron and folate deficiencies increase the risk of Pb poisoning. Pb-based traditional cosmetics and folk remedies are very commonly used in South Asian countries, thereby increasing the risk of Pb poisoning. Recent research has indicated that no blood Pb levels could be considered safe for humans. Low levels of blood Pb (2 mcg/dl) have been found to be associated with high rates of CVD. Pb pollution is indeed a risk to human health in Pakistan where there is a high prevalence of CVD. A couple of studies carried out in Karachi have shown that blood Pb is associated with plasma homocysteine, an established risk factor for atherosclerosis. Moreover, high blood Pb has been found to be associated with oxidative stress, another risk factor for CVD. Pakistani males are at a greater risk of developing hyperhomocysteinemia compared to females probably due to greater exposure to Pb. In another study carried out on primary school children in Karachi, an association was found between impaired learning and increased levels of blood Pb. Although growth stunting and anemia have been reported to be occurring in populations with high blood Pb, yet there are hardly any reports in the literature from Pakistan. In order to protect Pakistani population from the deleterious effects of Pb pollution, the following measures should be adopted: Impressing upon the Government to implement environment friendly measures to reduce Pb pollution; public advocacy for more awareness that even low exposure to Pb is not without risks to human health and high consumption of fruit juices to decrease levels of blood Pb.

DETERMINATION OF GENETIC DIVERSITY AND PREDATOR PREY RELATIONSHIP IN SOME SELECTED COLEOPTERAN AND HYMENOPTERAN SPECIES BY DNA/PCR BASED MOLECULAR ANALYSIS

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Genetic diversity and predator- prey relationship of eleven insect species was studied by RAPD technique. Three Coleopteran species *Coccinella septempunctata, Cheilomenes sexmaculata, Hippodamia convergens,* three Hymenopteran species *Camponotus pennsylvanicus, Solenopsis invicta, Formica rufa* and five Hemipteran species, *Macrosiphum miscanthi, Aphis maidis, Bemisia tabaci, Empoasca kerri* and *Drosicha mangiferae* were accessed. A total of 175 fragments were amplified by using 25 RAPD primers. 159 fragments were polymorphic showing 91% polymorphism. The number of amplification products varied from 6 to 11, with an average of 7.00 per primer. Genetic characterization was done with the help of cluster analysis constructed on the

basis of similarity matrix. Three main cluster groups were depicted. In one group only predator species were present along with an herbivorous prey species *Empoasca kerri*. Second group contained three herbivore prey species while third group was solitary containing a single prey species (*Drosicha mangiferae*). A single band of approximately 850bp was identified in DNA sample of *Cheilomenes sexmaculata*. Similarly another band of approximately 700bp was identified in DNA sample of *Macrosiphum miscanthi*. These fragments can be used as fingerprints for the identification of *C. sexmaculata* and *M. miscanthi*. Predator-prey relationship was observed on the basis of comparison among control predator, fed predator and prey species. Few unique fragments of aphid preys were identified in the fed coccinellid predators, suggesting a trophic link between studied predator and prey species.

DIGENIC INHERITANCE OF AN AUTOSOMAL RECESSIVE HYPOTRICHOSIS IN TWO CONSANGUINEOUS PEDIGREES

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Hypotrichosis is a human hereditary hair loss disorder in which affected individuals show sparse to complete absence of hair on scalp and/or on different body parts. To date at least eight isolated autosomal recessive and dominant forms of hypotrichosis loci have been mapped on different human chromosomes and the corresponding genes have been identified. Detailed clinical and molecular studies were undertaken of the hereditary hypotrichosis observed in the two consanguineous families (A and B), presented here. Human genome scan, using > 500 highly polymorphic microsatellite markers identified equal evidence of linkage of the hypotrichosis phenotype on chromosome 12q21.2-q22 and 16q21-q23.1 in both the families. The novel hypotrichosis locus on chromosome 12q21.2-q22 spans 16.3 cM (17.62 Mb), flanked by markers D12S326 and D12S101. At this locus maximum multipoint LOD scores of 3.68 and 3.31 were obtained in family A and B, respectively. The second hypotrichosis locus on chromosome 16q21-q23.1, identified in the two families, spans 5.58 cM (8.28 Mb) and flanked by markers D16S3031 and D16S512. Maximum multipoint LOD scores of 3.17 and 3.31 were obtained with markers mapped at this locus in family A and B, respectively. DNA sequence analysis of six candidate genes (*PLEKHG7, SLC6A15, VEZT, DUSP6, KERA, KITLG*), located in the linkage interval on chromosome 12q21.2-q22, failed to detect potential sequence variants in the affected individuals of the two families. However, DNA sequence analysis of *CDH3* gene, located on chromosome 16q21-q23.1, detected a single base pair homozygous insertion (c.1024_1025insG; p.342insGfsX345) in exon-9 in family A and deletion of four base pair (c.1859_1862delCTCT; p.620delSfsX629) in exon-13 in family B. We described for the first time digenic inheritance of an autosomal recessive hypotrichosis phenotype in two unlinked loci on chromosome 12q21.2-q22 and 16q21-q23.1 in two unrelated

consanguineous Pakistani families.

DIFFERENTIATION STUDY OF MURINE MESENCHYMAL STEM CELL INTO OSTEOBLASTS IN THE PRESENCE AND ABSENCE OF DEXAMETHASONE

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Bone marrow is rich source of multipotent stem cells that can be easily isolated and differentiated. Mesenchymal stem cells (MSCs) from the bone marrow have multilineage potential and have many therapeutic applications in the form of cellular therapy, tissue repair and regenerative medicine. MSCs differentiation into osteoblasts is a complex process that is regulated by many internal and external factors. We studied the differentiation ofMSCs into osteoblasts using osteoblast inductive medium in the presence and absence of dexamethasone. MSCs were isolated from Tibia and Femur of mouse and differentiated into osteoblasts using osteoblasts induction medium. Alkaline phosphatase (ALP) activity, total protein concentration, total cell number and mineralization was checked during differentiation process in the presence and absence of dexamethasone. Total protein of induced and un-induced cells was also analyzed. ALP activity was higher in induced cells as compared to un-induced cell but total protein concentration and cell number was higher in un-induced cells. After the differentiation process induced cells were positive for Alizarin Red stain even in the absence of dexamethasone and un-induced cells were negative. No difference in protein profile was observed in induced and un-induced cells by SDS-P AGE. MSCs were successfully differentiated into osteoblasts even in the absence of dexamethasone. Dexamethasone effect on cell proliferation but induce early differentiation of osteoblasts.

MICROBIOLOGICAL ANALYSIS OF WHEAT FLOUR SAMPLES TO DETERMINE THE FREQUENCY OF CONTAMINATION: A STUDY FROM KARACHI & HYDERABAD, PAKISTAN

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The excellent quality of Pakistani wheat impaired frequently during handling and

drying practices and method 6f transportation from field to storage. The main purpose of this study was the microbiological analysis of wheat flour samples collected from different parts of the cities including flour mills, retailer shops and storage :areas to determine the frequency of contamination. Wheat flour sample's were collected from flour mills, chakies and retailers' shops and Surveys' and inspections were carried out of flour roller mills, chakies and retailer shops of Karachi and \Hyderabad. Collected samples were brought in laboratory and physical quality characteristics were determined and then preceded for microbiological investigations. A total of 256 wheat flour samples were examined from September, 2009 to June 2010. All samples were found! negative for Salmonella. The presence of *A. flavus, A. candidus* in surface i disinfected kernels in evidence of poor storage in the past or of spoilage under way at present in store from which samples were taken. Overall, 45 'samples of wheat, 256 samples of wheat flour, 25 samples of Maida and 5' samples of barn were investigated for infestation of storage fungi. The sample of Atta showed the highest infestation of storage fungi and the maida showed the lowest fungal count. to maintain the quality andi characteristics we have to maintain the proper grain storage system in the city.

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

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Antioxidant activity of methanolic leaves extract of the hill toon, *Cedrela serrata* (Royle) was determined *in vitro* by DPPH free radical scavenging assay (using Ascrobic acid as standard) and DNA protection assay. All the analysis was made with the use of UV-Visible Spectrophotometer (DAD 8453, Agilent). The antioxidant activity of *C. serrata* leaves extracts was evaluated using 1,1-diphenyl-2-plerylhydrazyl (DPPH) radical scavenging assay in which all of the leaf extracts showed significant activities compared t standard. Inhibitory concentration (IC_{50}) of Ascorbic acid 5.54 ppm, whereas, IC_{50} for methanolic extract was 4.39 ppm. For n-butanol fraction IC_{50} was <1 ppm and for ethyl acetate fraction and aqueous fraction IC50 was 70 and 5.60 ppm, respectively. DNA protection against hydroxyl radical from hydrogen peroxide was assayed by agarose gell electrophoresis. Plant has no damaging effects on DNA and was able to reduce the hydroxyl radical-induced DNA damage. The results concluded that the extracts have a potential source of antioxidants of natural origin. These results suggest that *C. serrata* may act as a chemo-preventative agent, providing antioxidant properties and offering effective protection from free radicals.

IN SILICO STUDY OF PROMISCUOUS MHC I AND MHC II BINDING EPITOPES OF H1N1 PROTEOME OF ASIAN ORIGIN AND PHYLOGENETIC ANALYSIS

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The study was based on the novel influenza A virus (H1 N1) (swine flu) of *Orthomyxoviridae* Family, causes spiratory diseases and is known to be pandemic at phase 6 with 30,000 cases reported in 74 countries of the xld. Because of the development of resistance to drugs and antivirals *i.e.* amantadine, rimantadine, seltamivir, mamivir, etc. the only effective therapy against emerging influenza subtypes is the epitope based vaccine. Hence imunoinformatics studies were carried for the promiscuous epitope prediction and phylogenetic analysis of H1 N1 .oteome of Asian origin. The study provided an effective way to overcome the mutation rate and to determine the omiscuous binders to be used for epitope based subunit vaccine design.

COUSIN MARRIAGES AND THEIR EFFECTS IN OFFSPRING IN BAJAUR AGENCY, NORTHERN PAKISTAN

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The prevalence of genetic disorders in offspring increases with the passage of time. It is a big challenge for our society to compete this situation. To control genetic disorders it is necessary to get complete information of their prevalence and risk factors. The study was conducted from January to March 2010, in Bajaur Agency, Northern Pakistan. Data was collected from 123 consanguineous couples through questionnaire comprised of information about complete history of couples and risk factors of genetic disorder. Present study shows that sever effects of cousin marriage on offspring are mental retardation, deafness and speech disorder, increases risk of abortion. The genetic diseases if present- in consanguineous couples then the chance of malformation increased. The risk factors for these disorders are different, some are, age at the time of marriage, health condition during marriage, consanguineous. couples having cousin marriage in parents. Although cousin marriage has major affect on malformation of offspring but there are some environmental factor which are responsible for such type of congenital malformation.

ACUTE TOXICITY OF ZINC TO *CATLA CATLA* AND *CIRRHINA MRIGALA*

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Acute toxicity (96-hr LC_{50} and lethal) of zinc to fish viz. *Catla catla* and *Cirrhina mrigala* were performed, separately, at constant temperature (32°C), total hardness (250 mgL^{-1}) and pH (7) of water. Physcio-chemical variables *viz.* total ammonia, dissolved oxygen, carbondioxide, sodium, potassium, calcium and magnesium were monitored on daily basis. Statistically significant differences between LC_{50} and lethal concentrations of zinc for *Catla catla* were computed as 56.96 and 132.12 mgL^{-1} while the same for *Cirrhina mrigala* were remained as 63.09 and 158.04 mgL^{-1}, respectively. *Catla catla* was most sensitive to zinc than that of *Cirrhina mrigala.* The metal concentration of test medium showed positively significant correlation with total ammonia, carbondioxide, electrical conductivity, sodium and potassium for both fish species *(Catla catla* and *Cirrhina mrigala).* However, dissolved oxygen contents showed inverse relationship. The ammonia secretion by fish increased significantly with the increase of metal concentration in the test mediums while oxygen consumption by the fish also increased which result in decreased dissolved oxygen contents in the test medium.

IMMUNOASSAY BASED SCREENING OF PATHOGENIC *ESCHERICHIA COLIIN* FOOD SAMPLES COLLECTED FROM DIFFERENT LOCALITIES OF LAHORE AND GUJRANWALA

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Some bacterial pathogens cause food borne or water borne bacterial disease only when they are in large number while ten pathogenic *E. coli* are enough to cause human infections like diarrhea and other complicated diseases. Food poisoning caused by *Escherichia coli* is commonly caused by eating squalid vegetables or unwashed fruits. However most infectious agents do not multiply on foods, but use them as vector to gain entrance to human body. The aim of this study was based on the isolation and immunological detection of pathogenic bacteria present in hawkers foods i.e. dahi bhala and fruit chat. Dahi bhala and fruit chat samples were collected from different localities of Lahore and Gujranwala. Microbial screening of this pathogenic bacterium was performed, Nutrient agar and MacConkey agar was used for growth of bacteria Tryptic soy broth (TSB) and Tryptic soy broth modified with novobiocin was prepared for identification of pathogenic *E. coli. E. coli* was isolated by streak plate and pour plate method by using selective media. To perform immunoassays,antibodies against *E. coli* were produced by immunization of rabbit. Immunization was carried out by injecting

mixture of *E. coli* grown culture and Freund's complete adjuvant in first immunization. *E. coli* grown culture and Freund's incomplete in subsequent immunization. Immuno dot blot assay was performed to detect the concentration or intensity of *E. coli* isolates from the samples. ELISA was also performed to check the degree of pathogenic *E. coli* contamination in food samples (dahi bhala and fruit chat). For molecular level studies *E. coli* DNA, RNA and crude proteins were isolated. For the size determination of DNA and RNA agrose gel electrophoresis was performed. Proteins were extracted from the *E. coli* cells by using cell lysis solution and proteinase inhibitor that inhibit the degradation of protein. Then SDS PAGE (Sodium Dodesyl Sulphate Poly Acryl amide Gel Electrophoresis) was performed to examine crude proteins in pathogenic *E. coli*.

ANTIMICROBIAL PROPERTY OF *GLIRICIDIA SEPIUM* PLANT EXTRACT

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Antimicrobial activity of ethanol extract of *Gliricidia sepium* was studied *in vitro* against twelve pathogenic gram positive bacteria *(Staphylococcus aureus, Streptococcus intermedius, Streptococcus faecalis, Bacillus pumilus, Bacillus cereus* and *Bacillus subtilus)* and gram negative bacteria *(Eschrichia coli, Salmonella typhi, Salmonella para typhy B, Klebsiella pneumoniae, Proteus mirabi/us, Shigella flexneri)* and some fungi *(Fusarium sol ani, Trichophyton rubrum, Aspergillus effuses, Rhizomucor pusil/us, Trichophyton sclerosis, Macrophonia phaseolina, Rhizoctonia so/ani).* Ethanol extract of the leaf extract exhibited maximum antimicrobial activity against all tested gram +ve bacteria than gram-ve bacteria and fungi at concentrations of5, 10 and 20 mg/mt. Antimicrobial activity was compared with standard antibacterial and antifungal drugs that are ampicillin and nystatin respectively. The minimum inhibitory concentrations (MIC) were between 0.5-1mg/ml against gram-ve and gram +ve microorganisms and 2.5mg/mI for fungi.

STUDY OF CUS - OPER& PROMOTER OF *KLEBSIELLA PNEUMONIAE* FOR COPPER SENSITIVTY BY USING LACZ AS REPORTER GENE

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Copper is an essential trace metal for both prokaryotes and eukaryotes, and associated with various metal enzymes which play many essential roles in the living system. But excess of the metal is very toxic and even lethal for living organisms. In microorganis'ins the intracelhllarlevel of copper is maintained by many genes working as

an operon and induce by the presence of copper in their environment. One such operon is called ellS operon regulated by *cusRS* two component system in *Klebsiella pneumoniae.* This *cusS* (sensory region) and *cusR* (regulatory region) autoregulate the expression of structural as well as regulatory part of the cus operon by binding of CusR protein to the. bidirectional promoter part of the operon. We have isolated the genomic DNA from previously characterized strains of *Klebsiella pneumoniae* and amplified the whole regulatory cusRS region with promoter of almost 3.0 kb through PCR and cloned it in vector pTZ57R1T. Promoter region of almost 180bp was amplified by using this DNA fragment and transformed in DH5a after ligating in p-LacZ vector (promoterless). The confirmed transformants were used for expression analysis of beta-galactosidase reporter gene, by using Copper metal as an inducer for promoter. The expression was confirmed through SDS PAGE as well as by more sensitive method of assay for beta galactosidase gene using ONPG as substrate. Results confirmed that the cloned promoter is highly sensitive for copper metal induction.

MOLECULAR AND IMMUNOLOGICL STUDIES OF PATHOGENIC *ESCHERICHIA COLI* IN MEAT SAMPLES COLLECTED FROM DIFFERENT LOCALITIES OF LAHORE

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Escherichia coli (E. coli) is the predominant nonpathogenic facultative flora of the human intestine. A minority of *E. coli* strains are capable of causing human illness by producing several different toxins. *E. coli* 0157:H7, an emerging cause of food-borne diseases with the occurrence of an estimated 20,000 illnesses and 250 deaths each year in the USA, has now been reported from several developing countries including Pakistan. Meat contamination occurs during slaughtering, processing and storage due to ineffective sanitary and handling practices. This research work was based on Microbial, I~munological screening and Molecular study of pathogenic bacteria *E. coli* in meat samples (Beef, Mutton, Quail and Fish). Three hundred Meat samples were collected from different localities of Lahore. Tryptic soy broth (TSB) and MacConkey agar was used for growth and identification of *E. coli* while mEC supplemented with Novobiocin, broth was prepared for isolation of *E. coli* 0157:H7. Out of 300 samples, 225 samples were identified as contaminated with *E. coli* by culture method. Polyclonal antibody production against pathogenic *E. coli* 0157:H7 was performed by immunizing rabbits with pure grown culture of pathogenic *E. coli* 0157:H7. Immunological studies (Dot Blot assay and ELISA) were performed for all meat samples by using produced polyclonal antibody and verified the results by using commercial monoclonal antibody (anti *E. coli* 0157:H7) which confirmed the presence of pathogenic *E. coli* in collected contaminated meat samples. Molecular studies include DNA and RNA extraction by using commercial kits. To study genomic DNA band patterns and RNA analysis of pathogenic *E. coli*

0157:H7, agarose gel electrophoresis was performed. Crude proteins were extracted from pure culture of *E. coli* 0157:H7 grown on selective media and their band patterns were observed by resolving on SDS-Polyacrylamide gel electrophoresis.

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

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The aims of this study were to determine the effects of saline, hCG and GnRH on the progesterone and protein concentrations of the ovine follicles. For this study reproductive tracts were obtained from the slaughter house and immediately brought to the laboratory (Institute of Pure & Applied Biology, B. Z. University Multan). Ovaries were isolated and follicles were dissected and cultured with the addition of saline, hCG and GnRH for two and half hours at 37C^{o}. Progesterone and proteins were extracted and concentrations determined by ELISA and Folin-Lowery method. The concentrations of progesterone were 1.23±0.13, 0.75±0.17 and 0.89±0.12ng/ml in saline, GnRH and hCG group respectively. Significant reduction ($P < 0.05$) in the concentrations of progesterone was found as a result of GnRH and hCG treatment. Moreover, the decrease in progesterone level was large in GnRH group ($P < 0.05$) as compared with hCG group. The concentrations of proteins were 0.38 ± 0.01, 0.4±0.01 and 0.43±0.01mg/g in saline, GnRH and hCG groups respectively. Significant increase ($P < 0.05$) in the concentrations of protein was found as a result of GnRH and hCG treatment. Moreover, the increase in protein level was large ($P < 0.05$) in hCG group as compared with GnRH group. It is concluded that both hCG and GnRH effect the progesterone and protein concentrations in the follicles. In case of progesterone GnRH suppresses its level more effectively than the hCG, while hCG increases the protein concentrations in the follicle more than the GnRH.

THE GENOMIC DNA AND PROTEIN EXTRACTION FROM THE FRESH FEATHERS OF *LOPHURA LEUCOMELANOS* AND ITS ENDORESTRICTION ANALYSIS

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The genomic DNA extraction from avian blood is difficult because of their age and size, but a rapid and accurate protocol was used in current research to isolate high-quality genomic DNA from the fresh feathers of *Lophura leucomelanos*. Total genomic

DNA and protein extraction was carried out first time in Biotechnology lab, Department of Zoology, Azad Jammu and Kashmir, University to continue and establish the frontier techniques. DNA extraction from the feathers of *L. leucomelanos* includes collection, preservation and analysis steps, which differs in both temperature and time of incubation depending on the feather size. Purification of genomic DNA was also performed with phenol: chloroform: isoamyl alcohol extraction and ethanol precipitation methods. By using this protocol, a significant amount of high-quality genomic DNA was obtained from more than 20 samples of *Lophura leucomelanos*. The purity and quality of isolated genomic DNA was confirmed through endo-restriction analysis using the combinations of restriction enzymes to which further leads to the evolutionary relationships among the other pheasants. Total protein of *Lophura leucomelanos* was also observed through modified SDS-PAGE electrophoresis. On the other hand different genes of *Lophura leucomelanos* were also identified by using bioinformatics tools.

EFFECT OF SELECTED ANTIBIOTICS AGAINST DIFFERENT MICROBES THROUGH STANDARD DIFFUSION DISC METHOD AND ISOLATION OF GENOMIC DNA THROUGH MODIFIED METHODS

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Bacteria and fungi are associated with the soil, vegetables and fruits to perform important functions like nutrient cycling, disease suppression and water dynamics but some microbes are pathogenic that causes diseases in both humans and plants, respectively. Different types of chemicals, vegetable oils and plant extracts were used to control all these microbes but in present research some antibiotics were selected to control the mycorhizae fungus, SAZAK2010 and other bacterial species such as *Escherichia coli*, *Klebsiella pneumonia* and *Enterococcus faecalis* through standard diffusion disc methods. The antifungal activity of selected antibiotics against SAZAK2010 was shown that SAZAK2010 was sensitive against tobramycin, gentamicin, tetracycline, *ciprofloxacin*, streptomycin, and kanamycin and resistance against ampicillin, **a***moxicillin* and p*enicillin* G, respectively. On the other hand the antibacterial activity of selected antibiotics against *E. faecalis* indicated that it was susceptible to the various antibiotics such as *ciprofloxacin***,** streptomycin, kanamycin, gentamicin, tetracycline, and amoxicillin, whereas resistant to tobramycin and tetracycline. Interestingly results were observed in case of biotechnological bacterial 10 b strain *E. coli*, which indicated sensitivity against all used selected antibiotics on both MacConky agar and Nutrient agar medium. Whereas, it was observed that *K. pneumoniae* was susceptible to tetracycline, *ciprofloxacin***,** streptomycin, kanamycin, gentamicin, tetracycline, and amoxicillin but showed resistance against penicillin G, respectively. The genomic DNA was also extracted from SAZAK2010, *E. coli*, *K. pneumonia* and *E.*

faecalis through modified techniques by using proteinase K digestion, phenol/chloroform extraction and ethanol precipitation for amplification of various genes that have importance in preventing various related diseases. Through bioinformatics tools it was analyzed that that these bacterial strains carrying different genes which play an important role in replication, act as a protein precursors and transporters, respectively.

OPTIMIZATION OF GENOMIC DNA AND PROTEIN ISOLATION FROM MOLTED FEATHERS OF HIMALAYAN GRIFFON VULTURE THROUGH MODIFIED METHODS

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The Himalayan Vulture or Himalayan Griffon Vulture (*Gyps himalayensis*) is an Old World vulture in the family *Accipitridae.* In present research work the genomic DNA and total protein extraction of Himalayan Griffon Vulture feathers was carried out first time in Biotechnology lab, Department of Zoology, Azad Jammu and Kashmir, University, to continue and establish the frontier techniques. The samples of molted feathers of Himalayan Griffon were collected from different areas of AJ&K. The genomic DNA was extracted by changing the both temperature and time of incubation depending on the feather size but the significant amount of DNA was not observed. Later the purification of genomic DNA was also performed with phenol: chloroform: isoamyl alcohol extraction and ethanol precipitation methods but results were also failed. On the other hand the fault and inaccuracy of the genomic DNA was checked by isolating the DNA from the fresh feathers of domestic chicken, used as a positive control. It was concluded that the protocol was effective and operational and the problem was related to the molted or old feathers. It was analyzed that the genomic DNA extraction from the molted feathers were difficult but we will try to extract the genomic DNA through further modified protocols. Total protein of molted feathers of Himalayan Griffon was also tried to observe through modified SDS-PAGE electrophoresis but the results were significant. It was concluded that may be the DNA was degraded in the molted feather as compared to the fresh feathers. Second the duration of molted feathers, the size of feathers and the time of preservation and incubation temperatures are much important for the genomic DNA extraction.

ANTIBACTERIAL ACTIVITY OF SOME ANTIBIOTICS AGAINST *PSEUDOMONAS SYRINGAE* AND *STAPHYLOCOCCUS AUREUS* AND GENOMIC ANALYSIS THROUGH STANDARD DIFFUSION DISC METHOD AND BIOINFORMATICS TOOLS

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Staphylococcus are responsible for food poisoning through the production of an enterotoxin and Pseudomonads are natural residents of soil and water, may cause primary skin infections in healthy individuals, usually a self-limiting skin rash. Different types of vegetable oils and plant extracts were used to control *Pseudomonas syringae* and *Staphylococcus aureus* but in present research some antibiotics were selected to control these bacterial species through standard diffusion disc methods. Two types of mediums were used for the standard diffusion disc method such as MacConky agar and Nutrient agar medium, respectively and indicated that *P. syringae* was sensitive against tobramycine, *ciprofloxacin*, and ampicillin whereas it was resistant in the presence tetracycline, gentamycine, penicillin G, kanamycin, and streptomycin. It was also observed that *S. aureus* was sensitive to the kanamycin, gentamicin, tobramycin, streptomycin, and ampicillin, whereas it was susceptible to the various selected antibiotics such as ciprofloxacin, tetracycline, and amoxicillin, respectively. The genomic DNA was also extracted from both *P. syringae* and *S. aureus* through modified techniques by using proteinase K digestion, phenol/chloroform extraction and ethanol precipitation for amplification of various genes that have importance in preventing various related diseases. Through bioinformatics tools it was analyzed that that these bacterial strains carrying different genes which play an important role in replication, act as a protein precursors and transporters, respectively.

HISTOPATHOLOGICAL AND METAL PROFILES OF GILLS AND LIVER OF *OREOCHROMIS MOSSAMBICUS* SAMPLED FROM THE INDUS RIVER

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This study monitored the accumulation of selected metals (Mn, Pb, Cu, Zn, Hg and Cr) in gills and livers and their relevance to the histology of these organs in *Oreochromis mossambicus* from three sites (one clean and two polluted) of the Indus River in Mianwali district of Pakistan. Mn, Cu, Zn and Cr in fish tissues showed significant differences between sites ($P<0.01$). These metals showed tissue specific differences ($P < 0.001$) in gills and liver. While the Mn, Zn, Hg and Cr levels were higher in gills and liver of fish than the WHO standards, the Pb and Cu levels were within

the permissible limits for fish as a food for human consumption. More metal concentration was observed in livers than the gills. Most common gill abnormalities observed in *Oreochromis mossambicus* were desquamation of lamellar epithelium, hypertrophy of epithelial cells, lifting up of lamellar epithelium, intraepithelial oedema, aneurysm, hyperplasia, and haemorrhage in the gill filament. Histopathology of liver revealed the presence of heterogeneous parenchyma characterized by vacuolization, foci of necrosis, hypertrophy of nuclei and degenerated hepatocytes. In conclusion the evidence of pathological alterations in gills and livers of *O. mossambicus* appeared to be a useful bio-marker to assess the impact of metal pollution in water on the health of fish.

GENOTOXIC AND CYTOTOXIC ROLES OF CADMIUM AND SELENIUM IN MALE SPRAGUE-DAWLEY RATS

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This study aimed to assess the role of selenium and cadmium in male Sprague Dawley rats. The rats were given subcutaneous doses of 1mg/Kg body weight of either normal saline (Control group) or cadmium chloride (Cd group) or sodium selenite (Se group) or cadmium chloride plus sodium selenite (Cd+Se group) on alternate days for four weeks. For this purpose body and liver weights, comet and TUNEL assays, histological analysis as well as levels of lipid peroxidation and antioxidants in liver were determined in four groups of male Sprague-Dawley rats. The Cd group showed increased DNA damage, apoptosis and hepatic levels of lipid peroxidation and altered histology. Conversely, the levels of antioxidants in this group were decreased as compared to the control group. The Se group also showed DNA damage, apoptosis and altered histology as well as reduced catalase activity but it was less severe than the Cd group. In the Cd+Se group ameliorating effects of Se were observed in the Cd+Se group. It appeared that the Se was able to curtail the toxic effect of Cd but Cd and Se on their own appeared to be genotoxic as well as cytotoxic at the dose of 1 mg /kg body weight of rats.

EFFECT OF CHLORPYRIPHOS AND CYPERMETHRIN ON THE WEIGHT AND GROWTH RATE OF THE *GALLUS GALLUS* (CHICKS)

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Effects of Organophosphate (Chloropyrephose) and Pyrethroid (Cypermethrin) were observed on weight and growth rate of *Gallus gallus* (Chicks). One month old chicks were selected for experimental purpose. 0.5mg/individual and 1.0 mg/individual

doses of both pesticides were applied with seven days regular intervals up to 165days. Oral treatment method was adopted for pesticides application Mortalities were also noted through out experimental period. Weight, length and height of treated and untreated batches were taken. Significant weight loss and decrease in length were observed in treated batches with comparison to control. Antifertility effect also noted in treated batches.

PREVALENCE OF SUB-CLINICAL MASTITIS IN COWS AT MUZAFFAR GARH DISTRICT OF PUNJAB (PAKISTAN)

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Mastitis is the inflammation of milk gland with physical, chemical and microbiological changes characterized by increase in somatic cells especially, leukocytes in the milk and by pathological changes in the mammary tissue. In sub-clinical mastitis all the five cardinal signs of udder inflammation (redness, heat, swelling, pain and loss of milk production) are present, while the sub-clinical form is bereft of any manifestation of inflammation. The present study was carried out during the year 2008 to determine the quarter-wise and animal-wise prevalence of sub-clinical mastitis in cows at four tehsils i.e., Muzaffar Garh, Kot Addu, Ali pur and Jatoi of District Muzaffar Garh of Punjab. Milk samples were collected from apparently mastitis free 1600 quarters of 400 buffaloes and subjected to Surf Field Mastitis test (SFMT). The principle of the test is that when detergent is added into milk sample, it causes rupture of somatic cell and release DNA and other cell contents. DNA is acid in nature, while detergent contains alkyl-aryl-sulfonate, which is basic in nature. DNA and detergents unite to form a gel; consistency of gel depends upon the number of somatic cells. The more cells, more thick gel and *vice versa*. The results showed that overall quarter-wise prevalence was 47.5% while animal-wise prevalence was 57%. The maximum quarter-wise prevalence was found to be 10.6% in tehsil Muzaffar Garh followed by 7.9, 9.1 and 8.9% in the tehsils Kot Addu, Ali pur

and Jatoi, respectively, while the maximum animal-wise prevalence was 66.7% in tehsil Muzaffar Garh followed by 63, 47.1 and 51.1% in the tehsils Kot Addu Ali pur and Jatoi, respectively.

HEXACHLOROCYCLOHEXANE, CYCLODIENE, DIPHENYLALIPHATIC AND THEIR METABOLITES IN THE BREAST MILK OF WOMEN FROM KARACHI, PAKISTAN

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The levels of Hexachlorocyclohexane (HCH), Cyclodiene and Diphenylaliphatic residues in human milk collected from Karachi were analyzed. The milk samples were subjected to GC (Gas Chromatography) after extraction and clean-up procedure given by Compay *et al.* (1). ß and y isomers of HCH accounted for the major residue of total HCH excreted in breast milk. Among different Cyclodiene compounds Aldrin and a Endosulfan were . comrhonly detected compounds. 43% of the samples were found to be contaminated with (4,4 DDD). Total concentration of total DDT was higher than HCH. In accordance with their level of accumulation such toxicants in the milk samples can be written as <HCH > Cyclodiene Diphenylaliphatic.

SKIN DISORDERS AND ASSOCIATED DIABETES MELLITUS IN RURAL AREAS OF SINDH

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An attempt was made to study the skin manifestations in the diabetic patients of Insulin Dependent Diabetes Mellitus (100M) throughout sindh. The patients were examined by taking history about the skin disorders and the bacterial types. Both male and female patients were equally infected. The majority of the skin disorders were infected by a common bacterial organism ie Staphylococcus lugdunensis. The total skin disorders having 7 bacterial species.1, Hidradents suprativa: Staphylococcus lugdunensis. 2, Angulare cheilitis: Staphylococcus lugdunensis and Acinetobacter Iwoffii. 3, Psoriasis: *E. coli* type-1. 4, Atopic dermatitis: staphylococcus aureus. 5, Boils: Staphylococcus aureus and pseudomonas aeruginosa. 6, Necrotising fasciitis: Staphylococcus lugdunensis. 7, Sraphylococcus scaled skin syndrome (ssss): Staphylococcus lugdunensis. 8, Erysipelas: streptococcus species. 9, Impetigo: staphylococcus aureus. 10, Neurogenic ulcer (Diabetic ulcer): Morganella Morganii. 11, Granuloma annulare:

Staphylococcus aureus and E. coli Type-1 12, Cellutitis: Staphylococcus aureus streptococcus species were noted. It is concluded that skin manifestations"and organism involved are due to the negligence of the patient.

EFFECT OF HUMAN CHORIONIC GONADOTROPIN (HCG) ON *IN VITRO* OOCYTE MATURATION IN FRESHWATER CYPRINID, *BARILIUS VAGRA*

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Exposure of Barilius *vagra* ovarian follicles with human chorionic gonadotrpin (hCG) resulted in a maturation of oocyte but the response being time dependent. There was slight but significant ($P<0.05$) increased in germinal vesicle break down (GVBD, 12.42 % vs 2.94 %) at 24 hrs. Prolonged incubation further increased GVBD (29.8% at 72 hr). Moreover hCG treatment at 72 hrs enhanced the synthesizing capability of the vitellogenic oocytes. There was significant increase in the secretion of estrdiol- 17ß ($P= 0.0001$) and non significant increase in testosterone ($P= 0.07$), whereas 17α-hydroxyprogesterone (17-OHP: 17α-hydroxypregn-4-ene-3, 20-dione) and 17α-20β-dihydroxypregn-4-ene-3-dione (17,20βP: 17, 20β-dihydroxypregn-4-ene-3-one) were detected and identified only in the hCG treated incubation medium. Morphological changes in the granulosa layer of oocyte such as abundance of mitochondria with heavily coated matrix, increased number of free ribosomes, rough and smooth endoplasmic reticulum and retraction of microvillar process (contact between microvilli of granulosa cells and pore canals of zona radiata in response to hCG revealed the role of gonadotropin in the induction of maturation of oocyte.

THE EFFECTS OF COPPER SULFATE ON THE MORPHOLOGICAL AND PHYSIOLOGICAL FUNCTIONS OF THE JUVENILES OF TILAPIA, *OREOCHROMIS NILOTICA*

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Waterborne copper exposure can exert a variety of physiological effects in fish, including the disruption of sensory system function, which has wide-reaching implications for fish behavior. In developing fish larvae, copper is known to affect key parameters, such as survival and growth and more recently has been shown to interfere with the development of the young fish. The present study is aimed to take a combined view of morphological (e.g. effects on eyes, gills and other mouth parts) and functional (e.g. feeding activity, survival rate and swimming activity) in order to understand the

complex effect of copper on fish development. Environmental stress of copper sulfate assessed in laboratory reared juveniles of Tilapia, *Oreochromis nilotica* in glass aquarium. Juveniles were exposed in test solutions of 0.2, 0.3, 0.4 and 0.5µg/l. In 0.4 and 0.5µg/l all juveniles were died within 24hrs. In 0.2 juveniles survived for 10 days then died. Feeding activity partially reduced in 0.2 , and 80% reduction was seen in 0.3 compared to control. In 0.4 and 0.5 copper the juveniles did not feed at all. The morphological changes appeared in 0.3 and 0.4 copper such as Protrusion of eyes, cataract developed in both eyes, mouth widely open, swelling of operculum, and bleeding behind the operculum. The affected juveniles become blind and died of starvation.

ISOLATION AND CHARACTERIZATION OF CHITIN FROM EDIBLE BLUE CRAB *CALLINECTES SAPIDUS* AND ITS COMPARISON WITH THE PURIFIED CARBOHYDRATE

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Chitin is important constituent of the exoskeleton of crustacean and insects and cell walls of fungi. The carbohydrate found abundantly in waste form. Chitin is potentially useful for applications in pharmacy, agriculture, medicine and analytical purposes. Chitin was extracted from edible blue crab by demineralization (HCl) and deproteinization (NaOH). The prepared chitin was represented approximately 20% of the total weight of the crab shell. The extracted chitin compared with other market available analytical grade chitin (Sigma and Roth) by FT-IR spectroscopy and X.Ray diffraction. Owing to the similarity pattern of the isolated substrate with analytic grade, the crab chitin may be employed for screening of chitinolytic bacteria from natural samples.

CORROSION OF MILD STEEL SIMULATING LONG TERM SOIL BURIAL FIELD CONDITIONS DIFFERING IN NUTRITIONAL AND BIOTIC COMPONENTS

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Mild steel coupons (MSCs) buried for thirty months in soils inoculated with bacterial isolate *Bacillus cereus*-SNB4 resulted in microbiologically influenced corrosion (MIC) based average percent weight loss (APWL) and corrosion rate (CR) as 8.02% and 3.53 $mgdm^{-2}d^{-1}$, respectively with 18.85% moisture and 0.218% organic contents. For nutrient added soil the corresponding figures were -9.36 and -4.12 with 13.71% moisture

and 0.239% organic contents. The CR and APWL of MSCs due to MIC treated with *Bacillus thuringiensis*-SN8 were 4.77 $mgdm^{-2}d^{-1}$ and 10.65%, respectively with 17.52% moisture and 0.228% organic contents. For nutrient added soil, the respective values were -1.49 and -3.52 with 15.32% moisture and 0.19% organic contents. Corrosion rate and APWL of MSCs due to MIC in the co-cultured soils without added nutrients were 1.52 $mgdm^{-2}d^{-1}$ and 2.34%, respectively with 13.99% moisture and 0.206% organic contents. For co-cultured nutrient added soil, CR and APWL due to MIC were -2.82 $mgdm^{-2}d^{-1}$ and -6.69%, respectively with 16.85% moisture and 0.272% organic contents. XRD data of corrosion products expressed peaks of higher intensities of $Fe(OH)_3$, β-$Fe_2O_3.3H_2O$ and β-FeOOH While FTIR spectra depicted C–O, S=O, C–H, C–H, N–O, N–H, C=O, –SCN and O–H functional groups.

STUDIES ON STARCH DEGRADING ENZYME ACTIVITY FROM LOCALLY ISOLATED *BACILLUS* SPECIES

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The study was aimed to assess the production, optimization and characterization of starch degrading enzyme from locally isolated bacillus species. Among sixty bacillus species screened and isolated PCSIR-NL 11 and PCSIR-NL3 showed best production. Optimum temperature for the two strains was found 37°C, optimum pH was 7 and optimum substrate concentration was 2% (w/v) for 48 hours in shaking water bath at 140rpm. The enzyme was active. at wide range of pH and temperature. It retained 90% activity at 90°C and 84% at 100°C for 20 minutes.

TOXICITY ASSESSMENT OF IRON AND MANGANESE IN WATER AND SEDIMENTS OF RIVER RAVI AT BALOKI HEADWORKS, PAKISTAN

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Geochemical structure. urban sewage water und industrial wastes generate potential source of heavy metal contamination in river system. Therefore. this study was conducted to assess the variation in iron and manganese toxicity in the river Ravi. The water and sediment samples were collected from up- and downstream of river Ravi at Baloki Headworks. Six sampling campaigns were carried out in each site in six months period, during June to November. 2008. Samples of both water and sediments were analyzed for the iron and manganese concentrations by using Atomic Absorption

Spectrometry. Auxiliary data included physico-chemistry or river water. Significant seasonal variations were observed among pre-moonsoon (June). monsoon (July and August and post monsoon (September, October and November) season. Iron and manganese concentrations in sediments were higher during post monsoon season than pre- and monsoon seasons. The overall concentrations of iron in both river water and sediments were determined higher at downstream as compared to upstream of the river. Similarly concentrations of manganese in sediments were significantly (p<O.OS) higher at downstream than river's upstream while in water manganese was higher at upstream as compare to the river's downstream. Remarkable correlations were also recorded between metallic ions and physico-chemical variables. The seasonal variation in toxicity or these two metals was may be due to either anthropogenic source such as waste water discharge or natural causes such as climatic changes (rain) season) or variations in physico-chemical conditions of the river water.

COMPARISON OF BIOSAL AND PERMETHRIN TO ASSESS THEIR EFFECTS ON TRANSAMINASES ACTIVITIES (AST, ALT) IN VARIOUS ORGANS OF *CYPRINUS CARPIO*

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In this experimental work the comparative effects of permethrin [3-phenoxybenzyl (lRS)-cis, trans-3-(2, 2-dichlorovinyl)-2, 2-dimethylcyc1opropanecarboxylate] and Biosal (dried -neem whole fruit extract containing 0.32% azadirachtin) were studied on common carp *(Cyprinus carpio)* as a non-target aquatic organism. *Cyprinus carpio* (common carp) was exposed to 24 hours LCso ofpermethrin and Biosal (Neem based phytopesticides). Transaminases (AST, ALT) activities were calculated in various fish organs (brain, heart, liver, kidneys, gills and muscles) of Biosal and permethrin treated as well as untreated (control) fish samples. ALT activity was observed to elevate in all the organs and higher increase was noted in permethrin treatment than that in Biosal treatment. In case of organs, elevation in AL T activity was maximum in liver followed by kidneys and heart. AST activity was also found to increase in all the organs of Biosal and permethrin treated fish. This increase was more in permethrin treatment than that in Biosal treated fish. Elevation in AST activity was found to be maximum in brain followed by heart and gills.

HISTOLOGICAL ANOMALIES IN LIVER OF CYPRINIDS DUE TO UPTAKE OF WATERBORNE TOXICANTS FROM KEENJHAR LAKE

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The liver is an excellent indicator of pollution; hence, liver has a key role in accumulation and detoxification. The liver of *Cyprinids (L. rohita; C. mrigala* and *C. catla* H.) were selected for histological and trace metal examination from Keenjhar Lake. The accumulation of HM' s in liverofL. *rohitawas,* 1.69-0.77, 1.47-0.76, 1.02-0.51 and 1.89-1.01; liver of *C. mrigalaH.* possess highest metal burden 3.14-1.09,3.67-2.14,2.19-1.56 and 4.18-1.14 µg g^{-1}, liver of C. *catla* H. possess second high accumulation, 1.82-0.93, 1.56-0.84, 1.26-0.64 and 2.07-1.16 µg g^{-1} d. w. for Cd, Ni, Zn and Pb respectively, significantly difference at $p < 0.05$. Cyprinid's liver showed severe alterations, including hepatocytes with pyknotic nuclei; disruption of hepatic parenchyma; cytolysis; cirrhosis; hemorrhage; hyperemia; aneurysm; ischemia; proliferation of pile duct; macrovesicular steatosis; infiltration by leukocytes and hepatic vacuolation. Among the fish species examined, *C. mrigala* has the greatest number of cellular changes. *C. mrigala* is a bottom-feeder and comes into contact with contaminated sediments leading to the high content of trace and toxic metals accumulated. On behalf of our results the histological distortion increases with the increase in toxic influx.

EFFECT OF TOBACCO USE IN PREGNANT MICE AND THEIR OFFSPRINGS

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Use of tobacco is quite popular in various countries all over the world. The most common forms of smokeless tobacco (ST) available and used in Pakistan include; Pan, Naswar, Chhaalia, Gutka and Sweeteners. It is reported that use of chewing tobacco by female is quite common and tobacco smoking during pregnancy also increase risk for pre-term delivery. With view to see the effects of tobacco on pregnant mice and their offsprings, an experiment was conducted on 20 pregnant albino mice that were divided in 2 groups. Experimental group was treated with tobacco powder mixed in normal diet while control group was treated with normal feed for the same purpose. The results showed a significant ($p < 0.05$) decrease in the body weight of the offsprings of experimental mice when compared with control group. The initial weight of experimental male offspring mice was found to be 1.17 grams and the final weight in same group was 8.61 grams and in female mice the initial weight was 1.09 grams and final weight was 7.87 grams. In control group, male mice initial weight was 1.48 and final weight was 11.47 grams where as in female offsprings initial weight was 1.37 grams and final weight

was 11.04 grams. Experimental results suggest that use of smokeless tobacco during pregnancy has adverse effects on the offsprings manifested by the decrease in the body weight, decrease in the weight of liver, cellular injury to liver parenchyma specially the fatty infiltration and hydropic degeneration.

TERATOLOGICAL EFFECTS INDUCED BY METFORMIN IN DEVELOPING FETUSES

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An antidiabetic drug, Metfonnip. was tested for its adverse effects on developing fetuses of mice. Different concentrations of the drug 62.50, 250.00, 500.00 and 1000.00 µg/g B.W were prepared in such a way that each 0.1 ml contains the desired concentration. These doses were given orally to pregnant mothers on day 6 of the gestation. The treated pregnant mice were dissected on day 15 of gestation and fetuses were recovered. Morphological studies of the fetuses showed abnormalities such as hemorrhage, dysplasia, hydrocephaly, clubbed feet, brain bulges. The incidence of fetal resorptions also increased with higher dose concentrations. Comparatively higher doses proved more toxic and also caused more developmental defects. Morphometric studies of body weight, CR length, brain size, eye size, pinna size, hind limb and forelimb size and tail length of fetuses showed dose dependent significant decrease against controls. The doses of this drug given during present study proved dangerous to developing fetuses of mouse. It is quite apparent that this drug must be used with utmost care during critical developmental periods even in humans.

PREVALENCE AND DETECTION OF ANEMIA (IRON DEFICIENCY) IN FEMALE POPULATION IN KOHAT KHYBER PAKHTUNKHWA

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A total 200 blood samples of women of different ages have taken. Amongst these 108(54%) females were found to have iron deficiency (anemia). Prevalence rate of anemia in married (pregnant) female was (66.6%) which was more than the married (non-pregnant) females (55%). In these 108(54%) anemic females 32(29.6%) were young females, 38(35.1%) were married (non-pregnant) and 8(7.4%) were married (pregnant). While 30(27.7%) females were aged above 40 years. Majority of the females (46.2%) were aged 21-40 years including married pregnant and non pregnant and (29.6% &

24.0%) in age groups 12-20 years and 40 years and above age respectively. Based upon serum hemoglobin concentrations, most of the patients were found to suffer from mild anemia (77.7%), moderate anemia (20.3%) and severe anemia (1.85%).

CHROMIUM RESISTANT SULFUR OXIDIZING BACTERIA FROM A SOIL EXPOSED CHRONICALLY TO THE TANNERIES EFFLUENTS

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Biological conversion of elemental sulfur and sulfur compounds by sulfur oxidizing bacteria to sulphate is a major component of sulfur cycle. The sulphate formed improves the soil fertility, whereas acidity produced by such bacteria solubilizes plant nutrients and help metals bioleaching in contaminated soils as well. However, for in situ employment in such contaminated areas the bacteria be capable of metal resistance too. Soils sampled from tanneries effluents' influenced area of the city Kasur was screened for sulfur oxidizing bacteria employing the specific medium containing thiosulphate as sulfur source. Twenty eight isolates were found to be resistant upto 500 mg/L Cr(VI).The bacteria have a potential to leach out sulfur contents as well as heavy metals from contaminated soils and waters.

ISOLATION AND CHARACTERIZATION OF BACTERIAL STRAINS CAPABLE OF CARBARYL DEGRADATION

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Samples were collected from pesticides exposed soils of different agricultural fields of National Institute for Biotechnology and Genetic Engineering (NIBGE) and Ayub Agriculture Research Institute (AARI) Faisalabad. These were used for the isolation of carbaryl/1-Naphthol (a metabolite of Carbaryl) degrading bacteria (D1, D2, D3, D4), which were used for degradation studies. The isolate D1, having relatively higher carbaryl biodegrading capacity of 80% was used for further studies. It was characterized for colony and cellular morphologies. It was motile and Gram's negative having a cellular size of nearly 1-2 × 0.4-0.5 µm and was tentatively identified as *Yersinia* sp. Growth affecting parameters viz; pH, media composition, carbaryl concentrations, etc. were studied for the isolate D1. It showed growth up to 500mg/L of

carbaryl in MSM, when analyzed its utilization by HPLC. It displayed 91%, 85% and 76% degradation after 15 days in 100mg/L, 250mg/L and 500mg/L concentrations of carbaryl respectively, while no degradation was observed at 1000mg/L. The degradation observed was 85% in MSM (without any additional nutrient or carbon source), 60% in yeast extract, 73% in nutrient broth and 75% in glucose, when different nutrients composition *i.e.*, nutrient broth, yeast extract and glucose (0.1%) were applied in the presence of 50mg/L of carbaryl and 2% inoculum of isolate D1. In the same conditions, optical density (590 nm) of culture with nutrient broth and yeast extract was much higher than culture in glucose and MSM. Isolate D1 displayed the highest (89%) degradation of carbaryl at pH 8.5 while the lowest (55%) was observed at acidic pH 5.5 after one week. Rapid increase in O.D. was found after 24 hrs of incubation at alkaline and neutral pH values, while it was slower at acidic pH during 7 days. In MSM, containing 50mg/L of 1 Naphthol, isolate D1 degraded 84% 1-Naphthol in 6 days. Complete degradation of 50mg/L and 100mg/L carbaryl was observed in 4 days by D-consortium and it also completely degraded the 50mg/L and 100mg/L 1-Naphthol after 54 and 96 hours respectively. These results are reported from the indigenous pesticides exposed soil of Faisalabad for the very first time, which warrant extensive studies encompassing degradation of such highly important commercial pesticides. Moreover, some ecological implication related studies should also be carried out in this context.

CHROMIUM RESISTANT PHOSPHOROUS ACCUMULATING BACTERIA ISOLATED FROM SOIL SAMPLED FROM A SUBURBAN LOCALITY OF CITY KASUR

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Excess phosphate levels in the water cause eutrophication in the natural water bodies. Phosphate accumulating bacteria are a group of microorganisms which facilitate bioremoval of excess phosphorous from waste waters in a process called enhanced biological phosphorous removal (EBPR). The accumulated phosphorous is in the form of polyphosphate granules within the cells. However, owing to the fact of varied nature of pollutants being discharge into natural systems, bacteria intented for remediational measures be capable of resistant to the accompanying contaminants. Thus screening of such bacteria is very important in order to preserve natural environments. Soils sampled from tanneries effluents' influenced area of the city Kasur was screened for heavy metal Cr(VI) resistant polyphosphate accumulating bacteria employing a acetate mineral media. Five isolates proved to be resistant upto 500 mg/L Cr(VI). These bacteria may be employed for recovery of phosphates from metals and phosphate contaminated soils.

EFFECTS OF CHEMOTHERAPEUTIC DRUGS ON SERUM PROTEIN FRACTIONS IN BREAST CANCER PATIENTS

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Study concerns the analysis of serum total proteins. and fractions (albumin, total globulins, gamma and non gamma globulins) of breast cancer patients following chemotherapy (FAC regimen). For this purpose, blood samples of 52 women with breast cancer were collected from Institute of Nuclear Medicine and Oncology, Lahore before and after chemotherapy. These were compared with 30 age and sex matched healthy volunteers. Analysis of serum total proteins and fractions were carried out using commercially available kits by semi automated clinical chemistry analyzer. Results were analyzed, statistically, by one way analysis of variance and Turkey's post hoc multiple comparison test. Serum total proteins have shown a non significant increase in patients when compared with controls. Almost same degree of non significant increase was observed when patients were categorized on the basis of menopausal and surgical status, in overall, inter and intra group comparisons. A remarkable reduction in serum albumin level ($p<0.0001$) was observed in newly diagnosed patients and a significant recovery of this protein following chemotherapy was also indicated. On the other hand, serum total globulins, gamma globulins and non-gamma globulins levels have shown significant increases ($p<0.0001$, $p=0.0104$ and $p<0.001$, respectively) in newly diagnosed patients, whereas, following chemotherapy, a significant recovery was shown only by gamma globulins. Menopausal status and surgery did not show any role in changing senuil protein profiles. Our results provide preliminary information about total serum proteins and fractions of breast cancer patients indicating lowered serum albumin and higher serum globulins to compensate albumin reduction, in patients. Chemotherapy has been found to playa significant role in recovery. Although the given regimen is effective but a comparative analysis is mandatory to study the effectiveness of different drugs or therapeutic methods used for treatment of breast cancer.

BIOREMEDIATIONAL REMOVAL OF NITROGEN CONTENT FROM WASTEWATERS CONTAMINATED WITH HEAVY METAL

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Industrial effluents pollute natural water systems with organic and inorganic pollutants loads. Among the organic contaminants nitrogen is known for eutrophication problems. For its removal denitrifying bacteria can be employed. However, presence of

diverse pollutants make such processes more demanding for the microbes to be the pollutant resistant. For instance in case of presence of heavy metals, metal sensitive microbes can not be employed. In the present study twenty isolates of Cr, Pb, Hg, and Cu resistant denitrifying bacteria have been isolated on trypticase soy agar medium. These isolated bacteria were capable of resisting Cu upto the level 700 mg/L, Pb upto 800 mg/L, Hg upto 20 mg/L and Chromium upto 500 mg/L.These heavy metals resistant denitrifying bacteria may be used for removing nitrogen content from effluents of industries.

ISOLATION AND CHARACTERIZATION OF NITROGEN FIXING BACTERIA FROM TANNERIES EFFLUENTS' INFLUENCED SOILS

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Nitrogen fixing bacteria are well known for increasing soil fertility. Various industrial pollutants exert detrimental effects on the distribution and population densities of nitrogen fixers. Revival of such contaminated soils requires the pollutant resistant nitrogen fixing bacteria. Soils sampled from tanneries effluents' influenced area of the city Kasur was screened for the nitrogen fixing bacteria employing N-free glucose medium. Bacteria were tested for chromium resistance. Eighteen isolates were found to be resistant against 500 µg/ml of chromium Cr(VI) incorporated media. These bacteria appear potential candidates for rehabilitation programme in the metal contaminated soils.

ISOLATION AND CHARACTERIZATION OF CELLULOLYTIC BACTERIA FROM TANNERIES EFFLUENTS' INFLUENCED SOILS

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Discharge of industrial effluents and their dumping in the soils create localities exerting selective pressure for microbial communities. Soils sampled from tanneries effluents' influenced area of the city Kasur was screened for the cellulolytic bacteria employing a selective medium containing cellulose as major ingredient. The isolated bacteria were tested for chromium resistance. Seventeen bacterial isolates proved to be resistant upto 500 mg/L Cr(VI). The bacterial isolates yielded cellulase production upto 156 U. Their cellulolytic activity was assessed in the absence and presence of chromium. These bacteria have also been characterized for morphobiochemical features. Reduction of microbial cellulolytic profile of a soil is considered inversely related to the effect of

different pollutants. Under highly pollutants stressed conditions, the soil cellulolytic activity may be ceased or come to so lower profiles that biogeochemical turnover suffers to the level that disturb these ecosystems. For such locations cellulolytic isolates reported here appear potential candidates for soil rehabilitation programmes involving mobilization of cellulosic material in metal contaminated sites.

URBAN POLLUTANTS LOAD AFFECTS GROWTH FACTOR OF ROHU IN RIVER RAVI NEGATIVELY

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Rohu (*Labeo rohita*) were collected from four locations of the river Ravi during high and low flow seasons and were soon transported to the lab., where the specimen were analyzed for accurate measurements of length and weight. These data were then used for calculation of growth factor. The parameter had a value of 3.19 for a fish sampled from the upstream location; Siphon during low flow season. The growth factor decreased (b=2.49) at the second sampling point at Shahdera (downstream) and showed a further decrease (b=2.17) at the third sample location (Sunder). For the last downstream sampling locality (head Bolloki), the parameter (b=2.74) more or less stabilized and rather showed a small recovery as compared to second and third study areas. These results clearly indicate negative impact of urban pollutants for the riverine Rohu fish as envisaged by their lesser values of growth factor following the exposure. Further studies covering long distances downstream from the city Lahore may reveal pollutants stresses' recovery level or otherwise situation.

BIOCHEMICAL PARAMETERS OF *LABEO ROHITA* SAMPLED FROM UP AND DOWNSTREAM LOCALITIES OF THE RIVER RAVI

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The present study was conducted to evaluate the impact of urban pollutants' load on biochemical parameters of the fish muscle. Freshwater fish specimen, Rohu *(Labeo rohita)* were caught from Punjab University fish research farm (control) and four selected sampling locations; Siphon (A) Shahdera (B), Sunder (C) and head Bolloki (D) of the river Ravi during low flow season. Levels of the biochemical constituents viz., total carbohydrate, total protein, soluble protein, total cholesterol, total lipid, DNA and RNA were determined by standard biochemical protocols. Muscle sampled from sampling

location A (upstream) showed increase in total lipids by 87.22 % as compared to control. The parameter showed further increases at downstream localities B (142.52 %) and D (313.20 %). The trend was especially noticeable for the lipid contents which attained up to 462.41 % elevation for the fish sampled from the site C. Besides total lipid, total protein and soluble protein contents of the riverine fish samples increased at the downstream localities. Total carbohydrate and nucleic acid (DNA and RNA) contents decreased up to 70. 87%, 78.19 % and 42.05 % respectively for the downstream locations. 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.

HEAVY METAL RESISTANT BEACTERIA FROM GUT CONTENTS OF THAILA FROM UP AND DOWN STREAM LOCALITIES OF RIVER RAVI

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Removal of heavy metals from industrial effluent can be accomplished in an environmentally friendly way, through bioremediation by using heavy metals resistant and detoxifying microorganisms. Thaila, *Catla catla* were sampled from four selected sampling stations viz., Siphon (A), Shahdera (B), Sunder (C) and head Bolloki (D) of the river Ravi during high flow of the river. Twenty four heavy metals resistant bacteria were screened on the metal containing agar media. Of these 5, 8, 4 and 7 isolates were found to resist Cr, Pb, Hg, and Cu upto 300 µg/ml, 350 µg/ml, 10 µg/ml and 250 µg/ml respectively. Further, these isolates showed varying levels of multiple metal resistance.

DECOLOURIZATION AND DETOXIFICATION OF AZO DYE, SYNOZOL RED HF-6BN, BY *ASPERGILLUS NIGER* AND *NIGROSPORA* SP.

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In the present investigation the fungi, *Aspergillus niger* and *Nigrospora* sp. were employed for decolourization of Synazol Red HF-6BN. Decolourization study showed that *A. niger* and *Nigrospora* sp. were able to decolorize 88% and 96% Synazol Red 6BN, respectively in 24 days. It was also studied that 86% and 90% Synazol Red containing dye effluent was decolorized by *A. niger* and *Nigrospora* sp. after 28 days of incubation at room temperature. A protein with relative molecular mass of 70 kDa was partially purified and examined for enzymatic characteristics. The enzyme exhibited

highest activity at temperature ranging from 40-50°C and at pH 6.0. The enzyme activity was enhanced in the presence of metal cations. High performance liquid chromatography analysis confirmed that these fungal strains are capable to degrade Synazol Red dye into metabolites. No zones of inhibition on agar plates and growth of *Vigna radiata* in the presence of dye extracted sample indicate that the fungal degraded dye metabolites are nontoxic to beneficial micro-flora and plant growth. *A. niger* and *Nigrospora* sp. have promising potential in colour removal from textile wastewater containing azo dyes.

ISOLATION OF HEAVY METAL RESISTANT BEACTERIA FROM GUT CONTENTS OF ROHU COLLECTED FROM UP AND DOWN STREAM LOCALITIES OF RIVER RAVI

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Discharge of industrial and urban sewage effluents causes substantial environmental contamination disturbes health and distribution of aquatic fauna. River Ravi while passing through the city, Lahore is a prominent example in this regards. Rohu, *Labeo rohita* samples were collected from four sampling sites viz., Siphon (A), Shahdera (B), Sunder (C) and head Bolloki (D) of the river Ravi during high flow of the river. Fish specimen were brought immediately in the lab., dissected and from their gut contents heavy metals resistant bacteria have been isolated. In this study, twenty six metals resistant isolates were purified on select metals' incorporated nutrient agar media. Of these, 7, 8, 5 and 6 isolates showed resistance against Cr, Pb, Hg, and Cu up to 350 µg/ml, 400 µg/ml, 10 µg/ml and 250 µg/ml, respectively. Further these bacterial isolates showed multiple metal resistance to varying levels. These bacteria might have been playing a role in lessening the metals pollutions' allied negative impacts on the riverine fish.

RIVER URBAN POLLUTANTS ENHANCED CARBOHYDRATE AND NUCLEIC ACID WHILE DECREASED PROTEIN AND LIPID CONTENTS OF THE *CIRRHINA MRIGALA* SAMPLED FROM UP AND DOWNSTREAM LOCALITIES OF THE RIVER RAVI

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Freshwater fish Mori, *Cirrhina mrigala* were collected from four sampling sites viz., Siphon (A), Shahdera (B), Sunder (C) and head Bolloki (D) of the river Ravi during

low flow of the river and Punjab University fish research farm (control). Muscle tissue of the fish sampled from locality A showed 20.55 %, 25.89 % and 3.05 % decreases in carbohydrate, DNA and RNA contents respectively as compared to the control The corresponding decreases of the parameters for the fish sampled from the locality C were 82.71 %, 68.07 %, 89.20 % and 43.88 %, respectively. Total protein and lipids content of riverine fish increased, in general, progressively, upto third sampling locality. Highest increase was for the lipid contents which elevated up to 677 .58 % again for fish sampled from the site C. Varied nature of pollutants of the river Ravi exerted negative effects on growth and disturbed its balanced nutritional importance.

MUSCLE BIOCHEMICAL PARAMETERS OF *CATLA CATLA* SAMPLED FROM UP AND DOWNSTREAM LOCALITIES OF THE RIVER RAVI

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Freshwater fish Thaila, *Catla catla* were collected from four selected sampling stations viz., Siphon (A), Shahdera (B), Sunder (C) and head Bolloki (D) of the river Ravi during low flow season of the river and Punjab University fish research farm (control). The fish sampled from the locality A (upstream) showed 17.56 % less total carbohydrates as compared to the control fish. The parameter showed further decreases at downstream localities so that at sites B, C and D, the reductions turned out as 50.29 %, 68.52 % and 37.93 %, respectively. Besides the total carbohydrates, nucleic acids (both DNA and RNA) content showed decreases for the riverine fishes, which in general intensified at downstream locations. However, total protein, soluble protein, total cholesterol and total lipids contents increased in the riverine fishes progressively towards the downstream. This trend was especially noticeable for the lipids content which showed 770 % increase at the last downstream sampling point. These information reflects stresses of urban pollutants which interfered with normal growth processes of the fish and changed nutritional importance of the animals.

TO STUDY MOLECULAR DETERMINANTS OF HIV-1 NEURO INVASION AND PREVALENCE RATE OF HIV ASSOCIATED DEMENTIA IN HIV INFECTED PAKISTANI POPULATION.

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HIV-associated dementia (HAD) largely depends on continuous seeding of central nervous system with HIV infected immune system cells and direct invasion of HIV or

HIV proteins mainly gp120 and tat from the periphery. Since blood brain barrier (BBB) is responsible for restricting direct cross barrier movement of HIV or HIV particles therefore, it is suggested with evidence that BBB looses its integrity on direct exposure with HIV or HIV particles via mechanisms that are not well elucidated till date. However it is reported that HIV envelop glycoprotein gp120 is responsible for proteasome-mediated degradation of BBB's tight junction protein zonula occluden-1(ZO-1), but detailed molecular mechanism still needs to be figured out. HIV-associated dementia represents the most severe manifestation of HIV-associated Neurocognitive Disorders (HANDs) that leaves it's life lasting impacts on patient's cognitive, behavioral and motor functions therefore patients are consistently required to be monitored for initial symptoms of HAD but in Pakistan no studies have been persuaded to figure out prevalence rate of HAD in non- ART HIV patients. This research work is focused on two main domains; understanding molecular mechanism lying in proteasome mediated degradation of ZO-1 protein via gp120 protein and studying prevalence rate of HAD in non-ART HIV infected Pakistani population. Using bioinformatics techniques it is inferred that gp120 protein initially marks ZO-1 protein which then lead to proteasome mediated degradation. Residues of gp120 protein involved in this molecular mechanism are thr 22, thr 23 and ser 11 that resides in V3 loop of gp120 protein while that of PDZ-1 domain are gln 70 and asn 72. There exist a great probability that gp120 protein marks ZO-1 protein via interacting at multiple positions as Ile 1 of first highlighted peptide of V3 region shares the distances of 4.36 and 3.95 angstrom with ile 18 and trp 19 of PDZ-1 domain respectively. Enzyme-linked immunosorbent assay (ELISA) revealed that prevalence rate of HAD in Pakistan is 12.5% which is far greater than neighboring country India's prevalence rate of 1-2% and meets with westerns countries 10-24%. So during the treatment of HIV patient physician should also focus in detail on mental health of patient due to high prevalence rate of HAD in Pakistan.

PREVALENCE OF TUBERCULOSIS IN HUMAN IN PESHAWAR KHYBER PUKHTOONKHAWA

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Tuberculosis (TB) continues to be a persistent challenge for global health and development, a total of 153 sample were tested in which 49 (32.02%) were positive, A high prevalence was recorded 17(37.77%) in month of July, followed by months of May 18(34.61%) and the lowest 14(25%). was recorded in months of June, 2010. The age wise prevalence was also determined in which high prevalence was recorded 68.96%(20/29) in age ranges from 10-20 year and followed by18 (35.29%) age ranges from 21-40 years and lowest was recored 15.06% (11/73) in the ages of 40 years. Sex wise

prevalence was determined in the said study in which high prevalence was recorded in female 33.33 %(26/78) then male 30.66 %(23/75) It was revealed that a significant P<0.05 was recorded when analyzed statistically.

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 have some limitations. 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. rMSCs 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 rMSC was reduced from 47.93 h with FBS to 45.34 h in the presence of CBS. 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.

GENETIC STUDY OF HEREDITARY HEARING IMPAIRMENT IN FAMILIES OF AZAD JAMMU AND KASHMIR

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Hearing impairment is the most widespread sensory disorder worldwide holds the considerable prevalence of 1.9 per 1,000 infants at birth. Hearing impairment is common

at all ages but it is also a common birth defect. A hearing defect beginning in early childhood (infants) severely affects the speech acquisition of child and psychosocial development. When the hearing defect appears later in life it seriously disturbs the subject's quality of life. More than 50% of early-onset hearing impairment cases in humans have genetic etiology. In most cases hereditary hearing impairment occurs due to single gene mutations and is mostly prelingual and sensorineural (cochlear defects) in nature. About 70% of HHI cases are classified as non-syndromic deafness (isolated hearing loss) and 30% as syndromic deafness (hearing loss with other anomalies). The mode of inheritance of non-syndromic hearing impairment is autosomal recessive (80%), autosomal dominant (20%), X-linked (1%) and mitochondrial (<1%). Non-syndromic hearing impairment is highly heterogenous and about 50% of prelingual autosomal recessive hearing impairment is caused by mutation on the DFNB1 locus harboring *GJB2* gene. In the present study two families with autosomal recessive non-syndromic hearing impairment were ascertained from Azad Jammu and Kashmir. Family A has three affected individuals while family B has four affected individuals. To identify the causative genes, homozygosity mapping was carried out in both families A and B, by genotyping microsatellite markers linked to currently known DFNB loci. Homozygosity mapping of both the families was suggestive of linkage to autosomal region on chromosome 13q12. DNA sequence analysis of exon 2 of *GJB2* gene, located in thecandidate region on chromosome 13q12 identify a disease causing mutation in two families.

ASSESSMENT OF IODINE DEFICIENCY IN PREGNANT WOMEN AND NEONATES IN ISLAMABAD, PAKISTAN

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Background and objectives. Iodine deficiency is a major nutritional problem in Pakistan. The pregnant women and their neonates have been important target groups for the study of the prevalence of iodine deficiency in a community. The main goal of the present study was to assess the status of iodine deficiency in the pregnant women and their neonates, in Islamabad, Pakistan. Methods. A hospital-based, cross-sectional screening study was conducted on 261 full term pregnant women, 125 neonates born to them and 50 controls (non-pregnant healthy women) in the Maternity Unit of the Islamabad Hospitals. The overall iodine status of the pregnant and non pregnant women were estimated by measuring the urinary iodine concentration and neonatal thyroid function was estimated by measuring TSH levels in their cord blood, in Environmental Laboratory of Chemical and Materials Engineering Department at PIEAS, Nilore, Islamabad. Results. A total of 73% pregnant women showed urinary iodine concentration > 20 µg/L and 50 percent showed UIC between 20-49 µg/L. The median UIC values in

the pregnant women were found to be 30.37 µg/L. Statistically significant ($p<0.05$) difference was found between the UIC levels of pregnant and control women and non-significant difference was found in UIC levels between different age groups of pregnant women ($p > 0.005$). A total of 19 (14.84 percent) neonates had TSH values >10 mIU/L. Conclusions. It can be concluded from the present study that pregnant women of the study area were found to be moderately iodine deficient and the neonates were mildly iodine deficient. These findings indicate that the use of iodized salt should be maintained in the study area and neonatal screening by measuring TSH levels is recommended in this area.

EFFECT OF AQUEOUS AND ORGANIC SOLVENT FRACTIONS OF *MELIA AZEDARACH* (LINN) BERRIES ON BIOCHEMICAL PARAMETERS OF *ORYCTOLAGUS CUNICULUS*

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Dry fruit powder of *Melia azedarach.* linn (locally called Tora shandi) is commonly used by the residents of Malakand division of Khyber pakhtunkhwa (K.P.K) for the relief of stomach pain, fever and diabetes. Reports exist about the therapeutic use, toxicity and poisoning of *Melia azedarach* fruits both in animals and humans. To investigate its potential as an anti-diabetic and anti-hyperlipidemic agent, fruit extract was prepared in 50% methanol and fractionated into n-hexane, chloroform, ethyl acetate, butanol and aqueous fractions. The aim was to explore its antidiabetic and anti-hyperlipidemic properties. For this purpose, serum glucose, cholesterol, triglyceride, low density lipoprotein (LDL), high density lipoprotein (HDL), glutamate pyruvate transminase (GPT), alkaline phosphate (ALP) and creatinine concentrations were studied in normal rabbits after 20 and 40 days of oral exposure to these fractions, at the dose of 50 mg/kg b.w. The anti-hyperglycemic effects of crude extract and its fractions were also studied in glucose induced-hyperglycemic rabbits, at a dose level of 300 mg/kg body weight. A significant reduction to near normal levels occurred in plasma glucose level after 3 hrs in all treatment groups as compared to the glucose overloaded untreated control groups ($p < 0.05$). Marked reduction in glucose was found in glibenclamide and aqueous fraction treated animals. Reduction ($p <0.05$) in serum cholesterol, triglyceride and LDL was found. HDL was ($p<0.05$) elevated in chloroform (73.33%), n-hexane (61.66%) and ethyl acetate fraction treated groups (36.66%) after 20 days. After 40 days, all the fraction except n-hexane fraction resulted in significant elevation ($p <0.05$) of serum HDL level compared to untreated control group. LDL/HDL ratio was significantly reduced ($p <0.05$) in all treatment groups. Serum glutamate pyruvate transaminase (GPT) and alkaline phosphatase (ALP) were significantly elevated ($p <0.05$) in all fractions except the aqueous fraction treated group. Serum creatinine was significantly reduced in

the groups that received aqueous (23.07%) and n-hexane fraction (19.23%) for 20 days. After 40 days, aqueous fraction caused significant reduction (37.74 %; p <0.05) in creatinine levels. In conclusion, nearly all fractions of *Melia azedarach* berries had potent anti-hyperglycemic action in glucose-induced hyperglycemic rabbits, hypoglycemic, hypolipidemic and HDL boosting in normal rabbits. Notably, among these fractions only the aqueous fraction was found to be safe for liver and kidney, while all other fractions turned to be hepatotoxic and nephrotoxic. The present study therefore recommends cautious use of the *Melia azedarach* fruit powder in traditional medicine.

ANTIDIABETIC ACTIVITY OF *WOODFORDIA FRUITICOSA* CRUDE EXTRACT IN NORMAL AND ALLOXAN MONOHYDRATE TREATED SPRAGUE-DAWLEY RATS

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Diabetes is one such disease that is increasing alarmingly both in developed and developing nations. Herbal medicines to treat diabetes are in use but in depth investigations for their morphological and pharmacological effects are limited. The present study was designed on two plant species namely *Woodfordia fruticosa* (Linn), and *Vibernum cotinifolium* (D. Don). Crude extracts of plants were administered to alloxan monohydrate (150 mg/kg b.w.) induced diabetic rats at 3 and 5 g/kg b.w doses respectively. Control groups were distilled water treated, vehicle control treated with DMSO (only in case of *woodfordia fruticosa*), and positive control group treated with glibenclamide (10 mg/kg b.w). Cut off line for diabetes was plasma glucose levels > 200 mg/dl. Extracts were given 48 hrs after the rats were found to be fully diabetic. Fasting glucose levels were estimated at 0, 1, 3 and 5 hrs after the extract administration. Chronic experiments were set up in the next phase, however these were done with *Woodfordia* only, rats were made diabetic as above, and *Woodfordia* extract was given for continuous 21 days at the rate of 142 mg/kg b.w, controls were as above, glucose levels were estimated on day 1, 7, 14 and 21. Rats were sacrificed after 21 days, blood and pancreas were collected. Serum was assessed for creatinine, urea, ALT, AST, ALP, cholesterol and triglycerides. With acute administration of *Viburnum cotinofolium* extract glucose levels of diabetic rats decreased significantly ($p < 0.05$). *Woodfordia fruticosa* extract caused a comparatively greater reduction ($p < 0.05$). Rats treated with chronic doses had significantly reduced glucose levels ($p < 0.05$) when estimated on day 21 compared to day 1. All the three liver enzymes, ALT, AST and ALP, showed a significant reduction in the extract treated diabetic rats ($p < 0.05$). Serum urea was significantly elevated in the diabetic controls but was normalized in the extract treated diabetic rats showed a significant decline ($P < 0.003$). Pancreas showed cellular deformities including cellular lesions, reduced dimensions and loss of connective tissue that led to hyperplasia whereas

in extract treated groups there was restoration of normal cellular population size. This study demonstrates that both the extracts contain antihyperglycemic activity and show improvement of hepatic and renal damage induced by diabetes.

ANTIDIABETIC POTENTIAL OF *VIBURNUM COTINIFOLIUM* CRUDE EXTRACT IN LABORATORY RATS

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Since centuries, herbal medicines have been highly valued sources of ayurvedic medicine. Due to the therapeutic potential, medicinal plants are widely used in the traditional medical practice for cure of various ailments like diabetes, hepatitis, arthritis, cancer, infertility etc. Due to the ever growing rates of diabetes to the level of a serious epidemic, vigorous efforts are being taken at pharmacological, cellular, molecular and genetic levels to combat the disease. Although the primary cure for diabetes is available in the form of insulin and many other oral hypoglycemic agents, attention towards herbal and folk medicines is increasing because of the side effects of the primary therapies. Presently, we investigated the anti-diabetic effect of *Viburnum cotinifolium* (D. Don) plant extract in alloxan monohydrate (150 mg/k.g b.w) induced diabetic rats. The effect of plant extract on glycosylated hemoglobin (HbA1c), cholesterol, triglycerides, serum urea, creatinine, ALP, ALT levels were assessed. Oral administrations of crude extract (5 g/k.g b.w) for 20 days resulted in a significant lowering of plasma glucose levels compared to the positive control group treated with glibenclamide (10 mg/kg b.w). Marked decrease in HbA1c, urea, creatinine, ALT, ALP, cholesterol levels were the outcome as compared to the diabetic control group and paralleled the observations in the positive control group. The present study demonstrated the antihyperglycemic as well as the antihyperlidimic potential of plant extract of *Viburnum cotinifolium*, as evidenced by an improvement in renal and hepatic functioning.

MULTIPLE DRUG RESISTANT (MDR) BACTERIA IN MASTITIC MILK OF BUFFALOES

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Prevalence of multiple drug resistant was studied in buffaloes suffering from sub-clinical mastitis. In total 600 dairy buffaloes from various dairy farms located in four

districts of Punjab were included in the study. The cases of Sub-clinical mastitis were detected in 234 buffaloes by White Side Test. The bacterial strains belonging to nine genera were identified *i.e. Staphylococcus, Escherichia, Streptococcus, Pseudomonas, Salmonella, Bacillus, Klebsiella, Enterococcus* and *Corynebacterium* were subjected to antibiotic sensitivity test using Kirby-Bauer method against ten most commonly used antibiotics by the field veterinarians. Out of 94 resistant strains, 24 were found to be resistant to at least one antibiotic; 32 to two and rest of the 38 were resistant to more than two antibiotics. The isolates found resistant to more than two antibiotics were declared as multiple drug resistant (MDR) bacteria. The most prevalent MDR belonged to the genera of *Pseudomonas* and *Staphylococcus* (26.31% of the isolates) followed by *E. coli* (18.42%), *Streptococcus* (10.52%), *Salmonella* (7.89%), *Bacillus* (5.26%), *Klebsiella* (2.63%) *and Corynebacterium* (2.63%). Use of combination of various antibiotics to treat such cases was evaluated in vitro. The combinations tested include A (Penicillin + Streptomycin), B (Gentamycin + Ceftizide), C (Amoxicillin + Clavulanic Acid) and D (Oxytetracyclin + Tylosin). Combination C was found to be the most effective (93.25%) against MDR bacteria followed by B (84.56%), D (66.74%) and A (34.54%), respectively.

CNV ANALYSIS IDENTIFIED A NOVEL DELETION MUTATION IN *TUSC3* GENE IN A PAKISTANI FAMILY WITH INTELLECTUAL DISABILITY

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Autosomal recessive nonsyndromic intellectual disability is a serious handicap among the peoples of all ages with a prevalence rate of 1-3 % of general population in US. It's a heterogeneous disorder with no set clinical criteria with respect to its genetic make up. Autosomal recessive NSMR is the least explored disorder among the genetic MR and so far only 30 loci and seven genes (*PRSS12, CRBN, CC2D1A, TUSC3, GRIK2, TRAPPC9* and *TECR*) for this disorder is known. In the current investigative study, a Pakistani family (three affected individuals) segregating autosomal recessive nonsyndromic MR was analyzed. The genome wide SNP analysis by using Genechip mapping 500K array identified a single region of homozygosity on 8p22 locus. The common linkage interval among all affected individuals was between rs6989820 and rs2237834, which delineates a critical region of 12.494 Mb. The subsequent CNV analysis of the data revealed a homozygous deletion of 170.673 Kb which encompassed the *TUSC3* gene. TUSC3 is the subunit of oligosaccharyl transferase complex involved in N-glycosylation of protein. Previously, one deletion mutation (Iranian family) and one insertion mutation (French family) have been reported for *TUSC3*. Here we reported a novel deletion mutation in *TUSC3* gene in a Pakistani family with autosomal recessive NSID.

PREVALENCE OF MULTIPLE DRUG RESISTANT (MDR) BACTERIA IN INTESTINAL INFECTIONS OF DOGS

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Cats and dogs are the potential sources for spread of antimicrobial resistance in humans due to their close contact with them. From the collected 100 samples of entritis in dogs, a total of 210 different bacterial species, 127 *E. coli,* 50 *Salmonella enterica,* 12 *Proteus vulgaris,* 17 *Citrobacter diversus* and 04 *Psedomonas spp.* were identified. Among all of these *E.coli* was most prevalent 60.47% followed by *Salmonella enterica* 23.81%, *Citrobacter diversus* 8.095%, *Proteus vulgaris* 5.71% and *Psedomonas spp.* 1.904%. Out of 127 *E.coli* isolates 52 (40.94%) were declared as MDR-Bacteria following 50 *Salmonella enterica* isolates 17 (34.00%), 17 *Citrobater diversis* 06 (35.29%), 12 *Proteus vulgaris* isolates 06 (50%). Out of 52 MDR E.coli isolates 19 (36.50%) were found to be invasive. Different antibiotic combinatins were also applied on MDR bacteria. It was observed that 81 MDR isolates were sensitive (74.30%) to antibiotic combination Amoxicillin - Clavulanic Acid, followed by Oxytetracyclin – Tylosin (52.61%), Gentamycin – Ceftriaxone (41.48%), and Penicillin – Streptomycin (31.82%) respectively. Recommendations are made on prudent use of antimicrobial drugs in dogs, as well as on the need to develop science-based infection control programs in veterinary hospitals.

LINKAGE STUDY OF FAMILIES SEGREGATING AUTOSOMAL RECESSIVE MENTAL RETARDATION FROM AZAD JAMMU AND KASHMIR

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Mental Retardation/Intellectual Disability (MR/ID) causing limitations in the ability to conduct normal activities of daily life is significantly sub averaged intellectual functioning present from birth or early infancy. MR is an important unresolved problem in medical science and occurs either alone or associated with other maladies. MR with onset before the age of 18 years results in significant impairment of cognitive and adaptive functioning and estimated to occur in 1-3% of the total population. The etiologies of MR are extremely heterogeneous including both genetic and environmental factors. The mode of inheritance of MR can be autosomal dominant or recessive and X-

linked dominant or recessive. To date, 164 X linked genes involved in mental retardation (98 syndromic and 66 non-specific forms) have been identified while the list of autosomal recessive non-syndromic mental retardation known loci is thirteen with six genes discovered so far. Present study involves two consanguineous families (A and B), from District Bagh of Azad Jammu and Kashmir, demonstrating non syndromic autosomal recessive mental retardation. Affected individual were examined for the presence of malformations (other than mental retardation) and history of metabolic disorders. To identify the causative genes, homozygosity mapping was carried out in both families A and B, by genotyping microsatellite markers linked to currently known ARNSMR loci. For both families, data for all the genotyped loci was analyzed using easy LINKAGE plus version 5.02, which failed to yield significant LOD score, indicating the exclusion of known loci. Thus linkage to all known loci was conclusively excluded in both families, indicating involvement of potentially novel loci. The identification of novel genes by genome wide scanning using microsatellite markers or by whole genome SNP typing using microarrays may contribute to the better understanding, diagnosis, prevention and eventually even the treatment of mental retardation.

STATISTICAL ANALYSIS OF QUALITY CONTROL SAMPLES RUN AT CLINICAL LABORATORIES, INMOL, LAHORE

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The present study deals with statistical analysis of raw data taken from Clinical Chemistry Labs of Institute of Nuclear Medicine and Oncology for Quality Control check. Quality Control is a process which helps in ensuring the certain level of quality. One of the most important actions that can help maintain the quality of any process is to collect relevant data consistently over time, plot it, and examine the plots carefully. If analysis of control chart indicates that the process is under control then the data obtained from the process can be used to predict the future performance of that process. If the chart indicates that the process being monitored is not in control, analysis of chart can help in determining the sources of variation which can then be eliminated to bring the process back into control. The data from quality control samples of nine common chemical pathology tests was used and control charts were made. These clinical chemistry tests include: Glucose, Uric Acid, LDH, AST, ALT, ALP, Bilirubin, BUN and Creatinine. The results obtained from control charts were found satisfactory since maximum QC values lied within the specified limits. Thus, it proved the results of tests being performed at daily basis in Clinical Labs of INMOL are correct and reliable.

RESPONSES OF CONTACT LENS ASSOCIATED BACTERIAL ISOLATES TO COMMERCIALLY AVAILABLE CLEANING SOLUTIONS

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Present study was conducted to investigate antimicrobial efficacy of commercially available local and imported contact lens solution. A total of 43 strains of bacteria were isolated from contact lens and lens cases containing the used maintenance liquid. They were screened against 11 antibiotics belonging to all known groups. Multiple antimicrobial resistance was common in lens isolates. In comparison to gram positive, gram negative isolates were found to have significantly high survival and replication rate in various contact lens solutions. The solutions contained polyhexamethylene biguanide and polyaminopropyl biguanide as antimicrobials, no significant difference in the efficacy of lens solutions was notices due to the presence of two different antimicrobials. On the basis of their sensitivity to antibiotics, isolates were divided in three categories, sensitive (resistance to ≤ 1), Resistant (≤5 antimicrobia) and highly resistant (resistant to ≥10 antimicrobial). Responses of all isolates to lens solutions were found to be independent to their antimicrobial resistance level.

GENETIC STUDY OF FAMILIES WITH AUTOSOMAL RECESSIVE RETINITIS PIGMENTOSA IN AZAD KASHMIR

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Vision is the most crucial and fundamental sense which makes the life colorful and cheerful**.** Retina is an important component of eye and its abnormality directly affects normal vision process. Retinal dystrophy due to hereditary defects is an established fact which is genetically heterogeneous. Hereditary retinal dystrophies which have been associated with autosomal recessive, autosomal dominant or X linked inheritance and may result due to defect in genes responsible for normal function of eye. The most reported prevailing mode of inheritance of retinitis pigmentosa is genetically reside in autosomal recessive case, which is 20-25% of total known percentage (45-60%) of RP. The recessive mode of inheritance expresses its affects in siblings having normal parents. In this study two Pakistani consanguineous families (A and B), from Azad Kashmir, exhibiting severe early-onset RP have been explored for genetic defects. Clinical presentation of both families (A and B) showed severe visual impairment to all affected individuals, night blindness at their early childhood, early complete vision loss and

showing bone spicules with typical RP fundus changes. Homozygosity mapping of family A showed linkage to *LRAT (OMIM 613341)* gene which established early onset / juvenile RP in this family. However genotyping of family B did not showed linkage to tested loci of retinitis pigmentosa. Linkage analysis was performed by using easyLINKAGE plus version 5.02, which did not yield significant LOD score for family B, excluding the linkage to known loci. Sequencing of *LRAT* gene is in progress which may reveal any pathogenic change associated with RP. Because RP is a heterogeneous disorder so there may be a possible chance of presence of any other gene contributing its defective expression causing congenital blindness in family B. For better understanding and identification of gene responsible for early onset of RP in family B, a whole genome scan through microsatellite markers or whole genome SNP typing using microarrays may be the ultimate step.

PRODUCTION AND UTILIZATION OF BIOGAS EFFLUENT THROUGH RECYCLING OF VEGETABLE WASTE AS A BIOFERTILIZER FOR CROP (BARSEEM AND MAIZE) AND FOR FISH (*LABEO ROHITA*) PRODUCTION

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The present study aims at production and utilization of biogas effluent through recycling of vegetable waste co-digested with buffalo dung using the process of anaerobic digestion. The digested effluent generated during the process is further utilized for the growth of algae and plankton in ponds later to be used as a feed for Rohu*(Labeo rohita)* culture. The biogas affluent was also tested as bio-fertilizer for growing maize and barseem (fodder crops) for a period of three months. The methodology adopted was the collection of vegetable waste sample from fruit and vegetable market and buffalo dung from Gawala colony. Proximate analysis of nutritional values was done followed by biogas production. Finally, biogas effluent collected was utilized on crops as bio-fertilizer and in the ponds for algal and plankton growth for Rohu culture. The field application of the digested effluent was also carried out by dividing both types of crops (maize and barseem) into four groups. Group A; without any fertilizer application to crops, Group B; crops grown with application of vegetable waste effluent, Group C; crops grown with application of buffalo dung effluent and, Group D; crops grown with application of chemical fertilizer (urea). In case of Rohu culture (200 fish, each group contained 50 fish) of Rohu same segmentation was followed i.e. fish culture was divided into four groups. Group A *i.e.* fish fed with no supplementation, Group B fish fed with vegetable waste effluent, Group C fish fed with buffalo dung effluent and Group D fish fed on rice polish (fish meal). This trend was followed to make a comparison of four types of groups to check the efficiency of vegetable waste effluent with respect to controlled conditions, buffalo dung effluent, chemical fertilizer and fish feed (rice polish) in crops and in fish

production respectively. The result of the study showed progressive increase of 10-15% of crop yield grown with vegetable waste effluent and buffalo dung effluent as compared to crops grown under control conditions and urea application. For fish production the weight gain of Rahu reared with buffalo dung and vegetable waste effluent was more than the weight gain in fish reared without supplementation and rice polish.

PERCENTAGE OF AREA OWNED BY REGENERATED MUSCLE FIBRES WITHIN CROSS-SECTIONS IS A BETTER ESTIMATE OF STRUCTURAL RECOVERY OF MUSCLE TRANSPLANTS

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Morphological aspects of muscle fibres' regeneration following chemical/physical trauma and transplantation etc. have largely been described through average cross sectional area (ACSA) of and / or total number of regenerated muscle fibres. However, histologically varied nature of regenerated muscle fibres within a graft in terms of their diameter distribution as well as hyperplasia, dictate that ACSA may not be a good indicative of amount of regenerated sarcoplasm, on which the functional recovery depends. Further, the ASCA of regenerated muscle fibre approach does not reflects the connective tissue(C.T.) development with the transplants. The earlier parameter reflects positive , while the latter negative recoveries of a regenerating muscle tissue. Thus a factor which should derive from both the parameters i.e., strength of regenerated muscle fibres as well as C.T. would definitely be a good assessor of the quality of a muscle regenerate instead of ACSA of regenerated muscle fibre approach. Strength of regenerated muscle fibre, cannot solely be estimated from the ACSA. As in some transplants this parameter may indicate high profile, while in fact there may a few regenerated muscle fibre / area with a graft. On the contrary ACSA of regenerated muscle fibre may show lesser value and the graft may have a full complement of regenerated muscle fibre (hyperplasia),albeit of smaller diameters. In any case there may be less, moderate or elaborative C.T.development within the muscle regenerates. C.T.obviously is not a contratile strength of a skeletal muscle. The present study was intended to masure percentage of area within a cross-section of regenerated muscle owned by regenerated muscle fibre and to evaluate "regenerated sarcoplasmic intensity" as a parameter indicative of regenerated muscle strength while integrating the regenerated. muscle fibres hypertrophy, hyperplasia and C.T. development within freely grafted extensor digitorum longus muscle orthotransplants in control and insulin supplemented male rates at various stages post-transplantation.

UROPATHOGENIC CONTROLLING EFFICACY OF *LACTOBACILLUS* STRAINS ISOLATED FROM ROTTEN FRUITS AND VEGETABLES

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The aim of the present study was to identify predominant lactic acid bacteria (LAB) by molecular biology-based methods isolated from different spoiled fruits and vegetables. Samples were collected from eight different local markets of Lahore, Pakistan. A total of 13 Gram-positive and catalase-negative isolates were obtained from MRS. The isolates were grouped and identified by 16S rRNA gene sequencing and carbohydrate assimilation profiling. The isolates were identified as *Lactobacillus fermentum, L. plantarum, L. delbrueckii spp. bulgaricus, L. casei, L. helvetics, L. brevis, L. rhamnosus*, and *L. salivarius.* The bacterium *L. plantarum* was isolated from majority of the samples. Further, these isolates were subjected for determining their inhibitory effects against multi-drug-resistant uropathogens(*Candida, Klebsiella, Pseudomonas, E.coli* and *E. fecalis*). The antibiotics Augmentin, cefixime, ciprofloxacin and gentamicin were employed as control drugs. The *L. plantarum* and *L. brevis* strains demonstrated remarkable inhibitory activity against all the tested uropathogens. Based on these results it is conclude that there is a potential for using these LAB as a natural approach for the control of the described uropathogens.

PROTEASE PRODUCTION OF *BACILLUS* SP. EMPLOYING SOME NON-CONVENTIONAL LOW COST MEDIA

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Three bacterial species *Bacillus licheniformis, Bacillus cereus* and *Paenibacillus dendritiformis* were evaluated for alkaline protease production in some non-conventional low cost media. The optimum pH and temperature for the three bacteria were 9 and 37^{o}C ,respectively. Enzymes remained active over a broad range of pH (9-12) and temperature (50-100^{o}C). However, maximum activity was obtained at pH 10 for *Bacillus licheniformis* and pH 9 for *Bacillus cereus* and *Paenibacillus dendritiformis*. The bacterial enzymes exhibited maximum activity at 60^{o}C. Different carbon sources (glucose, waste bread, molasses and potato) and nitrogen sources (soybean meal, chicken feathers and whey) were used in different combinations to construct nine different media. These were evaluated for the bacterial cultivations and alkaline protease production at different incubation periods (48 h, 96 h, 144 h and 192 h). *Bacillus licheniformis* and

Bacillus cereus exhibited maximum activities i.e., 305.86 and 326.32 U/ml respectively in medium M3, comprising mainly of molasses and soybean meal after 48 h of incubation. *Paenibacillus dendritiformis* exhibited highest enzyme activity of 739.18 U/ml in medium M1, comprising mainly of glucose and soybean meal after 48 h of incubation. Varying levels of enzymatic activities were obtained in other media by the bacteria while minimum activity was obtained in medium M9, containing whey as main substrate. Conclusively, soybean meal in combination with suitable carbon source(s) appeared a promising recipe for cultivation of the bacterial strains and promising yield of thermostable alkaline proteases.

BLOOD DYNAMICS, CARCASS CHARACTERISTICS AND WEIGHT GAIN IN POST WEANED LOHI LAMBS RAISED UNDER TRADITIONAL AND HIGH INPUT FEEDING SYSTEM

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The present study was planned to check out the effect of differ protein with or without ionophore and Probiotic on Lohi lambs. For this purpose eighty male lambs (3 months of age) were randomly divided in ten groups with eight animals in each. Nine isocaloric diets with three levels of crude protein (18, 22 and 26%) with or without ionophores (@ 20ppm) and probiotics (0.1% of ration) were formulated. These diets were fed to nine groups of lambs while tenth group was offered fodder (berseem hay) only. The lambs were fed *ad libitum* and were weighed weekly to know the weight gain and its economics. In present trial, higher dry matter (DM) and crude protein (CP) intake and digestibilities were observed by goats fed LP, MP and HP diets than those fed FOD diet. The DM and CP intake increased linearly in goats fed diets with gradual increase in dietary CP concentration and similar trend was noticed by supplementation of ionophores and probiotics. Nitrogen (N) balance was higher in goats fed LPI, MPI, HPI diets than those fed LP, MP, HP and LPP, MPP and HPP diets. Higher T3 and T4 concentrations were noticed in goats fed HPI and MPI diets compared to those fed LP, MP, HP and LPP, MPP and HPP diets. Blood glucose concentration increased linearly with increasing the dietary CP in goats with or without ionophores and probiotics supplementation. Blood urea nitrogen (BUN) was lower in goats fed FOD diet than those fed diets with gradual increase in dietary CP concentration with or without ionophores and probiotics. Goats fed increasing dietary CP concentration gained more weight than those fed FOD diet. Cost of feed to produce one kg live weight and feed conversion ratio (FCR) were higher in goats fed FOD diet than those fed any other experimental diet with or without ionophores or probiotics.

LABORATORY SCALE COMPOSTING OF VEGETABLE AND FRUIT WASTES UNDER CONTROLLED CONDITIONS

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Solid waste management is one of the biggest environmental challenges the world is facing due to the increasing population and urbanization. Load of organic wastes as domestic and urban refuse produced in big cities is so great, that the natural systems cannot satisfactorily decompose even 50% of the total amount. Different treatment methods including composting, anaerobic digestion, incineration, thermolysis and gasification represent the most usual treatment methods. In order to select suitable substrate for composting, peels of apple, banana, oranges and potatoes were composted following autoclaving under controlled aerobic condition, at 50°C for 21 days. Samples were taken at weekly basis for analyses of pH and electrical conductivity (EC) ash and moisture contents, seed germination and bacterial C.F.U. pH and ash contents of all the four compost samples increased, while an increase in EC of sample B (banana peels) and D (potato peels) and decrease in sample A (apple peels) and C (orange peels) was observed. A significant increase in seed germination assay of sample D (potato peels) was observed. Significant reduction of *E. coli* was observed in all samples within 14-days of composting. These results explain benefits of composting under the described controlled conditions in terms of reduction in the coliform content and enhancement of seed germination process.

ANTIMICROBIAL EFFECTS OF SOME HERBAL EXTRACTS ON SKIN AND URINOGENITAL OPPORTUNISTIC PATHOGENS

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Aqueous, ethanolic and n-hexane extracts of eight plants viz, *Coriandrum sativum* (coriander) (seed), *Mentha sylvestris* (mint) (leaves), *Aloe barbadensis* (*aloe vera*) (leaves), *Zingiber officinale* (ginger) (rhizome), *Azadirachta indica* (neem) (leaves), *Camellia sinensis* (tea) (commertially available dried tea), *Melia azadirachta* (derek) (leaves), and *Allium sativum* (garlic) (bulb) were analysed for their antimicrobial activities against four opportunistic microbial pathogens by the disc diffusion method. The test organisms included *Staphylococcus aureus, Escherichia coli, Candida albicans* and *Saccharomyces cervicae.* A given material was loaded on presterile filter paper disc (10mm), which was subsequently placed on test organism inoculated nutrient agar plates. After incubation, zone of growth inhibition (ZGI) were recorded. *Coriandrum sativum*

was found effective against all the microbes except, *C. albicans*. *Mentha sylvestris* was effective against *S. aureus* and *C. albicans. Aloe barbadensis* showed growth inhibition zones against *E. coli, S. cerevicae*, and *C. albicans. Zingiber officinale* showed antimicrobial activity against *S. aureus*, *S. cerevicae* and *C. albicans. Azadirachta indica* and *Camellia sinensis* were effective against all the microbes. *Melia azadirachta* showed antimicrobial activity against *E. coli* and *S. cerevicae.* Herbal extracts showing positive results were mixed in 1:1 ratio, to evaluate their synergistic activity. Among the aqueous extracts, Mint + Ginger was effective against *S. aureus*. *Aloe vera* + Derek was effective against *S. cerevicae.* Among the alcoholic extracts, *Neem* + *Garlic* was effective against *S. aureus* and *E. coli*. Coriander + Neem showed antimicrobial activity against *S. cerevicae*. Among the n-hezane extracts, Ginger + Garlic proved to be effective against *S. aureus* and *S. cerevicae.*The test organisms were also screened for antibiotics sensitivity discs (Oxoid) *S. aureus* showed resistance to the antibiotics ceftriaxone, cefuroxime sodium, trimethoprim and erythromycin. *E. coli* and *S. cerevicae* showed resistance to cefuroxime sodium. *C. albicans* was resistant to ceftriaxone and cefuroxime sodium. These herbal preparations indicate potential of of controlling the microbes in an increasingly antibiotic resistance developing situation.

ISOLATION OF OLEAGINOUS YEASTS AND THEIR CULTIVATION IN AGRO INDUSTRIAL WASTES

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Oleaginous yeasts have been perceived as a promising source of biodiesel. In this study for yeast strains were isolated form a soil sample and cultivated in xylose broth, sugarcane bagasse, whey and the mixture of sugarcane bagasse and whey. Optimum temperature for all the yeast isolates in all the four media was 28^{o}C. The initial pH 4 was found optimum for LR1 and LR3 in xylose, LR1, LR3 and LR4 in M-II and LR1, LR3 and LR4 in M-III. The yeast isolates LR1 through LR4 in MI, LR2 in MIII and LR2 and LR4 in xylose showed maximum growth at initial pH 5 while in M-II the strain LR2 grew maximum at pH3. The yeast isolates LR1 through LR4 showed maximum growth at 10% inoculums in the presence of oxygen supply. The optimum incubation time for LR1, LR3 and LR4 in xylose, LR4 in MII and all the strains in MI and MIII were found to be 72 hours. In M-II, LR1 showed maximum growth at 96 hours, whereas LR2 in xylose and LR3 in M-II grew maximum in 120 hours. Biomass of different isolates ranged from 15 to 35 g/L in the four media. Whereas, the lipids were found maximum for cells cultivated in media having whey. In xylose, yeasts LR1 though LR4 produced 3.2, 5, 4 and 7% lipids of their dry weight, respectively. In sugarcane bagasse 3.2, 6, 6 and 5% lipids were produced by them respectively. The yeasts in whey medium produced 13, 8, 8 and 6.6% of lipids. In the mixture of whey plus bagasse the corresponding values appeared as

produced 5.3, 7, 13 and 10%. Physical non oily look of the extract intracellular lipids as well as FTIR data that dictated absence of double C=C bonds, the yeasts lipids appeared comprising of saturated fatty acids. Which in turn are considered higher energy compounds than unsaturated lipids.

REVASCULARIZATION AND REINNERVATION OF FREELY GRAFTED EXTENSOR DIGIRORUM LONGUS MUSCLES IN RATS REPOND POSITIVELY TO THE PROVISION OF TESTOSTERONE PROPIONATE

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Extensor digitorum longus (EDL) muscles were autotransplanted in gonadectomized, testosterone propionate replaced GTP-I (0.2mg/100bw/day) and GTP-II (2mg/100bw/dayf) rats and the grafts were recovered at 1st through 4th week post grafting periods. They were fixed in Bouin's fixative and routinely stained in Haemotoxylin and Eosin as well as processed for nerve staining. Diameters of the arteries (8.00µm) were found higher in GTP-I than control as well as GTP-II muscle grafts following one month post transplantation. This indicated positive role of appropriate amount of the hormone on the process of revascularization following intact muscle orthotransplantaiton. Thickness of the arterial wall (2.08µm) in the GTP-I grafts increased significantly at 4th week stage than the control. Likewise, diameter of regenerated nerves (6.93µm) as well as thickness of myelinated sheath (1.17µm) increased in GTP-I grafts at 4th week postgrafting than control as well as GTP-II grafts. Thus the anabolic hormone enhanced the process of skeletal muscle fibre regeneration by promoting the processed of revascularization and reinnervation.

ISOLATION AND CULTIVATION OF HYDROGEN (BIOFUEL) YIELDING BACTERIA IN FOOD INDUSTRIAL WASTES

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Purple non sulfur bacteria (PNSB) convert solar energy to H_2but under natural conditions they seldom occur in visible concentrations. However, this diverse group of microorganisms do form coloured microbial mats and blooms in some habitats. In present study, bacterial strains were isolated following enrichment in Pfenning's medium. All the strains grew well at 30°C and failed to grow above 40°C. Morphological and cultural

characterization of the isolated PNSB showed dark red to pink coloured colonies measuring from pinpoint to 2 mm in diameter. These gram –ve and non spore forming rods had bacteriochlorophyll **a** and carotenoids capable of utilizing sucrose, pyruvate, glucose, malate and cellulose as carbon and yeast extract as nitrogen sources. These bacteria appear as potential candidates for producing H_2 fuel gas from diverse carbon sources. The strain designated SS-8 yielded 66 ml of H_2 after 60h or start of the gas production phase, 100 ml of medium containing sugar cane bagasse as major substrate fermentation. The gas production was started after 18h of inoculation. Further work for describing their potential to use some food/agro industrial wastes as carbon/nitrogen source is in progress. Such endeavours are promising for utilizing the industrial wastes with concomittant yield of a value added product. Hence the proposed bioprocesses have inherent incentive for the industrialists to consider applications of biotreatment plants for getting rid of effluents which are polluting our environment.

DETECTION OF *SARCOPTES SCABIEI* VAR. *HOMINIS* CAUSING SCABIES IN LOCAL HUMAN POPULATION BASED ON PCR AND DNA FINGERPRINTING

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Infestation of the skin by the "itch mite" *Sarcoptes scabiei* var. *hominis* results in a contagious ecto-parasitic skin infection in humans, called "scabies". Scabies is ubiquitous and debilitating parasitic human dermatosis, characterized by superficial burrows and intense pruritus. Being prevalent worldwide; it is a major health hitch for people from all socio-economic levels in twin cities of Pakistan, where its epidemiology is related primarily to poverty, hygienic conditions and overcrowding. Moreover, a significantly higher (<0.05) scabies infection was recorded in infants and at early adulthood i.e. 21-39 years. The basic problems regarding this disease are the fact that, often the lesions are masked by eczema or impetigo or are atypical. So, by resolving etiology, morphology and cross transmission issues, a molecular marker system should be established, using an accurate, resulting and swift technique, in order to develop treatment and control programs for human scabies by improving our understanding of the epidemiology of *Sarcoptes scabiei*. Concomitant with this, the present study was designed to establish molecular diagnostic techniques for scabies infestation in human population and to collect skin scrapings and mites samples from scabies patients by visiting different hospitals and clinics of Rawalpindi and Islamabad. For molecular diagnosis, the preferred method for breaking the hard exoskeleton of sarcoptid mites was sonication in order to prepare *Sarcoptes* genomic DNA by commercial DNA extraction kit method. Two primers i.e. Sarms 15 F/R and 16S D1/D2 were used to amplify target

sequence by using PCR. The amplified products were then separated by agarose gel, electrophoresis and analyzed after staining and visualizing in UV trans-illuminator. Upon electrophoresis, a 178bp band was obtained with mite specific primer Sarms 15 F/R and band size of 460 bp was observed with 16S D1/D2, and it indicate the amplification of mite gDNA only, leading to the development of a PCR based diagnostic test for scabies. Thus, these investigations proved to be an initiative towards the development of taxonomic markers. This will undeniably improve our diagnostics leading to reduce scabies infestation from human population.

EFFECT OF CYSTEINE IN EXTENDER ON MOTILITY, VIABILITY, PLASMA MEMBRANE AND DNA INTEGRITY OF NILI-RAVI BUFFALO (*BUBALS BUBALIS*) BULL SEMEN

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This study was designed to determine effect of cysteine in semen extender on motility, viability plasma membrane and DNA integrity of buffalo (*Bubalus bubalis*) bull spermatozoa. Semen from three Nili-Ravi buffalo bulls of similar age group was collected with artificial vagina and qualifying semen ejaculates were split into six aliquots for dilution [37°C; having approximately 50×10^6 spermatozoa ml^{-1}] with *tris*-citric acid extender containing 0.0mM, 0.5mM, 1.0mM, 1.5mM, 2.0mM and 3.0mM cysteine. Semen was cooled to 4°C in 2 hours, equilibrated for 4 hours and filled in 0.5ml French straws at 4°C. The straws were kept on liquid nitrogen vapours for 10 minutes and later plunged into liquid nitrogen for storage. Sperm motility (%; visually), plasma membrane integrity (by supravital hypo-osmotic swelling test), viability (by dual staining procedure with Trypan blue-Giemsa stains) and DNA integrity (by acridine orange assay) was assessed at 0, 2 and 4 hour post-thaw incubation (37°C). Sperm motility, plasma membrane integrity and viability of buffalo semen were higher in extender containing 1.0 and 1.5mM Cysteine compared to extender containing other concentrations of Cysteine. Sperm DNA integrity was higher in extender containing all concentrations of cysteine compared to control. In conclusion, cysteine added in concentrations of 1.0mM and 1.5mM in semen extender improved the freezability of buffalo bull spermatozoa based upon post semen assay.

ANATOMICAL STUDIES ON JATTAL GOAT CERVIX FOR ARTIFICIAL INSEMINATION

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This study aimed at studying the cervix anatomy of local goat breed (Jattal) and its penetration by AI catheter. The animals were tagged before slaughter into two age groups; < 2y and > 2y. Reproductive tracts (n=141) were collected from local abattoir and transported in an ice box to laboratory for further studies. Each group was further categorized on the basis of cyclicity into luteal and non-luteal stage. The length and diameter of cervix and os type (flap, slit, duckbill, rose and papilla) were noted. The straight inseminating catheter was passed through cervix to the greatest point of penetration without exerting force. After measuring the depth of penetration of AI catheter, the internal anatomy of the cervix was studied and cervix was graded as grade I (less number of folds) and grade II (more complicated than grade I). The maximum depth of cervical penetration by AI catheter was affected ($P < 0.05$) by stage of oestrous cycle and was recorded high in NL > 2 y (53.16 ± 4.58 %) than L > 2 y does (20.70 ± 2.26 %). The cervical grade was affected by cyclicity and age of doe ($P < 0.05$). In luteal does the most frequent cervical grade was grade II cervices (35.71%) while in non luteal does the most frequent cervical grade was grade I cervices (49.49%). Grade I cervix was more frequent in < 2y while grade III cervix was more frequent in > 2y. The depth of cervical penetration was high in grade I cervix that was more frequent in non luteal does. It is concluded that the insemination is easier in NL > 2 y which have grade I cervix and slit type os.

EFFECT OF STREPTOMYCIN, NEOMYCIN, POLYMYXIN B AND E WITH PENICILLIN IN EXTENDER ON BACTERIAL CONTROL AND QUALITY OF BUFFALO SPERMATOZOA

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This experiment was designed to evaluate the effect of streptomycin, neomycin, polymyxin B and E with penicillin in extender on post thaw quality of buffalo (*Bubalus bubalis*) bull spermatozoa. Qualifying semen ejaculates from three Nili-Ravi buffalo bulls were splits into five aliquots and diluted (50×10^{-6} spermatozoa/ml; 37ºC) in

extender either containing streptomycin, neomycin, polymyxin B and polymyxin E with penicillin or without antibiotics (control), cooled to 4 ºC in 2 hours and equilibrated for 4 hours. Cooled semen was filled in 0.5 ml French straws at 4 ºC, kept over liquid nitrogen vapours (5cm) for 10 minutes and then plunged in the liquid nitrogen for storage. For the assessment of post thaw semen quality (motility, plasma membrane integrity, viability and normal apical ridge) and total aerobic bacterial count, thawing was performed at 37 ºC for 30 seconds. Sperm motility, plasma membrane integrity, viability, livability and normal apical ridge remained similar ($P > 0.05$) in all experimental extenders containing streptomycin, neomycin, polymyxin B an E with penicillin. However, the lowest bacterial count was recorded in extender containing polymyxin B with penicillin. In conclusion, polymyxin B with penicillin may be used in extender for cryopreservation of Nilli-Ravi buffalo bull spermatozoa to reduce bacterial load without compromising post thaw semen quality.

EFFECT OF ESSENTIAL AND NON-ESSENTIAL AMINO ACIDS ON *IN-VITRO* MATURATION AND FERTILIZATION OF BUFFALO OOCYTES

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In vitro embryo production (IVEP) is the technique that can be used to exploit the genetic potential of our dairy buffalo to the maximum extent. The composition of maturation media affects the development of oocytes *in vitro*, their ability to get fertilized and undergo embryonic development up to blastocyst stage. The present study was planned to observe the effect of amino acids supplementation in maturation media on *in vitro* maturation and fertilization of buffalo oocytes. For this purpose, buffalo ovaries were collected from a local abattoir and brought into laboratory. Cumulus-oocyte complexes (COCs; n=1567) were aspirated from follicles (2-8 mm in diameter) and randomly assigned to four groups for maturation: (1) Basic medium alone as control; (2) Basic medium supplemented with 2% essential amino acids solution; (3) Basic medium supplemented with 1% non-essential amino acids solution; and (4) Basic medium supplemented with 2% essential amino acids solution + 1% non-essential amino acids solution. COCs were incubated in 100 µl drops at 38.5°C in an atmosphere of 5% CO2 with 95% humidity under mineral oil and incubated for 24 h. Cumulus expansion was higher ($P<0.05$) in medium supplemented with essential, non-essential and both (essential + non-essential) amino acids compared to control. Nuclear maturation rate and penetration rate was higher in medium supplemented with non-essential amino acids. These results indicated that the presence of amino acids, especially non-essential amino acids in the maturation medium is beneficial for oocytes maturation and subsequent fertilization *in vitro*.

EFFECT OF THIOGLYCOL IN EXTENDER ON QUALITY OF BUFFALO (*BUBALUS BUBALIS*) BULL SPERMATOZOA

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This study was designed to evaluate the effect of thioglycol in extender on quality of Nili-Ravi buffalo (*Bubalus bubalis*) bull spermatozoa. Semen from three breeding buffalo bulls was collected and qualifying semen ejaculates were split into six aliquots for dilution with *tris*-citric acid extender containing either 0.0 or 0.5 or 1.0 or 1.5 or 2.0 or 3.0mM thioglycol at 37°C having approximately 50×10^6 spermatozoa ml^{-1}. Diluted semen was cooled to 4°C in 2 hours, equilibrated for 4 hours and filled in 0.5 ml French straws at 4°C. Then semen filled straws were kept on liquid nitrogen vapours for 10 minutes and plunged into liquid nitrogen for storage. Sperm motility, plasma membrane integrity, viability and DNA integrity was assessed at 0, 2 and 4 hour post-thaw incubation (37°C). Sperm motility, plasma membrane integrity and viability of buffalo bull spermatozoa were higher in extender containing >1.0mM thioglycol compared to 0.5mM and control. However, sperm DNA integrity was higher ($P < 0.05$) in all extenders containing thioglycol compared to control. In conclusion, thioglycol 1.0mM in extender improved the motility, plasma membrane integrity, viability and DNA integrity of buffalo bull spermatozoa.

MOLECULAR CHARACTERIZATION OF SEVEN DIFFERENT SPECIES OF FUNGI *(ASPERGILLUS)* THROUGH RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) AND ENZYME ANALYSIS

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The aim of this study was to characterize specie of the same genus at molecular level. Phenotypic and genotypic correlation was strong evidence in case of finding differences among species as all phenotypic variations always depicted differences at molecular and biochemical level, showing that genetic diversity on molecular basis is obvious in the environment. DNA was extracted from seven different species of *Aspergillus* followed by RAPD analysis. RAPD-PCR conditions were optimized for two primers of series B i.e. GL Decamer B-09 and GL Decamer B-I0 out of 10 total primers. RAPD results were evaluated by statistical software known as Minitab to form a phylogenetic tree. GL Decamer B-09 showed 38 bands and GL Decamer B-I0 gave 46 bands, with all seven species to show similarity pattern within genera as 50% and 75% with the two RAPD primers respectively. Biochemical characterization was done by screening of zones production with particular enzyme activity of each specie resulted in particular substrate degradation.

EFFECT OF KISSPEPTIN TREATMENT ON PLASMA ESTRADIOL CONCENTRATION IN SPINY TAILED LIZARD (*UROMASTYX HARDWICKII*)

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Kisspeptin, a neuropeptide product of the KiSS-1 gene, is known to regulate the release of gonadotropin releasing hormone (GnRH) from the hypothalamic GnRH releasing neurons that act as primary site of action for kisspeptin. Kisspeptin binds with a G protein coupled receptor, GPR-54, to mediate its action as a key regulator of hypothalamic-pituitary-gonadal axis and as a regulator of puberty onset. A number of studies have investigated kisspeptin's role in gonadal steroid release in mammals, however, similar work on submammalian vertebrates have not been reported. The present study was carried out to investigate the role of kisspeptin-10 in modulating serum estradiol levels in non-breeding season in male *Uromastyx hardwickii* following acute intraperitoneal doses of kisspeptin-10. Animals were grouped as: Group-I (normal control); Group-II, III, IV and V injected with 1 µg, 10 µg, 50 µg and 100 µg kisspeptin respectively; Group-VI (70 IU hCG); Group-VII (100 IU hCG); Group-VIII (50 µg kisspeptin antagonist+saline); Group-IX (50 µg kisspeptin antagonist+50 µg kisspeptin). Sequential sampling was done at time intervals of 0, 30, 60, 120 and 240 min. Blood was aspirated through cardiac puncture. Plasma estradiol concentrations were estimated through enzyme immunoassay (EIA). Comparisons were made through paired ANOVA and single factor one way ANOVA for within and between treatment groups and with the control. A highly significant increase was observed in estradiol levels on of kisspeptin-10 and hCG administration. In contrast, no significant increase was observed in the where kisspeptin action was blocked by its antagonist. Here we demonstrate that kisspeptin-10 profoundly affect estradiol concentration in male *Uromastyx hardwickii* and indicates that kisspeptin is fully active in reptiles and may nave roles in reptilian reproduction similar to those described in mammals.

EFFECT OF INSULIN ADMINISTRATION ON RECRUITMENT OF MYOBLASTS AND DIAMETER OF REGENERATED MUSCLE FIBRES IN FREELY GRAFTED EXTENSOR DIGfRORUM LONGUS MUSCLES IN RATS

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Extensor digitorum longus (EDL) muscles were transplanted in saline injected control and insulin administered (IU/0.1ml/l00gb.w./day) rats. The hor~one as well as saline were injected starting from the day after transplantation. The grafts were recovered

at 1 st through 4th week post grafting periods. They were fixed in Bouin's fixative and routinely stained in Haemotoxylin and Eosin. Longitudinal sections (8 µm) were observed in a calibrated ocular micrometer fitted microscope with the help of 100 oil immersion objective. Number of myonuclei within a regenerating/regenerated muscle fibre were counted upto 100 units of the micrometer. These data from replicates of a given group were averaged and calibrated as number of nucleil/100µm. At one week post grafting the number of myonuclei in insulin supplemented EDL muscle grafts was 7.53, while the control could attain a figure of 6.99/100 µm of regenerating myotubes. At 2nd week postgrafting the insulin treated grafts were characterized with 55.48% increased number of the myoblasts as compared to the control regenerating muscle fibres. This trend continued so that in 4-week hormone treated muscle transplants the parameter appeared 57.14 % elevated over the control value. These results clearly indicate positive role of exogenously supplied insulin in enhancing the activation/proliferation of myosatellite cells in regenerating muscle fibres. When diameter of regenerated muscle fibres was compared at first observational period, the insulin supplemented regenerating muscle fibres had 20.79% higher value than the control (17.70 µm). The parameter appeared comparable at the 2nd study period for the two categories of the grafts. While at 3rd and 4th sampling points the hormone treated regenerated muscle fibres had 25.16 % and 25.09% lesser diameters than the respective control fibres. It is concluded that additional supply of insulin increases the recruitment of myoblasts in regenerating muscle fibres. But the hormone could escalate development of regenerating muscle fibres only during the initial phases and later the catabolic effects of surplus insulin appeared.

INOCULUM DEVELOPMENT FOR ESCALATING COMPOSITING PROCESS OF HOUSE HOLD WASTE

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Compositing is aerobic biotransformation of wastes by microorganisms naturally present in/on the substrates. However, external microorganisms can be added to promote the compo sting process. Inoculation of compost with effective cellulolytic and lignoicellulolytic organisms fastens the process of compo siting. In the present study fifteen bacterial strains, were tested for their capability to grow on agricultural wastes. Inocula were prepared after optimizing the bacterial strains for inoculum size, mode of aeration and percent provision of the compost medium. These strains were also analyzed for their cellulolytic and amylolytic activities. All of the fifteen strains were able to grow on the compost medium (peels of potato and banana and wheat straw, 2:3:4). All the strains showed maximum growth in 2.5% of the compost medium. Inoculum size and mode of aeration varied in different strains, however, majority showed maximum growth with 20% inoculum size and 12 hrs aeration period. The bacterial strain exhibiting highest amylolytic and cellulolytic activities was selected for inoculum production for

compo siting of potato and banana peels. The bacterium can be employed for raising organic fertilizers form urban house hold wastes.

DESIGNING LOW COST MEDIA FOR THE CULTIVATION OF ANTIMYCOTIC CHITINOLYTI BACTERIA OF *BACILLUS* SPP.

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Antimycotic activity of chitinolytic bacteria was evaluated in low cost media. Primary screening of chitinolytic bacteria was done by cross streak method against *Saccharomyces cerevisiae,* three isolates of *Candida albicans,* and one each of *Trichophyton rubrum, Trichophyton mentagrophytes* and *Aspergillus spp.* Bacterial isolates showing antifungal activity were cultivated in low cost media; yeast extract (5%), bread crumps (5%), bagasse (2%) , whey and bagasse + whey (1:1), using nutrient broth as a standard. Growth inhibition effects of cell free culture fluids and their 5x concentrates were evaluated by filter paper disc diffusion method. Chitinolytic bacterial isolates showing diameter of growth inhibition zones (GIZ) around 12mm were optimized for temperature, pH, inoculum size and oxygen requirement. Growth curve was studied upto 45 hours. Cell free culture fluid of 12, 24 and 36 hours of bacterial isolates was evaluated for antifungal activity. Largest growth inhibition zone measured upto 12.67mm for 24 hours old cell free culture fluid of the bacterium It-29. A batch scale antibiotic production of selected bacterial isolates designated as It-08 and It-13 was carried out in a 2L jar glass mechanically agitated fermenter (MBF Eyela, Japan). The 5x "concentrates of harvested fluid were examined against the test organisms. The cell-free fluid of It- 08 yielded GIZ of 10mm for *Saccharomyces cerevisiae* and 11.67mm for *Aspergillus spp.* While the culture fluid of It- 13 yielded GIZ of 15.00mm for *Candida albicans.* Based on physiochemical and biochemical characterization, the bacterial isolates It-29, It-OS, It-18 and It-13 represented the genus *Bacillus* according to Bergey's manual of determinative bacteriology. The chitinolytic bacteria are required to be cultivated in more media formulations to identify more suitable substrates for obtaining the antifungal exoproducts.

EFFECT OF HUMAN CHORIONIC GONADOTROPIN (BCG) ON *IN VITRO* OOCYTE MATURATION IN FRESHWATER CYPRINID, *BARILIUP VAGRA*

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Exposure of Barilius *vagra* ovarian follicles with human chorionic gonadotrpin (hCG) resulted in a maturation of oocyte but the response being time dependent. There

was slight but significant (P<0.05) increased in germinal vesicle break down (GVBD, 12.42% vs 2.94 %) at 24 hrs. Prolonged incubation further increased GVBD (29.8% at 72 hr). Moreover hCG treatment at 72 hrs enhanced the synthesizing capability of the vitellogenic oocytes. There was significant increase in the secretion of estrdiol- 178 (P=0.0001) and non significant increase in testosterone (P= 0.07), whereas *17α*-hydroxyprogesterone (17-OHP: 17a-hydroxypregn-4-ene-3, 20-dione) and 17α-20ß-dihydroxypregn-4-ene-3-dione (17,20ßP: 17α-20ß-dihydroxypregn-4-ene-3-one) were detected and identified only in the hCG treated incubation medium. Morphological changes in the granulosa layer of oocyte such as abundance of mitochondria with heavily coated matrix, increased number of free ribosomes, rough and smooth endoplasmic reticulum and retraction of microvillar process (contact between microvilli of granulose cells and pore canals of zona radiata in response to hCG revealed the role of gonadotropin in the induction of maturation of oocyte.

EFFECT OF AN ANTI-THYROID DRUG, 2,8-DIMERCAPTO-6-HYDROXY PURINE ON REPRODUCTION IN MALE RATS

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This histomorphological study is designed to evaluate the peripheral action of 2,8-Dimercapto-6-hydroxypurine (an antithyroid drug) on male reproductive system. The drug was administered as i.p injection for 21 days to investigate its role on morphology of intratesticular cells and plasma testosterone level. Adult male rats (n=12), divided into three groups i.e. control, dimethylsulphoxide (DMSO) and 2,8-Dimercapto-6-hydroxypurine treated groups and treated with saline, DMSO and 2,8-Dimercapto-6-hydroxypurine for 21 consecutive days respectively. Blood samples were collected at day 1,7,14 and 21 and analyzed by using EIA systems. All the animals were scarified on 22nd day and testicular tissues were studied by histomorphpological assesment. 2,8-Dimercapto-6-hydroxypurine caused a significant decrease (P<0.0001) in mean testicular cell population, testicular cell diameter and resulted in arrested spermatogenesis. A significant decrease (P<0.0001) was observed in mean Sertoli and Leydig cell population and diameter in treated group. Similarly a significant decrease was observed in plasma testosterone level at day 1 (P<0.004), day 7 (P<0.05), day 14 (P<0.03) and day 21 (P<0.04) of drug treatment. The present study suggests that 2,8-Dimercapto-6-hydroxypurine is a negative modulator of reproductive system as it suppressed the plasma testosterone level and proliferation of different testicular cell types in adult male rats.

ANTISPER ANTIBODY ASSOCIATED WITH INFERTILITY IN MEN

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In this study role of seminal plasma antisperm antibodies (ASAs) in regards to semen parameters were investigated in human male subjects. The study population consisted of (n=lO) fertile (without any history of fertility problem) and (n=72) infertile male subjects. Semen samples were collected from each patient and seminal plasma was separated by centrifugation and stored at -20°C. Detailed medical information was obtained using prescribed Performa. Semen volume, sperm count, computer assisted sperm analysis (CASA) motility parameters: straight line velocity (VSL), Average path velocity (VAP), curvilinear velocity (VCL), amplitude of lateral head displacement (ALH), wobbling (WOB), linearity (LIN), percent sperm motility were measured according to WHO Criteria. Seminal Plasma antisperm antibodies (ASAs) were estimated by antisperm antibody enzyme linked immunosorbant assay (ELISA) kit from BIOSERV. In present study infertile patients with sperm agglutination (SA) had shown highly ~ignificant (P<0.00l) increased antisperm antibodies (ASAs) concentration in the seminal plasma. While asthenoteratozoospem; (ATZS) patients showed significant (P<0.05) increased in antisperm antibodies (ASAs) in their seminal plasma. Whereas patients with oligozoospermia (OZS), azoospermia (AZS), and oligoasthenozoospermia (OAZS) had decreased sperm concentration had shown non significant increased in ASAs in their seminal plasma. There was significant difference in semen volume (P<0.01) among fertile and AZS While oligozoospermia (OZS), oligoasthenozoospermia (OAZS), asthenoteratozoospermics (A TZS) and sperm agglutination (SA) infertile male subjects did not show significant difference in semen volume. In conclusion, this study suggests that seminal plasma antisperm antibodies (ASAs) is one of the important factors in male infertility especially, in sperm agglutination and asthenoteratozoospermics infertile male subjects and it can be used as a diagnostic tool for male fertility evaluation.

PATTERN OF GENETIC HETEROGENEITY AND GENE DIVERSITY AT *ABO* AND *RH* LOCI IN BAJAUR AND MOHMAND AGENCIES, FATA, PAKISTAN

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In order to understand the genetic heterogeneity and gene diversity indexes in the remote populations of Bajaur and Mohmand Agencies blood group phenotypic data was obtained from 2,219 umelated subjects. The allelic frequencies at ABO locus ranged from 0.188-0.324 for allele A, 0.165-0.309 for allele B, and 0.367-0.640 for allele O. At Rh locus the allelic frequencies ranged from 0.355- 0.811 for allele D. In order to analyze the

genetic differentiation within the populations of Bajaur and Mohmand Agencies, various indexes of gene diversity were established. Combined heterozygosity (Hs) at *ABO* and *Rh* loci in Bajaur sub-population was very low (0.47866) compared to Mohmand (0.51079), depicting more diversification in Bajaur population. Additionally, total heterozygosity (HT) was depleting in Bajaur (0.50158) compared to Mohmand (0.53043), demonstrating loss of heterozygotes a nd increased homozygotes in Bajaur Agency. Consequently, gene diversity index (GST) was higher in Bajaur (0.04570) contrasting to Mohmand (0.03703). Furthermore, the gene differentiation (GST) in total population of both agencies was remarkably higher (0.04508) than the similar estimates for Khyber Pukhtunkhwa population (GST = 0.00493). The se analyses using the classical immunogenetic markers clearly revealed more population stratification, fragmentation and diversification in both Agencies, the phenomenon being more pronounced in Bajaur. Further studies with the help of extended panel of highly informative micro satellite markers are required to understand the micro-evolutionary phenomenon taking place in these populations.

GENETIC HETEROGENEITY, GENE DIVERSITY AND CLINAL ANALYSIS AT ABO AND RH LOCI AMONG KHYBER PUKHTUNKHWA POPULATION

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ABO and Rh blood groups phenotypic data of 141,942 subjects originating from 14 Pukhtoon populations of Khyber Pukhtoonkhwa (KPK), Pakistan, was analyzed for genetic heterogeneity, gene diversity and geographic allelic variations. There was a wide variability in the proportions of blood types. Collectively, blood group B was most prevalent, 33.70%, followed by types O, A and AB, 29.15%, 27.87% and 9.77%, respectively. At the Rh locus, the fluctuations were not remarkable (92.75% of total sample was Rh+). Z-statistics employed to understand homogeneity among the blood group proportions among populations, revealed that the cosmopolitan and multi ethnic populations were more heterogeneous. The heterogeneous populations (ie, Mardan, Peshawar, DI Khan) nevertheless were observed to be deviating from Hardy-Weinber.g equilibrium expectations. Clinal analyses depicted only minor systems of regional trends. However, geographic allelic variations at larger latitudes were unremarkable. Gene diversity (GST) was observed to be 0.00493 and total heterozygosity (Hr) was estimated to be 0.393476. These analyses showed that there was substantial diversification among the populations and they were subdivided. Collectively, homogeneity analyses and gene diversity indices at ABO and Rh loci employed for meaningful grouping of Khyber Pukhtunkhwa populations revealed that a simple, straightforward pairing based upon, common language, geographic neighborhood, or long shared political boundaries, was not possible. Bootstrapping conducted through the genetic distances (DA) depicted that larger and cosmopolitan population were distinct and emerge as a separate cluster from

rest of the populations. Further studies are under way in order to understand the exact micro-evolutionary forces underlying this present heterogeneity in KPK population.

ASSESSMENT OF SELECTED PERSISTANT ORGANIC POLLUTANTS (POPs) IN DIFFERENT FISH SPECIES FROM STREAMS OF SIALKOT DISTRICT, PAKISTAN

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Samples of four fish species (*Cirrhinus reba, Oreochromis niloticus, Channa punctata and Puntius sophore*) were collected from the Nullah Aik and Palkhu, southern tributaries of the River Chanab, Sialkot and were analysed for eleven Persistent Organic Pollutants (POPs) viz., Beta-HCH, Lindane, Heptachlor, Heptachlor-Exoepoxide, Heptachlor-Endoepoxide, Dieldrin, DDD, DDE, Endrin, 2, 4-DDT and 4, 4-DDT. Residue of Beta –HCH, Lindane, Heptachlor, DDD, DDE, Endrin, 2, 4-DDT and 4, 4-DDT was present in four fish species. Increasind trend in concentration of POPs was observed from upstream to downstream in fish samples. The concentration of POPs was higher in *Cirrhinus reba* followed by *Oreochromis niloticus, Channa punctata* and *Puntius sophore. Cirrhinus reba*, a herbivorous species showed highest concentration of pesticide residues in upstream as well as in downstream. The results were compared with international guidelines values for pollutant concentration in fish. Concentration levels of studied POPs were found higher than the recommended limits set by World Health Organization (WHO) and United States Environmental Protection Agency (US, EPA) for human consumption. The results of this study highlighted its usefulness in the management of POPs in study area and its catchments.

COMPUTATIONAL PREDICTION OF FUNCTIONAL CONSTRAINTS TO ENGINEER MICRORNA FOR ANTI-HCV THERAPY

ATIYA MAHMOOD, SANA AIZAD, SHAHEEN SHAHZAD

MicroRNAs are the small moieties comprising of 20-22 nucleotides, firstly reported as the gene expression controller entities at post transcriptional level but the later research proved their function as the interacting layer between the host and the viruses. This feature has been empowered to silence gene expression either in host or in the virus, because they can cope with the heterogenetic forms of different viruses, as they require only partial complementarity to target the desired gene. Hepatitis C Virus occurs in

alternative genetic forms which even escape the RNAi, so the lack of effective treatment has associated high prevalence rate with it. The present research work focuses to make out the sequential constraints that are necessary to engineer a MicroRNA that can target the genetically variant forms of Hepatitis C Virus, so that a potential and economical therapy against Hepatitis C can be developed. Well established computational techniques based upon the so far predicted rules for MicroRNA-mRNA binding, have been used to predict the human MicroRNA targets in most prevalent genotypes of the HCV. Cut-off value and consensus scoring approach has been used to identify potential MicroRNA-mRNA duplexes. Keeping in view the artificially engineered MicroRNA therapy for multiple HCV genotypes, the microRNAs were identified which targeted same gene in more than one genotypes. Hsa-mir-1202 targeted E2-Env-Protein gene in genotype 1, 2, 5 and 6, while hsa-mir-196a* targeted core protein gene in genotype 1, 2, 4 and 5 and hsa-mir-551b found to target 5`-UTR in genotype 1, 3, 4 and 5. The target sites for the respective microRNAs were recognized as significantly conserved to engineer a microRNA that can target more than 3 genotypes unanimously. The identified conserved target sequence can be further used to synthesize artificial MicroRNA that can be used as a therapeutic against Hepatitis C.

TOXICITY OF ORGANOPHOSPHATE PESTICIDES ON *APHANIUS DISPAR* (KILLIFISH)

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Ecotoxicological methods are used to determine lethal limits of pesticides on aquatic organisms. The coastal waters of Pakistan receive pesticides from industrial and agricultural wastes from Indus River and other tributaries along Sind and Baluchistan coasts. Pesticides and the pollutants enter the food chain and tend to accumulate in organisms at higher trophic level. In the present study an attempt has been made to investigate the acute toxicity of organophosphate pesticide on *Aphanius dispar* (killifish). Acute toxicity of the pesticides to marine organism was estimated by determination of the 24 hour LC_{50} and biochemical effect of pesticides on Total Protein content was determined by Biuret method. The fish were exposed to five successive concentrations of pesticides for 24 hours. The results obtained from this experiment using probit analysis indicated that the LC_{50} values of two pesticides were 0.0025 for chlorpyrifos and 0.039 for methyl parathion respectively. *Aphanius dispar* when exposed to organophosphate pesticides shows that these pesticide inhibits Total Protein content. Low LC_{50} values for *Aphanius dispar* tested in the present study show that marine organism is highly sensitive to organophosphate pesticides.

GENETIC EPIDEMIOLOGY OF HEREDITARY MALFORMATIONS AND THEIR ASSOCIATION WITH CONSANGUINITY IN DISTRICT BHIMBER, AJK

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Congenital anomalies (CA) account for 3-5% of total live births globally and are a significant cause of postnatal mortality. To explore the prevalence of CA in District Bhimber Azad Jammu & Kashmir, an observation based epidemiological study has been conducted. Cluster sampling technique was employed to ascertain 1,731 female subjects from three *tehsils* of Disrtict Bhimber and data regarding demography, consanguinity and dysmorphology was obtained. Collectively, 79.99% marriages were consanguineous with 55.18% first-cousin type, 8.84% second-cousin marriages, and 15.97% distantly related marriages. The non-related marriages were only 20.10% of the total marriages. Overall inbreeding coefficient (F) was 0.0348. Consanguinity was positively associated with younger age, low marriage age, rural status and high education level of subject ($p \leq 0.05$). The estimates of consanguinity and inbreeding coefficient in Bhimber were significantly high than the previous reports for certain population in upper Punjab, Pakistan. A total of 73 CA were observed with a prevalence rate of 42.17/1,000. The most frequent CA were limb defects (n=50; 68.50%), followed by ear-neck-face problems (n=17; 23.29%), neurological disorders (n=5; 5.48%) and ectodermal defects (n=2; 2.74%). Among the limb defects, club thumb was predominant (n= 18; 36%), followed by brachydactyly (n=12; 24%), camptodactyly (n=8; 16%), clinodactyly (n=6; 12%), polydactyly (n=4; 8%), syn-polydactyly (n=l; 2%) and brachy-syndactyly (n=l; 2%). Collectively, 94% limb defects were sporadic while the rests (6%) were familial. In our data set, the disease status of subject was not associated with consanguinity level of the subject (X^2=0.8063; p=0.6682). This is a pioneer study to ascertain consanguinity and congenital anomalies in District Bhimber. Additional studies on extended sample size would be helpful in elucidating a comprehensive picture of consanguinity and dysmorphology in the area.

MICROBIAL ANALYSIS OF DIFFERENT TOP SOIL SAMPLES OF SELECTED SITES OF GILGIT

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A study was carried out from November 2010 to March 2011 in two main towns of Gilgit-Baltistan (GB) namely Gilgit city and Danyor, during this study the soil of both regions were analyzed for bacteria and fungi. The area was divided in to sub-sample areas according to fertilizers being used i.e. Gilgit where organic fertilizers are used

(GCD), Jutial where inorganic fertilizers are used (JIF) and Danyor where night soil was used (DNS) from each area soil samples were aseptically taken by X method from a depth of 1.3cm in laboratory soil type was determined according to USDA triangle, total viable cells are counted through dilution plate method, isolates were identified according to their cultural colonial morphology biochemical characteristics with reference to Bergey's manual of systematic bacteriology for bacteria and Talbot(1976) to fungi. The type of soil determined was loamy sand and total cell count for bacteria was between 6x 10^5-3x 10^6 cfu/g of soil while that of fungus was 10^2 to 10^4 cfu/g of soil. A total of seven bacterial species and four species of fungus were isolated and identified. In Gram negative was dominated by *Aromonas* and the fungal isolates were dominated by *Rhizopus.* Total bacterial count does not vary according to the fertilizers but high fungal count was observed in GCD. These results correspond that microbial diversity does not change greatly due to agricultural practices.

ISOLATION AND CHARACTERIZATION OF ACETYLCHOLINESTERASE (AChE) ACTIVITY FROM *PLANOCOCCUS CITRI*

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Acetylcholinesterase (AChE) hydrolyzes its physiological substrate acetylcholine at one of the highest known catalytic rates. In this study AChE activity was isolated and partially characterized from *Planococcus citri. P. citri* cause highly damaging effects on citrus plants. Crude protein extract was prepared by homogenizing the insect heads in Phosphate buffer pH 8.0. Crude enzyme contained the 740µg/ml of total protein which after acetone precipitation checked as 980µg/ml. AChE activity was screened. Total enzyme activity in the crude sample was 1.034 U/min/ml in crude homogenate. While in acetone precipitated sample activity was 1.634 U/min/ml. Gel filteration was performed by loading the 10 ml of crude sample. Active fractions were pooled out and subjected to ion exchange chromatography. Active fractions from IEC were further loaded on Native PAGE gel and molecular weight was calculated as 68.5 kDa.

ANALYSIS OF SERUM TRIGLYCERIDES LEVEL OF GOATS IN GILGIT-BALTISTAN

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Triglyceride is the most common form of fat in the body. Almost all the calories,

whether from fats or carbohydrates are converted into triglycerides and stored in the body. It is used as a source of high energy during food shortage and even in normal conditions. With an increase in age there is significant decrease in the triglyceride level. There is no significant correlation between serum cholesterol, triglycerides and lipoproteins. Blood Triglyceride analyses are extensively used as an indicator of energy status in the goats. The present study was proposed and conducted on Goat (both male and Female) having the age of two years based on teeth composition in three regions of Gilgit-Baltistan viz; Ghizer, Baltistan (Skardu, Ghanchey) and Hunza-Nagar. Samples of blood were taken randomly from 480 goats (120 No from each District) and serum was obtained by using centrifuge machine. Quantity of Triglyceride in mg/decilitre of serum was determined by analyzing the serum in Micro-lab 300. The results revealed that the average blood urea in male goats were 90.75±14.02mM/dl in Ghizer, 123.35±67.96mM/dl in Baltistan, 124.82±59.31mM/dl in Hunza-Nagar and triglyceride level obtained from female was 87.44±14.15mM/dl in Ghizer, 111.86±55.56mM/dl in Baltistan and 89.50±15.83mM/dl in Hunza-Nagar respectively. This study has shown a significant variance in the mentioned districts and for male and female.

ANALYSIS OF BLOOD GLUCOSE LEVEL IN THE GOATS OF GILGIT-BALTISTAN

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Glucose is used as fuel for production of energy and has a vital role for maintenance of physiological phenomena of the cell and in turn that of body. The blood Glucose level in ruminants is actively transported through blood stream. The ruminants are able to utilize Glucose as a source of energy and are a major form of circulating carbohydrate in blood. The blood glucose level is maintained by the action of two hormones insulin and glucagon. So far no study has been conducted to evaluate the blood glucose level in goats in Gilgit-Baltistan. The present study was proposed and conducted on Goat (both male and Female) having the age of two years based on teeth composition in three regions of Gilgit-Baltistan viz; Ghizer, Baltistan (Skardu, Ghanchey) and Hunza-Nagar. Samples of blood were taken randomly from 480 goats (120 No from each District) and serum was obtained by using centrifuge machine. Quantity of Triglyceride in mg/deciliter of serum was determined by analyzing the serum in Micro-lab 300.0. The aim of this study was to evaluate the glucose level as an indicator of energy in the goats of Gilgit-Baltistan feed on pure organic food at pastures of different regions. This study has reflected the pasture's potential in the four major regions of Gilgit-Baltistan. The average level of Glucose was obtained from male was 65.50±16.77 in Ghizer, 76.40±16.11 in Baltistan, 87.95±11.52 in Hunza-Nagir and Glucose level in female was 67.94±15.02 in Ghizer,70.92±15.01 in Baltistan,83.82±9.51 in Hunza Nagar respectively. This study has shown a significant variance with sex and region.

ANALYSIS OF UREA IN THE BLOOD SERUM OF GOATS IN GILGIT-BALTISTAN

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The urea present in the blood serum of ruminants appears to be actively transported across the rumen wall into the lumen and used as a nitrogen source. The ruminants are able to use the urea as source for food protein. Blood urea analysis is extensively used as indicator of protein nutritional status in goats. The present study was proposed and conducted on Goat (both male and Female) having the age of two years based on teeth composition in three regions of Gilgit-Baltistan viz; Ghizer, Baltistan (Skardu, Ghanche) and Hunza-Nagar. Samples of blood were taken randomly from 480 goats (120 No from each District) and serum was obtained by using centrifuge machine. Quantity of Urea in mg/deciliter of serum was determined by analyzing the serum in Micro-lab 300. The results revealed that the average blood urea in male goats were 65.08 ± 28.50 mg/dl in Ghezir, 70.6 ± 39.00 mg/dl in Baltistan and 57.15 ± 29.45 mg/dl in Hunza-Nagar respectively. And average blood urea in female goats was 56.80 ± 16.17 mg/dl in Ghizer, 54.76 ± 17.84 mg/dl in Baltistan and 54.16 ± 14.68 mg/dl in Hunza-Nagar respectively. The study also revealed that the differences observed in between male and female goats and region to region was nonsignificant.

COMPARISON OF BEETLE AND TERMITE CELLULASES ON THE BASIS OF THEIR STRUCTURE AND SEQUENCE

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Animals were thought to be dependent upon the intestinal bacterial flora for the cellulolytic activity but gene prediction made it possible to sort out some gene in lower animals responsible for such activity. Termites were the model lower animals to show the presence of cellulase gene. Latter, in other animals including beetle related gene were discovered. In present study, we have developed a comparison between beetle and termite cellulases. All available sequences belong to these animals were retrieved from the all databases. Then redundant sequences were removed. Some complete sequences were BLAST to get sequences related to cellulases and those one which function is not yet confirmed (Cellulase like activity sequences). Along with that, all reported three dimensional structure of cellulases belong to these animals were retrieved from Protein

Data Bank and Homology Modeled Databases. SwissprotModel Server was used for preparing a homology model for those sequences whose structures were not available. After that sequences Alignments and structure comparison was performed using the different bioinformatics tools including Clustal (W,X), Blocks Searcher, MOE, Swissprot Viewer and YASARA. Catalytic consensus regions were found and compared in the species and between the species. A structure comparison was performed showing the location of active site in the structures.

ANALYSIS OF SERUM PROTEIN LEVEL IN THE GOATS OF GILGIT-BALTISTAN

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Serum proteins have an important physiological role in the body. The evaluation of serum protein level provides useful information as an indicator on homeostasis of body. So for no research had been conducted to collect base line information about total serum protein level in goat found in Gilgit-Baltistan. The present study was proposed for determination of serum protein level in goat and conducted on both male and Female goats having the age of two years (based on teeth composition) in three regions of Gilgit-Baltistan viz; Ghizer, Baltistan(Skardu,Ghanche) and Hunza-Nagar. Samples of blood were taken randomly from jugular vein of 480 goats (120 No from each region) and serum was obtained by using Centrifuge Machine. Quantity of Protein in g/dl of serum was determined by analyzing the serum in Micro-lab 300. The study showed that the average blood protein level in g/dl of serum in male goat is 5.048±1.528 in Ghizer, 5.192± 0.695 in Baltistan, 5.714± 1.072 in Hunza-Nagir respectively and in female goat is 4.9000±0.7640 in Ghizer, 5.2606±0.6777 in Baltistan, 5.4253±0.7713 in Hunza-Nagir respectively. Beside determination of serum Protein level information regarding goat husbandry practices in the area is also collected and documented. The study also reveals that the protein level is significant in both male and female and for the region as well.

BIOMONITORING OF THE TRAFFIC RELATED ZINC AND COPPER POLLUTION USING ROADSIDE PLANTS AS POSSIBLE BIOINDICATORS

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Three roadside plants occurring widely in many regions Sargodha (Pakistan) were used as case study (as possible biomonitors) to access the potential effects of traffic pollution on accumulation of Zinc and Copper in leaves of Dalbergia *sissoo* Roxb.,

Prosopis juliflora L. and *Eucalyptus spp.* that were collected from different locations. Different roads selected based on traffic density and industrial pollution. The roads categorized then into five areas i.e. Urban (30,000 vehs/day), Suburban (15,000 vehs/day), Industrial (35,000 vehs/day), Roadside (40,000 vehs/day) and Rural (10,000 vehs/day). Eucalyptus showed highest and lowest concentration of Zn (86.39±48.39-27.98±4.83) in industrial and rural area respectively. But as for the Cu accumulation is concerned highest concentration was observed in *Prosopis* (74.82±16.11) in roadside area and lowest in *Dalbergia* (42.76±14.70) in rural area. All the selected plants proved effective biomonitors of heavy metal pollution in the study area.

BIOMONITORING OF THE TRAFFIC RELATED HEAVY METAL POLLUTION USING ROADSIDE PLANTS AS POSSIBLE BIOINDICATORS

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The degree of heavy metal pollution in Sargodha city was determined on different selected areas using three different plants as bioindicator. The leaf samples of *Dalbergia sissoo* Roxb., *Prosopis juliflora* L. and *Eucalyptus spp.* were collected from different sites including Industrial, Roadside, Urban, Suburban and Rural in Summer and Winter season and analyzed for heavy metals Fe, Mn, Co and Ni concentration. These elements were detected and estimated by using atomic absorption spectrophotometer. Results of correlation analyses showed significant correlations among Fe, Mn, Co and Ni contents in leaf samples. Among these Fe was present in highest concentration in plant leaves during summer and Ni was present in lowest concentration during summer. There exist a significant correlation between these heavy metals (Fe, Mn, Co, Ni) concentration in these plant samples. Thus leaves of *Dalbergia sissoo* Roxb., *Prosopis juliflora* L. and *Eucalyptus spp.* were found to be the most suitable bioindicator of heavy metal pollution.

COMPARATIVE ANALYSIS OF ZINC STATUS AMONG DISEASED PATIENTS USING HAIRS AS BIOMARKERS

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Zinc acts as a street traffic controller of human body because it directs and controls many important processes in the body by maintaining the appropriate state of

enzymes in the organs and cells. Zinc deficiency, imbalance or toxicity can lead towards progression of certain diseases. Zinc level fluctuates during different diseases such as tuberculosis, cataract, diabetes mellitus and renal failure. Zinc also plays curative and preventive roles in combating diseases such as dermatitis. These mineral incorporated in different tissues and in body fluid. By analysis of these tissues and fluids, one can estimate the body elemental imbalance. Hair sample analysis is a useful biomarker for detection of zinc imbalance in diseased patients. During present investigation study zinc status was found lower in patients suffering from dermatitis, renal failure and cataract as compared to normal healthy population.

TOXICITY OF A PYRETHROID INSECTICIDE, DELTAMETHRIN ON VARIOUS BIOCHEMICAL COMPONENTS OF *TROGODERMA GRANARIUM* LARVAE EVERTS

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Trogoderma granarium (Khapra beetle) is a stored grain pest of great economic importance all over the world including Pakistan. Efforts are being underway to control this pest in stored grain godowns by various techniques. In this study some biochemical effects of a pyrethroid insecticide, deltamethrin are being reported against 4th instar larvae of this beetle. For this purpose, larvae of five different populations (Khanewal, Lahore, Lahore old, Muzaffargarh and Sheikhupura) of *T. granarium,* were exposed to deltamethrin (10.5 EC), for evaluation of LC_{50} values, which were required to determine the sublethal doses, for biochemical analysis. The LC_{50} values shown by above five populations were 19.1, 18.51, 18.83, 19.63 and 18.99mg/ml, respectively. Muzaffargarh population with maximum LC_{50}, was named as "resistant", while Lahore population with lowest LC_{50} value was named as "susceptible population" against deltamethrin. Larvae of resistant and susceptible populations of *T. granarium* were exposed to (LC_{20} sublethal) dose of deltamethrin up to 192 hours, to evaluate the biochemical effects of the insecticide on glucose, lipids, soluble protein and total protein contents. After 192 hours insecticide exposure, significant increase in glucose content was observed in resistant population whereas change at 48 hours was non-significant. Lahore old (susceptible) population showed significant increase in glucose content at 192 hours treatment. The total lipid content also showed significant rise in both resistant and susceptible populations through out deltamethrin exposure period. Soluble protein content remained unchanged in both populations up to 96 hours treatment while highly significant increase was observed in resistant population after 192 hours treatment. The total protein content of resistant population showed significant decrease only after 24 hours treatment of

insecticide, whereas on further extending the treatment, nonsignificant change was observed. Lahore old (susceptible) population depicts nonsignificant change up to 48 hours treatment while 96 and 192 hours treatment resulted in significant increase in total protein content.

COMPARATIVE STUDY OF DRINKING WATER OF FAISALABAD AND RAWALPINDI CITIES ON THE BASIS OF BACTERIOLOGICAL ANALYSIS

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Water is the basic constituent of all living beings. Its deficiency or use of contaminated water may interfere with normal physiological functions of living beings. Therefore the aim behind this work was to monitor water quality in two major cities. Fifty water samples were collected by standard sampling technique from twenty five selected points of each city. The water samples were collected from twenty five locations covering the major supply sources of the city of Faisalabad i.e. (4) Tube well, (9) Tap, (2) Hand pump, (8) Injection pump and (2) Donkey pump. Water quality was determined by "Most Probable Number Method". Out of twenty five locations, eleven sources were found unfit for human consumption with 40% Total Coliforms and 36% *Escheria coli.* The water samples from Rawalpindi city were collected from twenty five locations covering the major water supply sources i.e. (21) Tube well, (2) Water supply and (2) Bore. Out of twenty five locations, Seventeen sources were found unsatisfactory with 68% Total Coliforms and 56 % *E.coli.* Analytical findings revealed that Rawalpindi water sources were more contaminated than Faisalabad city.

CONSANGUINITY AND INBREEDING CO-EFFICIENT ESTIMATES IN TWO MAJOR POPULATION ISOLATES (MUSLIMS AND HINDUS) IN DISTRICT RAHIM YAR KHAN

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Consanguineous marriages and inbreeding co-efficient *(F)* have been a source of major scientific interest worldwide due to their potential association with congenital defects and child mortality. To check the dynamics of consanguinity and inbreeding co-efficient, epidemiological data was collected from 2,204 Muslim and 296 Hindu married females of District Rahim Yar Khan. In the Muslim population the mean age of subjects

was 35.28 years and mean age at marriage was 19.17 years. Of the total Muslim subjects, the frequency of close marriages was 96.74% while the marriages in non-relateds were 3.26%. Within close marriages, cousin unions comprised 58.99% and marriages among distantly related were 37.75%. Mean inbreeding co-efficient calculated for the Muslim population was $F = 0.0317$. Inbreeding co-efficient *(F)* was observed to be higher in illiterate subjects, rural origin, Saraiki speaking respondents, and in younger age subjects. The comparison of Muslim and Hindu communities depicted marked differences in their demography and marriage patterns. For instance, in contrast to high prevalence of consanguineous marriages among Muslims, such marriages were totally absent (0%) among Hindus *(F=0).* This pilot study is helpful in getting insight into the socio-biological parameters of the two major populations isolates of Distrct Rahim Yar Khan and understanding the dynamics of consanguinity and inbreeding co-efficient *(F).*

STUDY OF ENZYMES OF *ZIZIPHUS* SP. AND MIXED-FLORA HONEY PRODUCED BY BEES

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Honey is an important nutritive food containing various kinds of sugars, acids, proteins and free amino organic acids with a fairly high caloric value. Honey also contains the trace elements, antioxidants, enzymes and vitamins. Enzyme type is fairly uniform across honey varieties but the amount present can vary widely. The balanced amount of the above components is important in quality criteria of honey. The present study was aimed at to assess the quality criteria of honey collected from different sources as compared to Punjab University (PU) standard honey. Nonavailability of sufficient scientific information on Pakistani honeys has been a major hurdle in effective legislation for the quality control of Pakistani honeys and consequently its export is limited especially of *Zizyphus* (sidder) honey according to the international standards. Among unifloral *(Zizyphus)* honey samples, PU honey produced by *Apis mellifera,* was collected from Oina area, Punjab province while to Unifloral honey samples (Islamic Shahed Centre, Lahore and Tayyaba's honeys) and one multifloral sample (Marhaba honey, Lahore) were collected from local market. The both unifloral and multifloral honey samples were analyzed for the quality parameters such as enzymes, pH, thickness, color and flavour. Among enzymes, diastase, acid phosphatase and glucose oxidase were evaluated according to the international prescribed methods, and compared with those of international honey quality standards (IHOS). Color of all thick unifloral honey samples was dark amber with stronger flavour as compared to light amber with mild flavour found in thin multifloral Marhaba honey. All honey samples showed pH in the range of 3.77 - 6.78. PU (standard) honey sample exhibited the highest diastase activity, 146.00 ON and Marhaba (multifloral) honey sample showed the lowest diastase activity (70.35 ON).

Again PU (standard) honey sample was showing the highest acid phosphatase activity (151.57 U/I) and Marhaba (multifloral) honey sample was found with the lowest acid phosphatase activity (19.12 U/I). Islamic center honey sample showed the highest glucose oxidase activity (13.00 µg/l/h) as compared to PU (standard) honey sample showing the lowest glucose oxidase activity 2.7 µg/l/h. On the basis of this analysis, it is concluded that PU honey is considered as a better as compared to market honeys because it is less acidic with high pH. Comparatively it showed the highest amounts of quality parameters. Less moisture content was, recorded in PU honey which resulted in low hydrogen peroxide (H_2O_2) as high H_2O_2 contents can damage the cells and tissues.

WONDER DRUG FOR THE CANCER

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This is an indigenous calx of *Raskapoor* (an impure Mercuric Chloride mineral both natural or. Synthetic). The carx of the rmneral is-maae in~sutpifur. It had proved its efficacy in every sort of CANCER. But it had adversely damaged liver and killed many patients on the spot. Owing to grave furtherance in the rational reflection I prepared its trituration in Lactose according to Hahnmannian approach. Its 1 X or 1\10 w\w trituration miraculously enhanced the efficacy and reduced adverse effects. While 2X or 1 \100 w\w reduced its medicinal power. In IX Trituration the dose of the drug was reduced from 300mg of raw calx to 70-l00mg of triturated calx. More over the repeated doses of raw calx was hazardous and a large amount of Lipid Food supplement was needed to counter the poisonous effects of the calx. On the other hand the doses of triturated calx were easily repeated without harming the patient.

ISOLATION OF PROTEOLYTIC *BACILLUS* SP., OPTIMIZATION OF ALKALINE, THERMOSTABLE PROTEASE PRODUCTION AND ITS CHARACTERIZATION

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Proteases is a class of enzymes which occupy a remarked position in variety of physiological, analytical and industrial applications all over the world. The use of protease is approximately 60% of the total enzyme sales in various industrial sectors. These enzymes are most widely used in detergents, food processing, leather industry, feather processes, silk gumming, bioremediation, pharmaceuticals, biosyntheses and

biotransformations. Most commercial proteases are produced by *Bacillus spp.* In the present study, an extra cellular, alkaline thermostable, protease producing strain was isolated from soil and identified as *Bacillus sp.* (PCSIRNP3) on the basis of biochemical characteristics. The most appropriate medium fm the protease production was a combination of casein, glucose, tryptone along with some salts. The optimum pH of the medium for protease production was 10 after 72 hours incubation at 30°C. Maximum protease production was obtained when casein was used at a concentration of 2%. The protease was highly active from pH 8-13 with an optimal activity at 10. The enzyme was active from 30100°C with maximal activity at 50°C. Substrates specificity tests indicated that casein at 1 % concentration was the best among the four substrates tested (casein, peptone, skim milk and BSA). line maximum activity time with fixed enzyme-substrate concentration was 10 minutes. The protease exhibited considerable thermostability from 30-100°C for 30, 60 and 120 minutes incubation and did not denature. It is concluded that this highly alkaline and thermostable protease may play an important role in detergent and leather industry.

PREVALENCE OF OLIGOZOOSPERMIA AND AZOOSPERMIA IN DISTRICT GILGIT, GILGIT-BALTISTAN, PAKISTAN

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Male infertility is a major reproductive health problem and prevalence of types of infertility is important to be studied for proper treatment. A prospective study was conducted to determine male infertility and prevalence of oligozoospermia and azoospermia in District Gilgit between July, 2010 to March, 2011. Semen samples from 104 suspected patients of infertility were obtained and semen volume, liquefaction time, sperm concentration, motility was studied. The results indicated that out of 104 suspected infertile patients 74% were found positive for infertility. The prevalence of oligozoospermmia was 30.8% (32cases), azoospermia 22. %. (23 cases), aesthinozoospermia 18.3% (19) and normozoospermia 28.9 % (30) respectively. From the present study, it was concluded that the percentage of Oligozoospermia is higher than other seminal abnormalities in the region. This invites further attention of research in this vital field.

PREVALENCE OF TUBERCULOSIS IN HUMAN IN KOHAT KHYBER PUKHTOONKHAWA

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A total of 132 samples were tested in which 47 (35.60%) were positive. A High prevalence was recorded in month of June and July 19(47.5%), 5(41.6%) in LMH hospital kohat respectively and lowest was recorded in month of June and July 12(24%), 11(36.6%) in DHQ hospital kohat. Age wise prevalence was determined in which high prevalence was recorded45.83 (48/22) in age ranges above 40 year and followed by 30.61 (49/15) age ranges from 21 to-40 years and lowest was recorded 28.57 (35/10) in the ages range from 10-20years.Similairly, Sex wise prevalence was determined in which high prevalence was recorded in female 37.5(72/27) then male 33.33(60/20).

EFFECT OF ORGANOPHOSPHATE INSECTICIDE, CHLORPYRIFOS ON SOME BIOCHEMICAL CoMPONENTS OF *TROGODERMA GRANARIUM* LARVAE EVERTS

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The stored grain pests are coming huge damage to stored grains world especially tropical regions. Different pesticides and fumigations are used for their control. In the present study, effects of an organophosphate, chlorpyrifos is being evaluated on Khapra beetle *(Trogoderma granarium)* which is a very serious pest in stored grain godones of Pakistan. For this purpose, the LCso of chlorpyrifos against 4th instar larvae of five different population like Khanewal, Lahore, Muzaffargarh and Sheikhupura of *T. granarium* were determined by residual film method. *T. granarium* the LC_{50} values shown by these population, respectively were, 1575.5, 2673.9, 1150.1 and 1790.0 glml. On the basis of LC_{50} value, populations Lahore strain considered as resistant against chlorpyrifos, whereas Muzaffargarh population was susceptible strain. Larvae of these two strains of *T. granarium* were exposed to sublethal dose (LC_{20}) of chlorpyrifos for the evaluation of the toxic effects on glucose, lipid, soluble protein and total protein content. The biochemical analysis was carried out following 24, 48, 96 and 192 hours of chlorpyrifos treatments. Following insecticide treatment significant increase in glucose content was observed in Lahore (resistant) and Muzaffargarh (susceptible) strains

throughout the treatment. Lipid content also showed similar change with high significance increase in almost all chlorpyrifos treatments in both strains. Soluble protein content depicted highly significant increase in both the strains after 24, 96 and 192 hours except Lahore strain, which showed non-significant rise after 48 hours';"r treatment. The total protein content of resistant and susceptible population showed highly significant increase in all treatments.

DETECTION OF WATER QUALTY, PARAMETERS AND HEAVY METALS IN RIVER JHELUM AT DOMEL, MUZAFFERABAD

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The water quality parameters and heavy metals of river Jhelum were studied from November 2007 to February, 2008 on weekly basis. Water quality parameters such as temperature, pH, alkalinity, hardness, dissolved oxygen, electric conductivity and levels of heavy metals (Ni, Cu, and Cd) were measured and analyzed by applying appropriate statistical tests. The mean values calculated as 18.06±0.03°C, 7.81±2.81, 148.33±15.48 mg/l, 271.33±6.49 mg/l,3.03±0.3 mg/l, 2,402±11.22µs/cm and 3.33±2.27mg/l,3.76mg/l, 0.35mg/l, 0.30 mg/l. The results of physical parameters as well as distribution of heavy metals were with in the range of safe water and river water was not much polluted.

PREVALENCE OF ANAEMIA AMONG PREGNANT WOMEN IN TEHSIL SARGODHA

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Anaemia is associated with increasing risk of maternal mortality in developing countries like Pakistan. Socioeconomic factors is important parameter for appearance of anaemia in pregnancy, it is therefore being hypothesized that prevalence of anaemia is higher in persons with low socioeconomic background. The aim of present study was to study the prevalence of anaemia among pregnant women in Tehsil, Sargodha and to compare it with that of non pregnant female and of male. Blood samples were collected from District Hospital Sargodha and Hilal-e-Ahmar Hospital Sargodha. Data was also collected about socioeconomic history of patients by using a questionnaire. Haemoglobin (Hb), pack cell volume (PCV) and mean cell haemoglobin concentration were used as haematological parameters to evaluate anaemia. On basis of haemoglobin estimation 79%

of pregnant women, 53% of non pregnant women and 53% males were anaemic whereas overall 81.4% pregnant women, 44.9% non pregnant women were observed anaemic on basis of pack cell volume. Among males no one was anaemic on the basis of PCV. Similarly the MCHC lower than normal was found in 66.2% pregnant women 64% non pregnant women and 55.5% in males. Results revealed that prevalence of anaemia among pregnant women in Tehsil Sargodha is very high as compare to non pregnant women and males. Most of the anaemic individuals among all three categories were with low socioeconomic background and low protein in diet.

IMPACT OF DIETARY AND LIFE STYLE FACTORS ON THE PREVALENCE OF BLOOD PRESSURE AND DIABETES MELLITUS IN AZAD KASHMIR

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Blood pressure and diabetes mellitus are major metabolic diseases, affecting the population of Azad Jammu and Kashmir. A cross sectional survey was conducted in three districts (Poonch, Bagh (Forward Khuta) and Kotli) of Azad Jammu and Kashmir during 2009, to collected information about their diets, particularly on the meat component of the daily diet in which proportion of fish meat was focused among the patients of blood pressure and diabetes. Our survey revealed that intake of fish meat was very meager, while the consumption of beef and poultry (chicken) was very high in proportion. It was observed that the peoples of age group 30 and above were the major victim of both diseases. Blood pressure was more common regardless of genders and 35.66% patients were suffering in high blood pressure, 10.66% having both high blood pressure and diabetes, and 12.66% were diabetic only. These two diseases were common in the area mainly due to unhealthy diet, and lack of awareness about other causes which are enhancing these diseases further.

PURIFICATION AND PROPERTIES OF THERMOSTABLE ALKALINE PROTEASES FROM PCSIR-NL35

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Proteases are highly exploited enzymes in food, leather, detergent, pharmaceutical, diagnostics, waste management, and silver recovery. Of all proteases, alkaline proteases produced by *Bacillus* species are of great importance in detergent industry due to their high thermostability and pH stability. The growth and production conditions for bacillus strain PCSIR-NL35 isolated from poultry waste were examined

during this study. The best protease production was achieved at 37°C, pH 9, after 48 hours of incubation with 2% (w/v) casein concentration. Extracellular proteases mixture was purified involving ammonium sulphate precipitation and centricon filtration. It was most active at 50°C using casein as substrate. It was stable between pH 8 and 12 with maximal activity at pH 10. The enzyme was almost 100% stable at 60°C even after 120 minutes of incubation. It retained half of its activity at 100°C. The enzyme has relatively low molecular mass (*M*) as revealed by polyacrylamide gel electrophoresis.

DRINKING WATER QUALITY IN DIFFERENT AREAS OF LAHORE: A COMPARISON WITH EPA AND WHO INTERNATIONAL STANDARDS

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The study was conducted to monitor the quality of drinking water consumed by urban population of Lahore. A total of 50 drinking water samples (16 from source and 34 from distribution system) were examined for physical, chemical and bacteriological parameters. The parameters including pH, turbidity, electrical conductivity, total dissolved solids, total hardness, calcium, magnesium, total alkalinity, carbonate, sulphate, chloride, nitrite, fluoride, sodium and potassium were analyzed. Sixteen out of fifty samples showed high values of alkalinity compared to EPA standards and WHO guidelines. Twenty eight samples were analyzed for heavy metals, chromium, iron, copper, zinc, cadmium and lead. Trace amounts of heavy metals were detected in some samples, however for most of the samples values were within the permissible limits although high concentration of zinc was detected in one sample collected from Mughal Pura. Fifteen samples were analyzed for arsenic. The results were unsatisfactory; around 73% samples showed exceeding values of As. WHO has suggested permissible limits of arsenic < 0.01 ppm, whereas 27% of samples have shown 0.05 ppm arsenic, which is five times greater than WHO highest permissible limits. All the samples were examined for *E. coli* bacteria. On the basis of bacteriological analysis, 42 % samples did not meet WHO guidelines and were unsafe for drinking.

MOLECULAR CHARACTERIZATION OF CYTOLYTIC PROTEIN GENE FROM LOCAL ISOLATE OF *BACILLUS THURINGIENSIS*

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Bacillus thuringiensis, a gram-positive endospore-forming, soil borne bacterium. It produces an insecticidal parasporal crystal inclusion(s) that are composed of several toxic polypeptides which fall into two classes: Crystal (Cry) and Cytolytic (Cyt). The Cyt

toxins are reported only in dipteran-specific *B. thuringiensis* strains, whereas the Cry toxins are found in all *B. thuringiensis* strains with specific toxicity. The Cytolytic-endotoxin gene from *Bacillus thuringiensis* subsp. *darmstadiensis* (SBS *Bt* 49) was amplified by PCR from genomic DNA using specific primers, sequenced and cloned in pTZ57 cloning vector. DNA sequence analysis revealed an open reading frame translating to 269-amino acid sequence. The cloned gene was designated *cyt2A*. The *cytolytic* gene was cloned in pT7-7 expression vector and transformed in BL21C+ for expression. This gene was highly expressed in *Escherichia coli* as inclusion bodies that could be solubilized in 50 mM Na2CO3, pH 10.5.Activation of the solubilized protoxin by proteinase K yielded the active fragment of about 23 kDa. Cyt2Aa showed high hemolytic activity against rabbit erythrocytes and was toxic to *Anopheles stephensi* larvae. Synergistic interactions were also observed between Cyt2Aa and Crys toxins (Cry11 and Cry4A) used in different combinations.

STATUS OF CODON 72 POLYMORPHISM OF *TP53* GENE IN PAKISTANI BREAST CANCER PATIENTS

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The tumor suppressor gene *TP53* encodes a nuclear protein that prevents the cells from dividing before DNA damage is repaired. Codon 72 polymorphism of *TP53* gene has effect on biological activities of cell. The objective of present study aims at determining the frequency codon 72 polymorphism of *TP53* gene in sporadic breast cancer patients, genetic lineage and normal controls. Genomic DNA was amplified by the allele-specific polymerase chain reaction (PCR), The PCR amplification produced a 199 bp fragment for the Pro allele and two (113 bp + 86 bp) fragments for Arg. The interpretation of bands was done with the help of DNA ladder run along with the samples. Three types of band patterns were observed after UV visualization of 4% agarose gel containing ethidium bromide. Single band of 199 bp fragment size corresponded to homozygous pro genotype, two bands of 86 bp and 113 bp fragment sizes corresponded to homozygous Arg genotype, whereas three bands of 86 bp, 113 bp and 199 bp represented the heterozygous arg/pro genotype. Both polymorphisms arg/pro and pro/pro, were found more significant in Pakistani breast cancer patients as compared to arg/arg with corresponding ratio of arg/pro (53.3): pro/pro (34.6): arg/arg (12). Normal controls showed about the same difference in ratio of arg/pro: pro/pro: arg/arg, (50:40:10). arg/pro and pro/pro alleles were also prominent polymorphisms in familial breast cancer patients. Although the polymorphism arg/arg, codon 72, exon 4 of *TP53* gene is reported as a functional relevant polymorphism that contributes to breast cancer development in western countries yet in the present study, genotype arg/pro and pro/pro remain more prominent in Pakistani sporadic breast cancer patients, genetic lineage and normal controls.

ISOLATION AND PHYLOGENETIC ANALYSIS OF METAL RESISTANT CILIATES

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Environmental contamination with metals through industrial wastes water is one of the major health concerns of developing countries. Metal pollutants can easily enter the food chain if heavy metal-contaminated soils are used for production of food crops. Farm productivity has been decreased in toxic metal polluted areas. Microorganisms with ability to grow in toxic industrial waste water have a potential use in bioremediation. During this study four different ciliates were studied from industrial wastewater of Sheikhupura and Kasur industrial waste. Protozoans from the industrial wastewater were cultured in lab in Bold-basal salt medium at pH 7.5 and 27^{o}C. Cultures were purified by the drop method. Two strains of paramecium (para300, paraGC), one strain of stylonychia (Sty-200) and a strain of tetrahymena (tera-1.7) were subjected to DNA isolation, 18SrDNA gene was amplified, cloned in T/A cloning vector and were sequenced by Macrogen Korea. The sequences of para300, paraGC, Sty-200 and tetra-1.7 showed 97% homology with *Paramecium multimicronucleatum*, 93% homology with *Paramecium tetraurelia*, 98% homology with *Stylonychia pustulata* and 98% identity with *Tetrahymena malaccensis* respectively.

EXPLORATION OF HOMOSEXUAL PAIRING IN DOMESTIC PIGEON (*COLUMBA LIVIA DOMESTICA*) IN CAPTIVITY

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Over 130 avian species worldwide are being documented to be engaged in same sex or homosexual behaviors. Pigeons (*Columba livia*) were also believed to be involved in homosexual behavior. Hence the present study was designed to investigate the homosexual pairing in domestic pigeons (*Columba livia domestica*) in captivity. For this purpose an experiment was designed to investigate the homosexual pairing in domestic pigeons. Three cages were developed having dimension of 6×6×6. All of these cages had roosting sites, nesting sites, feeding stations and water resource. Each cage had 20 pigeons, 1st cage had 20 male individuals, 2nd cage had 20 female individuals and 3rd cage had 10 male and 10 female individuals. The experiment was conducted during peak breeding season (April-June 2010) of pigeons. The breeding behavior of pigeons included; cooing, tail dragging, pair bonding, beaking, mating and building of nesting

site. Observations were taken at morning and evening by visual method. Results showed that in 1st cage (20 male individuals) 70% pair bond developed between male-male, in 2nd cage (20 female individuals) 60% pair bond developed between female-female and in 3rd cage (10 male and 10 female Individuals) 90% pair bond developed between male-female within 30 days. The first pair bond developed between male-female within 6 days in 3rd cage. Second pair bond developed between male-male within 9 days in 1st cage. Third pair bond developed between female-female within 12 days in 2nd cage. The male-male and female-female pair bond again and again repeated the same mating, nest building and hatching position after one clutch produce and male-female pair bond spend time to fed the squabs. It is concluded that the pair bond between (male-male), (female-female) existed in pigeons and homosexual pairing stronger in male-male then female-female.

IN VITRO INHIBITION OF INTRACELLULAR VEGF SIGNALING USING A NANOCONSTRUCT IMPROVES THE OUTCOME OF COMBINATION BASED PHOTODYNAMIC THERAPY IN PANCREATIC CANCER

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Purpose: Pancreatic cancer is the fourth leading cause of cancer-related death in United States. A major challenge in pancreatic cancer therapy is the cancer cells' propensity to upregulate compensatory cytokine signaling in response to therapeutic insult, thus promoting local cell survival and proliferation. This study investigates the potential for using a nanoconstruct to simultaneously deliver a phototoxic agent, while targeting a vital component of this compensatory signaling. Specifically the strategy is to disrupt the production and secretion of the multifunctional cytokine vascular endothelial growth factor (VEGF) using Avastin. The significance of this study is that improved drug delivery could produce more favorable outcomes for this deadly disease. Results: Intracellular delivery of Avastin was achieved through encapsulation into a nanoconstruct which resulted in an improved cytotoxicity compared to conventional delivery of each agent alone or in combination. Interestingly, the coencapsulation of the therapeutic agents prevented the recovery of AsPC-1 cells from PDT treatment. We observed that cells can recovered from the initial PDT, whereas coencapsulation of BPD and Avastin resulted in more pronounced cell death at 72 hours. This can likely be attributed to the intracellular delivery and retention of Avastin when packaged inside the nanoconstruct. Conclusion: The following study introduces new strategy to disrupt compensatory signaling pathways stimulated following photodynamic therapy. Future studies will aim to further interrogate the biological response of the approach described above.

MOLECULAR AND BIOCHEMICAL IDENTIFICATION OF GIT FRIENDLY BACTERIA ISOLATED FROM YOGURT SAMPLES OF FIVE DIFFERENT ENVIRONMENTAL RANGES OF PAKISTAN

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Lactobacilli are widespread in the environment including human gastrointestinal tract, where they may potentially function as probiotics. During the past 20 years identification of lactobacilli isolated from various sources has received great interest. This study has aimed at characterizing the lactobacillus strains isolated from yogurt samples collected from the local markets of five different cities of Pakistan i.e. Lahore, Sargodha, Faisalabad, Jamshoro and Islamabad. The strains were identified classically by their Gram reaction, growth at 15 and 45°C, fermentation of different carbon sources and growth in 7.5% NaCl. Molecular identification based on 16S rRNA gene sequences was also performed. The strains identified were *L. acidophilus*, *L. fermentum*, *L. plantarum*, *L. delbrueckii*, *L. bulgaricus*, *L. casei*, *L. helvetics*, *L. brevis*, *L. rhamnosus*, and *L. salivarius*. *L. acidophilus* was the most prevalent species (30%) among all isolates.

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

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The present study was carried out to assess glaucoma in relation to different factors and their associates among patients visiting eye OPD Nishter Hospital Multan Pakistan for the period of 12 months from January 2005 – December 2005. Patients of both genders (N= 401) were divided into three age groups i.e. (age 15-35 years), (age 36-55 years) and (age >55 years) and visual aquity, intraocular pressure, cup-disc ratio, hypertension and incidence of diabetes mellitus were analyzed. Patients were also divided into various groups on the basis of their nature of job and area of origin. The results indicated that 44.88% patients had mild, 25.18% had moderate and 3.99% patients had severe vision loss. 10.22% had no vision while 15.71% patients had normal eye vision. Mature males of age group (36-55) years were suffering maximum with the severe disease (6.36%) compared to old males (5.63%). Among the observed female patients a different trend was observed and old females of age group (56 & above) were suffering maximum (5.31 %) with the disease. Visual aquity disorder was maximum in left eye of

both male (22.53%) and female (14.89%) patients of age group greater than 55. Also when severity of disease was compared between both eyes, it was found that left eyes were suffering more severely (12.46 %) as compared to the right eyes (3.99 %). There was highly significant effect of age on intra ocular pressure (P = 0.000), cup disc ratio (P = 0.001) of left eye and visual aquity (P = 0.001) of both eyes among all age groups. Results also demonstrated that 11.5% glaucoma patients of age group >55 years were suffering from hypertension while 15.7% were diabetic with age between 36-55 years.

EFFECT OF NUTRIENT COMBINATION (PROTEIN, FAT AND CARBOHYDRATE) ON GROWTH AND BODY COMPOSITION OF SILVER CARP (*HYPOPHTHALMICHTHYS MOLITRIX*)

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An 8-week feeding trial was performed to determine the effect of different macronutrient combinations on growth and body composition of Silver carp (*Hypophthalmichthys molitrix*). Four isoenergetic (17.5kJ g^{-1}) experimental diets, control (C), protein rich (PR), fat rich (FR) and carbohydrate rich (CR) were formulated. Protein/fat/carbohydrate (P/F/C) levels in formulated feed were; C: P35/F8/C2, PR: P40/F8/C2, FR: P35/F10/C2 and CR: P35/F8/C5. 80 fish (10.7 $\pm$ 0.12 (g) and 11.2 $\pm$ 0.16(cm)) were stocked in fibreglass recirculating aerated system (RAS) tanks (152.4x61x61cm, 550L). The daily ration size was 5% body weight of fish. All the fishes were distributed in completely randomized way into four treatments with twenty replicates for each treatment. Results revealed that there were no significant ($P \geq 0.05$) differences in specific growth rate (SGR), weight gain (WG), feed conversion ratio (FCR) and protein efficiency ratio (PER) among treatments. In case of body composition, highly significant differences were observed for percent protein content. PR showed highest protein content. CR showed comparatively good growth and was cost effective.

STUDIES ON RELATIONSHIP BETWEEN SEASON AND INORGANIC METALS IN THREE LAKES OF AZAD JAMMU KASHMIR

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The present study was carried out at three lakes of Azad Jammu Kashmir, from June2007- Feb 2008. Study area was divided into three sites, site I (Chikar lake), Site II (Banjosa lake) and Site III (Bagh lake). Three samples from the surface of water were taken from these three sites on seasonal basis (summer, winter). Main objective of this

study was to evaluate water quality of these sites in relation to metal ion concentrations. Water samples were analyzed for inorganic metals (Cd, Cr, Ni, Cu, Pb, Ca and Mg) by using atomic absorption spectrophotometer. In site I Cd showed deviation from standard values of Pakistan Standard Institution (PSI) while other parameters are within limits. Metal ion concentrations in Site II, III were within favorable limits. ANOVA showed non significant difference between three sites.

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

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

perform various behavioral reflexes. The performance of knockouts was even worst following hypoxic ischemic insult. During long term phase, we observed that creatine supplementation improves the performance of both wild type and knockout mice in all HI as well as in no HI treatments. Knockout mouse even performed better than wild type littermates in some experiments. Performance of wild type as well as knockout GAMT mouse was significantly affected in experimental groups fed on creatine free diet. Creatine supplementation improves the over all performance of GAMT knockout as well as wild type mouse. GAMT knockout mouse is an ideal model to observe the effets of creatine supplementation as these mouse completely lack the creatine in their body. Creatine has a neuroprotective effect following the neonatal hypoxic ischemic insult and it can be used to reduce the damages of asphyxia.

FREQUENCY DISTRIBUTION OF *GSTM1* AND *GSTT1* NULL ALLELE IN PAKISTANI POPULATION AND RISK OF DISEASE INCIDENCE

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Glutathione S-transferases, *GSTM1* and *GSTT1* play a significant role in detoxification and bioactivation of a broad range of xenobiotic compounds known to be mutagenic and/or carcinogenic. Deletion polymorphisms of these glutathione transferases (*GSTM1* and *GSTT1*) predispose individuals to environmental carcinogenic compounds. Although a number of studies have shown the relationship between *GSTM1* and/or *GSTT1* deletion polymorphism and different cancers, these findings can't be extrapolated to other populations due to intra- and inter-ethnic variability. In order to assess the impact of differential ethnicity on the occurrence of different cancers in local population due to *GSTM1*, or *GSTT1* deletion polymorphism, 111 healthy male and female individuals of different age groups from Southern Punjab, Pakistan were genotyped using a multiplex polymerase chain reaction. From the results it is obvious that null alleles of *GSTM1* and *GSTT1* genes were found in 45% and 23% individuals, respectively. In 5% of individuals' simultaneous deletion of both *GSTM1* and *GSTT1* genes were observed. Frequency of *GSTM1* null allele is in concordance with those documented for Chinese, Caucasians, and Japanese populations. However, a significantly higher frequency for *GSTT1* null was reported in Chinese and Japanese population as compared to Pakistani population. It is the first ever report on frequency of *GSTM1* and *GSTT1* null allele in

Pakistani population which demonstrate the impact of ethnicity and provide basis for future epidemiological and clinical studies.

ISOLATION AND IDENTIFICATION OF VARIOUS PARAMECIUM SPECIES FROM AQUATIC ENVIRONMENT RECEIVING INDUSTRIAL WASTEWATERS

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Protists are the most abundant and diverse eukaryotic groups, yet current knowledge of these organisms is surprisingly sparse, compared with that for animals, plants and fungi. Among protozoans, the complex taxonomic structure of Paramecium species allows for an in-depth look into these organisms particularly for evolutionary and phylogenetic studies. Despite the fact that these organisms show incredible species diversity, research on the genome structure of paramecium is still largely incomplete. Only the complete mitochondrial genome for *Paramecium aurelia* and the complete macronuclear genome of *Paramecium tetra-aurelia* have been sequenced till now. The aim of present work is to isolate, identify molecularly through ribotyping and perform phylogenetic analysis of various Paramecium species. For this purpose water samples from tanneris and industrial effluents of different places (Shahdra, Kasur, GTR, Stalkot, Shekhupura, Gujranwala, Mansahra and Abotabad) were collected. Paramecium species from waste water were isolated by using drop method. These cultures were maintained in bold basal salt medium. Initial identification of protozoans was done by observing their morphology and movement patterns. Molecular identification of these paramecium species is in progress. It is based on homology of 18S rRNA gene sequence to those of already known paramecium species.

REGULATION OF COPPER INFLUX-EFFLUX THROUGH PLASMA. MEMBRANE IN *KLEBSIELLA PNEUMONIAE*

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Metals play an integral role in the life processes of microorganisms. Copper is an essential micronutrient both for eukaryotes and prokaryotes. It is also a potent cytotoxin when allowed to accumulate. in excess of cellular needs. Copper resistant *Klebsiella pneumoniae* isolated ftom the local environment has been shown to have accumulated. 35µg copper per mg cell dry weight. Amongst various genetic determinants present in *Klebsiella pneumoniae* to deal with copper processing is *cus* (copper sensing) operon. It

comprises two divergently occurring operons regulated by a single promoter. One operon, regulatory in nature, consists of two genes *cus R* (response regulator) and *cus* S (copper sensing), while the other operon consists of four structural genes including *cus* C (outer membrane factor), *cus F* (periplasmic binding protein), *cus B* (membrane fusion protein) and *cus A* (secondary transporter). These genes were amplified in three parts. One part consists of *cus A*, second *cus C, F* and *B*, while the third part consists of two regulatory genes *cus R* and *S*. These genes were then cloned in pTZ57RfI' and were used for transformation of *E. coli* DH5a. Sequence analysis revealed that the gene products were . highly homologous to a family of proton/cation antiporter complexes involved in detoxification of copper. Expression analysis at transcriptional level was carried out through real-time PCR in absence and presence of non-lethal and sub-lethal concentrations of copper. It was demonstrated that expression was significantly enhanced both under aerobic and anaerobic conditions. Moreover, copper sehsitivity of *cus* determinants promoter was determined by using *LacZ* as reporter gene. Promoter region of almost 180bp was amplified and transformed in DH5a after ligating in promoterIess p-LacZ vector. The confirmed transformants were used for expression analysis of ß-galactosidase reporter gene, by using copper metal as an inducer for promoter. The expression was confirmed through SDS-PAGE as well as by more sensitive method of assay for ß-galactosidase gene using ONPG as substrate. The results of the present investigation show that the cloned promoter is highly sensitive for copper metal induction and the *cus* operon is an important regulator in maintaining the homeostasis with reference to copper intake and efi1ux in *K. pneumonia.*

MOLECULAR ANALYSIS OF AGR2 PROTEIN IN P53 PATHWAY WITH REFERENCE TO DRUG DISCOVERY

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Breast cancer is the most common form of cancer diagnosed in women worldwide. One of the most commonly mutated and silenced gene in human cancers is P53. Oncogenic iI.Ihibition ofthe p53 tumor suppressor is a key feature of human cancer. AGR2 proto-oncogene is a novel type of p53 inhibitor, found upregulated in a number of different types of cancers including breast cancer and has been proved to attenuate p53 activity by suppressing its phosphorylation after DNA damage. It remains important to determine whether AGR2 is a potential drug target for reactivating the p53 pathway in cancer cells. The present study was done to explore the role of AGR2 in P53 pathway with reference to drug discovery. Breast cancer cell lines (MCF -7 cells) were treated with tamoxifen for different time intervals and induction of AGR2 expression was observed. Further to this experiment, a cumulative effect of tamoxifen and tricirbine was analyzed at transcriptional as well as translational level. In addition, the effects ofbioactive peptide aptamers that bind to AGR2, were analyzed on MCF-7 cells to

explore their role in p53 pathway proteins. Immunohistochemistry of AGR2 in breast cancer tissue was done and it was found that AGR2 is located in nucleus in cancer tissue.

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

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

SUPPLEMENTATION OF WHOLE GRAIN IN DIET ON LIPID PROFILE IN HYPERTENSIVE FEMALES OF VARIOUS ETHNIC GROUPS IN BALOCHISTAN

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The effects of supplemented whole grain consumption for 4 weeks on body mass index (BMI), total cholesterol. (CHO). triglyceride (TO), high density lipoprotein cholesterol (HDL) and low density lipoprotein cholesterol (LDL) in hypertensive female subjects of 4 major ethnic groups in Quetta. *i.e*. Pathan (P), Baloch (8), Hazara (H), and Punjabi (PU) were studied. Two groups each of 32 hypertensive female subjects and comprising subjects from each ethnic group were chosen. The subjects of the group taken as control used the conventionally available refined carbohydrates in meals and another batch reflected as treated subjects consumed 50g cereals in breakfast and whole grain chapattis in lunch and dinner for 4 weeks. Twelve hours fasting blood sample was taken from control and whole grain treated hypertensive subjects a day after the completion of experiment duration. The whole grain diet for 4 weeks showed insignificant decrease in

BMI in all studied groups. Reduction in systolic blood pressure was noticed in all ethnic groups; however, significantly (P < 0.01) in Punjabi group. It exhibited highly significant effect on diastolic blood pressure in all studied groups and decreased noteworthy in Baloch (P< 0.05), Pathan (P -< 0.01) and Punjabi (P < 0.01) group. total cholesterol was lowered significantly in Pathan (P < 0.05). Hazara (P <: 0.0 l) and Punjabi (P < 0.0) groups. Significantly lower concentration of low density lipoprotein cholesterol was observed in the subjects of Baloch (P <0.05), Pathan (P < 0.01) and Punjabi (P < 0.05) ethnic groups compare to their respective controls. Likewise triglycerides concentrations were also significantly decreased in all Baloch & Pathan (P < 0.001) and Hazara & Punjabi (P < 0.0 I) groups. Whole grain supplementation has exhibited promising results in the amelioration of lipid related heath profile, however, variedly in hypertensive females of various ethnic groups.

REDUCTION IN IODINE DEFICENCY DISORDERS: A PROSPECTIVE STUDY OF 2550 SCHOOL CHILDREN IN DISTRICT GILGIT

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To asses iodine deficiency disorders in 14 villages of district Gilgit, a survey was conducted in children aged 6-12 years.2550 school going children were surveyed for the purpose. Of 2550 children 07 male and 05 females were found to be suffering from goiter showing over all goiter rate(TGR) of 0.47%.This shows that prevalence of goiter among males I and females children is nearly same. Estimation of urinary iodine analysis revealed that 37 (7.83%), 24 (5.08%), 49 (10.3%), and 340 (75.5%) children were suffering from severe, moderate, mild and no iodine deficiency, respectively. On the basis of our studies we can conclude that iodine deficiency disorders in school going children has been reduced in the Northern areas presumably due to the utilization of iodized salt provided by Aga Khan health services and government of Pakistan.

THE NOVEL STRUCTURAL ANALYSIS OF GENETIC DETERMINANTS OF *KLEBSIELLA PNEUMONIAE* INVOLVED IN COPPER RESISTANCE

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Klebsiella Pneumoniae is one of the major micro flora found in stressed non-clinical environments. It has strong potential for bioremediation of some heavy metals such as mercury and cadmium and some organic compounds also. The aim of the present study was to explore its potential to resist copper, at molecular level. For this purpose, a

copper resistant *Klebsiella pneumoniae* was isolated from aquatic environment receiving industrial wastewater. Six genes namely cus A, cus B, cus C, cus F, cus R and cus S were amplified from its genomic DNA and cloned, followed by sequencing. Analysis of sequences obtained showed that these genes are present in the form of two operons; cus A, cus B, cus C and cus F forming one operon and cus R and cus S forming the other operon. Both these operons are regulated divegently by a same promoter and constitute cus regulon, a characteristic genetic determinant of family enterobacteriaceae, but may also found in some other bacteria. Primary structure of proteins of the six genes were deduced and subjected to blast analysis. To find out structural relationship of each protein with its related ones present in prokaryotic system, their phylogenetic positions were identified using web available programs. Tertiary structures of these proteins were also determined using homology modeling method. The structural and functional domains of each protein and important residues involved in specific functions were also identified.

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

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Enterotoxigenic *Escherichia coli* (ETEC) strains are leading causes of childhood diarrhea in developing countries. Adhesion is the first step in the pathogenesis of ETEC *E. coli* infections and ETEC pili designated colonization factor antigens (CFAs) are believed to be important in the biofim 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.

MOLECULAR CHARACTERIZATION OF *cry4B* GENE FROM LOCAL ISOLATES OF *BACILLUS THURINGIENSIS* ENCODING 70kDa CRYSTAL PROTEIN

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The toxic region 2.0 kb of *cry4B* gene amplified from the six different local isolates of *Bacillus thuringiensis* (DAB *B.t.* 1-6) was cloned in pTZ57R and transformed in DH5α. By double digestion with enzyme *EcoRI* and *Hind* III, the restricted fragment was ligated in the expression vector pT7-7 and transformed in *Escherichia coli* BL21C. The expressional profiles of the recombinant organism containing *cry4B* gene were studied and pT7-7 plasmid without *cry4B* gene was taken as control. The expression conditions were optimised with respect to IPTG concentration, time of induction and incubation temperature. It was found that the high level expression occurred at the concentration of 0.5mM IPTG incubated at 37°C for 3 hours. Biotoxicity assays against third instar of mosquito larvae *Anopheles stephensi* was done with genetically modified organisms and crude recombinant expressed Cry4B proteins. Biotoxicity assays showed the mosquitocidal activity of toxic region of Cry4 B crystal protein.

USE OF *AVICENNIA MARINA* (FORSK.) VIERH LEAVES EXTRACTS AGAINST SOME ATMOSPHERIC FUNGI

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Crude ethanolic extract of *Avicennia marina* leaves was tested against seven allergenic fungi viz., *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, Cladosporium herbarum, Penicillium notatum* and *Saccharomyces cerevisiae* using five

different solvents, DMSO, DW, chloroform, ethanol and acetone at 2000, 4000 and 6000 ppm doses. Dose dependent tendency in the increase or decrease in the growth of fungi was observed. Distilled water and DMSO were the most effective solvents as the % inhibition of fungal growth was 83.00%±4.73 in *A. niger*, 80.33%±5.60 in *A. flavus*, 78.58%± 3.18 in *A.alternata*, 72.91%±7.96 in *P. notatum*, 65.25%±3.55 in *C. herbarum* 63.25%±4.52 in *A. fumigatus* and 48.5%±7.89 in *S. cerevisiae*. Two synthetic drugs miconazole and amphotericin-B were used as positive control. Miconazole was 100% effective against *A. alternata, C. herbatum, P. notatum* and *S. cerevisiae* in the concentrations of 95.00±1.62, 78.00±4.99, 100.00±0.69 and 110.00±2.33(µg/ml of SDA medium) respectively. Amphotericin-B completely controlled the growth of *A.flavus, A.fumigatus* and *A. niger* in the concentration of 24.00±17.00, 30.00±15.66 and 18.00±18.34 (µg/ml of SDA medium) respectively. Statistically, the results were compared with negative control and found as highly significant ($p<.01$).

SECTION - II

PESTS AND PEST CONTROL

PESTICIDE RESIDUES IN VEGETABLES COLLECTED FROM NAWABSHAH, SINDH

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Pesticide residues in food commodities remain a fast growing global problem with serious repercussions on human health (Asmatullah, 1996). Furthermore, irrational use of pesticides has created new pests that have never been a problem before (Huque, 1990). The resurgence of pests, development of resistance (Zahoor, 1999), contamination of soil, water and air, destruction of predators, parasites and other non-target beneficial organisms including wildlife, brought about the realization that these chemicals are not exclusively doing the job they were intended to do but also have adverse effects on ecological systems with which human welfare is inseparably bound (Hussain *et al*., 1999). The dependency on pesticides is evident from the increasing trend in its consumption from 665 mt in 1980 to 45,680 mt in 1999 (Anonymous, 2000) and recently reduced to 25000 mt in 2006 (Economic Survey of Pakistan, ESP-2007), contrary to the experience in industrialized countries like USA, where herbicides make up 85% of total pesticides (USDA, 1987). At present more than108 types of insecticides, 30 types of fungicides, 39 types of weedicides, 5 types of acaricides and 6 different types of rodenticides are being used in Pakistan (PPSGPD, 2002) and the import of pesticides has decreased from 41,406 tons in 2003-04 to 20394 tons in 2006-07. The misuse of pesticides has led to tremendous economic losses and hazards to human health. Human exposure to pesticides is usually estimated by measuring the levels in the environment i.e. soil, water and food (Tahir *et al*., 2001, Ahmad 2004, Anwar *et al.* 2004, 2005). About 60-70% of pesticide poisoning cases were reported due to occupational exposure and female cotton pickers appeared to be at high risk of hazards (Ahmad, 1999 and Tahir, 2000) and recently Tariq *et al.* (2007) reviewed the pesticides poisoning cases in Pakistan. The present work was undertaken, to provide base-line data on pesticide residues level in vegetables samples for determination of toxicity level of pesticides in vegetables for the development of national codex standards. One kg of the sample was chopped and mixed thoroughly. A subsample of 25 gm was taken out and blended with 50 ml of acetone, 50 gm of anhydrous sodium sulphate and 50 ml of a mixture of cyclohexane and ethylacetate (1:1). The mixture was allowed to stand for some time until a clear

supernatant was formed and 30 ml supernatant was taken into a round bottom flask. A few drops of 10% propandiol in ethylacetate and about 4-6 glass beads were added. The solvent was evaporated at 40 °C under vacuum in rotary evaporator. The contents were reconstituted in 6 ml of cyclohexane and ethylacetate (1:1) and then passed through high-flow super cells. Two ml of this sample was applied on GPC column for further cleanup. After passing through GPC column, the samples were dried under vacuum and reconstituted in 1ml ethylacetate for analysis on Gas Chromatograph (GC). The retention time was within $\pm$ 2 percent of that of the standard. The method validation consisted of three sample sets. Each set included three levels of fortification (0.01, 0.05 and 1.0 ppb) and a method blank. All spikes and method blank samples were processed through the entire analytical method. Quantification was based on external standard calculation using the peak area. Gas Chromatograph, Perkin-Elmer, Autosystem, Microprocessor fitted with Electron Capture Detector (ECD-Ni^{63}) and Nitrogen Phosphorous Detector (NPD). Nitrogen and Air Generator Peak Scientific. Hydrogen Generator, Peak Scientific, Gel Permeation Chromatograph (GPC), Mikrolab Arhus A/S, USA. Rotary Evaporator, made Buchi R-114/A, Switzerland. Food Blender, Germany. FlASK Shaker SF1, Saltorius single pan analytical balance and Refrigerator/Freezer.

ARTHROPOD POPULATIONS' RESPONSES TO VARIOUS WEED PLANTS IN THE CULTIVATIONS OF FAISALABAD

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Dramatic and irreparable global changes caused by human activities are responsible for decline of biodiversity, Up till now the ecological impact of this change is poorly understood. The present studies have shown that biodiversity loss in the agro-ecosystem is feared to affect basic ecosystem processes like nutrient cycling and primary production. The weeds studied in sugarcane were *Cynodon dactylon, Amaranthus viridus, Convolvulus arvensis, Phalaris minor, Conyza ambiuga, Coronopus didymus, Chenopodium album, Cnicus arvensis, Malvastrum coromandelianum, Parthenum hystorophorus, Anagalliss arvensis, Dichanthium anulatum, Coriandrum spp., Anethum graveolens,* and *Sacchrum spp.* Total 712 specimens belonging to 91 species of predators and 1653 specimens of prey/pest belonging to 69 species were captured. The wheat weeds were *Anethum graveolens, Avena fatua, Ageratum conyzoides, Brassica campastris, Cynodon dactylon, Convolvulus arvensis, Cenchrus setigerus, Cnicus arvensis,Coronopus didymus, Chenopodium murale, Euphorbia prostrate, Ephedra spp., Malva neglecta, Phalaris minor, Polygonum plebejum, Rumex dentatus, Sonchus olearaceus,* and *Vaccaria hispanica.* Total 187 predator specimens belonging to 18 species as well as 699 specimens of prey/pest belonging to 28 species were captured

during the whole study period, decline in the diversity and abundance of weed species have dramatic affect and alter the richness and structure of arthropod communities. The results suggest that the loss of weeds may have profound effects on the diversity, abundance and richness of insect populations. The weed-crop complex seemed to be more successful. All the Coleopteran predators were abundant in this complex followed by *Oxyopes javanus,* and *Chrysoperla carnia*. Among pest species *Schizaphus graminum* was highly abundant followed by *Cernuella jonica, Dysdercus cingulatus, Mayetiola destructor, Pyrilla perpusila, Acyrthosiphon pisum* and *Acyrthosiphon gossypii* respectively.

RELATIVE ABUNDANCE AND ROLE OF BRACONID LARVAL PARASITOIDS IN CONTAINING LEPIDOPTERAN POPULATIONS IN DISTRICT FAISALABAD

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The ecological consequences of biodiversity loss have aroused considerable interest and controversy during the past decade**.** Major advances have been made in describing the relationship between species diversity and ecosystem processes. Functional diversity has been suggested to be the most important component of diversity, Lepidopteran, cereal stem borers are the most damaging group of insect pests and due to their economic importance they have been the subject of extensive research. Parasitic wasps are keystone species in many terrestrial ecosystems. These highly successful insects regulate herbivore populations and pollinate plants, and thus are valuable components in agro-ecosystems. The members of family Braconids are parasitoids of lepidopteran populations. Most of these are endoparasitoids, as well as ectoparasitoids. For being so specialized they are also considered as indicator of environmental richness and stability. The study was focused on determining the relative abundance and trophic status of braconid larval parasitoids and lepidopteran populations in district Faisalabad. For this purpose sampling was conducted for a period of 1 year. Data was collected with the help of handnets and by handpicking method from the selected quadrates of 1 meter square. The main species of lepidopterans were *Catopsilia pyranthe, Danaus chrysippus* and *Cepora nerissa* while *Cotesia flavipes* and *Bracon hebetor* were major species related to family Braconidae. The above discussed aspects were elaborated from these observations in studying the stability of agro-ecosystem.

PREDATOR-PREY ASSOCIATION AMONG SELECTED ARTHROPOD SPECIES IN THE CROPLAND OF PUNJAB, PAKISTAN

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In this paper, a simple mathematical formula was used to analyze the dynamics of predator-prey relationship. Arthropods are the major group of invertebrates found in the cropland, including many beneficial predators, parasitoids, pollinators and prey/pests. Crops like Fodder (Maize, Sorghum, Alfalfa), Wheat and Brassica were sampled round the year. The most abundant predator species *Coccinella septumpunctata, Cheilomenes sexmaculata, Hippodemia convergens, H. variegata, Chrysoperla carnea, Oxyopes javanus, Neoscona theisi* and *Araneidae nymph* while abundant preys/pests *Aphis maidis, Schizaphis graminum, Macrosiphum miscanthi, Empoasca kerri, Lepidopterous larvae* and *Musca domestica* were selected. Predator-prey ratios (p/p) were calculated from monthly abundance data and horizontal linear graphical pattern showed the probable association of a predator with selected preys. *A. maidis* showed the best probable association with all the beetles and *O. javanus* (spider), *S. graminum* with green lacewing while *M. domestica* seemed to be associated with all the spiders. Chi-square test was applied on the relative abundance of a predator and associated prey to check the significance of association. Such findings seemed to be helpful in ascertaining the species-specific biological control in the field.

CHEMICAL EVALUATION OF ASSOCIATED WEED SEEDS FREQUENTLY MIXED WITH WHEAT GRAINS AT HARVEST

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The study was concerned to evaluate the phytochemical potential of weeds associated with wheat crop. Fifteen weeds frequently occurring in the wheat crop were selected. Ethanolic extracts were prepared for qualitative and quantitative phytochemical analysis. Phytochemicals such as alkaloids, saponins, glycosides, terpenoids, anthraquinine, steroids, flavonoids and tannins were detected. It was found that tannins and alkaloids had wide spectrum and alkaloids ranged from 0.88 to 4.00%, tannins 7.97 to 24.17%, saponins 0.54 to 1.29% and flavinoids 3.91 to 15.55%. It is strongly

recommended that self-emerging weeds in the wheat crop fields can be extensively used for medicinal purposes. More investigation is needed to identify the phytochemical potential of other crop associated weeds.

CONTROL OF JASSIDS ON OKRA BY USE OF BIOPESTICIDE

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Field experiments were conducted at National Agricultural Research Center (NARC), This work was conducted with collaboration of Horticulture Research Institute (HRI) and Directorate of Organic Farming, NARC. Crude water extract of neem was prepared. This extract was tested in the field against jassid on Okra crop in three different concentrations (2%, 1%, and 0.5%). Data was recorded after 2, 5 and 7 days. Results indicated that the application of 2% neem extract was most effective (86 % mortality).

ROLE AND QUALITY PRODUCTION OF *TRICHOGRAMMA* ON LEPIDOPTEROUS EGGS UNDER LABORATORY CONDITIONS

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The present study was conducted for efficient and quality production of stingless wasp~ *Trichogramma chilonis* Ishii on grain moth, *Sitotroga cereallela* and rice meal moth, *Corcyra cephalonica.* Pesticides create a number of problems such as environmental pollution, pest resistance, and hazards for human beings and animals. Biological control offers effective alternatives for the control of many insect pests. To ~ort out the effect of rearing temperature, ho~t age, host density and parasitoid density on biological traits, parasitism, adult emergence, developmental period, female ratio and adult longevity of T. *chilonis.* The most favorable temperature was 28°C on which maximum rate of parasitism and adult emergence were obtained from S. *cereallela* eggs as compared to C. *cephalonica* eggs. Under no choice test the percentage of oviposition and adult emergence was highest at 2 hours eggs of both hosts. Host age had no significant effect on development time. For host density percent parasitism and adult longevity was highest at 20 eggs whereas emergence was highest from 10 eggs of S. *cereallela* while of C. *cephalonica* percent parasitism and emergence was highest at 10 eggs. For parasitoid density, Parasitism was highest at 5 pairs of *Trichogramma.* Percent emergence and longevity remained similar among all the 5 treatments: It is concluded

that S. *cereallela* eggs more suitable as compare to C. *cephalonica* eggs for mass and aualitv rearing of Darasitoid, *T. chilonis.*

CONTROL OF APHIDS ON CAULIFLOWER BY USE OF BIOPESTICIDE

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Field experiments were conducted at National Agricultural Research Center (NARC); This work was conducted with collaboration of Horticulture Research Institute (HRI) and Directorate of Organic Farming, NARC. Two concentrations of Neem extract (2% and 0.5%) were sprayed on cauliflower against Aphid (*Brevicoryne brassicae*) at Organic Farming field at NARC. The results indicated that after spray of 2% extract, per plant average population of aphid was 8.69 and after application of 0.5% extract the average per plant population was 17.63. In control plot, the average per plant population was 41.13 which indicated that the per plant average population of aphid in both treated plots were significantly lower than the control.

INTEGRATED PEST MANAGEMENT AGAINST DIAMOND BACK MOTH, *PLUTELLA XYLOSTELLA* IN CAULIFLOWER, *BRASSICA OLERACEA*

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The experiment on integrated pest management (IPM) techniques against Diamondback Moth (DBM) *Plutella xylostella* Linnaeus in cauliflower, *Brassica oleracea* Linnaeus was conducted at Agricultural Research Station Baffa (ARSB), Mansehra. The design used for the experiment was randomize complete block design with three roWs in different sequences. The treatments tested against *P. xylostella* were coriander, *Criandrum sativum* Linnaeus (inter cropping); lorsbin (synthetic chemical); neem seed extract (NEM); neem oil and *Trichogramma chilonis* Ishii (biological control). The results showed that NSE@ with 2 ml/L of water was the best followed by chemical and biological control. Intercropping and application of neem oil did not perform efficient control of *P. xylostella.* The least effect was noticed in the plots where no treatment were applied and showed the highest population of the pest. The effects of different treatments were proved reciprocal to the effects on pest population. Hence,

application of the NSE gave the highest yield (29.11 ton/ha) followed by lorsbin (28.06 ton/ha), *T chilonis* (19.55 ton/ha) and coriander (14 ton/ha), respectively. The lowest yield was obtained in the plats applied with neem oil (9.86 ton/ha) as compared to checked plots, which give only 293 ton/ha. Use of the NSE and biological method *T chi/onis* were found the cheapest treatment than any other strategies against *P. xylostella.* Use of the NSE and biological method *(T chilonis)* were found the cheapest treatment than any other strategies against *P. xylostella.* In the light of obtained results it was suggested that NSE can easily be used by farming communities against *P. xylostella* on *B. oleracea* in order to get high yield and low infestation of the pest.

DEVELOPMENT AND DISSEMINATION OF PARC DEVELOPED RODENT PESTS CONTROL TECHNOLOGY FOR URBAN AND AGRICULTURE STAKE-HOLDERS

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Rodent pests cause colossal damage to various commodities in'Cluding fixture and furniture, stored grains, poultry industry and field crops. It has been estimated that rodent pests, both commensal and field species, cause around 10 billion rupees losses annually to crops and stored grains. Rodent damage falls upon both the rich and poor, the home owner as well as the bu~iness. magnate. In business centre, rats cause damage to computer installation through cable gnawing, stop production by short-circuiting in electrical system, resulting in fire incidence in many cases. For effective and economical control of rats and mice infestation, the institute has developed a. package of rat baits after a serious of laboratory and field based trials. The rodent control technology is being disseminated to different institutions and organization, both in public and private sectors under which 37 food processing units, 21 Hospital and 71 Pharmaceutical companies were approached. Besides, 20 workshops and seminars were organized during 2009-10 to different stake holders to create awareness and motivation' for end users, thus to adopt PARC developed rat control technology.

PREVALENCE OF TICK INFESTATION IN SMALL AND LARGE RUMINANTS GRAZING IN POTOHAR REGION

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Livestock is the backbone of agricultural economy of Pakistan. The productive potential of livestock is being affected by several factors leading to reduction of its income-generating capacity. Among these factors, ectoparasitic infections cause severe

menace. Ticks are the most notorious threat to the livestock. There are more than 850 species of ticks reported throughout the world. About 1200 million animals are at risk from ticks and tick born diseases (TTBD). This study was conducted in different localities of Potohar region. The main study site was Barani Livestock Production Research Institute (BLPRI) Khairimurat, district Attock. Moreover, survey was also carried out in some private herds in different localities of Potohar region. The ticks were picked directly from different body parts of animals using forceps and rubber gloves without damaging their mouthparts and stored in 70 percent ethyl alcohol. Then they were carried to the laboratory for identification. The surveys were carried out in different seasons and months from October 2008 to August 2009. A total of 1804 animals (481 sheep, 1015 goats, 214 cattle and 94 camels) were examined for tick infestation. Out of the total 1804 animals 814 (45.1 percent) were found infested with ticks. A total of 700 tick specimens were identified belonging to six genera. The most abundant genus was *Haemophisalis* with a prevalence rate of 28.1 percent. It was followed by *Rhipicephalus* (24.8 percent), *Ixode* (20.0 percent), *Hyalomma* (14.1 percent), *Boophilus* (11.5 percent) and *Dermacenter* (1.3 percent). The highest infestation rate was found on cattle (58.8 percent), followed by camel (52.1 percent) and sheep (50.9 percent) while the lowest infestation rate (38.8 percent) was recorded on the goat.

AN APPRAISAL OF RESISTANCE OF OLD AND NEW WHEAT GENOTYPES TO RED FLOUR BEETLE, *TRIBIOLIUM CASTANEUM* (HERBST)

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In present investigation, thirty varieties of wheat were used to study their resistance against Red flour beetle, *Tribolium castaneum* H.The grains of all these varieties were obtained from Plant Breeding and Genetics Division, Nuclear Institute of Agriculture (NIA) Tandojam. Experiment was conducted in the laboratory at 29:!:2°C and 65:!:5% R.H. The standard of the samples of each variety used in the experiment was 1000 grains, kept in plastic jars (15 x 6 cm). Ten pairs of newly emerged adult flour beetles (both sexes in equivalent numbers) of uniform age from laboratory stocked culture were released in each jar. The results evaluated on the bases of adult population development, percent grain damage and frass production, revealed that none of the genotype was completely resistant to the infestation of *T castaneum.* All the varieties suffered losses, but their degree of resistance varied significantly. On the bases of pest population development, percent damage and frass production, the least damage was

noted in variety Barani-70 and Bhitai and the highest damage was recorded in T21 and T16; hence these varieties. were designated the most tolerant and the most susceptible varieties, respectively. The comparative resistance displayed by the wheat varieties, could be placed in the following order :< Barani-70 < Bhittai <T19 <T14 <T24< T13 < T20 < T9 < TII < TI5 <T25 < Tl < T12< T8 < TJ.-83 < T22 < T3 <T18 <T10 < T23 <T5 < Marvi-2000 < T17 < Mehran-89 < T6 < T7 < T16 < T21.

EFFECTS OF *GLIRICIDIA SEPIUM* AGAINST *AEDES AEGYPTI* (LINN) AND *TRIBOLIUM CASTANEUM* (HERBST)

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Gliricidia sepium is an exotic plant belongs to the family Fabaceae. Ethanolic extracts of *Gliricidia sepium* leaves were tested for their larvicidal and pupicidal activity against mosquito *Aedes aegypti* at the time interval of 24 hours. The plant showed highest mortality at 4.0% concentrations and that was larval mortality was 1000.10 and pupal mortality was 68% as compare to control. Repellent activity has been studied against stored grain insect *Tribolium castaneum,* the highest average repellency of 52.37% after 8 weeks was exhibited at 600llglcm3 as compare to control.

EFFECTS OF *SOLANUM NIGRUM* AGAINST *AEDES AEGYPTI* (LINN) AND *TRIBOLIUM CASTANEUM* (HERBST)

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Ethanolic extract of *Solanum nigrum* leaves were tested for their larvicidal and pupicidal activity against mosquito *Aedes aegypti*. The larval and pupal developments were inhibited by the treatment, at the time interval of 24 hours. Larval and pupal mortality were dose dependent with the highest dose of 4.0% plant extract evoking 100% and 96% mortality as compare to control. As naturally occurring insecticides, these plant derived materials could be useful as an alternative for synthetic insecticides controlling field population of mosquito. Repellent activity has been studied against stored grain insect *Tribolium castaneum* and the highest average repellency of 52.25% after 8 weeks at 300µg/cm^3 as compare to control.

EVALUATION OF RESISTANCE OF RECENTLY EVOLVED WHEAT VARIETIES TO KHAPRA BEETLE *TROGODERAMA GRANARIUM* (EVERTS)

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In laboratory investigation, thirty varieties of wheat were used to study their resistance against Khapra beetle, *Trogoderama granarium* E. The grains samples of all these varieties were obtained from plant breeding and Genetics Division, Nuclear Institute of Agriculture (NIA) Tandojam. Experiment was conducted in the laboratory of 29±2° and 65±5 Relative Humidity. The standard of the sample of each variety used in the experiment was 1000 grains, kept in plastic jars (15 x 6 em). Ten pairs of newly emerged larvae of Khapra beetles of uniform age from laboratory stocked culture were released in each jar. On the bases of pest population development, percent damage and frass production, the least damage was noted in variety Barani- 70 and TJ-83 and the highest damage was recorded in T -21, T -16; hence these varieties were designated the most resistant and the most susceptible varieties, could be placed in the following order:<Barani-70- <TJ-83 <Marvi-2000 <Mehran-89 <Bhittai <T-14 <T-22 <T-8 <T-4 :::T-9<T':6 <T-ll <T12 <T-25 <T-15 <T-7 <T13 <T-2 <T-l <T-20 <T-24 <T-I0 <T-18 :T-19 <T5 <T-23 <T-17 <T-3 <T-21 <T-16.

EFFECT OF PLANT OILS ON ADULT EMERGENCE, FECUNDITY AND WEIGHT LOSS OF STORED GRAIN BEETLE *COLLOSOBRUCHUS ANALIS* (FAB.) IN GREEN GRAM *(VIGNA RADIATA* L. ROXB).

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The experiment was carried out under laboratory condition in the post graduate laboratory, Department of Entomology, Faculty of Crop Protection, Sindh Agriculture University, Tandojam. The culture of stored grain beetle, *Callosobruchus analis* Fab.

obtained from grain storage laboratory, Tropical Agriculture Research Institute;Karachi University. Four different plant oils i.e. mustard *(Brassica Compestris* (L), Coconut *(Cocus nucifera* (L), sesame, *Sesamum indicum* (L.) and rocket seed *(Furica sativa* (Mill).The standard concentrations of each oil was *0.25* ml, mixed with the 250 grams of green gram *for* recording the adult emergence, fecundity and weight loss of stored grains. 250 gm of weighted green grams grains were kept in plastic jars covered with muslin cloth. The experiment was replicated four times. The culture was developed and *four* pairs adult beetles one day old of same age were released in each jar. The result indicated that the Rocket seed (J ambo) oil was the most effective followed by coconut oil sesame oil and mustard oil which resulted in low fecundity and prevented adult emergence for four (4) months. The minimum fecundity (1.47±*0.04)* was observed in rocket seed followed by mustard (17.27±2.18), sesame (25.27±3.23) and coconut (26. 70±:4.21), however, maximum fecundity (44.2±7.7) as compare to control. Similarly the minimum adult emergence (1.81±0.15) was observed in rocket seed followed by mustard (120.2±28.30), sesame (162.5±25.47) and coconut (192.61±:44.14) respectively. The minimum weight loss (20.67) was observed in rocket seed oil followed by mustard oil (51.43), sesame oil (64.08) and maximum weight loss was recorded on coconut treated 011 green gram (90.3), as compare to control treatment. The temperature 29° C and humidity 64% was found favorable for pest multiplication.

EFFECT OF HOSTS ON THE BIOLOGY OF CONVERGENS LADYBIRD BEETLE, *HIPPODAMIA CONVERGENS* (COLEOPTERA, COCCINELLIDAE) IN LABORATORY CONDITIONS

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Present studies on the effect of various hosts on the biology of ladybird beetle *Hippodamia convergence* (Coleoptera: Coccinellidea) was carried out under laboratory condition at the Department of Entomology, Sindh Agriculture University Tandojam. The adults were collected and reared in the laboratory. After hatching of eggs, fifteen neonate larvae were kept in the petridishes (15 cm dia) with five treatments and experiments replicated three times. The larvae were fed on different hosts such as nerium aphids, *Aphis nerii*, (Boyr), akk aphid, *Aphis nerii*, (Boyr). mustard aphid, *Lipaphis erysimi* (Ka1t), safflower aphids, *Uroleucon compositae* (Theobald) and wheat aphid, *Diuraphis noxia* (Linn) The result reveals that the nerium and akk aphids less preferred hosts for the development of *H. convergent* requiring maximum number of days while wheat and mustard aphids were more preferred. The result indicated that incubation period varied from 2.4 to 1.9 at variable temperature of 22.06± 1.15 to 30.31±1.08 when *H. converges*

was reared on different host's species. The longest mean larval period was 17.22±1.07 days recorded on akk aphid, *Aphis nerii*, and the shortest was 11.27±0.92 days when grubs were fed on wheat aphid, *Lipaphis erysimi.* Pupal period ranged between 2.42±0.36 to 4.76±1.25 days re"corded on wheat aphid, *D. noxia* and akk aphid, *Aphis nerii.* The highest male and female longevities were 24.6±3.9 days and 27.7±1.52 days recorded on mustard aphid, *L. erysimi* and lowest were 13.2±4.4 days and 17.0±1. 73 days on nerium aphid, *A. nerii.* The longest duration of life cycle of female was recorded 45.7 days on akk aphid, *A. nerii* while shortest duration of life cycle was recorded 36.4 days on nerium aphid, *A. nerii* respectively. The highest adult female body weight was recorded as 10.8±0.11 mg on wheat aphid, *D. noxia*, and lowest female body weight was recorded as 8.1±0.62 mg on akk aphid, *A nerii.* The highest fecundity was recorded as 391.7±25.1 eggs on wheat aphid, *D. noxia* and lowest fecundity was observed as 176.7±17.6 eggs on akk aphid, *A. nerii.* A significant positive correlation was found betweel1 adult female body weight and fecundity of, *H. convergens.*

EFFECT OF NEEM-SEED COAT FRACTIONS ON METAMORPHOSIS OF RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM*

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Red flour beetle, *Tribollium castaneum* is a major stored grain pest. Many pesticides are in practice for its control, which cause many problems, *e.g.*, environmental pollution, residual effect in food grains and resistance in insects. To minimize these problems certain indigenous plant derivatives are also in use. Neem plant is one of these useful God gifted plants and many bitter substances are extracted from its fruit, stem, bark, leaves, seed and seed coat. In the present study five Ethanolic neem seed coat extracts/fractions (NSCE-A, NSCE-B, NSCE-C, NSCE-D & NSCE-E) were tested for their efficacy as growth regulator against red flour beetle, *Tribollium castaneum*. The fractions were tested at the rate of 0.2%, 0.1%, 0.05% & 0.025% of whole wheat flour. Five grams of treated and untreated (control) wheat flour was kept in test tubes. Twenty (10 male & 10 female) ten days old adult insects were released in the test tubes and removed after three days. Each treatment was replicated four times. All experiments were conducted on the same laboratory conditions of 25-30 °C temperature and 60% $\pm$ 5% Relative Humidity. Larvae, pupae and adults were removed from the wheat flour by sieving (30 mesh) on 13th, 25th and 45th days respectively, counted and weighed. Treated wheat flour fed adult insects produced significantly different (less in number and under weight) progeny. Nearly all the fractions caused reduction in number or weight of larvae, pupae or adults during the metamorphosis of the insect. The fraction NSCE-B proved the

most effective fraction inhibiting reproduction and development of *Tribollium castaneum*, at all larval, pupal and adult stages. The fraction NSCE-C and NSCE-D were also quite effective growth inhibitor. It may be, therefore concluded that contrary to general thinking neem seed coat also contains insecticidal bearing compound and could be used purposefully.

HOST PLANT SURVEY OF APHIDS (HOMOPTERA: APHIDIDAE) IN HYDERABAD AND ADJOINING AREAS

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Aphids probably are best known as plant lice or "greenfly. They cause damage to the number of crops, vegetables and ornamental plant. Present study was conducted during November 2010 to February 2011. We have surveyed different fields of Hyderabad, Jamshoro, Matari, Tando Muhammad Khan and collected the aphids from different parts of plants, grasses and herb. They have been brought to the laboratory into plastic jars and preserved in **Glycerin** (**Glycerol**) .The host plants includes, Mandeville Vine (Mandevilla sp.), Cup plant (Silphium perfoliatum), China Rose (Rosa chinensis),Wild Rose (Rosa acicularis) ,Wheat (*Triticum aestivum*)and Mustard*(Brassica campestris esculenta*), Tomato(*Solanum lycopersicum*). They were present from November but their growth rate got faster from February and their number was increasing day by day. The preserved specimens were washed with 10% KOH then the slides were prepared for identification. The specimens were identified through literature and keys. Five species of aphids have been recorded which belong to family Aphididae (Aphids):*Schizaphis graminum*(Rondani 1852), *Brevicoryne brassicae* (Linnaeus, 1758), Green peach aphid *:Myzus persicae* (Sulzer, 1776) [1]), Brown aphids :*Toxoptera citricida* (Kirkaldy,1907). These species of aphids are newly recorded from Hyderabad and adjoining areas.

RODENT DEPREDATION AND ITS MANAGEMENT STRATEGY IN POULTRY FARMS

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Poultry sector is one of the most important segments of agriculture in Pakistan. This sector generates employment and income for about 1.5 million people. Poultry meat

contributes roughly 19 percent of the total meat production in the country. Whereas 10,712 million eggs are produced by poultry farms (*Economic Survey of Pakistan, 2007-08*). Poultry meat and eggs are superior form of animal protein as they contain relatively high amount of essential amino acids obligatory by human body. The large concentrations of poultry farms in the suburbs of big cities create attractive surroundings and food sources for rodents. Whereas enclosed and insulated commercial poultry buildings provide ideal habitat for supporting large population of commensal rats, which may cause direct or indirect economic losses to the producers (Shafi and Aziz, 1987; Corrigan and Williams, 1986; Surgeoner, 2007). Rodents pose serious threats to poultry by feeding on poultry feed. According to an estimate one rat eats approximately 11.0 kg of feed per year (Ahmed, 2003; Ahmed *et al*, 1984) resulting in substantial economic loss. They damage eggs, kill chicks and cause threats to poultry birds resulting in harmful impact on egg laying. Rats damage buildings, structures, electrical wirings and insulations of poultry farms. They eat and contaminate feed of poultry birds with their droppings, urine and hairs. Rodents are carriers of approximately 45 diseases, including *Salmonellosis, Pasteurellosis, Leptospirosis, dysentery, Trichinosis* and *Rabies*. Rodents spread *Salmonellosis*, which can be transmitted to poultry and related personnel resulting in severe food poisoning . The house rat *Rattus rattus* and Norway rat *Rattus norvegicus* are the most destructive species in poultry farms. Effort to minimize these rodents can significantly reduce the damage and disease transmission. The rodents infestation in poultry farms can be managed by using certain control methods, by trained personnel, such as, trapping (live and kill), chemical (acute and chronic poison), making sheds rat proof and improving sanitation conditions. Some research trails to evaluate a cost-effective model package and its adoption level by the poultry farmers were conducted at commercial poultry farms in Gadap area, District Malir, Karachi. The trials involved a novel rodenticide baiting strategy utilizing PVC anticoagulant bait stations. Bordifacoum (0.005%), a second-generation anticoagulant and Zinc Phosphide (2%), an acute rodenticide bait was formulated along with yeast additive and was named as "PARC RAT BAIT." Field trails of this baiting technique and formulation have resulted in rodent population reduction of 82.85% and 73.81% respectively.

FEEDING BEHAVIOR OF ASIAN RED PALM WEEVIL *RHYNCHOPHORUS FERRUGINEUS* ON DIFFERENT VARIETIES OF DATE PALM

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Red palm weevil *(Rhynchophorus ferrugineus* Olivier 1790) is also known as Asian red palm weevil, belongs to family Curculionidae. Red palm weevil is a major pest of date palm in the world, particularly in Middle East and Asian countries. It causes sever damage to all main varieties viz. Aseel, Fasly and Red palm (local / common names), which are commonly grown in Sindh province (usually in upper Sindh).

Presently we have observed the feeding behavior of the Red Palm weevil on all three varieties. This study was conducted from 20 August 2010 to 10 February 2011. Adult weevil *Rhynchophorus ferrugineus* were collected from the fields of Khairpur and Rohri, Sindh, and were reared in plastic jars for mating and oviposition in the laboratory on its natural diet, (stem of date palm). Eggs have been shifted to Petri dishes for hatching and larval development with a piece of stem (90 gm) of date palm of all varieties. It was observed that *Rhynchophorus ferrugineus* weevils fed voraciously on Aseel in comparison to two other varieties, they ate about 80 % of given stem of Aseel while 48% of Fasly and 45 % of Red palm

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SEASONAL PATTERN OF INFESTATION BY SPOTTED BOLLWORM *EARIAS INSULANA* (BOISD.) AND PINK BOLLWORM *PECTINOPHORA GOSSYPIELLA* (SAUND.) IN FIELD PLOTS OF TRANSGENIC Bt AND NON-Bt COTTONS

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A transgenic Bt cotton line IR-FH-901 carrying Cry1Ac toxic gene from *Bacillus thuringiensis* was evaluated for resistance against spotted bollworm *Earias insulana* (Boisd.) and pink bollworm *Pectinophora gossypiella* (Saund.) in Punjab province, Pakistan during 2007 and 2008. We found no significant differences in spotted bollworm egg density between Bt and conventional cotton but larval density was significantly lower in unsprayed Bt cotton than unsprayed non-Bt cotton plots and insecticides were applied in sprayed non-Bt plots to keep the pest population below the threshold level. The rosette flowers density caused by pink bollworm infestation and number of pink bollworm larvae found in bolls of Bt cotton were significantly lower than non-Bt cotton plots. We conclude that transgenic Bt cotton is more effective in season-long control of spotted and pink bollworm.

OCCURRENCE OF RODENT CONTAMINATION (FILTH) IN RICE GRAINS SAMPLED FROM MAIN GRAIN MARKETS OF KARACHI

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Rodents consume and contaminate food destined for humans, as well as livestock and other animal with their droppings (feces), urine and hair. Rodents pose a great threat to human health and economy. They are responsible for enormous losses of grain

commodities in storage, either by eating or by rendering, inedible by their filth. Rats and mice are known to spread over 35 diseases worldwide. Rodent-borne diseases can be spread to humans through rat bite, consuming food and water contaminated with rodent feces and urine or inhaling air polluted with germs from rodent urine or droppings. For assessment of rodent contamination (filth) in grain commodities sold/stored in main grain markets of Karachi, a study on rodent contamination in rice was carried out. Regular surveys of main grain markets of Karachi were carried out. Shopkeepers were interviewed to know the severity of the rat problem and control measures adopted by them. Rat signs (gnawing, odour, smudge marks and droppings) were observed in and around the shops. Data were recorded on printed proformas. Rice samples were collected from randomly selected shops in each market (10 samples from each). Samples (250g each) were analysed in laboratory to identify rat contamination (filth). The contaminants were weighed and data were recorded for statistical analysis. Analysis revealed rat contamination (filth) of 0.094 ± 1.40g in Super Market, Liaqatabad; 0.065 ± 0.90g in Gole Market, Nazimabad; 1.03 ± 2.60g in Empress Market, Saddar; 0.92g ± 1.01 in Cantonment Area, Saddar; 0.97±1.12g in Khudda Market, DHA; 1.08 ± 1.20g in Water Pump Market, Federal 'B' Area; 0.092 ± 1.20g in Shadman Market, North Karachi; 1.52 ± 1.03g in Ranchore Line Market; 1.28 ± 1.03g in Banarus Chowk; 1.85 ± 2.01g in Aligarh Market, Orangi; 1.92 ± 1.48g in Pak Colony; 1.60 ± 1.26g in Gulshan-e-Iqbal; 1.46 ± 1.09g in Gulistan-e-Jauhar ; 1.03 ± 0.95g in Safora Goth; 1.80 ± 1.26g in Jodia Bazaar; 1.90±2.05g in Machi Miani; 1.98 ± 2.14g in Quidabad; 1.48 ± 0 .82g in Landhi No.6 Market and 1.26 ±g 0.76 in Liaquat Market, Malir, Karachi. Trapping of rats in all the surveyed markets (for five successive trap nights) revealed, high population of Norway rat, *Rattus norvegicus* and Roof rat, *Rattus rattus;* whereas, house mouse, *Mus musculous*, were trapped from some markets of slum areas. Shopkeepers and residents of the area were randomly interviewed to investigate rat nuisance. Rat signs (gnawing, smudge marks, foot prints, odour and feces) and rats were observed roaming and feeding on spilled grains and poultry waste in the markets. Preliminary findings justify implementation of urgent, safe, economical and sustainable rat management in these markets, including creating their awareness among shopkeepers through technology transfer.

EFFICACY OF TRANSGENIC BT COTTON AGAINST *HELICOVERPA ARMIGERA* (LEPIDOPTERA: NOCTUIDAE) IN THE PUNJAB, PAKISTAN

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A transgenic Bt cotton line, IRFH-901 expressing Cry1Ac insecticidal protein from *Bacillus thuringiensis* Subsp. *Kurstaki* (Berliner) was evaluated for resistance to cotton bollworm, *Helicoverpa armigera* (Hubner) in the cotton planting region of the

Punjab, Pakistan. The results showed that there was no significant difference in egg densities between Bt and non-Bt cotton. However, larval densities were significantly reduced in Bt cotton as compared to conventional non-Bt cotton. No insecticide application was needed to control this pest in Bt cotton. The results indicate that transgenic Bt cotton can play a significant role in reducing the pesticide application for control of *H. armigera*. However, increased larval densities at later stages of crop may be a critical factor in the non-adoption of Bt cotton in Pakistan.

BIOACTIVITY OF A CHITIN SYNTHESIS INHIBITOR, TRIFLUMURON, AGAINST RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM*, (HERBST) (COLEOPTERA: TENEBRIONIDAE)

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Red Flour Beetle, *Tribolium castaneum* (Herbst), is a worldwide serious pest of dried, stored, durable agricultural commodities, and of many value-added food products and non-food derivatives of agricultural products. A laboratory study was conducted to determine the ovicidal and reproduction inhibitory effects of a chitin synthesis inhibitor (CSI), triflumuron, against two field strains of *T. castaneum*. At concentrations of 0.01, 0.02, and 0.04 ppm, triflumuron did not killed the adults but caused significant reduction in oviposition and egg hatching of adults exposed to triflumuron-treated flour. Subsequent development of F_1 larvae, pupae and adult from hatched eggs was also severely inhibited. In a separate experiment, direct exposure of eggs laid by unexposed beetles to triflumuron also caused reduction in egg hatching and subsequent development of larvae, pupae and adult emergence was also inhibited. However, the inhibition effects due to direct egg treatment were not as strong as those obtained due to the adult treatment. Results show that triflumuron can be a useful addition for pest management programmes in mills, warehouses and food storage facilities.

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

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The climate of Khyber Pakhtunkhwa is very useful for the production of stone fruits such as peach, plum & apricot. The total area under these fruit orchards in NWFP was 11.7 thousand hectares, which produced 108.4 thousand tones of fruit (Anonymous,

2008-09). Various insect pests attack these fruit trees but peach flat-headed borer is the serious one causing gummosis, a major problem resulted in declining fruit growing area in the province. Our farmers solely relied on the frequent use higher doses of agric-pesticides and the pest control situation are quite alarming. Current project is aimed to develop IPM model based on chemicals/cultural practices, determination of proper insecticides/fungicides & proper application timings based on weakest link of pest life cycle towards better management in stone fruit orchards. Peach flat-headed borer, *Sphenoptera dadkhani*, Oben. (Buprestidae: Coleoptera) is an economic pest of stone fruits in Pakistan. Monitoring the adult emergence and their population density were carried out through conventional plastic strip wrapped on plum tree trunks. Maximum adult peach flat-headed borers were recorded during the last week of April i.e., 2.3 adults/ plastic strip trapper and afterwards with inconsistent variation in population in the .months of July (1.7), June (1.1), May (0.40) and November (0.20) during (2009). Maximum borers were also recorded during April i.e., 9.6 adults/plastic strip trapper followed by July (8.0), June (2.5), May and November (0.80) respectively during (2010). Field spray efficacy of different synthetic insecticides with same fungicide (Copper oxychloride) was recorded after adult emergence of peach flat-headed borer. The results showed that the minimum level of gummosis due to borer infestation was recorded in Cyren and Tenekil Plus i.e. 0.05-0.09 gum points/m2 followed by Thiodan & Perfekthion (0.470.49), Decis-D & Triazofos (0.70-0.74) and Control (2.43) in April. The field sprays of Cyren & Tenekil plus with Copper oxychloride were found very effective as compared to other insecticides after adult emergence. During dormant season, the efficacy of different synthetic/ botanical pesticides in Bordeaux mixture showed that the minimum level of gummosis due to borer infestation were recorded in treatments of Cyren and Chlorpyrifos i.e., 0.05-0.06 gum points/m2 followed by Tenekil Plus (0.11), Neem oil 1% (0.12), Emamectin (0.14), Hing 1% (0.21) and Control (0.44) respectively in the month of January. Cyren and Chlorpyrifos were found very effective as compared to other tested insecticides in Bordeaux mixture against Peach flat-headed borer.

DIVERSITY OF FRUIT FLIES SPECIES AND THEIR PARASITOIDS FAUNA ON DIFFERENT HOSTS IN KHYBER PAKHTUNKHWA

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Tephritid fruit flies are the serious pest attacking fruits and vegetables in Khyber Pakhtunkhwa. Farmers usually rely on pesticides to combat the problem, which are not only ineffective as the larvae are concealed inside the fruit, but also result in insect resistance, pesticides residues, toxicity, environmental pollution and cost economics problems. Therefore, it is quite imperative and pertinent to devise pest control strategies, which are not only effective and economical, but also environment friendly. Biological

control is one such area to be explored and exploited as a component of the IPM program. Different orchards and fields in the target sites were surveyed by collecting infested/dropped fruits using fruit- lab samples method to check fruit flies and their parasitoid's fauna in the target site. The survey was conducted on different hosts plants; guava, musk melon, pear, bitter gourd, and hybrid gourd in Kohat, Haripur and Peshawar. Results spowed the presence of three fruit fly species i.e *Bactrocera cucurbitae* Coq., *B. dorsalis* Hend. and *B. zonata* Saund in the target sites. The last two were recorded from Kohat and Haripur on guava, while, *B. cucurbitae* was present in Peshawar on musk melon and cucurbits vegetables. *B. zonata* and *B. dorsalis* were dominant in Kohat and Haripur respectively. Pear and cucurbits especially hybrid gourd was found as the suitable hosts for *B. zonata, B. dorsalis* in Peshawar. Regarding the parasitoids; *Diachasmimorpha longicaudata* Ashm and *Trybliographa daci* Weld were found in Kohat, Haripur and Peshawar. The highest parasitism rate of these parasites ranged. as 10.8-18.6% in the months of August to November. While the lowest parasitization range (02.4%) was found in the months of December and January to July.

INFESTATION OF COTTEN BOLLWORMS IN HYDERABAD DIVISION DURING 2009

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Cotton bollworms, *Earias insulana* (Spiny bollworm), *Earias villella* (spotted bollworm) *Pectinophora_gossypiella* (pink bollworm) and *Helicoverpa armigera* (American bollworm) have been consider the major Lepidopteran pest of cotton in Sind, Pakistan. Study of infestation of cotton bollworm *was* carried out in cotton growing localities of Hyderabad Division Such as Saeedabad, Hala, Tando Allahyar and Jamshoro during 2009. The data of infestation collected through weakly visiting of fields. During present study we have observed the infdation of *Ear.as* species mld *Pectinophora gossypiella* recoded high among all localities and highest damage reooded in month of September 8.50 by *Earias* species and 9.10 by *Pectinophora gossypiella* while infestation *Helicoverpa armigera* was receded only in Hala locality. Highest infestation of all bollworm species recoded in the month of September and October. The *Helicoverpa armigcra was* present in Hala locality and not other in other localities because the field selected in Hala locality situated near the river Indus so vegetation host alternative host plants present throughout the year

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COMPARATIVE EFFICACY OF SOME INSECTICIDES AGAINST LENTIL POD BORER (*HELICOVERPA* SPP) UNDER NATURAL FIELD CONDITIONS

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Comparative efficacy of five commonly used insecticides *viz*., emamectin benzoate, chlorpyrifos, profenophos, spinosad and lamda cyhalothrin against pod borer (*Helicoverpa* spp), on lentil crop under natural field condition. Highly significant differences were observed amongst insecticides used. Results showed that emamectin benzoate was the most effective insecticide against lentil pod borer among the tested insecticides followed by spinosad, chlorpyrifos and profenophos. Lamda cyhalothrin was found to be the least effective insecticide among the five tested insecticides. The maximum increase in lentil seed yield per acre was obtained with emamectin benzoate, whereas lamda cyhalothrin showed minimum increase lentil seed yield over control.

POPULATION COUNT OF *TRICHOGRAMMA* SPP. (HYMENOPTERA: TRICHOGRAMMATIDAE) IN SUGARCANE UNDER NATURAL FIELD CONDITIONS

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Sugarcane (*Saccharum officinarum* L.), the cash crop of Pakistan that meet the raw material demands of local sugar industry. Insect pests of this crop are the causing factor of decline in quality and low yield. Pests of sugarcane includes, Gurdastpur borer, *Acigona steniella* Hampson, top borer, *Scripophaga nivella* F; stem borer, *Chilo infuscatellus* Snellen and root borer, *Emmalocera depressella* Swin. Injudicious and indiscriminate use of insecticides not only creates resistance among insect pests but also becoming one of the major causes of killing the naturally occurring fauna of parasites. Among the parasitoids, *Trichogramma* spp. is the widely known egg parasitoid of these borers under biological control. Experimental field trial was carried out at district Vehari on 4 locations as, Luddan, 65/KB, Khadar Adda and Machi Wal to estimate the naturally occurring population of *Trichogramma* in sugarcane crop sown in cotton growing areas during the year 2007. Five sugarcane fields at each four locations were selected. Counted fresh eggs of *Sitotroga cerealella* were pasted on paper cards and installed on leaves of

canes in each field by making six repeats. Next day, egg cards of *S. cerealella* were collected and after seven days carefully examined to record the parasitism. Results revealed very low population of *Trichogramma* observed in the form of parasitism on eggs of *S. cerealella* with overall 0.5 to 1.3% parasitism. It is concluded from the present study that naturally occurring population of parasitoids, *Trichogramma* has been severely affected in sugarcane crop present in cotton area due to heavy use of insecticides and unable to combat with pest and need inundative releases.

INVESTIGATING THE PERFORMANCE OF SOME PREDATORS AGAINST COTTON MEALYBUG, *PHENACOCCUS SOLENOPSIS* TINSLEY (STERNORRHYNCHA: COCCOIDEA: PSEUDOCOCCIDAE): AN INVASIVE MEALYBUG DAMAGING COTTON IN PAKISTAN

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The present study was carried out to evaluate the predatory potential of four predators viz., *Menochilus sexmaculatus*, *Coccinella septempunctata*, *Brumoides suturalis* and *Hippodamia convergens* against cotton mealybug under laboratory and field conditions. Significant differences were found in the performance four predators under laboratory as well as under field conditions. First instar of the all the predators consumed minimum number of mealybug nymphs (in the range of 31±0.57-25 ±2.5 nymphs, being significantly higher in *M. sexmaculatus* and lower in *H. convergens.* Fourth instar of the all the predators consumed maximum number of mealybug nymphs (in the range of 216.3±2.2 -251.7±2.9 nymphs, being significantly higher in *M. sexmaculatus* and lower in *B. sutusralis*). Percentage consumption was observed in the range of 70-98% for all instars of four predators. Per day consumption was observed minimum for 1st instar (in the range of 12.50±1.2-15.33±1, being statistically similar for all tested predators) and maximum for 4th instar (in the range of 37.3±45.67±0.9 nymphs, being significantly higher in *M. sexmaculatus* and lower in *B. sutusralis*). Fourth instar of all tested predators had maximum search rate (2 mealybug nymphs/hr); whereas, rest of the predators instars had ≤1 mealybug nymph/hr. Over all, *M. sexmaculatus*, *C. septempunctata* and *B. suturalis* statistically similar and better performance (106.8±23.31-111.8±20.58 mealybug nymphs/life span). However, *M. sexmaculatus* and *B. suturalis* with 62-66% consumption (15.2-16.5 of the released mealybug nymphs) performed better than rest of the tested predators in the field experiment. In conclusion, these two predators have the potential to manage mealybug and should be mass reared and released to reduce the number of insecticide sprays in the mealybug infested field crops or vegetables.

DETERMINATION OF AN EFFECTIVE PREDATOR-PREY RATIO FOR SOME PREDATORS OF COTTON MEALYBUG, *PHENACOCCUS SOLENOPSIS* TINSLEY (STERNORRHYNCHA: COCCOIDEA: PSEUDOCOCCIDAE): A CASE STUDY IN PAKISTAN

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The present study was carried out to determine an effective predaor-prey ratio of four predators viz., *Menochilus sexmaculatus*, *Coccinella septempunctata*, *Brumoides suturalis* and *Hippodamia convergens* against cotton mealybug under field conditions. Nine predator- prey ratios (1:10, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40, 1:50 and 1:60) were maintained on the confined plants for nine weeks with a control (no predator) for each ratio and total consumption by predators on mealybug as well as growth rate of mealy bug was recorded. The result showed increasing trend in the consumption of predators on mealybug from 1:10 to 1: 35 (predator: prey) in case of *M. sexmaculatus*, *C. septempunctata*; but the same trend was found from 1:10 to 1: 40 in case of *B. suturalis* and *H. convergens*. However, decreasing trend was observed in the consumption of predators on mealybug after above-mention predator-prey ratios. *B. suturalis*, *M. sexmaculatus* and *H. convergens* consumed mealybug nymphs in the range of 21.05-24.61 nymphs/adult/day at predator-prey ration of 1:35 and 1:40, being statistically similar and comparatively maximum consumption than other ratios. In term of percentage consumption, however, percentage consumption decreased with an increase in predator-prey ratio in all predators. *M. sexmaculatus* showed more than 80% consumption for 1:10 and 1:15 predator-prey ratio. All tested predators resulted in more than 50% consumption for all predator-prey ratio except 1:50 and 1:60, where less than 40% consumption was recorded. In conclusion, 1:10 and 1:15 can be efficient predator-prey ratio for effective management of cotton mealybug in the cotton-cropping system, where releases of *M. sexmaculatus*, *C. septempunctata* and *B. suturalis* are to be made for integration.

EVALUATION OF SOME BOTANICAL EXTRACTS FOR THEIR DETERRENCE TOWARDS MELON FRUIT FLY

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Kernel and leaves of neem, *Azadirachta indica* (Meliaceae) and 'dhrak'/bakain, *Melia azadirachta* (Meliaceae), peel of sweet orange fruit, *Citrus sinensis* (Rutaceae), chilli fruit, *Capsicum annuum* (Solanaceae), ginger rhizomes, *Zingiber officinale*

(Zingiberaceae) and garlic cloves, *Allium sativum* (Alliaceae) were evaluated for their effect on the deterrence/attraction of melon fruit fly towards treated fruits under the laboratory and treated crop under field conditions. The olfactometeric studies revealed that garlic, ginger and neem extracts deterred the maximum number of released fruit flies, from oviposition, (94.54 % to 92.8 % of released flies) for a longer period (8 days) and resulted in least fruit infestation (3.4±3.3% to 7.13±4.5%). After eight days, 13-15 fruit flies (26-30% of released fruit flies) were attracted to the fruits, placed in the small jars of an olfactometer and 16.67% to 33.3% of the fruits, placed in small jars, were found infested on 10th day. 13.4±3.12 (26.8% of released flies), 8.47±2.18 (16.94% of released flies) and 7.93±2.47 (15.86% of released flies) fruit flies were attracted to and 40.47±12.9, 56.67±12.7 and 63.33±10.3% fruit infestation was observed in those fruits, which were treated with chilli, 'dhrak' and citrus, respectively. Field studies also showed that spray of garlic, ginger and neem extracts resulted in fruit infestation ≈ 1.8-4.5 times less, yield loss/plant ≈ 2.1-4.3 times less, marketable fruits/plant ≈ 4.1-6.4 times more and marketable yield/plant ≈1.8-2.8 times more than control. However, the spray of other botanical extracts resulted in more than 40% fruit infestation, less than 4 marketable fruits/plant, more than 150g yield loss/plant and less than 350g marketable yield/plant. Conclusively, garlic, ginger and neem extracts, which were found comparatively more effective, can be integrated with other non-chemical fruit fly control tactics in cucurbits cropping system to reduce the number of cover spray with insecticides and ensure the provision of cucurbits free of toxic residues of insecticides.

EVALUATION OF SYNERGISM/ANTAGONISM IN THE TOXICITY OF INSECTICIDES AGAINST COTTON MEALYBUG THROUGH COADMINISTRATION WITH SILICON DIOXIDE UNDER LABORATORY CONDITION

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Cotton mealy bug, *Phenacoccus* sp. (Homoptera: Pseudococcidae) is a soft-bodied insect and has emerged as an important pest of cotton in Pakistan recently within 2-3 years. The cover spray of insecticides causes significant mortality in initial instars which lack waxy covering; but non significant (nominal) mortality in later instars and adult females which have very thick waxy covering. In this study, silicon dioxide was used that damages the waxy layer of insect body and insect die due to dehydration. Silicon dioxide was used with the combination of different insecticides to check its efficacy against cotton mealybug. The results revealed that higher concentrations of silicon dioxide synergized the toxicity of all tested insecticides many fold. Intensity of synergistic effect on the toxicity of insecticides increased with increasing concentrations of silicon dioxide. The quantity and percentage of wax removed from the body of adult female increased

with an increase in concentrations of silicon dioxide coadministrated with tested insecticides. The treatments, having silicon dioxide admixed with insecticides, removed many fold wax from the body of adult female than the treatments having only insecticides. Percentage mortality was found higher in the adult female as well as at ovisac stage at higher ratio and maximum at 1:3 (insecticide:silicon dioxide). Coadministration of silicon dioxide at higher concentration in insecticides resulted in many fold higher mortality at ovisac and adult female stage than the insecticide application alone.

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

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The most effective melon fruit flies management tactics, screened, through different experimentations, were integrated into different IPM-Models, which were evaluated at two localities, under field condition. The results of these tests showed that variations in the biotic and abiotic factors, of each locality, did not affect the performance of each IPM-Model. The performance of each IPM-Model was found to be similar at Harappa and Faislabad. However, an IPM-Model-7 (HSM + P x P 45 cm + Early Sowing (15 February) + Sanitation + Resistant variety + Baited pheromone + GF-120 + Food-baited chemical) proved to be a significantly best model, with a fruit infestation ≈ 53.5 and 12.1 times less; yield loss/plant ≈ 27.9 and 6 times less; marketable fruits/plant ≈ 112.9 and 2.6 times more; marketable yield/plant ≈ 7.2 and 2.7 times more; expected marketable yield in tonnes/ha ≈ 7.2 and 2.7 times more than that of IPM-Model-8 (control) and the conventional IPM-Model-1, respectively and a cost benefit ratio of 1 : 7. Similarly, an IPM-Model-3 (HSM + P x P 45 cm + Early Sowing (15 February) + Sanitation + Resistant variety + Baited pheromone + GF-120 spray), with a fruit infestation ≈ 39.8 and 9 times less; yield loss/plant ≈ 25.1 and 5.4 times less; marketable fruits/plant ≈ 97.5 and 2.3 times more; marketable yield/plant ≈ 6.3 and 2.4 times more; expected marketable yield, in tonnes/ha ≈ 6.3 and 2.4 times more than that of IPM-Model-8 and that of a conventional IPM-Model-1, respectively and a cost benefit ratio of 1: 6.8, also, gave significantly better results and was found, statistically, at par with IPM-Model-4 and IPM-Model-6. IPM-Model-4 (HSM + P x P 45 cm + Early Sowing (15 February) + Sanitation + Resistant variety + Baited pheromone + Botanicals spray) and IPM-Model-6 (HSM + P x P 45 cm + Early Sowing (15 February) + Sanitation + Resistant variety + Baited pheromone + Spray of GF-120 6 + Spray of Botanicals) yielded a fruit infestation ≈ 30.2-39.9 and 6.8-8.9 times less; yield loss/plant ≈ 19.4-23.5

and 4.2-5 times less; marketable fruits/plant ≈ 96.7-79.5 and 2.3 times more; marketable yield/plant ≈ 6.3 and 2.4 times more; expected marketable yield, in tonnes/ha ≈ 6.2-6.3 and 2.4 times more than that of IPM-Model-8 and that of a conventional IPM-Model-1 and a cost benefit ratio of 1 : 6.8. IPM-Model-5 (HSM + P x P 45 cm + Early Sowing (15 February) + Sanitation + Resistant variety + Baited pheromone + Food-baited chemical) yielding fruit infestation ≈ 14.5 and 3.3 times less; yield loss/plant ≈ 8.7 and 1.9 times less; marketable fruits/plant ≈ 93.3 and 2.2 times more than; marketable yield/plant ≈ 6.1 and 2.3 times more; expected marketable yield, in tonnes/ha ≈ 6.1 and 2.3 times more than that of IPM-Model-8 and that of the conventional IPM-Model-1, respectively and a cost benefit ratio of 1 : 6.5 also performed better. IPM-Model-2 (Optimum sowing time + Resistant variety + FSM + Chemical control + No sanitation), with a fruit infestation ≈ 5.8 and 1.3 times less; yield loss/plant ≈ 5.3 and 1.1 times less; marketable fruits/plant ≈ 55.4 and 1.3 times more; marketable yield/plant ≈ 3.3 and 1.2 times more; expected marketable yield, in tonnes/ha ≈ 3.3 and 1.2 times more than that of IPM-Model-8 and that of the conventional IPM-Model-1, respectively and a cost benefit ratio of 1 : 6.2, also, gave good results and was found to be, statistically, different from the conventional IPM-Model-1 and the control IPM-Model-8. The descending order of the performance of tested IPM-Models is: IPM-Model-7 > IPM-Model-3 > IPM-Model-6 > IPM-Model-4 > IPM-Model-5 > IPM-Model-2 > IPM-Model-1 > IPM-Model-8. In conclusion, IPM-Model-3, IPM-Model-4, IPM-Model-5, IPM-Model-6 and IPM-Model-7, can be exercised, in the areas, where there are hot spots of the melon fruit flies.

INSECTICIDAL AND ANTIFEEDANT ACTIVITIES OF PROTEINS SECRETED BY ENTOMOPATHOGENIC FUNGI AGAINST *PLUTELLA XYLOSTELLA* (LEPIDOPTERA., PLUTELLIDAE)

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The diamondback moth *Plutella xylostella* L., (DBM) is an important and cosmopolitan pest of cruciferous crops. The toxic crude proteins produced by *Isaria fumosorosea* were checked against DBM for its effectiveness as insecticidal and antifeedant characteristics. The crude proteins produced in the Czapek Dox liquid medium were used against 3rd larval instars of DBM. The results indicated a considerable insecticidal and antifeedant activity by crude protein extracts. Notably the most active crude protein extract belong to CNZH that showed 83.3% mortality of 3rd larval instar after six days of treatment. CNZH was tested for its toxicity at different concentration of crude protein and a maximum mortality percentage of 91.6 was recorded six days post treatment. A significant level of increase in the antifeedant index was recorded as the

concentrations increased and also the time duration as the time of exposure increased, significant results were obtained ($P<0.05$). The more time the larvae were exposed, the better results were obtained.

PREVALENCE OF ENTOMOPATHOGENIC FUNGI IN DIFFERENT LOCATIONS OF SOUTHERN PUNJAB, PAKISTAN

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Entomopathogenic fungi are an efficient tool for the biological control of a wide range of insect pest particularly related to agricultural crops. The soils from diverse sources (agricultural field crops, vegetables, orchards and plantation) were collected from different locations of Multan and Bahawalpur Districts for the isolation of different entomopathogenic fungi by using selective medium. The soils from the cotton fields harbor more number of *Metarhizium anisopliae* than other soil sources with recovery percentage of 18.12% and 7.5% for *Isaria fumosorosea.* In contrast to this soil samples from different sources of Bahawalpur recovered 11.6% of *Metarhizium anisopliae* and 1.66% of *Isaria fumosorosea*. The presence of these fungi in the soils of different fields requires further work to be done for their efficacy against different insect pests.

COMPARATIVE FIELD EFFICACY OF NEW CHEMISTRY INSECTICIDES AGAINST BOLLWORM COMPLEX OF COTTON IN FIELD CONDITIONS

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Cotton, *Gossypium hirsutum* L. the white Gold, the economy dependent crop of Pakistan. Its yield for the last few years has been reduced by the heavy havoc of pest attacks. Cotton pests are categorized into, sucking and bollworms pest complex. Control strategies of bollworm pests are mainly depends upon chemical methods by the use of pesticides. Present trial was conducted in Entomological field area at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad by sowing of commercial cotton cultivar NIAB 777 during 3rd week of May 2010. Standard agronomic practices were adopted through out the crop period. Six candidate insecticides were screen and evaluated for their efficacy against bollworm pests American bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) and spotted bollworm, Earias *vittella* (Fabricius) (Lepidoptera: Noctuidae) in comparison to standard check and control. It was found that

helmet 40% (Chloropyrfos) gave comparable results against bollworms, the candidate insecticides, Belt 480 SC and Explorer 1.9 EC (Emamectin benzoate) proved equally effective to that of standard insecticide in controlling the bollworm infestation.

COMPARATIVE EFFICACY OF NEW CHEMISTRY INSECTICIDES AGAINST SUCKING INSECT PESTS IN FIELD COTTON

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Cotton, *Gossypium hirsutum* L. is the white Gold, the economy dependent crop of Pakistan. Its yield for the last few years has been reduced by the heavy attack of insect pest that are categorized into, sucking and bollworms pest complex. Control strategies of sucking insect pests are mainly depending upon chemical methods by the use of pesticides. Present study was conducted in Entomological field area at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad by sowing of commercial cotton cultivar NIAB 777 during 3rd week of May 2010. Standard agronomic practices were adopted through out the crop period. Six candidate insecticides of new chemistry were compared with each of standard insecticide and a check and evaluated for their efficacy against cotton whitefly, *Bemisia tabaci* (Gend) (Homoptera: Aleyrodidae) and jassid, *Amrasca devastans* (Distant). Result showed that insecticides result 55% (Imidacloprid), mospilan 20 SP (Acetamiprid) gave comparable results against sucking insect pests to that of standard insecticides.

ECOLOGICAL STUDIES OF BOLLWORMS OF COTTON THROUGH PHEROMONES BAITED TRAPS

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Cotton, *Gossypium hirsutum* L. is vulnerable to sucking and bollworm pest complex. Heavy attack of these pests has reduced its yield for the last few years. These pests are categorized into, Bollworm complex includes American bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae); spotted bollworm, *Earias vittella* (Fabricius) (Lepidoptera: Noctuidae); and pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae). Information on their appearance before time can leads towards effective control strategies under integrated pest management (IPM) of cotton. IPM strategies of these bollworms are mainly depend upon chemical methods by the use of insecticides. Failure in pest control by the insecticides is due to lack of

information about the pest occurrence. Ecological study of pests through pheromone baited traps provides first hand information on the appearance of pests through the catch of their moths. In present study, pheromone baited traps of respective pests were installed in Entomological field area at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad during whole the months of the years 2009 and 2010. It was inferred from the two years field data that the pink bollworm moths remained active throughout the year and the highest peaks were observed in the month of April and September, however, moth population of other bollworms were recoded the highest during the period of cotton crop and observed low after the termination of cotton.

RESIDUAL TOXICITY OF INSECTICIDES USED IN COTTON AGRO-ECOSYSTEM TO COTTON MEALYBUG PARASITOID, *AENASIUS* SP. (HYMENOPTERA: ENCYRTIDAE) UNDER LABORATORY CONDITIONS

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These experiments were carried out to study the residual toxicity of insecticides used in cotton agro-ecosystem to cotton mealybug parasitoid, *Aenasius* sp. (Hymenoptera: Encyrtidae) under laboratory conditions. The main objective of the present study was to evaluate the hypothesis "If the recommended doses of insecticides used in cotton against the insect pest complex are lethal or safe for *Aenasius* sp." Nine concentrations including recommended dose of each of the twelve insecticides (Acetamiprid, Emamectin benzoate, Buprofezin, Chlorpyrifos, Cypermethrin, Esfenvalerate, Imidacloprid, Lufenuron, Profenofos, Pyriproxyfen, Nitenpyram, and Thiacloprid) were evaluated against free living adults and immatures (present in mummy cases) of the *Aenasius* sp. under laboratory conditions. Adults were exposed to pesticide residues by placing them in apparatus containing pesticide residues while the immatures were sprayed by the nine concentrations in petri dishes. The data regarding adult mortality of the parasitoid were recorded at an interval of 6hr for 24hr after treatment application but for immature (mummies) at an interval of 24hr for 25 days. The results revealed that mortality in adult and immature parasitoid increased with increase in concentration as well as exposure period for each insecticide. Significant variations in the residual toxicity of different insecticides against the parasitoid were observed. Chlorpyrifos showed maximum residual toxicity against adult parasitoid after an exposure time of six hours, causing 64.8% mortality to the subject population. Rest of the pesticides caused <50% mortality during this exposure time. After 12hr exposure, Chlorpyrifos, Acetamiprid, Thiacloprid, Emamectin benzoate, Nitenpyram, Cypermethrin, Profenofos and Esfenvalerate caused >50% mortality. The mortality after an exposure of 18hr revealed that all tested pesticides caused >50% mortality in the subject population to the adult parasitoid except the Buprofezin. Chlorpyrifos,

Acetamiprid and Pyriproxyfen even caused >75% mortality. After 24hr, all the tested insecticides caused >50% mortality, where Chlorpyrifos was the most toxic (100% mortality) and Buprofezin was least toxic (69.14% mortality) insecticide. Lethal toxicological effect on immature stages of the parasitoid for the 1/16, 1/8, ¼, ½ concentrations of the recommended dose of all tested pesticides were not >50%, Profenofos caused highest mortality but rest of the pesticides resulted in <50% mortality. Whereas, at 8x and 16x of recommended dose rate, Profenofos Esfenvalerate, Emamectin benzoate, Buprofezin, pyriproxyfen, Cypermethrin, Chlorpyrifos and Thiacloprid caused >50% mortality while rest of the pesticides caused less than 50% mortality to the subjected immature population of the *Aenasius* sp.

TUNNELING BEHAVIOR OF *HETEROTERMES INDICOLA* (WASMANN) AGAINST IMIDACLOPRID AND PVRETHROID INSECTICIDES

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Heterotermes indicola (Wasmann) is one of the most destructive subterranean termites. Tunneling behavior of *H indicola* (Wasman) against imidacloprid and pyrethroid insecticides was studied. *H indicola* (Wasman) penetrated effectively at 1.09375, 2.1875, 4.375, 8.75, 17.5, and 35 ppm concentration of Bouncer. In Mirage treated soil tunneling was recorded at 15.625, 31.25, 62.5, 125, 250 and 500 ppm. Mirage (Imidacloprid) proved to be more toxic as compared to bouncer (Pyrethroid) against *H. indicola.* (Wasmann).

LARVICIDAL ACTIVITY OF SOME CITRUS ESSENTIAL OILS AGAINST RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM* (HERBST) (COLEOPTERA: TENEBRIONIDAE)

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Red flour beetle, *Tribolium castaneum* (Herbst) is most common and destructive pest throughout the world and is found in association with stored commodities in granaries, mills and warehouses. In the present study, essential oils extracted from four *Citrus* species (*Citrus sinensis*, *Citrus reticulata*, *Citrus aurantioidea* and *Citrus paradisi*) were evaluated against grubs of *T. castaneum* under laboratory conditions. Twenty grubs of *T. castaneum* were exposed to the filter papers treated with *Citrus* essential oils at concentrations of 2, 4 and 6% at 3, 5 and 7 days exposure periods in each treatment. Mortality of grubs varied significantly with increase in concentration and due

to the effect of *Citrus* species. At concentration of 6%, *C. reticulata* exhibited maximum larvicidal activity (53.3%), followed by *C. paradisi* (50%), *C. sinensis* (36.5%) and *C. aurantioidea* (26.6%) at exposure period of 7 days. It is concluded that *C. reticulate* and *C. paradisi* essential oils exhibit excellent larvicidal activity against *T. castaneum* and these oils can be a useful addition in pest management programmes in mills, warehouses and food storage facilities.

STUDIES ON CONTROLLED RELEASES OF 1ST AND 2ND INSTAR *CHRYSOPERLA CARNEA* (STEPHENS) (NEUROPTERA: CHRYSOPIDAE) LARVAE UNDER LABORATORY CONDITIONS

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Lacewings, *Chlysoperla carnea* are important generalized biological control agents to reduce the population of sucking pests and neonate lepidopterous larvae. Different larval stages of *C. cornea* are feeding voraciously their prey including their own younger instars. For field augmentation either eggs of lacewings on paper cards or their larvae are adhered on plants with some soft means. In these practices very few larvae reach to their prey due to starvation and non-stability on plant. In present studies straw tubes of 2 different sizes filled with different quantities of food have been used on two different positions for first two instars of predator to minimize their cannibalism and effective dispersal on the plant. The overall results showed that straw tubes of size 0.3 cm X 8 cm proved better than size 0.3 cm X 12 cm in dispersing the 2nd instar larvae within 24 hrs with less cannibalism. Horizontal placement of straw tubes dispersed all (100%) 2nd instar larvae within 24 hrs followed as compared to 60% dispersal in vertical placement of tubes. The minimum cannibalism (10%) and eggs food consumption (30%) were recorded in horizontal placement than vertical placement showing high cannibalism (40%) and more egg food consumption (52%).

FEEDING PREFERENCES OF *HETEROTERMES INDICOLA* (WASMANN) UNDER NO CHOICE AND CHOICE LABORATORV AND FIELD TRIALS

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Ten species of wood were tested for their natural resistance and to reveal feeding preferences of *Heterotermes indicola* (Wasmann) under no choice and choice laboratory and field trials. The impact of drying temperature (60°C, 70°C, 80C, 90°C & 100°C) was

studied. The amount of wood consumed in general, increased with increase in drying temperature indicating that heat contributed to the loss of natural resistance components of the woods. In no choice laboratory experiments, *C. deodara* and *D. sissoo* were found highly resistant and *P. euramericana* highly palatable. When a choice was given and the . wood was offered in combination of two, *H. indicola* repeated its instinct, easily identified the most preferred wood and consumed it more. Consequently *H indicola* showed maximum feeding on *P. euramericana* and the minimum on *C. deodara* and *D. sissoo.* The mean feeding propensity was significantly different (P<0.000). Based on the feeding propensity, the woods are arranged in descending order of preference: *Populus euramericana* > *Mangifera indica* > *Pinus roxburghii* > *Acacia arabica* > *Morus alba* > *Abies pindrow* > *Tectona grandis* > *Azadiracta indica* > *Dalbergia sissoo* > *Cedrus deodara.* Although *H. indicola* fed aggressively on *A. pindrow,* the wooden blocks had undesirable effect on the survival of the species, manifesting toxic nature of the wood.

EFFECT OF BOUNCER (BIFENTHRIN AND CVPERMETHRIN) ON *COPTOTERMES HEIMI* (WASMANN)

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The toxicity of Bouncer (Bifenthrin and Cypermethrin) applied to soil was recorded against *Coptotermes heimi* (Wasmann) according to Smith (1979). Mortality during 8 hour exposure for Bouncer at 2.73,5.46, 10.93,21.87,43.75, 87.5, 175 and 350 ppm was 3.33%, 6.66%, 13.33%, 63.33%, 80%, 83.33% and 96.66% respectively. The LC_{50} values for *C. heimi* (Wasmann) exposed to soil treated with Bouncer were 40.73 ppm. In repellency test, *C. heimi* (Wasmann) showed repellent result in soil treated with Bouncer at 2.731 5.46, 10.93, 21.K7, 43.75, 87.5, 175 and 350 ppm. *Coptotermes heimi* (Wasmann) penetrated in Bouncer treated soil at 10.93, 21.87, 43.75, 87.5, 175, and 350 ppm.

FEEDING POTENTIAL AF HUNTING SPIDERS ON WHEAT PESTS

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The potential of three hunting spiders viz., *Lycosa terrestris, Pardosa birmanica* and *Oxyopesjavanus* (collected from wheat crops in Punjab, Pakistan) as predators was studied in the laboratory. Wheat insect pests i.e., adult and larvae of Armyworm *(Helicoverpa armigera),* adult and larvae of pink graminous borer *(Sesamia inferens)* and nymph and adult of aphid *(Sitobion avenae)* were used in the laboratory experiments.

Results of no choice feeding test revealed significantly different (P < 0.001) consumption rate of the three hunting spiders. O. *javanus* consumed significantly more prey items than *L. terrestris* (t = -6.90 P = 0.000) and P. *birmanica* (t =-4.71; P = 0.00). Consumption rate of females (all species) spiders was higher than males (t =-4.32; P =0.002 for *L. terrestris;* t = 3.80; P = 0.004 for P. *birmanica* and t = 4.54; P = 0.001 for O. *javanus).* In choice feeding experiment when a constant number of pests were offered, consumption and prey preferences of hunting spiders w as not different from choice experiments. The investigation indicated that the these predatory spiders may be an important natural enemy of wheat pests because of their polypredacious habit and efficient prey-searching ability.

MOLECULAR SURVEILLANCE OF MALARIA IN SOUTH PUNJAB WITH HIGHER PROPORTION OF MIXED INFECTIONS

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The present study was conducted to determine the Surveillance of malaria in South Punjab by microscopy and Polymerase chain reaction (PCR). One hundred clinically symptomatic patients for malaria fever were studied by using microscopy and nested PCR. Samples from 40 out of 100 patients were found positive for malarial parasites. One patient was found with mixed infection of *Plasmodium falciparum* and *Plasmodium vivax,* where as *P. falciparum;* and *P. vivax* infections were detected in 17 and 22 patients respectively. In nested PCR, genus-specific primers for *Plasmodium spp.* in round 1 and species-specific primers for *P falciparum* and *P. vivax* in round 2 were used. By the application of PCR, 41 % were found to be infected by *Plasmodium spp.* Among *Plasmodium* positive patients, mixed, *P falciparum* and *P. vivax* infections were detected in 10, 15 and 16 patients, respectively. Thirty nine microscopically positive patients confinned to have *Plasmodium spp.* Infection and 1 turned out negative, by PCR. Two microscopically negative patients had shown *Plasmodium spp.* infection (1 *P falciparum* and 1 *P. vivax)* by PCR. In total samples, *P falciparum, P. vivax* and mixed infection *(Pfalciparum* and *P. vivax)* accounted for 36.6%, 39.0% and 24.3%, respectively. A greater proportion {24.3%) of mixed infections detected by nested PCR signified me sensitivity o f PCR assay over microscopic diagnosis. In this study, microscopy was found deficient for interpretation of mixed infections, low parasitaemia, and species specific diagnosis. The sensitivity, specificity and efficacy of nested PCR was calculated 95%, 98% and 97% respectively, showing PCR as a more effective and efficient diagnostic tool for of malaria.

SCREENING INSECTICIDES AS TOXICANT IN LURE, METHYL EUGENOL FOR MALE AN INHILATION OF FRUIT FLIES

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Fruit flies *(Bactrocera)* are one of the main pests of fruits and vegetables, infesting hundreds of cultivated and wild hosts. Male annihilation using lure baited traps is a good alternative of pesticide spray for the control of fruit flies. Methyl eugenol attracts males of a large number of fruit flies. A lure toxicant bait is widely used to detect & suppress flies population. Due to ban on existing lure toxicants, it is necessary to replace these by other easily available toxicants. For this purpose 12 insecticides viz. Chlorpyrifos, Thiodan, DDVP, Laser, Decis-D, Saprofan, Cypermethrin, Karate, Methyl parathion, Dimegro, Fyfenon, and Amicon were mixed @ 5% with 85 % Methyl eugenol and 10 % sugar. Five ml of test bait was used /trap on cotton wick. Traps were installed in the month of August in CR design in guava orchard at Kohat. Data were recorded on number of flies captured /week/trap for 15 weeks. The results indicated that all test insecticides caused fly mortality, none of them out classed Saprofon (standard) however, 4 insecticides, Decis-D, Cypermethrin, Karate and Fyfenon were found as effective as standard and hence can be used as good alternate toxicants in lure bait for male annihilation of two important fruit flies species i.e. *Bactrocera dorsalis* and *B. zonata.*

COMPARING EFFICACY OF MIXED LARVAL DIETS ON THE DEVELOPMENTAL ATTRIBUTES OF *ANOPHELES ARABIENSIS* PATTON

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A comparison test was conducted on three mixed diets consisting of ingredients from natural grains and commercial brand aquaculture foods. *Anopheles arabiensis* Patton larvae were reared on these diets separately under standard rearing conditions at the Insect Pest Control Laboratories (IPCL), IAEA, Seibersdorf, Austria. Time to pupation, time to emergence, survival to pupal and to adult stages, and adult wing length measurements were recorded and taken into account as parameters for diet comparison. All three diets resulted in development of larvae to pupae and to the adult stage. Significant reductions in time to pupation and to emergence were recorded for larvae fed diet 1 (NIPA1) composed of natural ingredients. Diet 1 resulted in the shortest larval duration, highest survival rate and largest wing lengths for both male and females. Body length of the 4th instar (L4 stage) was also largest for diet 1, but was not significantly different from other diets. Addition of vitamin mix to diet 1 did not improve its quality as measured by larval duration, survival and emergence success.

EVALUATION OF DIFFERENT INSECTICIDES AND DIFFERENT CONCENTRATIONS AGAINST SUBTERRANEAN TERMITE, *HETEROTERMES INDICOLA* WASMANN UNDER LABORATORY CONDITIONS

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Experiments were conducted under laboratory conditions at Entomology Division, Nuclear Institute for Food and Agriculture, Peshawar. Three pesticides viz. Chlorpyrifos, Fipronil and Cadusafos were tested against *Heterotermes indicola* Wasmann. Five concentrations of each pesticide @ 25ppm; 50ppm; 100ppm; 200ppm and 400ppm were made. Mortality was recoded after different intervals of time i.e. 8hrs to 48 hrs. Chlorpyrifos was found most effective; it caused 100% mortality even at 200 ppm, while Cadusafos was found least effective at all the concentrations. Mortality was found directly correlated with insecticide concentration. When the insecticide concentrations were increased mortality was increased. The medium treated with all insecticides and different concentrations were re-tested for their effectiveness after 15 days and found least effective. It is concluded from the results that Chlorpyrifos @ 200ppm is effective for the control of *Heterotermes indicola.*

EFFECT OF CHEMICALS ON THE TUNNELING BEHAVIOUR OF *HETEROTERMES INDICOLA* WASMANN (ISOPTERA: RHINOTERMITIDAE) UNDER LABORATORY CONDITIONS

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Experiments were conducted to see the tunneling behaviour of termites when blocked by treated sand barrier. Three pesticides viz. Chlorpyrifos, Fipronil and Cadusafos were tested against *Heterotermes indicola* Wasmann under laboratory conditions in 30cm long glass tubes. Five concentrations of each pesticide were made @ 25ppm; 50ppm; 100ppm; 200ppm and 400ppm. Three parameter studied were cumulative tunneling distance (cm), longest tunnel length (cm) and Number of tunnels. The results showed that Chlorpyrifos @ 400ppm stopped the tunneling completely and termite workers did not manage to cross the treated sand barrier which might be due to its repelling fast acting nature. At 200ppm shorter tunnel distances were observed but as the concentration decreased; all the three parameters get increased. Although *H indicola* tunneling was significantly affected by higher concentrations of all the insecticides but in Cadusafos and Fipronil termite workers able to cross the treated sand barrier even at higher concentration of 400ppm. At lower concentrations. of pesticides the insect penetrated to longer distances and made more number of tunnels.

FIELD TESTING OF NEEM OIL FOR MANAGEMENT OF CHICKPEA POD BORER, *H. ARMIGERA* ON CHICKPEA CROP

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Helicoverpa armigera (I-lb.) is a major economic insect pest of chickpea, tomato, cotton and tobacco crops of Pakistan. Each host crop is severely damaged by *H. armigera* and causes losses to the tune of 20-96% in chickpea crop. farmers increasingly rely on synthetic insecticides to manage this pest in different crops leading to develop insect resistance to pesticides. When the larvae cross third instar, none of the pesticides give effective control. Continuous and indiscriminate usage of insecticides has resulted in development of insect resistance problems to the chemicals. Neem derivatives having bioactive compound such as Azadirachtin and salamin are bitter tasting makes the crop plants . unattractive for pest oviposition and have repellant action. Keeping in view the non-hazardous property of neem oil, a trial was laid out to evaluate the efficacy of different concentrations of neem oil against pod borer on chickpea crop. Chickpea variety NIFA-95 was planted during November at experimental farm of NIFA. There were five treatments including control. Each treatment had four replications with a plot size 12 m^2 in RCB design. Neem Oil treatment concentrations were 1, 2, 3 and 4(% along with control (without treatment). Data were recorded on pre & post-treatment larval population, (% pods damage & grain yield. The results showed that among treatment concentration 3% of neem oil was found effective in reducing pod borer larval population (6.5 larvae/ 5 plants) with minimum pods damage (40%) and maximum grain yield (200 kg/ha). In control (untreated) high larval population (22.7 larvae/5 plants), maximum % pods damage (72.4%) and low grain yield (100 kg/ha) was recorded in chickpea crop. It is concluded from the results that neem oil having 3% concentration proved effective in reducing pod borer crop losses, larval population and enhance grain yield of chickpea crop.

DETERMINATION OF PESTICIDES (ACARICIDES) RESIDUES AND HEAVY METAL DETECTION IN HONEY

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A number of mite infestation control product (Apistan, Amitraz and Formic acid) were used for 4 weeks period in September 2009. The objectives of the research work

were to determine the infestation of varroa, distribution level of selected heavy metals like Copper (cu), Zinc (Zn), Iron (Fe), lead (Pb) and Magnesium (Mg) and to investigate the residual levels of various pesticides. Treatments were given to different apiaries located in Kohat Karak and D I Khan regions. The residual concentrations determined in the honey, apiston residues were in the following range of 0.004 -0.066 ppm, amitraz in 0.011 - 0.108 ppm and formic acid was found high i.e (15-75 mg/kg) respectively. Some honey samples showed higher residual level of formic acids then world health organization (WHO) limit of 1mg/kg. Heavy metal in honey is of interest not only for quality control but the determination of environmental contamination. The mean value for heavy metal Cu, Zn, Fe, Pb and Mn were 0.146, 0.191, 4.244, 0.056 and 0.030 ppm respectively. The result suggested that honey may be useful for assessing the presence of environmental contamination.

INVOLVEMENT OF BARK BEETLE *HYPOCRYPHALUS MANGIFERAE* AS A VECTOR OF MANGO SUDDEN DEATH SYNDROME AND ITS INTEGRATED MANAGEMENT IN ORCHARDS

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Mango sudden death syndrome (MSDS) is a serious threat to mango industry and bark beetle *Hypocryphalus mangiferae* is also involved as a wounding and facilitating agent for transmission of mango decline disease pathogens. In this study, establishment of *H. mangiferae* as a potential vector of mango sudden death syndrome was also investigated and its integrated management strategy was devised in southern Punjab, Pakistan. Initially, bark beetle host selection behavior towards mango plants prevailing under different conditions; healthy, symptomatic, diseased, stressed (due to salts) and dried plants was determined. The adult beetles were periodically released in the enclosed insectary and number of holes was counted for attraction towards specific condition of host. It was found that *H. mangiferae* preferably attacked on diseased, symptomatic followed by stressed plants. Whereas, healthy and dried plants showed less significant attraction. It revealed that *H. mangiferae* is a true disease vector due to its consistent association with diseased as well as healthy plants. Chemical insecticides have been used against the bark beetles as curative as well as preventive measure. It was carried out to evaluate the toxicity of deltamethrin, bifenthrin, chlorpyrifos, emamectin benzoate, imidaclopred and spinosad in laboratory as well as in field. This study demonstrated an increased toxicity of systemic insecticides and chlorpyrifos compared with deltamethrin and bifenthrin suggesting these insecticides could be an alternatively tool in a comprehensive *H. mangiferae* management program. Therefore, it is highly important to develop integrated management systems for mango production to minimize the risk of and the damage through Mango Sudden Death Syndrome.

ANOTHER EFFECT OF METHANOLIC EXTRACT OF *ELAEAGNUS ANGUSTIFOLIA* AGAINST THIRD INSTAR LARVAE OF *DROSOPHILA MELANOGASTER*

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Drosophila melanogaster Megin (Diptera/ Drosophilidae) were used as experimental model for toxicological evaluation. To get the larvicidal effect of the *Elaeagnus angustifolia* the third instars larvae of *Drosophila melanogaster* were exposed to ethanolic extract of *Elaeagnus angustifolia* by contact method. The percent concentration of 1, 2 and 3 after 24,48,72,96 and 120 hours caused 10%, 43%, 50%, 53% and 56% mortality respectively while the pupal delation observed at the same concentration. The larvae failed to pupate and mortality was caused up to great extant after 120 hours of treatment depending upon variation of concentration. Along with mortality and pupal delation the non significant, anti feedant and insect repellent activity were also observed. At 1 percent concentration of *Elaeagnus angustifolia* the rapid emergence of adults of *Drosophila melanogaster* were also observed. This pupal delation and mortality revealed that *Elaeagnus angustifolia* has good larvicidal properties or deleterious effect on dipterous larvae. As this plant is widely distributed and can be used for further investigation.

MANAGEMENT OF *BREVICORYNE BRASSICAE* AND *LIPAPHIS ERYSIMI* (HOMOPTERA: APHIDIDAE) ON CANOLA, *BRASSICA NAPUS* THROUGH SEED TREATMENT WITH IMIDACLOPRID AND THIAMETHOXAM IN SOUTHERN PUNJAB (PAKISTAN)

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Brevicoryne brassicae L. and *Lipaphis erysimi* (Kalt.) are the devastating insect pests of canola, *Brassica napus* in Pakistan. Our ten years of studies have revealed that these species cannot be controlled without application of insecticides. Present study was therefore undertaken to determine the effect of imidacloprid and thiamethoxam on population of both the species. Experiments were conducted at Multan during crop years 2003-04 and at Bahawalpur during crop year 2004-05 in a Randomized Complete Block Design. Both locations are 100km apart from each other. Crop was sown with the seed treated at three different doses of imidacloprid and thiamethoxam at Multan and with single dose at Bahawalpur. Canola variety Oskar was planted at Multan on 5th November

2003,whereas Westar variety was planted on 23rd October, 3rd and 13th November 2004 at Bahawalpur. All the experiments were planted by following recommended method of sowing having plot size of 8.32m^2. Number of aphids were recorded from initiation of flowering to maturity of the crops.We could not find significant differences in weekly population of two aphid species in plots sown with treated seeds and in plots where seeds were not treated. We report effect of seed treatment on population development of aphids from *Brassica* spp. first time from this region.

EVALUATION OF *VARROA DESTRUCTOR* CONTROL USING NEEM AND BARBAKA PLANT EXTRACTS

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The study assessed the comparative efficacy of different miticides including Neem (*Azadirachta indica*) and Barbaka (*Vitex trifolia*) plant extracts against the *Varroa destructor* mite in Pakistan. A total of 3960 colonies from 90 apiaries in the districts of Karak, Kohat, and Bannu had infestation levels of 73.26%, 60.90%, and 53.47% respectively, before treatment. This indicates that Varroa is a major problem for beekeepers in these areas. Using 54 colonies five different compounds were tested; groups A (Check mite), B (Flumethrin), C (Amitraz), D (Neem) and E (Barbaka). In Groups A, B, and C percent mite mortality was 62%, 55% and 47.72%, respectively, compared to 2% in control colonies. Group D (neem) was 45% while group E (Barbaka) treated colonies group E, the mite drop was only 26% and not significant different (T-test, P>0.05) from the control colonies.

EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ON INFESTATION OF BRINJAL FRUIT BORER (*LEUCINODES ORBONALIS* GUEN.)

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Effect of temperature and relative humidity on infestation of brinjal fruit borer (*Leucinodes orbonalis* Guen.) was studied on four brinjal varieties viz HLSF, Nirala, Dilnasheen and Round Black. The experiment was planted in a Randomized Complete

Block Design in the field. Temperature and relative humidity data were obtained from observatory of the University of Agriculture, Faisalabd, where the study was conducted. Data on infestation by brinjal fruit borer was recorded from ten plants in each treatment. Percent infestation was recorded by counting total and infested fruits on these plants. Temperature had no effect on infestation of brinjal fruit borer, whereas relative humidity significantly affected the infestation. Maximum infestation (66%) was noted at 54.3 % rh and minimum was zero percent at 20% rh.

EFFICACY OF BIOLOGICAL CONTROL AGENTS WITH INTEGRATION OF SELECTIVE INSECTICIDES AGAINST *EARIAS* SPP. OF COTTON

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The efficacy of biological control agents (*Chrysoperla carnea* and *Trichogramma chilonis*) integrated with selective insecticides was assessed against the *Earias spp* of cotton in the field. There were six treatments T1 (Chemical control), T2 (*Chrysoperla carnea*), T3 (*Trichogramma chilonis*), T4 (*Chrysoperla carnea* +*Trichogramma chilonis*), T5 (chemical+ *Chrysoperla carnea*),T6 (Control). There was no significant difference in the population of *Earias spp.* in all the treatments before releases. *Chrysoperla carnea* and *Trichogramma chilonis* eggs were taped in the cotton leaves and stems. The augmentative releases of biological control agents were made on weekly basis. T5 was found significantly less damaged by *Earias spp.* than the level observed in the other treatments. Thus releases of *Chrysoperla carnea* with integration of chemical control in cotton plots have potential for suppressing the population of *Earias spp.*.

MORTALITY RESPONSES OF *SPODOPTERA LITURA* (FAB.) (LEPIDOPTERA: NOCTUIDAE) AGAINST SOME CONVENTIONAL AND NEW CHEMISTRY INSECTICIDES UNDER LABORATORY CONDITIONS

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The new chemistry and conventional insecticides, viz., Curacron 50EC, Lorsban 40EC, Lannate 40SP, Larvin 80DP, Match 5EC, Agrimec 1.8EC, Proclaim 1.9EC, Tracer 24SC, Steward 15EC and Runner 24SC, were used to determine their efficacy against the armyworm, *Spodoptera litura* (Fab.) in the laboratory. All the insecticides used under laboratory conditions, proved to be toxic to *S. litura*, but chlorpyrifos, profenofos,

emamectin benzoate, spinosad, indoxacarb, methoxyfenozide and lufenuron proved to be highly toxic for the pest, as the exposure time was extended. After 3 days of the insecticide treatment, a maximum mortality of 100.00% was observed in emamectin benzoate @ 100 and 110 ml/acre, which was followed by that of 96.67% with chlorpyrifos @ 1100 ml/acre, of 86.67% with leufenuron @ 55 ml/acre, of 83.33% with methomyl @ 440 ml/acre, chlorpyrifos @ 1000 ml/acre and emamectin benzoate @ 90 ml/acre, as against that of 80.00% with thiodicarb @ 440 ml/acre. However, the insecticides, like, chlorpyrifos, emamectin benzoate, at all the three doses, leufenuron at a higher and recommended dose and thiodicarb, spinosad and methoxyfenozide, at higher doses, were ranked highly toxic-as a maximum mortality of 100.00% was observed in chlorpyrifos @ 900, 1000 and 1100 ml/acre methomyl @ 400 and 440 ml/acre leufenuron @ 50 and 55 ml/acre and emamectin benzoate @ 90, 100 and 110 ml/acre, which were followed by a mortality of 93.33% in thiodicarb @ 440 ml/acre, in leufenuron @ 45 ml/acre, in spinosad @ 44 ml/acre and in methoxyfenozide @ 110 ml/acre.

ESTIMATION OF GERMINATION LOSSES CAUSED BY MITES IN WHEAT DRAWN FROM FARMER'S HOLDINGS OF TEHSIL TOBA TEK SINGH AFTER THREE MONTHS OF STORAGE

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The mites are important pests of stored grains and other stored commodities. They are responsible of both qualitative and quantitative losses in stored grains. These mites feed on embryo thus resulting in germination loss in the grains along with deterioration in quality of seed as well as flour prepared from the infested grains. The present studies were conducted to determine the impact of mite population on germination in seeds of wheat drawn from farmer's holdings of Tehsil Toba Tek Singh after three months of storage. The samples were collected from Tehsil Toba Tek Singh viz., Toba Tek Singh, Janiwala, Dabawala, Jalalpur, Dulum, Rajana, Bairianwala, Pairra and Chatiana. Mite population, germination and infestation were recorded initially and after three months of storage. The results revealed highly significant differences between pest mite populations at different places. Maximum populations of harmful mites were recorded from Rajana at initial and final stages of experiment which was 2.66 and 7.6 respectively. Significant variations were recorded in initial and final germination percentage which ranged 87.00-92.3 and 78.12 -82.12 respectively. Negative correlation was observed between harmful mite population and final germination percentage of wheat with correlation coefficients of -0.486.

RICE LEAFFOLDER (*CNAPHLOCROSIS MEDINALIS* GN.) AND HOST PLANT RESISTANCE IN THE PUNJAB, PAKISTAN

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Elite rice lines (both medium and fine grain) were evaluated for susceptibility to the rice leaffolder. Susceptibilities were assessed in this regard by evaluation of infestation as folded/ damaged leaves caused by larval feeding of the notorious pest. Results of the screening evaluations during 2009- 10, following standard evaluation system for rice, out of 60 fine lines, one was found moderately susceptible, six susceptible and 53 highly susceptible while amongst 23 medium grain lines two were observed as moderately susceptible, 11 susceptible and 10 highly susceptible. This evaluation data of lines help in the selection of rice lines and varieties in the continuation of the breeding programme of evolving new rice varieties not susceptible to the attack of rice leaffolder.

POPULATION DYNAMICS OF MEALYBUG (*PHENACOCCUS SOLENOPSIS* TINSLEY) AND ITS PARASITOID (*AENASIUS BAMBAWALEI* HAYAT) IN FAISALABAD, PAKISTAN

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A survey was conducted in Faisalabad through out the year to determine the population dynamics of mealy bug (*Phenacoccus solenopsis*) polyphagous in nature and its severity level (percent infestation) on major crops, weeds, ornamental and vegetable plants. In relevance the occurrence of parasitoid (*Aenasius bambawalei*) in regulating population of mealybug was also recorded. Mealybug was found attacking 35 different plants including 14 weed plants, 8 ornamental plants and 13 crop and vegetable plants. Mealy bug peak infestation was recorded in months from July to August followed by a decline from October to December due to temperature and humidity fluctuations and nature of host plants in these months. Maximum infestation of mealybug was noted in weed plant *Abutilon indicum* (86%), in ornamental plant *Hibiscus rosa-sinensis* (94%), in crop and vegetable plant *Gossypium hersutum* (67%). The over all infestation of mealybug in all host plants ranged from 2 to 94%. The maximum parasitoid activity was noted on *Abutilon indicum* (58%) in weed plants, ornamental plants *Hibiscus rosa-sinensis* (50%), in crop and vegetable plants *Abelmoschus esculentus* (62%). However, the range of parasitism against mealybug on different host plants was 0 – 62%. The

survey was useful to document some carryover of mealybug from weed to ornamental, main crops and vegetables side by side. Weeds and ornamental plants bridged the population to attack major field crops. Average parasitism (14%) against mealybug on crop and vegetable plants showed that natural population of parasitoid was not up to acceptance level and need further augmentation of same parasitoid and introduction of other bio-control agents to manage the pest.

CHEMICAL CONTROL OF *MELOIDOGYNE INCOGNITA* THROUGH ENVIRONMENT-FRIENDLY PESTICIDES ABAMECTIN AND ABAMECTIN BENZOATE ON EGGPLANT CULTIVAR BAIGAN XLANGZUE-6

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Abamectin and Abamectin benzoate are semi-fermented and semi-synthesized pesticide/insecticide of avermectin kind. These less phytotoxic and environment-friendly pesticides were tested for their efficacy against Meloidogyne incognita, a serious pest of vegetables. For the purpose, Baigan Xlangzue-6, the most susceptible cultivar of Brinjal found in our cultivar screening trials against *M .incognita* was used. Chemicals, Abamectin and Abamectin benzoate were used at the concentrations of 5g, 10g and 15g/100 mL water for the control of the root knot pathogen. Abamectin and Abamectin benzoate were most effective and statistically at par in their efficacy @10g/mL water with respect to plant growth parameters and number of knots/ plant root system. This study recommends usage of both the chemicals keeping the environmental perspective in view.

POPULATION FLUCTUATION OF SUGARCANE STEM BORER *CHILO INFUSCATELLUS* SNELLEN ON DIFFERENT VARIETIES OF SUGARCANE IN SINDH

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Chilo infuscatellus Snellen cause a regular colossal damage to sugarcane in Pakistan it causes significant damage during early stage of the crop when it is young. But it's exact economic significant had not been studied yet. To check the growth and population of this pest present study is being reported for the first time from this region. Present study has been carried out during the year 2009 from June to October. Observation has been collected from the three selected varieties i-e Thatta-10, CPF-237 and SPF-234. Infestation of *C. infuscatellus* was reported significantly highest during the

month of May 2.02% followed by 1.57 & 1.55 in April and August respectively. However, there was no significant difference was found in the month of September 1.45% & October1.2%.

EFFECT OF ANTIPROTOZOAN CHEMICALS (MENTHOL, SULPHONAMIDES AND RESOCHIN) ON *COPTOTERMES HELMI*, *HETEROTERMES INDICOLA* AND THEIR HIND GUT FLAGELLATES

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Termite infestation to economically important plants and household wood, fabrics in addition, paper is a devastating problem throughout the world. A number of insecticides are in use for controlling this insect pest. Presently, we investigated the effect of menthol, sulfonamids (sulfaquizanoxline sodium, sulfadimidine sodium, diaviridine) and resochin on mortality of termites and their gut flagellates, *Coptotermes heimi* and *Heterotermes indico/a.* Extracted oil by using steam distillation method from fresh leaves of *Mentha arviensis.* Crystals were separated . from mint oil through chilling process and kept at 26° C Exposed termite's workers and soldiers to three different dosage regimens of menthol, 25 mg, 50 mg and 100 mg, each tested for a maximum of 6 hours duration. Quantitative data on rate of mortalities in workers and soldiers recorded and analyzed statistically. Results demonstrated dose-dependent significantly increased mortality of termite workers, soldiers and their gut flagellates in both termite species. In another experiment, the termite workers and soldiers of same species fed on filter paper, dipped, and then dried with air in, 2%, 10ml solution of sulfonamides and resochin at 25°C. There was a gradual and significant decrease in flagellate's population in the hind gut of termites during 72 hours of feeding on the drug impregnated filter paper, and after 72 hours all the flagellates died followed by the death of workers in each case. Menthol and anti protozoan drugs used in experiments could be the best replacement of insecticides for the termite control.

REPELLENT ACTIVITY OF SOME BOTANICALS AGAINST RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM* (HERBST) (COLEOPTERA: TENEBRIONIDAE)

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Red flour beetle, *Tribo/ium castaneum* is an economically important pest of stored grain commodities throughout the world. A laboratory study was conducted for

determining the repellency of essential oils at 2, 4, 6, 8 and 10% concentrations against *T. castaneum.* These concentrations of tested oils were applied to half of the filter paper and remaining half was treated with acetone alone as control in three replicates in each treatment. In each treatment twenty insects were released and data were recorded after 24 hours. At concentration of 6%, musambi showed the maximum repellency (37%) followed by sugarcane bagass (30%), datura (29%), neem (26%) and narangi (25%). All other concentration exhibited less repellent activity. Rresent results show the potential usefulness of plant derived materials for the management of *T castaneum* in mills, warehouses and godowns.

REPELLENT EFFECT OF FOUR MEDICINAL PLANT EXTRACTS AGAINST *TROGODERMA GRANARIUM* (EVERTS) (GOLEOPTERA: DERMESTIDAE)

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The study was conducted to evaluate the repellent effect of four medicinal plants viz., *Azadirachta indica, Curcuma tonga, Piper nigrum* and *Nigella sativa.* Acetone was used as solvent to obtain and apply the extracts of these plants efficiently. The extracts were evaluated for their repellent effect against *T. granarium* at 5, 10, 15 and 20% concentrations. Three replications were used for each dose of all the plant extracts and observed in petri dishes. Extracts of *Azadirachta indica* showed highest repellent effect, which was 80% and minimum repellent effect showed by *Nigella sativa* which was 48.51 % at 20% dose level. It was also found that rate of repellency enhanced with the increase in dose rate, at 20% dose rate all plant extracts showed highest repellent effect.

EFFECTS OF THIODON (INSECTICIDE) ON THE SURVIVAL AND AVOIDANCE BEHAVIOUR OF SPIDERS

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Present study was designed to evaluate the potential effects *of* Thidon (insecticide) on the survival and avoidance behaviour *of* salitcid spider e.g., *Plexippus paykulli* which was found to be susceptible to Thiodon. We observed 10% mortality at field rate concentration during residual toxicity experiment. There was significant difference in the time spent by *Plexippus paykulli* on the insecticide or water treated part of filter paper.

EFFECTIVENESS OF FENPYROXIMATE AND BIFENTHRIN AGAINST RHIZOGLYPHID (ACARI: ACARIDAE) MITES ON STORED WHEAT

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Acarid mites are abundant arthropod fauna stored wheat. There are many species belonging to family Acaridae which infest stored grains and stored products of all kinds. These mites penetrate the seed and initially feed on the embryo, consequently the seed looses the viability. Two pesticides viz., fenpyroximate (Unique M) and bifenthrin (Talstar) were tested in different concentrations (6.25, 12.5, 25, 50, 100 and 200 ppm) for population inhibition of stored grain mites after 1, 2, 3, and 4 weeks. Both of these inhibited the population even at very low dose of 6.25 ppm. Fenpyroxymate inhibited maximum population after 1st week at 200 ppm (90.97%). While at lowest dose of 6.25 ppm it inhibited 35.63 %. The population inhibition decreased with the passage of time but maximum population was inhibited at 200 ppm at all the test intervals. Bifenthrin was less effective as it inhibited 56.64 % population after one week at 200 ppm. At 6.25 ppm it inhibited only 10 % population. The effective ness of bifenthrin, however increased with the passage of time.

STUDIES ON THE EFFICACY OF TWO BENEFICIAL INSECTS INTEGRATED WITH SELECTIVE INSECTICIDES TO CONTROL COTTON THRIPS

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Field studies were carried out in cotton to investigate the efficacy of predator *(Chrysoperla carnea)* and parasitoid *(Trichogramma chilonis)* integrated with selective insecticides. Biological control agents were released after two weeks regularly. The population dynamics of Thrips were monitored weekly. There were six treatments Tl (Chemical control), T2 *(Chrysoperla carnea),* T3 *(Trichogramma chilonis),* T4 *(Chrysoperla carnea +Trichogramma chilonis),* T5 (chemical+ *Chrysoperla carnea),* T6 (Control). The combination of the release of beneficial insects with selective insecticides resulted in a difference in efficacy. *Chrysoperla carnea* along with selective chemical was able to keep the population of thrips below the level of the solely pesticides treated area and others. Thus *Chrysoperla carnea* was able to suppress the population of thrips.

USE OF *AVICENNIA MARINA* (FORSK.) VIERH LEAVES EXTRACTS AGAINST SOME ATMOSPHERIC FUNGI

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Crude ethanolic extract of *Avicennia marina* leaves was tested against seven allergenic fungi viz., *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, Cladosporium herbarum, Penicillium notatum* and *Saccharomyces cerevisiae* using five different solvents, DMSO, DW, chloroform, ethanol and acetone at 2000, 4000 and 6000 ppm doses. Dose dependent tendency in the increase or decrease in the growth of fungi was observed. Distilled water and DMSO were the most effective solvents as the % inhibition of fungal growth was 83.00%±4.73 in *A. niger,* 80.33%±5.60 in *A. flavus,* 78.58%±3.18 in *A.alternata,* 72.91%±7.96 in *P. notatum,* 65.25%±3.55 in *C. herbarum* 63.25%±4.52 in *A. fumigatus* and *48.5%±7.89* in *S. cerevisiae*. Two synthetic drugs miconazole and amphotericin-B were used as positive control. Miconazole was 100% effective against *A. alternata,* C. *herbatum, P. notatum* and *S. cerevisiae* in the concentrations of 95.00±l.62, 78.00±4.99, 100.00±0.69 and 110.00±2.33(µ/ml of SDA medium) respectively. Amphotericin-B completely controlled the growth of *A.flavus, A.fumigatus* and *A. niger* in the concentration of 24.00±17.00, 30.00±l5.66 and 18.00±18.34 (µg/ml of SDA medium) respectively. Statistically, the results were compared with negative control and found as highly significant (p<.0l).

EFFECT OF SECONDARY PREDATORS ON THE ESTABLISHMENT OF THE EGGS PARASITOID *TRICHOGRAMMA CHILONIS,* WITH REFERENCE TO ITS PLACEMENT AND TIME OF RELEASE IN SUGARCANE FIELD

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Secondary predators can hamper the establishment of released biocontrol agents hence reducing the efficacy of applied biological control *Trichogramma chilonis* is effectively used to control sugarcane borers. Present study was conducted to assess the best time and position of placement of Tricho cards (containing mature pupae of the parasitoid) in sugarcane fields to avoid predation by ants and spiders etc. The cards were placed at three different positions, i.e., top, middle and bottom of the sugarcane crop and the experiment was repeated in morning, afternoon and evening times. The experiment

continued from February to October throughout the growing season. The activity of secondary predators was observed during whole season of sugarcane but maximum damage was observed from August-September attributing to the favourable environmental conditions prevailing during this time for predators to thrive. Results indicated that the release time had no significant impact on predation by secondary predators, while, placement of cards showed significant variation. Least damage was done when cards were placed at top position (morning-3.69, afternoon2.16% and evening-2.71 %) followed by the middle position (morning-6.22, afternoon5.96 and evening-5.47%). Maximum damage was observed in the bottom position for all release times (morning-14.49%, afternoon-14.31 %, and evening-14.27%). The study showed that placement of cards at top position can help in avoidance of secondary predators, hence contributing towards better establishment of the parasitoid in the field.

DO THE TOXIC CARDENOLIDES REDUCE THE SUITABILITY OF *APHIS NERII* FOR PREDATORS? A CASE STUDY WITH TWO PREDATORS *MEPOCHILUS SEXMACULATUS* AND *CHRYSOPERLA CARNEA*

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Presence of allelochemicals in the host plant can adversely affect natural enemies through the channel of plant toxins up the trophic pyramid. It is known that the aposematic aphid, *Aphis nerii* commonly known as oleander or milkweed aphid, sequesters toxic cardenolides from its host plants in the family Asclepiadaceae and Apocynaceae and uses these chemical defences effectively against a range of natural enemies. The aphids' bright colouring warns predators of the toxic cardenolides (cardiac glycosides) produced by the host plants, that are then incorporated into aphids' tissues. Generalist predators feeding on these aphids suffer the effects of the cardenolides. Previously, it has been reported in different studies that fewer lacewing larvae survive to become adults and reproduce. Ladybirds develop deformed wings and spiders weave strange disrupted webs. Here we report the suitability of *A. nerii* as a prey for two predators; zig-zag ladybird beetle *Menochilus sexmaculatus* and green lacewing *Chrysoperla carnea.* Both predators were reared in laboratory on *A. nerii.* Eggs of Angoumois grain moth *Sitotroga cerealella* were used as control for C. *carne a* and corn leaf aphid *Rhopalosiphum maidis* for *M sexmaculatus.* Mean development time for C. *carnea* on *A. nerii* was 36.56 days with 54.32% larval survival as compared to control (8. *cerealella,* 13.42 days and 92.45%). For *M sexmaculatus* it took 17.21 days for development from egg to adult with 60.86% larval survival on *A. nerii* as compared to *R. maidis* (15.94 days, 61.87%). Once pupated, the adult emergence was 90.23 and 85.13% for zig-zag beetles and lacewings respectively. Results reveal that cardenolides present in *A. nerii* had detrimental effects on lacewings but zig-zag beetles remain unaffected, hence

showing good potential to be used as biocontrol agent against this aposematic aphid species.

IMPLICATION OF PROVIDING SUPPLEMENTAL HOSTS FOR INITIAL SURVIVAL OF THE PARASITOID *TRICHOGRAMMA CHILONIS* IN RICE

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For reliable pest management, providing supplemental host to the released parasitoids may play an important role in the initial survival and establishment of the beneficial insects. Experiments comprised treating rice crop with *Trichogramma chilonis* released @ 10, 0000 per acre during cropping season (using rice variety Shandar) along with fresh & chilled eggs of *Sitotroga cerealella* as supplemental host *(25000* per acre), and without provision of any host. Both the treatments on initial establishment of the parasitoid were compared with the crop where no bio-control agent was applied. The infestation of stem borers (dead hearts and whiteheads) was observed at fortnightly intervals. Results showed that the initial establishment of *T. chilonis* was superior in the treatment where bio-control agent was released along with supplemented host S. *cerealella* eggs compared to the treatment where no supplemental host provided. Whereas, density of *T. chilonis* was slightly lower in the crop without application of bio-control agent. As a consequence, the use of supplemental host to increase the abundance and impact of natural enemies of arthropod pests with other compatible elements of an integrated pest management strategy will form a part of future biological control programme.

DETERMINATION OF PREDATOR, *CHRYSOPERLA CARNEA* REQUIREMENTS TO MANAGE PEST INFESTATION DENSITY OF APHIDS IN CANOLA, *BRASSICA NAPUS* L.

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Biologically based pest management by reducing the need for frequent pesticide use, is an excellent element of any integrated pest control technique. Field studies were undertaken on effectiveness of *Chrysoperla carnea* released @ 15000, 25000 and 35000 eggs! acre during cropping season against aphid infesting canola crop (Wester). Observations on plant sampling showed that application of predator significantly reduced the incidence of pest and increased the grain yield compared to control in all treatments.

The treatment with high release rates followed by medium and low rates recorded significantly higher grain yield over the non treated canola that led to higher pest populations or damage. The populations of egg, larva and adult stages of predator were significantly predominantly analyzed within higher C. *carnea* released treatments to adversely affect populations of target pest. It is counted that application of C. *carnea* @ 35000 eggs! acre reduced the highest incidence of aphid and increased the yield in canola during the trial. Consequently, the knowledge of releasing density requirements of natural enemies will be helpful to increase suppression of the pest herbivores.

INSECT PESTS MANAGEMENT IN BT COTTON THROUGH MANIPULATION OF DIFFERENT ECO-FRIENDLY TECHNIQUES

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Transgenic cotton with expression of crystalline insecticidal protein of *Bacillus thurengensis* has become a major contributor for the management of cotton bollworms. *Bt* cotton has an adverse effect on the larval mortality, growth and development of bollworms. To study the management of insect pests of Bt cotton and impact of Bt cotton on beneficial insects, Bt (IR-1524) and conventional cotton (NIA-Ufaq) were grown in randomized complete block design having six treatments (Bt + biocontrol, Bt + colour traps, Bt + insecticide, Bt cotton alone, conventional + biocontrol and conventional cotton alone) replicated three times. Data on the establishment of released predators and other beneficial insects along with the infestations of sucking and bollworms insect pests were recorded weekly. Results indicated that infestation of sucking pests varied in different treatments. Per leaf population of Jassids in bio-control treated Bt cotton was found non significant with bio-control treated conventional cotton but was significantly lower than all other treatments where as highest was recorded in untreated conventional cotton. Population of Thrips was non significant between Bt cotton alone and conventional alone but was significantly higher from the rest of treatments. Lowest Thrips population was observed in bio-control treated Bt cotton treatment followed by bio-control treated conventional cotton where as highest was recorded in untreated conventional cotton. No bollworms infestation was recorded in transgenic cotton however, significantly higher bollworms attack was found in untreated conventional cotton as compared to bio-control treated conventional cotton. Comparatively biocontrol-Bt cotton produced significant results in term of least infestation by sucking pests and maximum yield while conventional alone was found susceptible to sucking and bollworms complexes producing lower yield. Population of *C. camea* (eggs, larvae and adults) was significantly higher in bio-control-Bt cotton and biocontrol-conventional cotton treatments in relation to other treatments. Percent parasitism by *Trichogramma* was found significant in bio-control-conventional cotton treatment. Over all study

revealed that transgenic cotton is a bio-control friendly technology which effectively preserves local population of various important bio-control agents.

FIELD PERFORMANCE OF EGG PARASITOID *TRICHOGRAMMA CHILONIS* AT DIFFERENT RELEASING DENSITIES AGAINST RICE STEM BORERS A KEY PEST OF RICE

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Field studies were undertaken to evaluate the efficiency of egg parasitoid *Trichogramma chilonis* against rice stem borers using different release doses (50000, 75000 and l00000 per acre) during cropping season. The performance of parasitoid *T. chilonis* for managing borers was determined by planting the rice genotype Shandar (MSB) and the parasitoids were released at fortnightly intervals after six weeks of crop planting when incidence of the borer began. The performance of *T. chilonis* was evaluated based on the incidence of borers and paddy yield. Irrespective of the parasitoid released densities, the performance of *T. chilonis* in treatment where parasitoid was released proved superior over untreated crop. Experimental results indicated that release of egg parasitoid at the rate of 100000 per acre proved significantly the best and most practicable for pest control over the lower doses. Significantly lower infestation of borers was recorded in crop imposed with parasitoid released @ of 75000 followed by 50000/ acre. The highest pest incidence was recorded in control where no treatment was applied. Similarly, the experiments validated optimum paddy production per acre with a density of 100000 *T. chilonis* release rate. So, is it appropriate to adopt this technology more widely to ensure parasitoid release density as a part in the successful future of rice pest management programme.

ECO-FRIENDLY MANAGEMENT OF INSECT PESTS OF COTTON CROP USING BIO-CONTROL AGENTS IN CONJUNCTION WITH OTHER TACTICS

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Application of pesticides are continuously affecting the biotic and a-biotic factors of the eco-system, that increases the scope of using biological and behavioral control against the sucking and bollworms complexes in within cotton. Four treatments (bio-control agents comprising *Chrysoperla carnea* and *Trichogramma ch iion is,* sticky color traps for sucking pests and pheromone traps for bollworms were used separately or in

different combination) were evaluated in comparison to the untreated control for the management of insect pests of cotton. Different traps were applied in yellow and blue colors to attract sucking insects, while, for bollworms delta and funnel traps were used baited with synthetic sex pheromones as male annihilation tactics. Results revealed that lowest populations of jassids, thrips and whiteflies were observed where bio-control agents were released in combination with colour sticky and pheromones traps followed by the separate treatment of bio-control agents. The population of pink bollworm remained comparatively low throughout the season and as the infestation was not significantly different in all treatments except untreated check where it was higher than economic threshold level. The infestation of spotted bollworm was significantly low in combine treatment of bio-control agents, colour and pheromone traps. Similarly the seed cotton yield was also significantly higher in treatment where bio-control agents were released in conjunction with colour and pheromones traps followed by the separate treatment of biocontrol agents. Collective populations of C. *carnea* (eggs, larvae and adults) were higher in Hio-control treated blocks weather these were released separately or in combination with colour and pheromone traps. Parasitism of *Trichogramma* was significantly higher in biocontrol treated blocks.

EVALUATION OF RESISTANCE IN WHEAT AGAINST *RHOPALOSIPHUM PADI* (L.) (HOMOPTERA:APHIDIDAE) UNDER LABORATORY CONDITIONS

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All over the world aphids are pest of economic importance, they affects all varieties of plants like cereal crops, vegetables, ornamental plants and fruits. Aphid damage plants either directly or indirectly. Aphids are one of the major agricultural pests. There are only few plants that are not liable to be infested by this destructive pest. They cause direct damages by sucking sap of leaves, block photosynthesis which results in leaf distortion, gall production, discoloration, stunting, leaf curling, wilting, twisting and premature leaf falls. They are directly involved in transmission of plant viruses and indirectly by depositing honey dew that reduce photosynthetic activity and induce sooty mould production and premature leaf senescence. Hosts were wheat, there was twenty varieties of National Uniform Wheat Yield Trials (NUWYT) Normal (N) of year 2004-05, and twelve varieties of (NUWYT) Rain Fed (R.F) of year 2004-05. All these varieties were evaluated against bird cherry oat aphid R. padi L pest. Evaluation for resistance was done by seedling bulk test and by comparing the results of components of resistance like

antibiosis, antixenosis and tolerance tests. These experiments were performed under controlled environmental conditions. Experiments were of randomized complete design and replicated. The data obtained was statistically analyzed of variance (ANOV A) and the mean values was compared by Duncan's new multiple range test (DMRT) at 5 percent level of significance (Steel and Torrie, 1980). Among the tested germplasm one variety and 11 lines of wheat), V -5 line was found resistant and NRL-2017 line was found susceptible to Rhopalosiphum padi. The results of Antixenosis Test indicated that two wheat lines, V-5 and PR-83 were least preferred and three lines viz., NRL-2017, V-00055 and V-00BT004 were highly preferred by the test insect. The results of Antibiosis Test showed that PR-83 line was least fecund whereas NR-241 and V -00055 lines were highly fecund. The results of Tolerance Test indicated that four varieties, PR-83, V-5, SN-128 and NR-241 were highly tolerant, while only one line, V -00BT004 was least tolerant. To avoid harmful effects of this insect pest, only resistant, least preferred, least fecund and highly tolerant lines must be used in wheat breeding programmes.

EFFICACY OF *DIRHINUS GIFFARDII* ON BIOLOGICAL CONTROL OF FRUIT FLY IN LABORATORY CONDITIONS

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Studies were carried out to find the efficacy of *Dirhinus giffardii* on biological control of fruit flies in laboratory conditions. Pupae of fruit flies were used as a host for Dirhinus giffardii. There were four replications, each contained four treatments. Fifteen pupae were provided to one pair of *Dirhinus giffardii*. To find percent parasitism, adult emergence and longitivty, four treatments e.g.: 1, 2, 3, 4 and 1 pair of *Dirhinus giffardii* was compared with 15 pupae of fruit flies. Percent parasitism was highest (28.3%) at 15 pupae with one pair of *Dirhinus giffardii* in R_2, whereas percent emergence was highest (13.3) in R_2.The longitivty was highest in R_2 that is 40 days, so 15 pupae with one pair of *Dirhinus giffardii* were suggested for rearing at laboratory conditions.

MECHANISM AND CROSS-RESISTANCE OF DELTAMETHRIN IN *SPODOPTERA EXIGUA* FROM PAKISTAN

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Spodoptera exigua (Hubner), a polyphagous pest of vegetable and field crops. A field population of *S. exigua* (H) from Lodhran was found to be highly resistant to

deltamethrin but relatively low resistant to cypermethrin, chlorpyrifos, profenofos, spinosad, indoxacarb and abamectin compared with Lab-PK population. Two sub-populations were maintained in the laboratory, one was left unselected and second was selected for six generations at the dose equal to $LC_{50.}$ Bioassays at G_7 gave resistance ratios of >700 fold compared with Lab-Pk. When DEL-SEL strain was tested for other insecticides, it showed high cross-resistance with cypermethrin, little cross resistance was found for chlorpyrifos and profenofos while no cross resistance was found for abamectin, indoxacarb and spinosad. Deltamethrin resistance in DEL-SEL strain of *S. exigua* was suppressed with the synergists, piperonyl butoxide (PBO) and *S,S,S*-tributylphosphorotrithioate (DEF), suggesting the involvement of monooxygenase and esterase in the development of resistance in *S. exigua*.

COMPARATIVE EFFICACY OF FOUR INSECTICIDES AGAINST SUCKING AND CHEWING INSECT PESTS OF COTTON

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Four insecticides when used alone or in combinations *viz.* Cypermathrin 10EC, Deltaphos 360EC (deltamathrin + triazophos), Deltaphos 360EC + Confidor 200SL (deltamathrin + triazophos + imidacloprid), Cypermathrin 10EC + Confidor 200SL (imidacloprid) were tested against sucking insect pests such as jassid, *Amrasca biguttula biguttula* Dist.; thrips, *Thrips tabaci* Genn.; whitefly, *Bemisia tabaci* Lind. and spotted bollworm, *Earias insulana* Boisd. on cotton under field conditions. All insecticide whether used alone or in combinations were applied at their recommended doses with the help of knapsack sprayer three times with an interval of a week. The results of the present study revealed that all insecticides or combinations were almost equally effective to control *E. insulana*. Deltaphos 360EC + Confidor 200SL combination was comparatively most effective against *A. biguttula bigutulla, T. tabaci* and *E. insulana.* None of the insecticides or combinations was found effective for the control of *B. tabaci*.

COMPARISON OF HOST-PARASITOID SCENARIO IN DIFFERENT VARIETIES OF COTTON

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Cotton is attacked by a complex of insect pests; both sucking and chewing type. These pests cause substantial losses during cotton production. Many types of natural enemies (predators, parasitoids and diseases) also exist in the cotton crop. Natural field populations of these insect predators and parasitoids alone, if undisturbed, can

substantially reduce pest populations in cotton. The most severe constraint to realizing the potential of natural enemies in field crops is disruption through the widespread use of insecticides. Over/misuse of insecticides can lead to the elimination of natural enemies and give rise to phenomena such as pest resurgence, occurrence of secondary pests, and selection of population of resistant insects. Host Plant Resistance (HPR) plays an important role in controlling insect pests and protecting natural enemies in an agro-ecosystem. The Integrated Pest Management (IPM) concept advocates both chemical and biological control in agricultural systems. Five varieties of cotton viz. CIM-496, CIM-534, NIAB-111, MNH-786 and Bt-121 with four replications were used for the experiment. From the present study it can be concluded that Bt-121 and MNH-786 were the most tolerant varieties against both the sucking and chewing insect pests of cotton but the application of insecticides would still be required to keep the population of insect pests below the economic damage.

LABORATORY SELECTION FOR *SPODOPTERA LITURA* (LEPIDOPTERA: NOCTUIDAE) RESISTANCE TO SPINOSAD

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Tabacco budworms *S. litura* were artificially selected in the laboratory for resistance to spinosad having a novel mode of action. A field collected papulation of *S. litura* was divided in two cohorts, one selected with spinosad for up to 6 generations, while the second reared in the laboratory for up to 11 generations without any selection pressure. After 6 generations of selection, the resistance of *S. litura* to spinosad was increased to 1.5-folds as compared to field population and 15.1-folds as compared to susceptible laboratory population which was generated by rearing for up to 11 generations without any selection pressure. The result of our study shows that *S. litura* has the potential to develop a low to medium resistance against spinosad when having selection pressure for few generations.

COMPARATIVE EFFICACY OF BIO-INSECTICIDE AGAINST DIFFERENT STRAINS OF *HELICOVERPA ARMIGERA* (HUBNER) UNDER LABORATORY CONDITIONS

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The present study was conducted to determine the toxicity of commercial formulation of Cry 1 AC against *Helicoverpa armigera* collected from three Districts of

the Punjab viz. Multan, Dera Ghazi Khan and Sahiwal under laboratory conditions at University College of Agriculture, B.Z. University Multan. The strains collected from the field were reared on artificial diet in lab and F_2 generations of those strains were tested by incorporating various doses of commercial formulation of Cry 1 AC in artificial diet. The 1st and 2nd instar larvae were exposed to the artificial diet having various levels of that bio-insecticide. It was observed that D.G. Khan and Sahiwal strains showed the significantly same mortality trend which was quite higher than that of the Multan strain. The 1st instar larvae of Sahiwal strain had high resistance level than that of Multan and D.G. Khan Strains where as the 2nd intar of D.G. Khan strain was more resistant as compared to the other ones. The resistance level in 1st instar was higher than that of 2nd instar in Multan strain where as it is lower in case of D.G. Khan and Sahiwal strains.

COMPARISON OF PREDATOR-PREY SCENARIO IN DIFFERENT VARIETIES OF COTTON

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Cotton is the main cash crop of Pakistan. Successful production of this crop requires heavy applications of insecticides as most of the insect pests of cotton have developed resistance against several insecticides which results in high cost of production. Five varieties of cotton i.e. CIM-496, CIM-534, NIAB-111, MNH-786 and Bt-121 were compared in a split plot manner. It was inferred that the Bt-121 and MNH-786 were the most tolerant varieties against both the sucking and chewing insect pests of cotton but the application of insecticides would still be required to keep the population of insect pests below the economic damage. It is, therefore, suggested that only such insecticides should be used which posses minimum toxicity on non-target organisms. Use of environmental friendly insecticides is accordingly required to be encouraged in Pakistan to avoid deleterious effects of poisons on beneficial insects.

EVOLUTION OF RESISTANCE TO INSECTICIDES IN BEMISIA TABACI IN PAKISTAN: IMPACT ON COTTON LEAF CURL VIRUS DISEASE

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The cotton whitefly, *Bemisia tabaci*, is an important pest of field crops, vegetables and ornamentals worldwide. Neonicotinoids are considered an important group of insecticides being used against *B. tabaci* for several years. *Bemisia tabaci* has developed

resistance to some of the compounds of the group. This study was designed to investigate if the selection of *B. tabaci* with acetamiprid would give a broad-spectrum of cross-resistance and to genetically classify the resistance. At G_1 a low level of resistance to acetamiprid, imidacloprid, thiamethoxam, thiacloprid and nitenpyram was observed with resistance ratios of 3-fold, 8-, 9-, 6- and 5-fold, respectively, compared with laboratory susceptible population. After selection for eight generations with acetamiprid, resistance to acetamiprid increased to 118-fold compared with laboratory susceptible population. Selection also increased resistance to imidacloprid, thiamethoxam, thiacloprid, nitenpyram, endosulfan and bifenthrin but no change in susceptibility to fipronil was observed. Furthermore resistance in field population was stable in the absence of acetamiprid selection pressure. Genetic crosses between resistance and susceptible populations indicated autosomal and incompletely recessive resistance. Further genetic analysis suggested that resistance could be controlled by a single factor. The high level of cross-resistance and stability of incomplete resistance in the field population is of some concern. However lack of cross-resistance between acetamiprid and fipronil or unstable resistance in the resistance population could provide options to use alternative products which could reduce acetamiprid selection pressure.

OPTIMIZATION AND STANDARDIZATION OF OPERATIONAL PARAMETERS OF HOT WATER TREATMENT SYSTEM FOR THE POST-HARVEST CONTROL OF FRUIT FLIES IN MANGOES

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Fruit flies infestation and its resultant consequences in the shape of pesticide residues, insecticide resistance and quality deterioration are putting adverse effect on the economy of farmers and traders. Some of the fruit which could fetch valuable foreign exchange are not being exported due to the infestation of fruit flies. Four varieties of mangoes (Chaunsa, Fajri, White Chaunsa and Sensation) were artificially infested with fruit fly under laboratory conditions and treated with hot water at different temperature and durational regimes in hot water treatment system for its optimization and standardization of operational parameters for the control of fruit flies. Fruit flies were best controlled at 48°C for 50min in Chaunsa and Fajri; 48°C for 60min; 48°C for 65min. Before the recommendation of these operational parameters further studies are required to measure their impact on the quality of mangoes.

STUDIES ON THE BIOLOGY OF THE WHITE-BACKED PLANTHOPPER, *SOGATELLA FURCIFERA* (HORVATH) ON TWO RICE VARIETIES IR-6 AND DR-92

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Biological studies of the of the White-backed Planthopper, *Sogatella furcifera* (Horvath) on rice varieties IR-6 and DR-92 were determined under laboratory condition at Rice Research Institute, Dorki during the year 2009. The eggs of white-backed planthopper (WBPH) were collected from the experimental area of the Institute and introduced to the rice varieties. The rice varieties were transplanted in wooden boxes in the laboratory. Hatching period of WBPH was recorded 5.40±0.30 and 6.055±0.60 on the rice varieties IR-6 and DR-92, respectively. Nymphal period of WBPH was completed 14.47±1.90 days. The total duration of life cycle of WBHP was noted 13-19 and 16-22 days on the variety IR-6 and DR-92, respectively. The longevity of adult male and female of WBPH was 5 and 7 days on IR-6 and DR-92. The result shows that WBPH preferred feeding on IR-6 and DR-92.

IMPACT OF DRY WEATHER ON THE INFESTATION RATE OF APHIDS (HOMOPTERA, APHIDIDAE AND ADELGIDAE) ON HIGH LAND CONIFERS IN AZAD KASHMIR

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Conifers the cones bearing trees are widely grown forest of Himalayan foothills within a natural geographical range of 5000-6500ft altitude. An unusual high infestation of two aphid species *adelgids* and the *Cinara* has been a big challenge for the last five years. The highest attack rate has been observed during the early winter from mid of November to the end of January or until the first installment of snowfall/winter rain shower. The principal aim of study conducted was to identify the aphid pest spp. and to find out the factors that contributes in the severe infestation during dry winter and the species variation from six natural forests sites. The interaction between aphids and their host trees were thoroughly studied and there was no apparent indication of genetic variation among the conifer trees. Uniform attack rate was seen on the tree affected by water stress Some of the pine trees from same study site were not attacked at all, less attacked depends on the severity of physiological drought. Soil moisture around the affected and none affected tree was estimated and there was a significant difference

between the availability of gravimetric soil moisture between the attacked (0-25%) and none attacked trees (5075%). The most of the startling affect of the damage occurred on terminal portions of upper stems of those trees severely affected by the water stress and true firs that brought by the family Adelgidae. None of the natural enemy of pest was found on a single study site during the peak infestation period of early winter. The most promising biological agent of the conifer aphis were reared in the laboratory to measure their requirement of upper and lower temperature for their survival and for the prey searching activity.

EFFECT OF NEEM WATER EXTRACT ON CABBAGE AND CAULIFLOWER APHID POPULATIONS IN THE FIELD

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Experiments were conducted at NARC fields Islamabad on Cabbage to evaluate the effect of Neem water extract against Aphid (*Brevicoryne brassicae)* population**.** Four different concentrations i.e. 2%, 1%, 0.5% and control (water) of Neem extract were sprayed on cabbage plants. Average total population of aphids was 32.9, 27.8, 30.2 and 21.6 at the four fields. These fields were sprayed three times with the water extract of neem, in four concentrations 2%, 1%, 0.50% and control (no spray) respectively. Data was recorded after each spray, after 3rd spray the population of aphids decreased and the best control is showed by 2% concentration of water extract of neem and the aphid populations were decreased to the average of 2.3.

INTERACTION OF PREDATORS WITH INSECT PESTS OF WHEAT CROP

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The present findings was aimed to determined the interaction of predators with insect pests on different wheat varieties was laid out at Experimental Area of Entomological Section, Agriculture Research Institute, Tandojam during rabi wheat growing season of 20092010. Ten wheat varieties were sown in a complete randomized block design plot. The varieties include SKD-I, Imdad, Moomal, Indus, Pak-81, TJ-83, TD-1 Kiran, J.S. and S.B. The results indicated the maximum mean population of insect pest and predators were recorded on different varieties of wheat crop. The maximum mean Black aphid population was recorded on variety of PAK-81 (3.19/leaf) and

minimum on Moomal and SB (2.57/leaf). The maximum mean number of green aphids were recorded on variety of Indus (6.60/leaf) and minimum mean population of variety of TJ-83 (3.92/leaf). The American bollworm was recorded on variety of Moomal (0.51/leaf). Its population was increase in the mid to end of season. The maximum thrip population appeared on variety TJ-83 (6.10/leaf) and lowest on Moomal (3.16/leaf). The thrips increases slowly at the start the season continued at mid to end of season. Among predators, the maximum population of Brumers was recorded on variety Indus (0.06/plant) and lowest population observed on variety gf SKD (0.03/plant). Similarly the lacewing was recorded on variety of SKD (1.48/plant) and its lowest population was on variety of Imdad (0.04/plant). Maximum Zigzag beetles population was on variety Indus (0.13/plant) and the lowest population on SB (0.04/plant). While the maximum mean population of 7-spotted beetles was recorded on variety of Indus, Pak-81, TJ-83 (0.08/plant) and the minimum population on Kiran and JS (0.05/plant).

ROSE-RINGED PARAKEET, *PSITTACULA KRAMERI (SCOPOLI)*, DAMAGE TO MAIZE CROP IN SUBURBS OF KARACHI

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Maize is a major cereal crop after wheat and rice, which is used as food grain, raw material for edible oil production and fodder for animals. NWFP and Punjab are main maize growing regions. It contributes 5.67% value of the major crops (Economic Survey of Pakistan 2008-09). Maize was grown over an area of 1,118,000 hectors and its production peaked to 4,036,000 tons in 2008-09. However, A considerable part of this crop is damaged by pests. Rose-ringed parakeet, *Psittacula krameri (Scopoli)*, is amongst the most destructive pests and inflicts heavy damages to standing maize crop. To observe extent of pestilence of the parakeet for maize crop, a study was carried out in commercial maize fields in suburbs of Karachi. Five fields (approximately ½ acre each) were selected for this study. Overall 76% of the fields had parakeet damage. Average loss of grains from damaged cobs was 29.61% in the fields. Loss in weight of grain was positively correlated with the length of damaged cobs.

SECTION - III

ENTOMOLOGY

BIODIVERSITY OF LEPIDOPTEROUS FAUNA (BUTTERFLIES) IN DISTRICT SARGODHA PUNJAB, PAKISTAN

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Five tehsils of Sargodha *i.e*. Sargodha, Shahpur , Sahiwal , Silanwali and Bhulwal were selected to observe the biodiversity of adult butterflies in urban and rural areas from September 2009 to April 2010. During the experiment 566 specimens was identified into 4 families, 14 genera and 20 species. Biodiversity was calculated by using Shannon-Wiener diversity index. The calculated values indicated that urban areas were more divers as compare to rural areas. From all five tehsils Bhalwal showed maximum biodiversity in both urban and rural areas while Silanwali showed minimum biodiversity. None of the reported species was found to be threatened to become extinct or found to be favored by enriched flora.

A REWIEW OF CAYSTRINE SUBGROUP *CAYSTRUS LANGEI* BREDDIN (1899) WITH SPECIAL REFERENCE TO THE SPECIES DESCRIBED BY LINNAVUORI *i.e., C. HIPPONAX, C. LANGEI, C. NIOKANUS* AND *C. PSEUDOBRUNNESCENS* (HEMIPTERA: PENTATOMIDAE: PENTATOMINAE) FROM ETHIOPIAN AND PALAEARCTIC REGIONS

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Ahmad and Zahid (2000 and 2009) described Linnavuori's species of the genus *Caystrus* Stål *i.e*., *C. hipponax*, *C. niokanus* and *C. pseudobrunnescens* which were shown closely related with each other but in this subgroup of the genus *Caystrus* Stål another species *C. langei* Breddin has only been known by superficial characters of coloration. Originally *langei* was described by Breddin (1899) under the genus *Neodius*, followed by Oshanin (1906), Kirkaldy (1909) and Gaedike (1971). Bergroth (1908) cited *langei* under *Caystrus*, followed by Oshanin (1912), Bodenheimer (1937), Linnavuori

(1960), Stichel (1961, 1962), Hariri (1971) and Linnavuori (1974 and 1975). Whereas the detailed morphological features of Linnavuori's species of this subgroup (*i.e., C. langei* subgroup) as noted above are fully known and redescribed by the present author with special reference to metathoracic scent auricle and male and female genitalia. Only superficial mostly color characters of *C. langei* are known in the literature to date. Presently with the help of original description and those of Linnavuori and by the studies of their holotypes its phylogenetic relationships within its subgroup are briefly discussed here. Linnavuori (1974 and 1975) separated *C. langei* with his own species *C. deserticolis* known from Saudi Arabia probably erroneously considering it related to his own species.

POPULATION DIVERSITY OF MOSQUITO FAUNA IN TANDOJAM, SINDH-PAKISTAN

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Research work was undertaken to investigate the prevalence of mosquito genera in Tandojam and its surroundings from May to June 2010. The data was collected from survey and field observation, while further examination leading to genera identification was carried out in Laboratory of Parasitology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam. Collections of samples were taken from 48 different localities of 6 habitat types viz; marshy/swampy areas, lavatories, ditches, grassy areas/ plants, animal pens and houses/ containers. A total of 2316 mosquito samples were collected which belongs to five genera viz; *Culex*, *Anopheles*, *Aedes*, *Uranotaenia* and *Psorophora* comprising 49%, 16%, 1.4%, 11.7% and 21.8% respectively. A total of 563 mosquitoes were collected from marshy/swampy areas, *Psorophora* spp. were the most abundant 218 (38.7%) were collected followed by 138 (24.5%) *Uranotaenia* spp., 123 (21.8%) *Anopheles* spp. and 84 (14.9%) *Culex* spp.. A total of 277 mosquitoes were collected from lavatories among which *Culex* spp. were dominant 239 (86.3%) followed by 23 (8.3%) *Anopheles* spp. and 15 (5.4%) *Psorophora* spp. were collected. There were 186 mosquitoes collected from ditches and *Culex* spp. were the most prevalent as 105 samples (56.5%) were collected followed by 81 samples (43.5%) *Anopheles* spp. A total of 552 mosquitoes were collected from grassy areas comprises 5 genera. *Anopheles*, *Culex*, *Aedes*, *Uranotaenia* and *Psorophora*. Of total genera *Psorophora* were the most abundant that is 273 (49.5%) followed by *Uranotaenia* spp., *Culex* spp., *Aedes* spp. and *Anopheles* spp. with abundance of 133 (24%), 94 (17%), 32 (5.8%) and 20 (3.6%) respectively. A total of 629 adult mosquitoes belongs to 2 genera were collected from animal pens. The most common genus was *Culex* comprising 524 samples (83.3%) and least common was *Anopheles* spp. 105 samples (16.7%) were collected. A total of 109 adult mosquito samples were taken from houses/containers

belongs to 2 genera. *Culex* spp. were by far the most abundant 89 (81.7%) followed by *Anopheles* spp. 20 samples (18.3%) were collected.

REDESCRIPTION OF MYROCHEINE GENUS *ERACHTHEUS* STÅL 1861 (HEMIPTERA: PENTATOMIDAE: PENTATOMINAE) AND ITS PHYLOGENETIC RELATIONSHIPS

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Stål (1861) described the genus *Erachtheus* to accommodate his (1853) species *Paramecocoris lutulentus* (which was subsequently designated by Kirkaldy (1909) as the type species) and followed by him (1876), Lethierry and Severin (1893), Kirkaldy (1909), Villiers (1952) and Gross (1975). But Linnavuori (1982) established a new subgenus *Alomaella* under the genus *Erachtheus* and designated *Sciocoris tibialis* as its type species. Linnavuori (1982) gave a short description of both the sub genera *i.e.*, *Erachtheus erachtheus* the nominal subgenus designating *S. boris* Dallas (1851) as its subspecies. Presently the two subgenera of *Erachtheus* with their type species are briefly described with special reference to their general features metathoracic scent auricle and male and female genitalia and in this light the phylogenetic relationship of *Erachtheus* within its tribe myrocheini Stål is also briefly discussed.

BIODIVERSITY OF ARACHNIDS IN LOW AND HIGH INPUT WHEAT (*TRITICUM AESTIVUM* L.) FARM AGRO-ECOSYSTEMS

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The study was focused to collect, identify and compare the species richness and abundance of spiders with respect to their ecological importance in wheat fields with low (organic) and high (conventional) inputs in the four major zones of Punjab (Pakistan). The LIP fields of all the systems except RFZ were highly diversified with respect to the abundance of spider fauna. By and large the sample size tended to decrease with time in the pair fields of all the zones with few exceptions that showed minor increase. Spiders of

MCZ, CWZ and RWZ districts were equally diverse except RFZ district. Nonetheless, the total diversity of spiders in LIP fields was significantly higher than those of HIP fields.

RELATIVE ABUNDANCE AND ROLE OF SYRPHIDS IN THE CROP SYSTEM OF FAISALABAD

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Majority of the agro-ecosystems are unfavorable for natural enemies due to high levels of disturbance. For this purpose habitat management is an ecologically based approach aimed at favoring natural enemies and enhancing biological control in agricultural systems. Global biodiversity declines is an important point which has focused attention on the implications of species losses for the maintenance of ecosystem functioning. Animal pollination contributes to 35% of global food production. Widespread distribution of Diptera and differences in their requirements of larvae are features that promote Syrphidae as potentially good bioindicators. As indicated by high mobility of adults, Syrphids are probably most suitable for environmental evaluation at a larger scale. The study was focused on determining the synergetic role of syrphids in the crop system of Faisalabad**.** Data was collected with the help of hand nets and by handpicking method from the selected quadrates of 1 meter square. For this purpose sampling was conducted for a period of 1 year, Major specie of this family was *Episyrphus balteatus* with some other species and the above discussed points were viewed in detail for study of stable agro-ecosystem.

STUDIES ON SILK POTENTIAL OF A NEW STRAIN (K_2) OF SILKWORM *(BOMBYX MORI* L.) UNDER DIFFERENT SETS OF TEMPERATURE AND HUMIDITY

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Silk is a natural protein fiber. Silkworm rearing is an applied science based on theory of anatomical physiology of silkworm and silk worm diseases. As a result, a lot of

work has been done to know the environmental effects on silkworm growth, development and fecundity. With an ultimate aim of producing more and better quantity and quality of silk and to expand the areas for its production country wise regions many strains are introduced date to date. A new strain of silkworm (K) was evaluated to include it in the efforts to raise our national productive potential of natural silk. For this purpose the biology of this strain was determined under different sets of temperature and humidity. I he stud\ regarding the rearing of silkworm for testing the effects of environment on its growth, development and fecundity was carried out in the Old Insectary, Department of Agri. Entomology, University of Agriculture, Faisalabad. Pakistan. The eggs of silkworm were brought from Directorate of Sericulture. Muzaffarabad, Azad Jamrnu & Kashmir. The eggs were shifted to four different sets of temperatures20, 25, 30 ºC and at room temperature as reference. The relative humidity (R. H.) at each temperature was also different respectively 60, 70 and 80 % for first three temperatures. The temperature for each group was decreased 1± ºC per instar and the R. H. was decreased *5* % per instar. In each group equal number of larvae was placed and the groups were assigned as: T_0 = Room temperature and R. H. 30 ºC with 60 % R. H., T_2 25 ºC with 70 % R. H., T_3 20ºC with 80 %/ R. H. The experiment was laid out in completely randomized design. It was observed that the longest period for mounting or spinning period (8 days 10 minutes) was observed when larvae were subjected to 16 ºC and 60 % R.H; whereas the shortest period (6 days 1 hour and 7 minutes) was noted when larvae were subjected larvae were subjected to 26 ºC and 40 % R.H. As far as about the larval mortality at 5th instar in each group, the highest (60%) mortality rate was observed when larvae were subjected to room temperature and R. H. and the lowest (10%) mortality was observed when larvae were subjected to 21 ºC and 50 R. H. The highest numbers of eggs (591) were laid when moths were subjected to 25 ºC and 70% R. H.; whereas the lowest number of eggs (254) were laid when they were subjected to 30 ºC and 60% RH. on an average to complete their total life durations. The comparison of the treatments showed that the average cocoon weight shell weight and pupal weight at 30 ºC and 60% were 0.98g at 20 and 80% were 1 .41g. 0.35g and 1 .05g and at room temperature were 0.93g 0.10 g and 0.74g. The heaviest value of cocoon weight was I .75g at *25* and the lowest was *0.93g* at room temperature. The highest shell weight was 0.40g at *25* and the lowest was 0. 1 9 both at 30 V and room temperature conditions. Pupal weight was the highest at *25* ºC and it was estimated 1.35 g while the lowest pupal weight was recorded at room temperature and it was 0.74g. The heaviest value of cocoon shell ratio was at 30 ºC and 60% R.H.

BURRIA CANA INGRISCH (PACHYMORPHINAE: HETERONEMIIDAE: PHASMID) A NEW RECORD FROM PAKISTAN

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Burria cana Ingrisch is recorded for the first time from Balochistan provinces of Pakistan. Present study based on the single female and male show the same character as

given by Ingrisch (1999). The previously un-described male also show the same character except it is smaller in size.

NEW RECORD OF GENUS *EREMOPEZA* SAUSS. (ORTHOPTERA: PAMPHAGIDAE) FROM SINDH, PAKISTAN

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Eremopeza Saussaure comprise seven species which are peculiar to Iran and adjacent countries, these are divided into a numbers of subspecies. This genus previously was reported from Balochistan province of Pakistan presently, I have recorded from Sindh Province. The species is very close to *Eremopeza gigas* (Kirby) and has been compare with the specimen from Balochistan and difference has been noted in the shape of pronotum. Should this character prove to be distinct for the Sindh population, it would justify recognition of this phenon as distinct subspecies (or species), but it is premature to make a decision, based on single specimen.

A NEW SPECIES OF GENUS *HILETHERA* UVAROV (OEDIPODINAE: ACRIDIDAE : ORHOPTERA) FROM P AKISTAN

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Hilethera Balucha n. sp is described from Balochistan Province of Pakistan.

DISTRIBUTION OF *SCHIZODACTYLUS MONSTROSUS* (DRURY) (ORTHOPTERA) ALONG THE RIVER INDUS SINDH, PAKISTAN

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A large series of specimens of *Schizodactylus monstrosus* (Drury) was collected from the River Indus during the year 2009-2010. It is large, robust, nocturnal insect has a great economic importance. During the present study population dynamic in *S. monstrosus* influence of its physical factors on the distribution pattern has been reported. Further, its population fluctuation in various months of the years was also recorded for the first time from this region.

AN ANNOTATED CHECKLIST OF NEUROPTERA OF PAKISTAN

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A checklist of Neuroptera recorded from Pakistan along with their geographical distribution is prepared. Surveys were carried out throughout the country including Azad Jammu and Kashmir (AJK) during summer season of 2005 - 2009.\Help was also taken from published literature of the country and also the specimens housed in National Insect Museum (NIM). A total of 20 species were recorded. For each recorded species synonyms and geographical distribution has been given, except for the species housed in NIM which were collected from Pakistan but their exact localities were not cited.

COLLECTION OF DRAGONFLIES (ANISOPTREA: ODONATA) HOUSED AT NATIONAL INSECT MUSEUM, ISLAMABAD-PAKISTAN

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Specimens of dragont1ies housed at National Insect Museum, National Agriculture Research Centre, Islamabad were reviewed. In museum, Anisoptera specimens are placed under two different sections *i.e.* pre-partition and post-partition. Data of pre-partition section is based on specimens collected from different localities of the subcontinent deposited in Pusa museum while post-partition collection includes specimens collected from Pakistan after partition. A check list of 100 species is reported herein.

OCCURRENCE OF INSECT POLLINATORS OF BITTER GOURDS AND THEIR RELATIONSHIP WITH QUALITATIVE AND QUANTITATIVE YIELD OF FRUITS IN TAXILA

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The experiment was done in the *Momordica charantia* field in the village Gangu, Taxila. One hundred and fifty flowers were tagged in the last week of August 2010. The

insect visitors coming on the bitter gourd flowers were counted for one and half minute during the flowering periods between 0800 and 0100 hours. The counting was made till shedding of flowers. Just before the opening of flower buds, card tags were hanged on the nodes. Each card was labelled with the flower number and the plot number. The crop field was distributed into five plots and total 150 flowers were tagged @ 30 flowers per plot. The male flowers were tagged just to determine that whether the visitors on male flowers were also visiting female ones or not. The use of Tele counters were also done by working with two of them at a time. Every visual observation of each plot was done on the other alternate time to see the variation occurring in the number of visitors. The data was recorded from last week of August till end of September. Each flower remained under observation for one and half minute. The total number of insect visitors according to their species and frequency of each visit was noted on the respective day. Some of the insects were caught with the help of aerial net as a sample and then sent to National Insect Museum, NARC, Islamabad for identification. The counted insects were placed in various taxonomic groups. When the fruits were mature and farmer was about to harvest the tagged fruits, they were purchased from him. The data was recorded for percentage fruit setting, length, width, weight, seeds per fruit and seed weight. The number of pollinators recorded in each plot was 219, 182, 223, 199, 184 in plot-I, II, III, IV and V respectively. The maximum no. of pollinators/ insect visitors was in plot-III. These pollinators consisted of family Apidae, Scoliidae (Hymenoptera), Hesperidae, Papilionidae, Nymphalidae, Pieridae (Lepidoptera), Empidae (Diptera), Cleridae and Chrysomelidae (Coleoptera). The study showed that more than 10 species of insects visited flowers of selected crop during flowering periods. The maximum -7 no. of insect pollinators belonged to Apidae family. The fruit setting was 75, 65, 70, 65 and 60% in plot-I, II, III, IV and V respectively. Fruit length was 20.63, 20.09, 20.76, 19.20, 20.09 cm, Fruit width; 3.66, 3.29, 3.42, 3.32, 3.45 cm, Fruit weight; 123.67, 121.31, 122.21, 109.38, 118.08 g, Seeds per fruit; 18.93, 18.38,19.07,18.38,18.42, Seed weight; 0.17, 0.15, 0.17, 0.25, 0.24g respectively. These parameters cannot be significantly correlated with no. of pollinators visiting the flowers. There may be some other parameters which may affect them.

A REVIEW OF SUBHIMALAYAN GENUS *CAHARA* GHAURI (HETEROPTERA, PENTATOMIDAE, HALYINI) AND ITS CLADISTIC RELATIONSHIP

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The species of genus *Cahara* are distributed in Himalayan region. Ghauri (1978) described the genus *Cahara* to accommodate the type species *Dalpada brevivtta* Walker and two other *Dalpada* species, *D. jugotaria* Lethierry and *D. confuse* Distant on the basis of paraclypei longer than clypeus, labium extends beyond the third abdominal

segment and male and female genitalia. Presently here the genus *Cahara* is revised with its nine world known species by brief distinguished features and zoogeographical distribution. The characters of each taxon are scanned from the present description and those given in the literature to date. Their apomorphic states are recognized on the bases of out group comparison within the tribe Halyini at large. A cladogram is constructed based on the principle of parsimony to throw light on the evolutionary relationship of the included taxa.

NEW RECORD OF BUTTERFLY SPECIES OF FAMILY PIERIDAE (LEPIDOPTERA) IN HYDERABAD DIVISION

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Butterflies belong to order Lepidoptera. They are very delicate and look very beautiful and attractive due to their colourful wings. Butterflies are important pollinators for many varieties of plants because they fly for long distance, but at the same time some of them are very serious pest of many crops and fruits. Most of the butterflies in the family Pieridae are in medium to small size. They usually have white or yellow colour, wings with black edges. Caterpillars of most Pieridae feed on various varieties of mustards (family Brassicacae), legume and cabbage.Presently we have surveyed Jamshoro, Tando Jam, Matiari localities of Hyderabad division and more than 300 specimens have been collected. Identification has been done by the colouration of fore wings, mouth parts and external and internal male and female genitalia. Their wings have been expanded on the stretching board and leave for twenty four hours and mounted in wooden boxes. Seven species of family Pieridae have been recorded, which includes four species of subfamily Coliadinae viz. *Anteos clorinde* Godart 1824, *Catopsila pomana* Frushstorfer 1908, *Catopsila florella* Fabricius 1775, *Eurema hecabe* Fabricius 1775, two species of subfamily Dismorphinae viz. *Colotis amata* Fabricius 1775, *Colotis vestalis* Vestalis 1876 and one species of subfamily Pierinae viz. *Anaphies aurota* Fabricius 1793). It has been a new record of these species from surveyed localities of Hyderabad, Sindh, Pakistan.

A NEW RECORD OF GENUS *NEOSCONA* (ARANEAE: ARANIEDAE) FROM SINDH, PAKISTAN

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Four species of genus Neoscona are described for the first time form Sindh, Pakistan.

INTERACTION BETWEEN *LYCOSA TERRESTRIS* (LYCOSIDAE: ARANEAE) AND *COCCINELLA SEPTEMPUNCTATA* (COCCINELLIDAE: COLEOPTERA)

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Among invertebrate predators, intraguild predation and cannibalism are antagonistic interactions that diminish the top down impacts on shared prey. Two predators *i.e*., wolf spider *Lycosa terrestris* and lady bird beetles *Coccinella septempunctata* co-occur abundantly in the agroecosystem of Pakistan. Experiments in the laboratory were designed to assess the functional response of adults of both predators on the second or third instars larvae of *Helicoverpa armigera.* Intraguild predation (IGP) and cannibalism activities in the adults of both predators were also assessed at different densities and in the presence or absence of extraguild prey *i.e.*, *H armigera.* Polynomial logistic equation reported a type I functional response of *L. terrestris* and *C. septempunctata* to the number of extraguild prey and type II functional response to the . proportion of extraguild prey offered. Data showed a pronounced cannibalism activity in the adults of *L. terrestris* compared to *C. septempunctata*. However adults of both predators showed high cannibalism activity in the absence of food and increase density of specimen. IGP showed asymmetry in favor of *L. terrestris* both in presence and absence of prey. When sufficient number of *H. armigera* larvae was present, mortality of *C. septempunctata* decreases significantly in IGP experiments, indicating that adult coccinellids is less preferred or less valuable prey to *L. terrestris* as compared to *H. armigera* . However, when density of both predators increased in experiment, IGP pressure decrease on *C. septempunctata* due to increased cannibalism activity in the specimens of *L. terrestris.*

EFFECT OF DIFFERENT TEMPERATURE ON DEVELOPMENT AND SURVIVAL OF IMMATURE STAGES OF *BACTROCERA ZONATA* (SAUNDERS) (DIPTERA: TEPHRITIDAE)

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Bactrocera zonata (Saunders) (Diptera: Tephritidae) is the most important and well known pest among fruits and vegetables in Pakistan. The development and survival of *B. zonata* from egg to adult stage was studied under laboratory at 15ºC, 20ºC, 25ºC, 30ºC and 35ºC±1ºC with photoperiod of L12:D12. Rapid embryonic development observed at 35ºC but there was no pupal and adult development at this temperature. The

slowest development of immature stages was recorded at 15ºC. The highest percentage of adults (70) was obtained at 25ºC. The developmental time of eggs was 5.25 days at 15ºC which decrease to 1.21 days at 35ºC. Larval development period of 34.20 days at 15ºC which decrease to 6.24 days at 35ºC. Pupal development at 15ºC was 35.25 days while no pupa formation and adult emergence at 35ºC, this temperature was observed as most lethal temperature. The optimum temperature for development and survival of immature stages of *B. zonata* was 25 ºC among the five tested temperatures.

COMPARATIVE BIOLOGY OF TRICHLORFON RESISTANT AND SUSCEPTIBLE STRAIN OF *BACTROCERA ZONATA* (SAUNDERS) (DIPTERA: TEPHRITIDAE) UNDER LABORATORY CONDITION

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Bactrocera zonata (Saunders) considered one of the most destructive fruits and vegetables pests which spread in several region of the world. In Pakistan this pest caused 25-50% losses in guava fruits only. Repeated and indiscriminate use of some insecticides against fruit flies creates resistance among them. Comparison of trichlorfon resistant strain to susceptible strain of *B. zonata* was made to determine the rate of increase of resistant population. Some developmental and reproductive parameter such as emergence from pupae, oviposition preoviposition, fecundity and longevity of male and female was observed. Trichlorfon resistant strain had longer pupal and preoviposition period and more mean generation time as compared to the susceptible strain of *B. zonata*. Low fecundity, shorter male and female longevity was observed in trichlorfon resistant strain as compared to susceptible strain. While duration of eggs, larval stages and pre-mating period had no remarkable difference between the both strains of *B. zonata.*

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

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Mites belonging to the family stigmaeidae are well known predators against the phytophagous mites and small soft bodied insects and often collected from aerial plant parts and some times from soil leaf litter. A survey was conducted to explore the predatory mite fauna of Punjab (Pakistan), a new species of genus *Pseudostigmaeus* was

collected from Jhang on Lehli weed (*Convolvulus arvensis*) and has been described in this research article. Collected specimens were mounted on the glass slides with the help of Hoyer's medium. The drawings of different body parts were made with the help of an ocular grid high power microscope. These specimens were compared with the already described species of the world. Ceremonial description, illustration of main body parts, host range and comparison remarks are also given. Seven paratypes were collected from the same collection data and six were collected from Khanewal on cotton crop. All specimens were deposited in the Acarology Research Laboratory, Department of Agri. Entomology, University of Agriculture, Faisalabad, Pakistan.

EFFECT OF SIZE AND DENSITY OF PREY ON THE WEB STRUCTURE OF *NEOSCONA THEISI* (ARANEAE: ARANEIDAE)

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Orb-web spiders show remarkable flexibility in their foraging behaviour and adjust their web building behaviour according to prey availability. The present study is conducted to investigate the effect of prey size and prey density on web structure (web area, mesh height, number of radii and anchors, horizontal and vertical diameters, upper and lower spirals) of *Neoscona theisi.* For experiment, three sizes of prey *i.e.* small «05 mm), medium (5-10 mm) and large (>10 mm) were selected. Results showed a significant difference in the web structure due to the variation in the prey size. In the presence of larger prey, we noted increase in mesh height, anchor number, horizontal and vertical diameter but decrease in web area, radii number and spirals. For each prey size, we studied two density parameters *i.e.* high and low density. ANOV A showed that density affects web area, number of radii, horizontal and vertical diameter, and upper and lower spirals in the presence of medium sized prey.

A NEW SPECIES OF GENUS *ERYTHRAEUS* LATREILLE (ACARI: ERYTHRAEIDAE) FROM PUNJAB, PAKISTAN

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Erythraeus (Erythraeus) layyahensis sp. nov. (Acari: Erythraeidae) has been described in detail along with illustrated diagrams from a holotype larva collected from foxtail grass (*Setaria viridis* L.). from district Layyah, Punjab, Pakistan**.** In subgenus *Erythraeus* 29 species are known from Europe and Asia. This new species differs from

all previously described species by having unique characters like basifemoral setal formula 4-4-3, AL=PL=80, very long ASE and PSE both 90 m and surrounded by thin cuticular lines. Paratypes 9 larvae, collection data of 6 larvae same as holotype while 3 paratypes were collected from district Bakhar from madhana grass (*Dactyloctenum aegyptium* L.). All specimens have been deposited in Acarology Research Laboratory, Department of Agri. Entomology, University of Agriculture, Faisalabad.

FLORAL RESOURCE UTILIZATION BY BUMBLEBEES IN AGRICULTURAL ECOSYSTEM OF NORTHERN PAKISTAN

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The observations on the bumblebees and their floral association were carried out from March to September in agricultural ecosystem from cropped and non- cropped area on two consecutive days *i.e*., on day first in cropped area and on day second in non-cropped area within the search area of 100m radius and counted the bumblebees during the bloom of the target crop/ plant for one hour with six observation turns. Significant variations were observed in the species richness of non cropped area than the cropped one which reflected the importance of non cropped area as a refuge area for the development of bumblebee colonies in agricultural habitat which ultimately play a vital role in the pollination of crops, increasing their quality and quantity. *Trifolium alexandrinum, Medicago sativa* and *Solanum tuberosum* were the only plant species which were visited by all bumblebee species in the cropped area.

A NEW RECORD OF PREDATORY MITE OF GENUS *AGISTEMUS* (STIGMAEIDAE; ACARI) FROM PUNJAB, PAKISTAN

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Mites belonging to the Family Stigmaeidae are well known bio-control agents for the Phytophagous mites and small soft bodied insects. A survey was conducted in district

Narowal Punjab (Pakistan) to explore the fauna of this family. A new species *Agistemus;zahdi* of genus *Agistemus* was collected from Narowal from Neem (*Azadirechta;indica*). This is described in this paper. Specimens were mounted on the glass slides with the help of Hoyer's medium. The drawings of different body parts were prepared with help of an ocular grid in a high power microscope. These specimens were compared with the already described species. The description and illustration of main body parts, host range and comparison remarks are also given.

FIRST RECORD OF THE GENUS *RHIZOGLYPHUS* (ACARI: ACARIDAE) FROM PAKISTAN

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Storage mites are important pests of all types of stored commodities. They are not only responsible for direct damage in form of weight reduction but also imply the indirect damage in form of germination loss of the grains, deterioration of the nutrients and quality of the stored grains and other stored products. Mites of the genus *Rhizoglyphus* are very important pests of stored grains, plant bulbs and tubers. One new species *Rhizoglyphus tritici* was found in abundance from wheat of different localities of Tehsil Toba Tek Singh. This is the first species of the genus *Rhizoglyphus* (Acaridae) recorded from Pakistan. The Types were deposited in the Acarology Research Laboratory, University of Agriculture, Faisalabad.

IMPACT OF WEATHER IN POPULATION FLUCTUATION OF FRUIT BORER, *HELICOVERPA ARMIGERA* (HÜBNER) IN TOMATO

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The larval-population of the fruit-borer *Helicoverpa armigera* (Hübner) and its fruit-infestation, obtained from the initial and final varietal screening trials, during 2007 and 2008, under Faisalabad ecological conditions, were processed for correlation and Multiple Linear Regression analysis, along with the coefficient of determination (R^2) values. Maximum, minimum and average temperature, during 2007 and 2008, each had a

significant and positive correlation with the larval population of the tomato fruit-borer. Relative humidity, exerted a negative and highly significant correlation with the larval-population of fruit-borer, during both the study years, but, on an average, it exerted, positive and significant correlation ($P \leq 0.05$) with the larval-population/ plant. Maximum, minimum and average temperature, each had a significant and highly positive correlation with the fruit-infestation; whereas, the relative humidity showed a negative and significant correlation ($P \leq 0.05$) during the year 2008, whereas, during 2007, as well as on the basis of an average for both years, all the factors showed a non-significant correlation with the infestation.

A NEW GENUS, THREE NEW SPECIES AND FOUR NEW RECORDS OF POULTRY LICE (PHTHIRAPTERA: AMBLYCERA AND ISCHNOCERA) REPORTED FROM KARACHI, PAKISTAN

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A new genus, *Gallimenopon* gen. nov. consists of two new species, along with a new species of the genus *Menacanthus* Neuman, of the family Menoponidae (Amblycera) were described in detail from Karachi, Pakistan, with reference to their chaetotaxy, and male and female genitalia. These were collected from different breeds of domestic fowl, *Gallus gallus* L. from different localities of Karachi region. The new genus and new species were also compared with its closest allies. The present paper also includes four new records of poultry lice (Phthiraptera: Amblycera and Ischnocera) from Karachi region. All the poultry chewing lice found in the world were keyed out to understand their closed relationship and approaching to the specific level of identification.

RESEARCH METHODOLOGY: SAMPLING METHODS AND SAMPLE SIZE

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The importance of statistics in biological sciences can hardly be overemphasized. Conceptual understanding is required on the relationship between sample and population, concept of probability in drawing inferences, need for sampling, the importance of a representative sample, risks in using un-representative sample, risks in a random sample (simple random samples, stratified samples, systematic sample, cluster sample, etc). Practical approaches involved in taking a random sample will be shared with the audience using a couple of examples. A field researcher needs to know how to use table of random numbers for random sampling. An investigator should have a clear concept of

the requirement of an adequate sample size. Methods for calculating the required sample size will be discussed.

A NEW SPECIES OF GENUS *APODIPHUS* SPINOLA (HEMIPTERA, PENTATOMIDAE, HALYINI)

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A new species of the Genus *Apodiphus* Spinola is described from Queeta, Pakistan with special reference to metathoracic scent gland ostiole and male and female genitalia and compared it with its close allied species A. *integreceps* Horvath. The description is supported by illustration.

ANOPHELES MOSQUITOES DENSITY IN URBAN AREAS OF KARACHI - PAKISTAN.

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Mosquitoes are very dangerous insect on the earth. It is difficult to estimate the involvement of mosquitoes in the different diseases. Mosquitoes are the most medically important insects in the entire world, thus it is significant to understand to the professionals about the mosquitoes density, biology and their control. Different genera and species of mosquitoes found in Pakistan, many are known to be vectors (carriers) of important diseases such as Malaria, Dengue hemorrhagic fever, West Nile virus and Chikenquinea etc. Mosquito control has an imperative role in maintaining public health even in the absence of disease transmission. Different species of Anopheles are responsible to transmit malaria. About 24 species and sub species of Anopheles mosquitoes are recorded in Pakistan, among these many are known to be vectors (carriers) of Malaria diseases. Adult *Anopheles* mosquitoes (Diptera: Culicidae) surveillance was conducted during 2002 to 2004 at Urban areas of Karachi including 05 districts. Adult mosquitoes were collected through netting, aspirating and by using artificial resting station from indoor and out door in and around the residence, hotel, school and cowsheds. *An. culicifacies, An.stephensi, An. subpictus* and *An. pulcherimus* were found in all districts. Total 68055 mosquitoes were collected from all five districts

of Karachi, among those 5194 were documented as *Anopheles* mosquitoes including 2967 male and 2227 female. *An. culicifacies* was the most abundant species found 32.7%, 18.60% and 34.61% whereas *An. pulcherimus* was the least common 5.19%, 1.44% and 1.92% were during the surveillance period of 2002, 2003 and 2004 respectively. It was concluded that present investigation on mosquito surveillance will provide arrangement, operation, and evaluation of any effective mosquito control program. As well as Mosquito surveillance programs will provide a record of local mosquitoes and the effectiveness of control strategies.

OEDIPODINAE (ACRIDINAE: ORTHPTERA) OF AZAD JAMMU & KASHMIR, PAKISTAN

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The present study lime light the identification of 15 species of sub family Oedipodinae under 13 genera. This study has added 4 species in the previous record identified from the collection area. In addition, the key for the local genera has also been constructed.

DISCOVERY OF A NEW SPECIES OF GENUS *LEPTUS* LATREILLE (ACARI: ERYTHRAEIDAE) FROM PAKISTAN

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[2]*University of Agriculture, Faisalabad, Pakistan*

A new species *Leptus aphidus* (Acari: Erythraeidae) has been described in detail along with illustrated diagrams from a larva collected as a parasite from *Aphis gossipii* (Aphididae: Homoptera) in rice fields from district Sialkot, Punjab, Pakistan**.** *Leptus aphidus* sp. nov. belongs to the species group with two palpgenualae and four setae between coxae II-III. This group includes: *L. lomani* Oudemans, 1912 from Chile, *L. anomalus* Southcott, 1946, *L. benzaliensis* Fain and Elsen, 1972 from Congo (Zaire), *L. aggoratus* Haitlinger, 1990 from Zambia, *L. fortei* Southcott, 1991, *L. waldockae* Fain, 1991, *L. faini* Southcott, *L. utheri* Southcott, *L. halli L. truncates* Southcott, Southcott, 1993 all from Australia, *L. fathipeuri* Haitlinger and Saboori, 1996 from Iran, *L. hringuri* Haitlinger, 2000 from Peru, *L. iguacuicus* Haitlinger, 2004 from Brazil, *L. dinekaicus* Haitlinger, 2006 from Ethiopia, *L. elminus* Haitlinger, 2006 from Ghana, *L. pakistanensis* Kamran *et al*. from Punjab, Pakistan. This new species differs from all

above mentioned species by having AW=78, PW=95, PSE =34, AL= PL, ASE and PSE with strong ciliations on distal halves of their lengths, fD 54, fV=20, GL=187, L=78, W=100, ornamentations on scutum and IP=1608.

SECTION - IV

PARASITOLOGY

A NEW METACERCARIAL FORM *EUCLINOSTOMUM ROBUSTUM* (TREMATODA CLINOSTOMIDAE : EUCLINOSTOMINAE) FROM A FRESH WATER FISH OF SINDH, PAKISTAN

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A new metacercaria *Euclinostomum robustum* is described here from the liver of the fish *Channa gachua* of Haleeji lake, Sindh. This new form is characerised by having flattened, stout body, flat anteriorly and rounded posteriorly with bluntly pointed tip, with narrow preacetabulal region and gradually widening in postacetabular region, widest at the level of anterior testis. Oral sucker transversely elongated with a downward projection in the centre. Pharynx is absent, esophagus small bifurcating anterior to acetabulum. Unequal cecae branched, lateral branches emerging immediately at the base of acetabulum, 10-11 in number, reaching to near posterior end of the body. Acetabulum large, muscular, rounded in shape situated in anterior half of the body. Testes two, relatively large, in posterior half of the body, far posterior to acetabulum and near to posterior end of the body, anterior transversely elongated with anteriorly curved ends, posterior smaller, y-shaped. Ovary very small, submedian, close to posterior border of anterior testis. Vagina tubular, uterine sac long, reaching to acetabulum. Vitellaria consist of minute follicles, extending laterally in the postacetabular region to posterior extremity of the body. Confluent behind posterior testis. Excretory pore median, subterminal.

DESCRIPTON OF A NEW SUBFAMILY HECKMANNINAE (MONORCHIDAE (ODHNER, 1911) NICOLL, 1915) WITH A NEW GENUS AND SPECIES FROM A FRESHWATER FISH OF SINDH, PAKISTAN

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A new subfamily Heckmanninae is described with a new genus and species *Heckmannia channai* n.gen., n.sp. from the intestine of *Channa striatus* (Bl.). The subfamily is characterized by having elongate body, oral sucker subterminal, rounded, prepharynx very small, pharynx notched in the center, pharyngoesophageal gland cells present between pharynx and esophagus, terminal organ is absent, cirrus sac is

preacetabular, genital pore submedian, postbifurcal. Acetabulum is pre-equatorial. Testis single, large, almost L-shaped, postacetabular, postovarian and postequatorial. Ovary is large, pretesticular, rounded with slightly irregular outline. Vitellarial consist of numerous follicles, scattered laterally from the level of ovary to posterior end of body, confluent in the post-testicular region. Uterus not visible.

A NEW SUBFAMILY ASYMMETRINAE (TREMATODA: FELLODISTOMIDAE) WITH *ASYMMETRA MAGNACIRROSA* N.GN. N.AP. FROM THE FISH *SPARUS BERDA* (FORSK.) OF KARACHI COAST

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A new trematode subfamily Asymmetrinae with *Asymmetra magnacirrosa* n.gen. n.sp. is described here from the intestine of the fish *Sparus berda* of Karachi coast and is accommodated in the new subfamily Asymmetrinae. The new trematode is characterized by having a small, elongate to elliptical body, slightly subterminal, large, rounded, oral sucker, prepharynx prominent, pharynx small, esophagus of moderate size, ceca reaching behind middle of the body and acetabulum is round, smaller than oral sucker, in anterior third of the body. Testes are two, rounded to oval in shape, submedian, oblique, one anterolateral and other posterolateral to acetabulum containing seminal vesicle and pars prostatica, genital atrium tubular, cirrus sac long with a bulb-like terminal reaching posterior to cirrus sac at the anterior level of posterior testis. Genital pore is postbifurcal, submedian, with a saccular outgrowth, ovary is smaller, submedian, pretesticular. Vitellaria is in acetabular and preacetabular zone, postovarian to post-testicular, consisting of 8-9 large rounded to oval follicles. Uterus long, coiled inbetween post vitalline zone, reaching to near posterior end of body, anteriorly reaching to genital pore. Eggs numerous, small. Excretory vesicle large, Y-shaped.

MICROTETRAMERES SP. (NEMATODA: TROPISURIDAE) FROM THE GREAT EGRET *EGRETTA ALBA* IN KARACHI, SINDH

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Seven *Egretta alba* Syn. *Ardea alba* were autopsied in the laboratory for helminth parasite infection, three out of seven, were found infected with thirty five nematodes. These were studied in detail, after fixing in hot 70% alcohol and later cleaning in a

mixture of 70% alcohol and glycerin. The nematodes were identified as belonging to the genus *Microtetrameres* Travassos, 1917. These are characterized by a: small cylindrical bucal capsule, Esophagus divided into a narrow, anterior muscular and a longer wider glandular posterior portion. Excretory pore just behind nerve ring. Cervical papillae asymmetrical. Body of female usually spirally twisted, Tail elongated, conical. Vulva close to anus. Eggs small, embryonated. Male without spines; tail tapering to a sharp point; spicules relatively very long, larger spicule one half to two third of the body length, rounded at the tip; smaller one sharp- pointed, slightly chitinized. Two pairs of preanal and two pairs of postanal papillae present. The genus *Microtetrameres* has not been reported earlier in Pakistan. This is therefore a first record in Pakistan.

SOME CELLULAR CHANGES IN THE LIVER OF *POMADASYS OLIVACEUS* (DAY) DUE TO NEMATODE LARVAE INFECTION

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Pathological changes in the liver of marine fish *Pomadasys olivaceus* (Day) due to nematode larvae is described here. Severe destructive effects have been observed resulting into loss of architectural morphology of the hepatocytes, inflammatory host tissue response, various necrotic abnormalities, degenerating bile duct. Clogging of hepatocyte was also observed. Dilated sinusoid is another important finding by this infection. Vacuolar degeneration of hepatocytes was prominent.

PSEUDOPROLEPTUS SP. (NEMATODA: PHYSALOPTERINAE) FROM THE FRESH WATER FISHES IN KARACHI, SINDH

MAJEEDA RUK

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Seven fresh water fishes were autopsied in the laboratory for helminth parasite infection, three out of seven, were found infected with five nematodes. These were studied in detail, after fixing in hot 70% alcohol and later cleaning in a mixture of 70% alcohol and glycerin. The nematodes were identified as belonging to the genus *Pseudoproleptus* Khera, 1955. These are characterized by a: month with two lateral lips, each bearing a truncate tooth and two submedian papillae. Cuticular cephalic collar present, bucal capsule cylindrical, with transversely striated walls, Esophagus long, undivided. Male posterior extremity spirally coiled. Caudal alae absent. 4 pairs of preanal

and 6 pairs of postanal papillae: last two of the latter are at the tip of the tail, whereas the first line close to the cloaca, and the remaining three are grouped at about the middle of the tail. In the ventral precloacal region there are unequal dissimilar; left spicule alate, transversely striated, right one much slender; gubernaculums-like. Female tail abruptly narrowed toward middle, and ending, and ending in a short blunt digit form process. Vulva post-equatorial. Uteri" opposed oviparous, egg thick-shelled, embryonated when deposited. Parasites of freshwater fishes.

A RECORD OF *STRIGEA FALCONIS* SZIDAT, 1928 (TREMATODA: STRIGEIDAE) FROM THE BIRD *EGRETTA ALBA* (UN.) IN SINDH, PAKISTAN

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During on autopsy study of some birds, *Egretta alba* in the Laboratory six small trematodes were recovered from the small intestine of a bird. These were fixed, dehydrated, stained and mounted permanently for detail study. The trematodes were identified as belonging to the genus *Strigea* Abildgaard, 1790 and species *falconis* Szidat, 1928, the specimens are characterized by having: Body bipartite, fore body cup shaped, hind body cylindrical. Oral sucker, Pharynx well delveloped. Ventral sucker comparatively larger, lobes of holdfast organ well developed. Testes :lobed in median part of hind body, ovary pretesticular, smaller, oval to rounded. Vitellaria extends into fore body while in hind body it extends posterior up to the copulatory bursa. Copulatory bursa and genital cone delimited from body parenchyma, a short ejaculatory duct opens at apex of genital cone.

BRACHYLECITHUM QADRII SP.N. (TREMATODA: DICROCOELIIDAE) ODHNER, 1910, FROM THE BIRD *EGRETTA ALBA* (LINNAEUS), IN KARACHI SINDH, PAKISTAN

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Three birds *Egretta alba* (Linnaeus) Great Egret or Large Egret Syn. *Ardea alba* were purchased from Empress market, Karachi. The birds were anesthetized, autopsied and examined for helminth parasite infection. Three trematodes were recovered from the small intestine of a host. These were identified as belonging to the genus *Brachylecithum* Shtrom, 1940. The specimens were fixed, dehydrated, stained and mounted permanently according to the standard procedure. These are characterized by a: slender and elongate

body. Anterior region more attenuated than the posterior which has a bluntly pointed spike like structure at the end. Maximum width is acquired in the posterior half of the body. Oral sucker sub terminal, muscular, rounded. Pharynx small, slightly broader than long. Esophagus short. Ceca not obvious due to profuse uterine coils. Ventral sucker sub equal to oral sucker, oval and rounded in shape, situated in the anterior-third of the body. Genital pore at the level of Pharynx at the anterior extremity. Testes diagonal, irregular in shape, unequal in size, situated in the anterior half of the body, obliquely tandem, separated from each other through the uterine coils. Anterior testis larger than the posterior. Cirrus pouch elongated claviform in front of the ventral sucker. Vesicula seminalis appear to occupy full space of the cirrus sac. Ovary irregular in shape, Post testicular, pre equatorial, smaller than the testes. (The vitellaria are few, well developed, larger in size, lie in the vicinity of the ovary in the pre equatorial area, extending in a short zone, and lateral in position. Uterus arising from the ootype runs posteriorly, filling the entire hinder part of the body, it runs further in the anterior region across the ovary and the testes, reaching up to the level of the acetabulum to open at the genital pore at the level of Pharynx in the prebifurcal region below the oral sucker. Eggs are small, double walled, oval to elongate.

GENERAL OBSERVATIONS ON *RAILLIETINA (PARONIELLA) REYNOLDSAE* (MEGGIT, 1926) KHAN AND HABIBULLAH, 1967 (CESTODA: DAVAINEIDAE) AND SCANNING ELECTRON MIRCROGRAPHIC STUDY OF SCOLEX OF THE SAME

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40 male 30 female *Passer domesticus* were purchased from the Empress market in Karachi city at random intervals. The birds were anesthetized/ autopsied and examined for helminth parasite infection; twenty cestodes were recovered from the small intestine of fifteen hosts. The specimens were fixed, dehydrated, stained and mounted permanently according to the standard procedures. These were identified as belonging to the genus *Raillietina (Paroniella) reynoldsae,* these are characterized by a: strobila 60-70mm long, composed of numerous immature, mature and gravid segments. Maximum width is attained at the level of gravid segments. Scolex simple, globular, rostellum with a single circle of minute hammer shaped hooks. Suckers spinose, Spines on the suckers appear larger while the rostellar spines are smaller in size. Neck long, mature segments broader than long. Ovary median, bilobed. Vitellaria post ovarian, compact. Seminal vesicle and seminal receptacle are quite obvious. Testes numerous, mostly surrounding the female gonads, lateral and posterior with few on the anterior margin. 20-26 on aporal side and 10-12 on poral side. Cirrus sac small, funnel-shaped. Genital pore marginal, slightly in front of middle of the lateral margin of the segments, back- wardly directed in position. Vas deferens and vaginal ducts parallel. Gravid segments much broader than long. Uterus

breaks down into several egg capsules, each capsule contains several eggs. Scanning electron micrograph of the scolex and its enface-view was prepared by the curtsey of Central Science Laboratory, Karachi University Campus, Karachi, 75270, according to the standard orocedures. Scanning electron micrographs of the Scolex of the genus *Raillietina (Paroniella) reynoldsae* appears to be flower-shaped at a glance. The en-face view of the scolex shows a larger rounded rostellium in the middle with a longitudinal-slit like opening, this is surrounded by rows of numerous leaf-like structures which are covered with several minute spiny out growths. The suckers appears to be highly muscular cushion-shaped provided with rows of spines which are quite larger than the spines on the scolex. The portion immediately below the scolex appears to have has rows of frill-shaped structures up to some distance below, which are devoid of spines or minute spiny out growths.

HISTOLOGICAL STUDIES OF CHICKEN LIVER INFECTED WITH AVIAN LEUKOSIS IN KARACHI

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The commercially important neoplastic diseases of poultry avian leucosis is caused by retrovirus. This disease causes severe economic loss due to mortality of birds and depressed performance. The avian leukosis virus (ALV's) are prevalent throughout the world, new strains from one location may spread across borders, thereby undermiring the disease control measures. Four commercial egg type white dead birds (33 weeks old) were received from a commercial layer poultry farm of Karachi. On postmortem examination, all birds showed necrosis in the liver. The daily mortality out of 14,000 birds was 30-45 (0.2-0.3 percent). All birds were vaccinated against Marek's disease at one day old. The infected liver specimens were preserved in 10% formalin and processed for histological studies. Dehydration was carried out in serial concentrations of known volume of ethanol. Dehydrated tissues were then infiltrated and embedded in paraffin wax at 52°e for 10 days. During the wax infiltration process, air bubbles were removed from the tissue under vacuum. Using a rotary microtome 10 !lm thick sections were cut and stained with haemotoxylin and eosin. Photomicrographs were taken using an automatic photographic camera mounfed on a research microscope Nikon Optimphot-2 in the Department of Zoology, University of Karachi. The sections of liver showed dilation of the sinusoids. The sinusoids became extremely distended, resulting in atrophy 'of parenchyma. Digeneration was visible. Similarly, degeneration of cells around central veins due to local anoxia were obvious.

A NEW PARASITIC NEMATODE SPECIES *RHABDOCHONA PAKISTANICA* N. SP. DESCRIBED FROM THE CARP FAMILY FISH (OSTEICHTHYES: CYPRINIDAE) IN CENTRAL BALOCHISTAN

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During the course of study conducted for helminthes species of fish,a new parasite of nematode genus Rhabdochona Railliet, 1916 (Nematoda: Rhabdochonidae) is described here from the stomach of inland fish *(Cyprinion watsoni* Day, 1872) caught at river Bolan, Sibi division, Balochistan. The new nematode species *Rhabdochona pakistanica* n. sp. is differentiated from all known species of the genus *Rhabdochona* in general and its closest allies (having 8 prostomal teeth) mainly in body size, length ratio of spicules, number and distribution of caudal papillae, shape of the egg and position of vulva and excretory pore.

ON A NEW SPECIES OF SPIRURID NEMATODE *RHABDOCHONA (RHABDOCHONA) ANNAI* (SPIRURIDA: THELAZIOIDEA) FROM PUTITOR MAHSEER, *TOR PUTITORA* (HAM.)

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A new species of *Rhabdochona* (Thelazioidea: Rhabdochonidae) is described here as intestinal parasite of Mahseer, *Tor putitora* (Ham.) from a river basin at Bolan of Balochistan province, Pakistan. *Rhabdochona (Rhabdochona) annai* n. sp. is characterized by a combination of diagnostic features that include the presence of prostomal teeth, simple (not bifurcated) deirids, and length ratio between spicules. The combination of these features easily differentiated the new species *R. (R.) annai* n. sp. from all its congeners including those species possessing 8 teeth in the prostome. The new species appears to be specific to freshwater cyprinids as previously twelve species of *Rhabdochona* Railliet, 1916 have been described from this group of pisces only from Balochistan.

RHABDOCHONA HASPANI NEW SPECIES (THELAZIOIDEA: RHABDOCHONIDAE) FROM STREAM FISH (ACTINOPTERYGII KLEIN, 1185) OF SIBI DIVISION, BALOCHISTAN

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A new nematode *Rhabdochona (Rhabdochona) haspani* n. sp. is described on the basis of the specimens recovered from the intestine of a fish, *Cyprinion watsoni* (Day, 1872) from fresh waters in Harnai, Balochistan. The new nematode specimens are clearly different from all the previously reported species of *Rhabdochona* Railliet, 1916 by the relevant length of esophagus, shape and size 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 measurement of eggs (0,013-0.035), morpholcgy of vagina, position of vulva and excretory pore. The new specimens have 8 teeth in the prosotme; additionally these have 13 pairs including 8 preanal and 5 postanal caudal papillae.

HAZARDS OF *ENTAMOEBA HISTOLYTICA* IN KARACHI CITY

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In the present study, prevalence of *Entamoeba histolytica* was observed in the population of different areas of Karachi city complained with intestinal infection. During two years i.e. January 2009 to December 2010, a total 441 stool samples of infected patients were collected from different hospitals of Karachi including Map hospital, AI-Haider memorial medical center, Baqai hospital, Shan hospital, Nimra hospital, Sindh heart and care hospital and Sindh government hospital, The stool examination has done by stained and unstained slide microscopy techniques. Most of the positive cases reported in Map hospital and Nimra hospital of New Karachi town i.e. 59 (26.46%) and 55 (12.47%) respectively. However less percentage of cases registered in Sindh government hospital of Liqatabad town i.e. 35 (7.94%). The rate of *E.histolytica* infection more commonly recognized in male patients i.e. 232 (52.61%) while less in female patients i.e. 209, (47.39%). Major reasons behind the prevalence of *E. histolytica* in studied area are lack of health education and poor sanitation.

FIBROSIS AND BROWN ATROPHY IN THE PORTAL TRACT AREA OF SHEEP LIVER INFECTED WITH *FASCIOLA HEPATIC*

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Damage of portal tract area associated with *Fasciola hepatica* infection in the liver of sheep is reported here. Whole liver was affected by this infection but more prominent injury and alteration of tissue was noted in the portal tract area which showed total destruction and morphological changes.Main findings were bile duct hyperplasia and proliferation' of bile duct with fatty degeneration and severe fibrosis of bile duct as well as artery. Shrinkage of tissue and brown atrophy of portal tract area was obvious. Brown atrophy was not reported previously in this area of liver by this infection in sheep.

CASE OF LYMPHOID LEUKOSIS IN CHICKEN-SPLEEN

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Lymphoid leukosis has been reported in both captive and free living wild birds. It is caused by retrovirus. This disease causes severe loss due to mortality of poultry birds and depressed performance. The avian leukosis virus (ALV's) are prevalent throughout the world, new strains from one location may spread across borders, thereby undermining the disease control measures. Four commercial egg type white dead birds (33 weeks old) were received from a commercial layer poultry farm of Karachi. These birds had no previous sign of ill health. On post mortem the spleen was diffusely enlarged and white in colour. A portion of the infected spleen tissue was preserved in 10% formalin and processed for histological studies. Dehydration was carried out in serial, concentration of known volume of ethanol. Dehydrated tissue were then embedded in paraffin wax at 52°C for 10 days. During the wax infiltration, process air bubbles were removed from the tissue vacuum. Using a rotary microtome 10 µm thick sections were cut and stained with haemotoxylin and eosin. Photomicrographs were taken using an automatic photographic camera mounted on a microscope Nik9n Optiphot-2 in the Department of Zoology, University of Karachi. The action of spleen showed massive infiltration of tumour cells.

Lymphoid cells of varying size were observed. The differential diagnosis of lymphoid leukosis is made on a number of factors including age, clinical signs, incidence, macroscopic studies and histology. In the present study Neural enlargement was absent while both liver and spleen were consistently involved and this is of considerable diagnostic significance in lymphoid leukosis.

HISTOPATHOLOGY OF SKIN OF *PROTONIBEA DIACANTHUS* INFECTED WITH PROTOZOAN PARASITES

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Histopathological observations are made on the skin of the fish *Protonibea diacanthus* infected with protozoan parasites *Oodinium* sp., *Dermocystidium* sp., *Trichodina* sp. and *Au/acantha* sp. For this purpose histological sections :were prepared by usual technique, stained with haematoxylin and ,eosin, dehydrated in graded series of alcohols, cleared in clove oil and xylene and mounted permanently in Canada balsam. Photographs were prepared with Nikon (Optiphot-2) photomicroscope using a Fuji. colour film. This infection severely damaged the skin tissue, common findings were abscess formation, dislocation, shrinkage and atrophy of muscle fibres which produced large spaces in between the 'muscles fibers. Muscle fibres were severely damaged and fragmented. Hyaline degeneration of muscles. fibers was also prominent in some sections.

NEW HOST AND LOCALITY RECORD OF *EUCLINOSTOMU~VASTROCAECUM* BILQEES, 1972 (TREMATODA)

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A new host and locality is recorded for the metacercarial form *Euclinostomum gastrocaecum* Bilqees, 1972 and is reported here. Nineteen metacercariae were collected from the liver of fish *Channa* (0) *striatus* of Manchar lake, Sindh which is a new host and locality record. Originally this form was described from a related fish. species *Channa* (0) *marulius* of Haleji lake of Sindh. Morphometric variations were observed in the number of lateral branches of caeca and sizes of various structures.

A NEW SPECIES *TUBULOVESICULA MAGNACIRROSA* (TREMATODA: HEMIURIDAE LOOSS, 1899) FROM THE FISH *PSEUDOSCIAENA DIACANTHUS* OF KARACHI COAST

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A new species of the genus *Tubulovesicula* Yamaguti, 1934 is described here and named *Tubulovesicula magnacirrosa* n.sp. From the stomach of the fish *Pseudosciaena diacanthus* (Sciaenidae) off Karachi coast. The new species can be . differentiated from the previously described species in having long soma and small ecsoma. Oral sucker terminal, small, pharynx is globular, adjacent to the oral s"ucker. Prepharynx absent. Intestinal caeca long, reaching to the posterior end of ecsoma. Ventral sucker is large situated in the anterior-fourth of the body. Seminal vesicle tubular, extending posteriorly to the posterior margin of ventral sucker. Pars prostatica is long, with numerous prostatic gland cells. Sinus sac large, globular. Genital opening is preacetabular. Ovary is in the middle of the soma. Vitellaria consist of 7 tubules radiating posteriorly from the ovary, postequatorial in soma and far anterior to ecsoma. Uterus occupying most part of the body, not reaching into the ecsoma.

MANAGEMENT OF NEMATODES ASSOCIATED WITH POMEGRANATE USING OIL-CAKES IN BALOCHISTAN

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The use of organic matter into soil for improving fertility is an old agricultural practice. However, the influence of this practice on plant parasitic nematodes became known only a few decades back. The efficacy of the organic amendments vary depending on nature of soil type, organic matter, crop and nematodes. An attempt was therefore made to evaluate the efficacy of mustard and castor oil cake at 40, 80, 120 days and 1 year after application against nematodes associated with pomegranate-trees. For comparison a chemical nematicide Carbofuran was used. Pomegranate *(Punica granatum* L.) trees were selected at Wadh, district Khuzdar, Balochistan. The organic amendments were applied at a rate of 14.6 kg/tree (800 kg/ha) while the chemical nematicide Carbofuran was applied at 150g/tree (15 kg/ha). There were 4 replicates for each treatment. Untreated trees were kept as control. The experiment was in complete randomized block design. Five ml aliquots (15 replicates) of nematode suspension were used for nematode counts and value converted to number of nematodes per 200 ml of soil samples. To estimate the yield, total fruit/tree were weighed. Data was subjected to

factorial analysis of variance (F ANOV A) following Zar (1996). The results showed population density of three nematodes namely *Helicotylenchus indicus; Xiphinema basiri* and *Meloidogyne javanica* differed significantly from the control (P <0.001). The population density of nematodes varied significantly with time (P < 0.001). Among the two-way interactions nematode x treatment and treatment x time were found significantly (P < 0.001). The tripar.i;ite interaction of nematode x treatment x time was also significant (P < 0.01). The yield of pomegranate was not significant influenced by the treatments (F = 0.37, n.s.).

IN VITRO FIGHT OF *EUGENIA JAMBOLANA* (JAMEN) AGAINST GIT HELMINTHS

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Livestock sector is contributing approximately 53.2% of the agriculture value added and 11.4% to national GDP during year 2009-10. This profitable livestock industry is shoddily affected by gastro-intestinal (GIT) helminthiasis. Chemical control of heliminths cause different hazards like drug resistance, side effects, food residues and environmental pollution. The use of plants extracts for the treatment of GIT helminths is unusual option. For this purpose, the present study was conducted to assess the in vitro anthelmintic activity of *Eugenia jambolana* against *Haemonchus contortus* (present in GIT of ruminants). Plants material was collected from local area, identified and authenticated by a botanist from Department of Botany, University of Agriculture, Faisalabad, Pakistan. In order to check the anthelmintic activity, adult motility assay and egg hatch tests were performed. The crude aqueous methanol extract (CAME) was used at the concentration of 50, 25, 12.5, 6.25, 3.125, 1.50 mg/ml while Phosphate buffer saline (PBS) and levamisol@1.5 mg/ml were also compared with CAME. The mortality was recorded after 0, 2, 4, 6, 8, 10 hours interval. The study described that CAME at the concentration of 1.5 and 3.125 mg/ml exhibited mild effect on the mortality of worms when observed on 4 and 6 hours; whereas plant extract at the amount of 6.25 mg/ml and 12.5 mg/ml was effective to induce the worm's mortality after two hours onward post exposure, while all worms showed 100% mortality when levamisol was applied. The anthelmintic activity of

plants extract @ 50 mg/ml was as good as that of levamisol from two hour onward exposure. All the worms remained alive in PBS upto 10 hours. Similarly the effects of CAME at the concentration of 8000, 4000, 2000, 1000, 500 and 250 µg/ml with control were observed. The lowest egg hatching (7.00%) was recorded when 8000 µg/ml of CAME was used while the highest egg hatching (93.00%) was found in oxfendazole at the concentration of 0.0027 µg/ml. From the results it can be concluded that CAME of Eugenia jambolana were effective against the hatching of eggs than oxfendazole as demonstrated by LSD_{50}. One the basis of results, it is recommended that *Eugenia jambolana* (Jamen) can be a productive tool for the control of worms in the intestine of sheep.

FIRST REPORT OF *DESPORTESIUS EQUISPICULATUS* WU & LIU, 1943 (NEMATODA: ACUARIIDAE) FROM PAKISTAN IN BIRD LITTLE EGRET *EGRETTA GARZETTA* (AVES: ARDEIDAE) OF JAMSHORO, SINDH, PAKISTAN

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During survey of helminth parasites of Little Egret *Egretta garzetta* (Aves*:* Ardeidae) of Jamshoro and Hyderabad Districts of Sindh Province Pakistan; one hundred eighty four (62 ♂ and 122 ♀) specimens of nematodes were collected from gizzard of 16 hosts. Worms embedded in gizzards of the hosts were removed with soft brush, killed in hot 70% ethanol and preserved in solution of 70% ethanol and glycerol. The present species have close resemblance with *Desportesius equispiculatus* Wu & Lui, 1943 in all essential features and is therefore identified as such. This is a first report of *Desportesius equispiculatus* Wu & Lui, 1943 from Pakistan.

FIRST RECORD OF *CONTRACAECUM YAMAGUTI* MAWSON, 1956 (NEMATODA: ANISAKIDAE) FROM PAKISTAN IN BIRD LITTLE EGRET *EGRETTA GARZETTA* (AVES: ARDEIDAE) OF JAMSHORO, SINDH, PAKISTAN

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During 2006-2009 a helminthological survey of bird Little Egret *Egretta garzetta* (Aves*:* Ardeidae) of Jamshoro and Hyderabad Districts of Sindh Province Pakistan; 45

(18♂ and 27♀) nematodes belonging to genus C*ontracaecum* Railliet & Henry, 1912 *(*Nematoda: Anisakidae) were collected from gizzard of 9 hosts. The live nematodes were killed in hot 70% ethanol and preserved in alcohol-glycerol solution. Temporary mounts were prepared in glycerol and lactophenol for a detail study. The present specimens resemble *Contracaecum yamaguti* Mawson, 1956 in all essential features and are therefore identified as such. Mawson (1956) described *Contracaecum yamaguti* from intestine of the bird *Mergus mergenaser* in Japan while, the present species recorded from gizzard of bird Little Egret *Egretta garzetta* in Jamshoro, Sindh, Pakistan. *Egretta garzetta* is the new host record for the genus *Contracaecum* Railliet & Henry, 1912. However, this genus is being reported for the first time from Sindh, Pakistan.

FIRST RECORD OF GENUS *DIPLOSTOMUM* VON NORDMANN, 1832 (TREMATODA: DIPLOSTOMIDAE) FROM PAKISTAN IN AVIAN HOST HERRING GULL*, LARUS ARGENTATUS*

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During helminthological examination of 10 Herring Gulls, *Larus argentatus* (Aves: Laridae) 45 trematodes belonging to the genus *Diplostomum* von Nordmann, 1832 were collected from intestine of 06 birds. These specimens have close resemblance with *Diplostomum spathaceum* (Rudolphi, 1819) but differ in having larger size of ventral sucker, more elongated holdfast, shape of anterior testis and bursa. On the basis of diagnostic characteristics, present specimens are identified as *D. spathaceum* (Rudolphi, 1819). This is first record of the genus *Diplostomum* von Nordmann, 1832 from Pakistan.

NEW HOST RECORD FOR THE GENUS *CONTRACAECUM* RAILLIET & HENRY, 1912 (NEMATODA: ANISAKIDAE) FROM BIRD LITTLE EGRET *EGRETTA GARZETTA* (AVES: ARDEIDAE) IN HYDERABAD, SINDH, PAKISTAN

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During an investigation of helminth parasites of bird Little Egret *Egretta garzetta* (Aves*:* Ardeidae) of Jamshoro and Hyderabad Districts of Sindh Province Pakistan; 98 (27♂ and 71♀) specimens of nematodes were collected from gizzard of the 19 hosts.

Specimens were first fixed in steaming 70% alcohol and later stored in glycerin-alcohol solution. Among all species of the genus *Contracaecum* Railliet & Henry 1912, present nematodes have close resemblance with *Contracaecum bubakii* Akram, 1996 in all essential features and is therefore identified as such. Akram (1996) described *Contracaecum bubakii* from stomach of the bird *Phalacrocorax niger* in Manchar Lake, Sindh, Pakistan while, the present species recorded from gizzard of bird Little Egret *Egretta garzetta* in Hyderabad, Sindh, Pakistan. *Egretta garzetta* is the new host record for the genus *Contracaecum* Railliet & Henry 1912. However, this genus is being reported for the first time from Hyderabad and is a second report of genus from Sindh, Pakistan.

A NEW SPECIES AND NEW HOST RECORD FOR THE GENUS *MEDIORHYNCHUS* VAN CLEAVE, 1916 (ACANTHOCEPHALAN: GIGANTORHYNCHIDA) IN PAKISTAN.

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A single acanthocephalan of the genus *Mediorhynchus* Van Cleave, 1916 was collected from intestine of the *Riparia paludicola*. Present specimen differs from its close allies in having smaller body size, number of longitudinal rows of proboscis hooks, number of hooks per row and some other differentiating characteristics. On the basis of these differences, a new species *Mediorhynchus ripariae* is proposed. This is third record of the genus *Mediorhynchus* Van Cleave, 1916 from Pakistan and *Riparia paludicola* is the new host record for this genus.

NEW HOST RECORD FOR THE GENUS *PORROCAECUM* RAILLIET AND HENRY, 1912 (NEMATODA: ANISAKIDAE) FROM BIRD LITTLE EGRET *EGRETTA GARZETTA* (AVES: ARDEIDAE) IN HYDERABAD, SINDH, PAKISTAN

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During survey of helminth parasites of bird Little Egret *Egretta garzetta* (Aves*:* Ardeidae) of Jamshoro and Hyderabad Districts of Sindh Province Pakistan; 28 (10♂ and 18♀) specimens of nematodes were collected from gizzard of 4 hosts. Specimens were first fixed in steaming 70% alcohol and later stored in glycerin-alcohol solution. Among

all species of the genus *Porrocaecum* Railliet and Henry, 1912, present nematodes have close resemblance with *Porrocaecum angusticolle* Molin, 1860 in all essential features and is identified as such. Molin (1860) described *Porrocaecum angusticolle* from stomach of the bird *Egretta alba* in Ukraine, Europe while, present species is recorded from gizzard of bird Little Egret *Egretta garzetta* in Hyderabad, Sindh, Pakistan. *Egretta garzetta* is the new host record for the genus *Porrocaecum* Railliet and Henry, 1912. However, this genus is being reported for the first time from Sindh, Pakistan.

NEW HOST RECORD FOR THE GENUS *PATAGIFER* DIETZ, 1909 (DIGENEA: ECHINOSTOMATIDAE) FROM BIRD LITTLE EGRET *EGRETTA GARZETTA* (AVES: ARDEIDAE) IN JAMSHORO, SINDH, PAKISTAN

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During (2006-2009) a helminthological survey of bird Little Egret *Egretta garzetta* (Aves*:* Ardeidae) of Jamshoro and Hyderabad Districts of Sindh Province Pakistan, 21 specimens of the genus *Patagifer* Dietz, 1909 were collected from intestine of the host. The specimens were flattened under slight cover glass, fixed in AFA, stained with Borax carmine, dehydrated in graded series of ethanol, cleared in clove oil and xylol and mounted permanently in Canada balsam. Morphometric studies specify that the trematodes understudy resemble *Patagifer hyderabadense* Dharejo, 2006 in all essential features, hence, identified as such. Dharejo (2006) described *Patagifer hyderabadense* from the intestine of bird Black Coot *Fulica atra* in Hyderabad, Sindh, while present species is recorded from the intestine of bird *Egretta garzetta* in Jamshoro, Sindh. Hence *Egretta garzetta* is first host record and Jamshoro is new locality. Dharejo (2006) reported species of genus *Patagifer* Dietz, 1909 for the first time from Sindh, while present species is the second of this genus from Sindh.

FIRST RECORD OF GENUS *METORCHIS* LOOSS, 1899 WITH DESCRIPTION OF A NEW SPECIES *METORCHIS PAKISTANENSI* IN *FULICA ATRA* OF PAKISTAN

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Trematodes belonging to genus *Metorchis* Looss, 1899 were collected from gallbladder of Black Coot, *Fulica atra*. After detailed study and comparison with other

species of genus *Metorchis* Looss, 1899, a new species *Metorchis pakistanensi* is proposed. Present new species have close resemblance with *Metorchis orientalis* Tanabe, 1920 in body shape but differs in having smaller body size, larger size of ventral sucker, deeply lobed and roughly outlined contiguous testes, more elongate seminal receptacle, roughly lobed ovary and smaller size of the eggs. On the basis of aforementioned diagnostic differences, a new species *Metorchis pakistanensi* is proposed to accommodate the present worm. However, this genus is being reported for the first time from Pakistan.

FIRST RECORD OF THE GENUS *TETRAMERES* CREPLIN, 1846 FROM PAKISTAN

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During present studies on the helminth parasites of Black Coot, *Fulica atra* nematodes of the genus *Tetrameres* Creplin, 1846 were recovered from gizzard of the host. After detailed study and comparison with other species of genus *Tetrameres* Creplin, 1846, the present specimens were identified as *T. globosa* (Von Linstow, 1879). This is first record of the genus *Tetrameres* Creplin, 1846 from Pakistan.

FIRST RECORD OF GENUS *NEOHARVARDIA* GUPTA, 1963 (TREMATODA: DIPLOSTOMIDAE) FROM PAKISTAN IN AVIAN HOST LITTLE CORMORANT, *PHALACROCORAX NIGER*

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18 trematodes of the genus *Neoharvardia* Gupta, 1963 were collected from intestine of *Phalacrocorax niger*. Present specimens have close resemblance with *Neoharvardia pandubi* Gupta, 1963 in body shape but differ in having larger body size, more elongated pseudosuckers and smaller eggs. On the basis of these characteristics, present specimens are identified as *N. pandubi* Gupta, 1963. This genus is being reported for the first time from Pakistan.

STUDY OF PLANT PARASITIC NEMATODES ON POMERGRANATE TREES IN SISTAN

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In order to identify the plant parasitic nematodes of pomergranate*(punica granatum*)trees in area of Sistan,14 samples soil were collected during years 2009-2010. Nematodes extracted from roots by Coolen and D`herd method (Coolen and D`herd, 1972) and soil by centrifugal-flotation technique (Jenkins, 1964)and then they were fixed and transferred to glycerin following De Grisse method(De Grisse1969). Morpholgical and Morphometrical characters were studied by light microscope, for identification and disccription of nematode specimens,species keys and refrences were used In this study 6 species belonging to 4 genera were identified as follows: *Pratylenchus penetrans Ditylenchus medicaginisis Ditylenchus dipsac Helicotylenchus vulgaris Meloidugyne javanic Meloidogune incognita*

OCCURRENCE OF HELMINTH PARASITES IN SHEEP, *OVIS ARIES* LINNAEUS IN PESHAWAR DISTRICT, PAKISTAN

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Sheep, *Ovis aries* Lihnaeus is raised for fleece, meat (lamb, hogget or mutton), milk, important for wool, pelts use in agriculture, as dairy animals and as model organisms for science. Sheep are also occasionally sacrificed to commemorate important events in Islamic cultures. A survey, occurrence of helminthes parasite in sheep in Peshawar district was conducted during September-December 2010. Helminthes Parasites have been shown to adversely affect on milk production, reduce breeding efficiency, reduce weight gains, decrease hair quality, reduce feed efficiency and negatively affect the immune system by decreasing the animal's ability to fight off other health problems. In this survey from 356 sheep, O. *aries* the feacal samples were collected from the different areas of Peshawar. The laboratory diagnoses of the samples were .conducted in VRI, Peshawar. During the diagnoses 274 sheep were positive for different internal helminthes parasite and 82 were negative. In the present study 8 species of internal helminthes parasites were identified. During study 1 specie of cestodes with 1.68% of *Moniezia expansa* and 1 specie of trematodes with 0.56% *Fasciola hepatica* were identified. There are 6 species of nematodes were reported. Among nematodes with 53.37% of *Haemonchus contortus,* the high worm load was observed. The other nematodes *Trichostrongylus vitrinus* 12.92%, *Nematodirus spathiger* 1.68%,

Strongyloides papillosus 3.93%, *Ostertagia sp.* 2.24% and *Trichuris ovis* 0.56% were also identified. It is concluded that there was low to moderate parasitic infestation w~re found in O. *aries.* Further research is required to prevent parasitic infestation in *O. arzes.*

MESOOPHORODIPLOSTOMUM LATEROVARIUM NEW SPECIES (TREMATODA: DIPLOSTOMIDAE) FROM POND HERON *ARDEOLA GRAYII (*AVES*:* ARDEIDAE) OF JAMSHORO, SINDH, PAKISTAN

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A new trematode *Mesoophorodiplostomum laterovarium* n. sp. is described from intestine of Pond Heron *Ardeola grayii* (Aves: Ardeidae) collected from Jamshoro, Sindh, Pakistan. The present specimen resembles to *Mesoophorodiplostomum anterovarium* Dronen, 1985 and *M. pricei* (Krull, 1934) Dubois, 1936; in external body shape and few morphological characters, but differs from both in several characters. It differs from *M. anterovarium* in smaller size of the oral sucker, esophagus, acetabulum, holdfast organ, anterior testis, posterior testis and ovary; copulatory bursa evaginable and vitellaria are arranged scattered as compared very dense in *M. anterovarium.* While it differs from *M. pricei* in position of ovary and arrangement of vitellaria. The present specimen cannot be included in any of the previously existing species of the genus *Mesoophorodiplostomum* because of above mentioned morphometric differences from its congeners. Hence, a new species (*Mesoophorodiplostomum laterovarium)* is proposed to accommodate present specimens. The name of new species refers to the position of the ovary in this trematode.

LOPHOSICYADIPLOSTOMUM RIZWANAE NEW SPECIES (TREMATODA: DIPLOSTOMIDAE) FROM POND HERON *ARDEOLA GRAYII (*AVES*:* ARDEIDAE) OF JAMSHORO, SINDH, PAKISTAN

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A new trematode *Lophosicyadiplostomum rizwanae* n. sp. is described from intestine of Pond Heron *Ardeola grayii* (Aves: Ardeidae) collected from Jamshoro, Sindh, Pakistan. The present specimen, when compared with the previously reported (*L. nephrocystis* and *L. saturnium)* specimens, were found different in several morphological

characters even in change of the host. It differs from *L. nephrocystis* in possessing a short body length. In *L. nephrocystis* and *L. saturnium* shape of the body is oval, while globular in present specimen. Considerable differences were also noted in fore and hindbody as well as hold fast organ of these trematodes. Forebody is smaller than hindbody in *L. nephrocystis;* forebody and hindbody are about equal in *L. saturnium,* while forebody is longer than hindbody in present specimen. On the basis of above morphological as well as morphometric differences between present and previously described species it is concluded that this fluke is new to science and therefore designated as *Lophosicyadiplostomum rizwanae* n. sp. The new species is named after Late Rizwana M. Khan Parasitologist, Department of Zoology University of Sindh, Jamshoro.

NEW HOST AND FIRST RECORD OF *CENTROCESTUS FORMOSANUS* NISHIGORI, 1924 (TREMATODA: HETEROPHYIDAE) FROM PAKISTAN IN BIRD POND HERON *ARDEOLA GRAYII (*AVES*:* ARDEIDAE) OF JAMSHORO, SINDH, PAKISTAN

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Eighteen worms collected from intestine of eight Pond Herons *Ardeola grayii* (Aves: Ardeidae) in Jamshoro, Sindh, Pakistan. Body of the worm is small, ovoidal with broad posterior half and spinose. Oral sucker sub terminal, armed with 32 circumoral spines arranged in two alternate rows. Prepharynx short. Pharynx muscular, followed by short esophagus. The fluke under study resembles *Centrocestus formosanus* Nishigori, 1924 in all essential features therefore identified as such. However, this species being reported for the first time from Pakistan and also a new host record.

COMPARATIVE PREVALENCE OF PEDICULOSIS *CAPITIS* BETWEEN GOVT. AND PRIVATE SCHOOL' CHILDREN ACROSS ALL DISTRICTS OF KARACHI

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Present study was conducted during the year 2005 - 2008. A total of 4025 students out of which 2025 Govt. and 2000 Private school students, were examined through head combing for head louse infestation from 80 schools in Karachi.. Overall infestation of

head louse was found to be 25.3%. Highly significant difference in intensity of infestation was found between Government and Private schools. (F=48.488, P<0.001). Infestation in Govt. schools (32.90%) was found to be higher as compared to the Private schools (17.60%). In both Govt. and Private schools, rate of infestation was found highest in District Central (41.88%) and (22.25%) respectively, the lowest rate of infestation in Primary schools was found in District Malir (22%) and in Secondary schools, it was found in District South (13.75%). Average infestation was found more in Govt. schools (2.662) as compared to the Private schools (1.655).Average infestation of adults (0.35), nymphs (0.37) and nit (0.17) were found higher in Government schools than the Private school children. In Private schools average infestation was found with adults (0.16), nymphs (0.11) and nits (0.02).

STOMACH HISTOPATHOLOGY OF *PLECTORHINCHUS CINCTUS* INFECTED WITH *ANISAKIS* SP. LARVAE

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The scientific studies on fish diseases remained largely unexploited but recently fish diseases are one of the important problems and great attention is being paid to get more and more information about it. Because fish is one of the important export and food item. The consumption of raw fish is deeply established in the culture of mainly predominantly rural societies. Parasite control can only be dealt with in the frame work of the overall social and economic development of affected societies. During the present work histopathological changes in various organs of *Plectorninchus cinctus* associated with nematode larvae were studied. For this purpose infected organs of fish were randomly collected and after visual examination, the piece of infected organs were fixed in 10% aqueous formaline for preparation of histology slides. Sections of the preserved organs were processed by using standard histological techniques (Bilqees and Fatima, 1993). Sections of 3-6 micron thickness were prepared with paraffin embedded tissue blocks. . The sections were stained with hael~toxyline and eosin and mounted permanently in DPX. The damage caused by these larvae in stomach is observed and presented here Photomicrographs of selected parts of sections were prepared in support of observations. Histopathological changes in stomach of fish were observed infected with *Anisakis* sp. larvae which causes disintegration in cellular organization, atrophic condition, degeneration of muscularis mucosa. Nematode larvae with tunnel formation is the specific pathology seen in this stomach of the fish *Plectorhinchus cinctus.* In this fish the larvae were found in the body cavity, attached to the mesenteries on the stomach. Some of the arteries were blocked with blood cells and fibrinoid tissue, necrosis of blood vessel walls with deposition of dark stained material was very prominent in stomach associated with *Anisakis* sp. larvae.

AN OCCUPATION BASED SURVEY REGARDING PREVALENCE OF INTESTINAL PARASITES IN MALE AND FEMALE SHEPHERDS OF SWAT, PAKISTAN

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Preliminary survey was conducted dealing with shepherding as occupation aimed to determine the percentage and prevalence of intestinal parasites in different seasons in male and female shepherds of swat district. This occupation represents a very sensitive sector for parasites distribution by the way that they are in direct contact with their animals. Swat is one of the district of Pakistan with approximately 1.2 million population. The local people mostly are related to agriculture followed by live stock. A total of 256 members (159 male and 97 female) of shepherds families were visited and convinced to collect faecal samples. These samples were brought to Medical Zoology section, VPCI/SARC, PARC, Karachi for final examination. Microscopic examination was carried out by wet mount technique, floatation and sedimentation technique. Of these 203 (79.2%) harboured parasites. Out of 203 infected samples 103 (50.7%), 57 (28.0%), 31 (15.2%) and 12 (5.91 %) showed one, two, three and four parasite species respectively, overall helminth infection rate was 310 (86.59%) and protozoan was 47 (13.12%). Males were found more infected than females.

PREVALENCE OF MALARIAL PARASITE IN HUMAN BLOOD

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In a survey from June 2005 to December 2005, a total of2457 samples of blood were collected in EDT A tube and were then brought to the Toxicological Laboratory, Department of Zoology, University of Karachi. The samples were observed for the presence of any stage of *Plasmodium* species (Leishman's stain according to routine practice). Out of2457 samples, 311 of the samples were found to be positive while the rest of 2146 samples were negative with reference to any species of malarial parasite. The dominating species was of *P. falciparum* which was present in 283 of the samples, *P. vivax* was in 28 samples only. The *P. malariae* and *P.ovale* were not found in any of the sample. As far as the stage of the parasite is concerned it was in an order of Sch izont < Trophozoite < Gametocyte. From the above findings, it can be concluded that malarial parasite exists in human blood to healthy persons. The less number of positive samples

indicates the awareness of the people towards Malaria, however the existence of *P falciparum* species among most of the positive samples is alarming as this species causes more threat to the people including organ failures or abnormalities in patient's blood which may often leads to Cerebral Malaria.

FIRST RECORD OF THE *CONTRACAECUM RUDOLPHII* HARTWICH, 1964 (NEMATODA: ANISAKIDAE) FROM PAKISTAN IN BIRD POND HERON *ARDEOLA GRAYII* (AVES: ARDEIDAE) OF HYDERABAD, SINDH, PAKISTAN

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During an investigation of Helminth parasites, of bird Pond Heron *Ardeola grayii* (Aves*:* Ardeidae) of Jamshoro and Hyderabad Districts of Sindh Province Pakistan; Ten male and forty female specimens of nematodes recovered from intestine of the host. The present specimen resembles with *Contracaecum rudolphii* Hartwich, 1964 in all essential features and identified as such. However, this species is being reported for the first time from Pakistan and also a new host record.

HEAD LOUSE: COMPARATIVE ACCOUNT OF INFESTATION BETWEEN GIRLS AND BOYS SCHOOL CHILDREN ACROSS ALL DISTRICTS OF KARACHI

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During the year 2004 - 2008, a total of 4025 students out of which 2025 boys and 2000 girls students, were examined through head combing for head louse infestation from 80 schools in Karachi. Overall infestation of head louse was found to be 25.3%. Prevalence of infestation between Boys and Girls schools did not differ significantly ($P>0.05$). The prevalence of head louse infestation in Girls (27.80%) was found to be higher as compared to the Boys, (22.90%). Rate of infestation was found highest in District Central (41%) in Girls schools and in Boys schools it was found highest in District West (25.5%). In both Girls and Boys schools the lowest rate was found in District South (19.5%) and (18.5%) respectively. Overall average infestations in Boys (2.31533) and in Girls (2.31531) are almost equal. Average infestation of adults (0.30)

and nymphs (0.25) were found higher in Girls schools than the Boys schools. In Boys schools average infestation of adults were (0.21) and of nymphs were (0.22), but average infestation of nits were found equal (0.10) in both schools.

CATENOTAENIA QADRII. SP.N. (CESTODA: CATENOTAENIIDAE) FROM THE HOUSE MICE *MUS MUSCULUS* IN KARACHI, SINDH

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During studies on hell11inth parasites of rodents in Karachi, Sindh, three mice, *Mus musculus* were live trapped. These were later autopsied in the laboratory for helminth Parasite infections, one out of three (mice) was found infected with specimens of tape worms. The worms thus recovered were first relaxed in refrigerator, later fixed in 0 70% alcohol, pressed lightly and kept in F.A.A. solution for 24 hrs. The specimens were prepared as permanent mounts according to the standard procedures for detailed study. These are medium sized tape worms, scolex simple, four-large muscular suckers. The anterior segments are shorter, followed by rectangular ones which in the gravid region become much longer than broad. Genital pores are irregularly alternating, situated at the anterior third of the margin. Number of testes varies in most of the segments, posterior and lateral to the ovary, cirrus pouch small, simple, ovary bilobed, branched, slightly poral. Vitellaria compact eggs are numerous and smaller in size. The present species was studied comparatively with the previously reported species and found it unmatched therefore it is regarded new and designated as *C. gadrii* sp.n. *Mus musculus* is a new host record.

EFFECTS ON MILK PRODUCTION OF *BUBALUS* SP. DUE TO HELMINTHIASIS

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Analysis of milk production in *Bubalus* sp. was carried out to estimate rate of decrease in milk production in buffaloes due to helminth infection. As buffaloes serves as major economically important animal for dairy and meat industry of Pakistan, Total 150 buffaloes were checked randomly. They were observed grossly. after sacrifice and also by fecal sample examination to find out types of helminth infection. 70 buffaloes were infected out of which 42 had trematode infection mainly in liver and 28 were parasitized

by cestode infection in intestine. Both infections lead to lean health of animal along with 3-5 kg/day less milk production as compared to normal buffalo producing 5-7 kg/day. Buffaloes with liver infection stopped or greatly reduced their milk production as flukes pathogenic activity in organ altered the normal architecture and function of cells and make them unable to take part in filtration which is main step for initiation of milk production. Therefore, deworming of buffalo species is greatly recommended to overcome losses in milk production and to enhance the quality of meat as for which helminth infection has been major cause during present investigation. Data observed has been presented in the form of tables and histograms.

NEW HOST AND FIRST RECORD OF *PARVITAENIA IBISAE* SCHMIDT AND BUSH, 1972 (CESTODA: DILEPIDIDAE) FROM PAKISTAN IN BIRD POND HERON *ARDEOLA GRAYII (*AVES*:* ARDEIDAE) OF JAMSHORO, SINDH, PAKISTAN

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Seven worms collected from intestine of two Pond Herons *Ardeola grayii* (Aves: Ardeidae) in Jamshoro, Sindh, Pakistan are described. The present specimen resembles with *Parvitaenia ibisae* Schmidt and Bush, 1972 in all essential features, it is therefore identified as such. However this is the first record of the genus *Parvitaenia* from *Ardeola grayii* and this species reported for the first time from Pakistan.

MANAGEMENT OF *MELOIDOGYNE INCOGNITA* INFECTING OKRA (*ABELMOSCHUS ESCULENTUS* (L.) MOENCH) WITH SOME ORGANIC AMENDMENTS

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The insidious root-knot nematodes are ubiquitous in distribution and inflict colossal losses. Most of the noxious chemicals used for the control of these pests are over expensive and perilous for humans and environment. Organic amendments being

innocuous and environmentally benign have gained much attention for their potential as an alternate to chemicals. Keeping in view the beneficial effects of organic amendments, the present investigations were carried out to evaluate the efficacy of leaves of *Azadirachta indica*, *Calotropis procera*, *Datura stramonium* and *Tagetes erecta* as soil amendments at various dosages for the management of *Meloidogyne incognita* on okra. The dried leaves of these plants were incorporated with formalin sterilized soil @ 0, 25, 50 and 75 g / Kg of soil. Two weeks after amendment, 3 seeds of okra cv. 'Punjab selection' were sown and after germination one healthy seedling was maintained. The plants were inoculated with 5000 J2s of *M. incognita* 10 days after germination. Each treatment was replicated five times. The pots were arranged in a completely randomized design in the green house at 25°C±2 for six weeks. After stipulated period data regarding growth parameters and nematode infestations were recorded. It was observed that all the amendments caused significant reductions in number of galls, egg masses and reproduction factors (Rf) resulting into increases in various growth parameters. Maximum reductions were observed with *A. indica* and *D. stromonium*. Similarly, higher concentrations of amendments caused maximum reductions as compared to others. The reductions in nematode infections were found directly proportional to the concentrations resulting into corresponding increases in growth parameters.

EFFICACY OF BIO-CONTROL AGENTS AGAINST ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA*

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The root-knot nematode, *Meloidogyne incognita*, is a sedentary endoparasitic plant pathogen with a very wide host range and causes annual loss of crop revenue amounting to millions of dollars. One of the viable alternatives to chemical nematicides is the use of biological control agents for the management of these nematodes. In the present studies the efficacy of four biocontrol agents was tested against M. incognita at different doses. The bio-control agents *Pasteuria penetrans*, *Pochonia chlamydosporia*, *Paecilomyces lilacinus* and *Trichoderma harzianum* were mass produced and were mixed with the formalin sterilized soil at the rate of 2×10^3, 4×10^3, 6×10^3, 8×10^3, and 1×10^4 endospores / chlamydospores / cfus per g of soil. The okra seeds were sown in these pots and ten days after germination, the plants were inoculated with 2000 J2s of *M. incognita*. The antagonists varied significantly in enhancing various growth parameters

and reducing nematode infestations. *Pochonia chlamydosporia* and *Pasteuria penetrans* were found equally effective at a concentration of 8×10^3 chlamydospores / endospores per gram of soil.

LICE INFESTATION IN BUFFALO (*BUBALUS BUBALUS*) AND CATTLE (*BOS INDICUS*) IN DISTRICT KHAIRPUR MIR'S PAKISTAN

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Lice are most prolific permanent ectoparasites of domesticated animals cause pediculosis and transmit some pathogens. Lice infestation is very common in buffalo and cattle throughout Pakistan. In order to establish data the field collection were carried out to investigate the lice infestation in buffalo and cattle from district Khairpur Mir's during 2009. Out of 500 animals (250 buffalo and 250 cattle) 243 (48.6%) infested, among these 53.2% and 44% buffalo and cattle infested with lice respectively. The relationship between age and lice infestation showed that adult animals was (50.6%) against 45.5% in young animals. Three species of lice *Damalina bovis*, *Hayaloma tuberculus*, and *Haematopinus* species were identified in examined animals. Month wise infestation revealed that high in January (44.4%) followed by February (30.8%) and (22%) in March.

A SURVEY ON BOVINE COCCIDIOSIS IN YOUNG CALVES IN AND AROUND TANDOJAM

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Bovine coccidiosis is primarily disease of young calves usually between 3 weeks to 6months of age. So keeping that in view the survey was done to collect data on losses due to bovine coccidiosis. Randomly 300 young calves were observed from different localities of Tandojam. History, clinical examination taken and faecal samples from calves collected and processed for coccidiosis. Out of these 189 calves were positive for coccidiosis after faecal examination. Calves have diarrhea, dysentery, loss of weight, loss of appetite some possess nervous signs also. The disease is usually associated with stressful situation such as shipping, overcrowding, and change in feed and severe weather as described by owner.

IN VITRO SCREENING OF *AZADIRACHTA INDICA* (NEEM) SEEDS FOR ANTHELMINTIC ACTIVITY

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Helminthiasis has been recognized as a major constraint in livestock production. The greatest losses associated with the nematode parasite infection show financial cost of internal parasitism are enormous. Chemical control of heliminths coupled with improved management has been the important worm control strategy throughout the world. However, the synthetic anthelmintics are not cost effective and unavailable to farmers in rural areas. In addition to this, drug resistance, food residues and environmental pollution have also been reported in western countries. Ethno veterinary medicine has been emerged as a challenging field in most recent times that promises to benefit to rural and peri-urban stock raisers. Some primary work has been done in this regard; however still there was room to do much more. For this purpose seeds of *Azadirachta indica* (Neem) was tested to evaluate the anthelmintic activity. Plants material was collected from local area, identified and authenticated by a botanist from Department of Botany, University of Agriculture, Faisalabad, Pakistan. In order to check the activity of anthelmintic, adult motility assay was conducted with crude aqueous methanol extract (CAME) @ 50, 25, 12.5, 6.25, 3.125, 1.56 mg/ml, levamisol @ 1.5 mg/ml and PBS. The mortality was observed on 0, 1, 2, 3 and 4 hours post exposure. CAME and levamisol exhibited 100 % anthelmintic activity where as PBS had no effect on worms. Correspondingly, egg hatch test was conducted with three replications for each concentration of CAME 8000, 4000, 2000, 1000, 500 and 250 micro g/ml and control. It was interesting to note that CAME of Neem plant were more potent in inhibiting the egg hatching than oxfendazole as indicated by LD_{50} recorded from these treatments. It is therefore suggested that Neem could be a promising alternative to conventional anthelmintics for the treatment of gastrointestinal nematode of small ruminants.

RESPONSE OF CUCUMBER CULTIVARS TO ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA*

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Root-knot nematodes (*Meloidogyne* spp.) are one of the most destructive pathogens of vegetables. The cultivars resistant to root-knot nematodes have comparatively better crop yield as compared to susceptible varieties and can be employed as a component of integrated nematode management. In pot experiments fifteen cultivars of cucumber (*Cucumis sativa*) were tested for their resistance against *M. incognita* by inoculating with 3000 2nd stage juveniles of the nematode. All the cultivars varied significantly in causing decreases in growth parameters over control. Likewise, all the cultivars behaved differently regarding formation of galls and egg masses. It was found that none of the cultivar was found tolerant or highly resistant, resistant and moderately resistant. Maximum galls (> 100) were found on Mehran, Mirage, Thamin II and Royal Sluis and were found to be highly susceptible. Also maximum reductions in growth parameters were observed in case of these cultivars. The cultivars Babylon, Cobra, Falcon-560, Green Wonder and Cucumber Cetriolo were found to be susceptible. The reductions in growth parameters of these cultivars were less severe as compared with those observed in case of highly susceptible cultivars. The remaining cultivars viz. Market More, Long Green, Dynasty, Pioneer-II, and Summer Green appeared as moderately Susceptible. This is also evident by the reduction in growth parameters of these cultivars.

ECTOPARASITIC FAUNA OF IMPORTED GOLDFISH *CARASSIUS AURATUS* (L.)

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Ornamental fishes are imported to Pakistan from South East Asian countries. These fishes are brought live and sold to the hobbyists. One hundred imported goldfish, *Carassius auratus* (veiltail variety) were studied to assess their ectoparasitic fauna. These fishes were examined by standard parasitologcal procedures in Fish Disease and Health Management Laboratory, Department of Zoology University of the Punjab, Lahore. Total length and weight of fish ranged from 8.5 to 9.8cm and 14.8 to 17.5g respectively.

Almost half of these fishes (47 %) were having parasitic infection. Four groups of parasites, protozoan *(Tricodina* sp; *Ichthybodo* sp; *Ichthyopthirus* sp) monogenea *(Dactylogyrus* sp. *Gyrodactylus* sp); crustacean *(Lernaea* sp. *Argulus* sp) and annelid *(Piscicola* sp) were recorded from various sites on the body of the fish such as, skin, fins and gills. *Dactylogyrus* sp. *Gyrodactylus* sp (80%); *Argulus* sp (40 %) and *Tricodina* sp *(AO%)* were more prevalent. Whereas *Ichthybodo* sp; *Ichthyopthirus* sp *Lernaea* sp and *Piscicola* sp were less prevalent (all <10%). Infection level, fish health status and control measures of these diseases in goldfish are discussed.

PATHOGENICITY OF CITRUS NEMATODE, *TYLENCHULUS SEMIPENETRANS,* ON *CITRUS JAMBHIRI*

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The citrus nematode, *Tylenchulus semipenetrans*, associated with slow decline is a universal problem including Pakistan. It limits production of citrus fruits under a wide range of environmental and edaphic conditions. In Pakistan up to 98.4 % infestation of this nematode .has been reported. Pot experiments were conducted on *Citrus jambhiri* inoculated with 1 x 103, 2 x 103, 4 x 103 and 8 x 103 juveniles of *T. semipenetrans* per kg of soil to establish the damaging threshold level of the nematode. Data regarding reductions in growth parameters and influence on nematode populations in roots and soil and reproduction factor were recorded. A significant reduction in plant height, fresh and dry weight of shoot and root was observed at all inoculum levels. The nematode build up was the maximum at lower inoculum level. An inverse relationship was observed between growth parameters and inoculum densities of the nematode.

PREVALENCE OF *PLASMODIUM FALCIPARUM* IN DIFFERENT AGE GROUPS IN KARACHI-PAKISTAN

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Malaria is dangerous and universal infectious disease among tuberculosis, dengue, HIV and AIDS and is responsible to 2 million annual deaths especially in African countries, and international scientist trying to eradicate and eliminate it from the world.

The study areas were located in Karachi region consists of five different districts including South, East, West, Central and Malir. Residents from these areas were informed and consent was sought from the adults and parents of young children from randomly selected houses. The Performa was provided and filled at the time of survey based on name, occupation, age, sex, previous health, disease in past, disease at present, number of family members, family history, medicine used in past and present, patient temperature and symptoms & complications were noted, details about the diets were also taken. Each house was visited once a week and participants having high temperature and history with severe headache and chill were thoroughly examined and blood smear was taken. A total of 3821 including 2299 males and 1522 females participants were selected during the investigation from January 2002 to December 2004. Thick and thin blood smears were examined and the thin blood smears were fixed in 100% methanol and stained in 2% Giemsa. Different stages of *Plasmodium species especially P. falciparum* and *P.vivax* were investigated under the oil immersion as well as the number of parasites/ 200 white blood cells (WBCs) was counted and the density of parasites per micro liter of blood was also calculated. Total 1249 individuals were infected with *Plasmodium falciparum.* Out of these 321 were infected during January to December 2002, 337 were found infected during January to December 2003 and 591 were infected during January to December 2004 consequently. Three years investigation periods and clinical diagnosis revealed that the children below two years of age showed low *P. falciparum* infection. The rate of infection was the highest in district South amongst the five districts (78.04 %) during the 2004 from under the age of 2-12 years, although the rate of infection in above 12 years children was 57.50% during 2004 recorded from district West. It was concluded that variation of infection is found not only on districts level but on different age groups as well as clinical malaria varies with immune status play a vital role in comparison of malaria intervention. It also reveled that more specific questionnaire and physical examination can be used to exclude the risk of malaria.

SECTION - V

FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY AND MARINE BIOLOGY

STATUS OF JELLYFISH FISHERY IN PAKISTAN

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Jellyfish has become an important fishery commodity of Southeast Asian countries. All the edible jellyfish belongs to the order Rhizostomeae because the body of these are large, tough and rigid with thick umbrella. The worldwide catch of jellyfish is approximately 3210000 metric tones, while the average annual catch during1988-1999 in South east Asia was estimated to be 169000 metric tones in wet weight.Atleast 11 species are known to be exploited world wide where as the 8 species of edible jellyfish harvested in Southeast Asia. In the present paper the status of jellyfish fishery in Pakistan will be discussed with respect to neighbouring countries.

MARINE FISHERIES, POVERTY AND RURAL LIVELIHOOD: PORT IBRAHIM HYDRI, KARACHI, PAKISTAN

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The objective of this paper is describe the livelihood of the rural population of the Port Ibrahim Hydri in general and to assess the role of marine fishing activity in these livelihoods in particular. For this purpose a combination of activity and wealth ranking exercises were carried out in eight villages of the Port Ibrahim Hydri, completed by a series of comparative analysis of the ethnic composition, accessibility to fishing grounds and fishing-gear ownership across the different socioeconomic strata of the populations. The analysis shows that for the entire area households irrespective of their wealth levels still rely largely on subsistence-based economy, where the three major activities (fishing, farming and herding) are closely integrated. With respect to fishing activity, the survey demonstrates the central role of this activity (in terms of income, labor and food supply) for all wealth groups. However, the analysis also reveals that there is no one-to-one relationship between the contribution of fishing activity and the wealth (poverty) level of the households. In particular the analysis suggests that well-known adages such as " the

fishermen are the poorest of the poor or the fishery is the activity of last resort do not reflect the complexity of real situation.

IDENTIFYING THE PREDATORS OF GREEN SEA TURTLES TO SUGGEST MEASURES FOR REDUCING IT: A CASE OF THE SONERA BEACH, KARACHI

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Attempt is made to investigate measures to reduce the predators of green sea turtles at Sonera Beach of Arabian Sea at Karachi; and suggest measures to reduce predators. Sonera beach is one of the most attract full beach for green sea turtles to lay eggs. Observation of the authors is that on average two females came to lay eggs daily at this beach during the season. During the visit of authors it is found that female during the nesting process were entangled at the tide poles during the low tide. Since, 15 yeas the one of the authors is visiting this site for collecting fishes and invertebrates and observed some of the following predators, which should be controlled: (i) Local Dogs (ii)Local children (iii) Aquarium fishes traders. It is found that the local doges of the Mubrark Village traveled the distance of 4.5 km to be feed on the eggs in the early morning. They open; the nest and eat the entire eggs. Local children are taking eggs for playing and very rarely for taking as food. This study reveals that that if enough food may be provided to the dogs than they can easily avoid feed on eggs. The people of Mubarak village are poor and having the monthly income of Rs.5,000./= pm. in this income, they cannot feed to the street dogs. It is suggested that the awareness campaign and some monetary support for this community can stop the children to collect eggs for playing and taking as food. Aquarium fish-traders can easily be controlled by increasing the frequency of raid on the aquarium fish shops. The early projects on the saving of eggs of marine turtles are confined to the Hawke's-bay and Sandspit. Hence, the authors found enough room to work at the Sonera beach of 2.5 km long.

MORPHOMETRIC ANALYSIS OF THE TWO SPECIES OF GENUS *LUTJANUS* (FAMILY LUTJANIDAE) COLLECTED FROM KARACHI FISH HARBOUR

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A total of 116 specimens of the two species including 68 individuals of *Lutjanus johnii* and 48 of *Lutjanus malabaricus* were collected during August to December 2009 from the landing specimens at Karachi fish harbour. Each individual was measured using five morphometric variables including Total length (TL), Forked length (FL), Standard

length (SL), Head length (HL) and body depth (BD). Pearson linear equation and coefficient of correlation (r) values were also calculated to find the linear regression relationship between these variables. The morphometric measurements among these two species show some variations enough to make a submission that these species were genetically or morphologically different.

FREQUENCY DISTRIBUTION PATTERN OF THE FIVE SPECIES OF GENUS *LUTJANUS* (FAMILY LUTJANIDAE) COLLECTED FROM KARACHI FISH HARBOUR

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Frequency distribution pattern for the five species (*L. johnii, L. lutjanus, L. malabaricus, L. russellii, L. fulvus*) of fishes belonging to the genus *Lutjanus* of family Lutjanidae were determined. About 370 samples of the five species were collected from the Karachi fish harbour during period of June 2009 to November 2010. The total catch samples in years 2009-2010 contained 24.8% individuals of *L. johnii*, 24.8% of *L. lutjanus*, 22.8% of *L. russellii*, 14.9% of *L. malabaricus* and 12.9% of *L. fulvus*. During the survey of Karachi fish harbour it was observed that *L. johnii* and *L. lutjanus* were abundant throughout the year but *L. malabaricus* and *L. russellii* were abundant in the months of September to December, while the fishes belonging to the species *L. fulvus* were very rare.

HABITAT ANALYSIS, POPULATION ESTIMATES AND NESTING ECOLOGY OF THREE FALCON SPECIES IN DISTRICT CHAKWAL, PAKISTAN

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Nine falcon species are reported from various parts of Pakistan; some of these are residents, some winter visitors, some vagrants and others passer migrants. However, very little studies have addressed their population and ecology in this part of the world. The current study, therefore, aimed to investigate habitat, population status and nesting ecology of three falcon species; Red-headed (merlin Falco chicquera*)***,** common kestrel **(**Falco tinnunculus**)** and saker falcon (Falco cherrug*)*, in district Chakwal, Pakistan. Eight potential sampling sites were selected through surveillance surveys, and line transects were established for data collection which were visited fortnightly from May 2009 to June 2010. For habitat analysis, data about trees were recorded using "Point Centered Quarter" method, while shrubs and herbs were estimated using "quadrate method". Data

were analyzed in terms of density, and frequency of trees, shrubs and herbs and also "Importance Value Index" (IVI) for tree species. Nesting ecology of falcons was investigated by searching for active nests utilized by falcons in the selected sites. Red-headed merlin was recorded in five sites with average population of 0.238 ± 0.06 birds /km^2, common kestrel also occurred in five sites with mean population density of 0.246 ± 0.05 birds /km^2, whereas saker falcon was found in only two sites having average population of 0.245±0.06 birds /km^2. Red-headed merlin and common kestrel utilized unused or abandoned nests of other bird species like kites and crows, built on trees whereas saker falcon used cavity nests for its breeding. Two active nests utilized by red-headed merlin were found located on *Acacia nilotica*, while one active nest used by common kestrel was found on *Tamarix aphylla* tree. Similarly, two active nests used by saker falcon were found in site V in cavities of a ledge at a height of 600m from ground level. Red-headed merlin was found resident, common kestrel as winter visitor as well as resident while saker falcon as resident species in the study area.

COMPARATIVE STUDY OF THE MORPHOLOGY OF CTENOID SCALES IN FIVE DIFFERENT FAMILIES OF TELEOST FISHES COLLECTED FROM KARACHI FISH HARBOUR

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An analysis of the ctenoid scales from five types of teleost fishes belongs to the different families like Cynoglossidae, Bothidae, Solidae, Gobiidae, Haemulidae was accomplished by using scale characters i.e. scale shapes and different numbers, shapes and arrangement of ctenii (spines) on the posterior margin of ctenoid scales. The detailed study of the structure of fish scales can be helpful in the distinguishing these families from each other. Therefore the scale character is an important tool in identification of major taxonomic groups.

SEASONAL EFFECT ON THE MEASUREMENT DYNAMICS OF DIFFERENT BODY PARTS OR ORGANS OF FIELD RATS (*RATTUS RATTUS*) IN FAISALABAD, PAKISTAN

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A study was undertaken to see the impact of seasonal variation on measurement of different body parts in field rats of Faisalabad, Pakistan. For this purpose rats were

captured from the fields or godowns from different areas of Faisalabad, Pakistan. They were kept in cages separately in comfortable cages at department of Zoology and Fisheries, University of Agriculture, Faisalabad, Pakistan. Feed and water were offered *ad libitum*. During the peak days of each season they were subjected to different measurement and the results revealed that total body length was significantly higher in spring and summer as compared to winter and autumn. Head and body length increased during winter and spring. Hind foot length significantly increased during spring and summer. In male rats the testes size and seminal vesicle length did not altered significantly during all the seasons of the studied area. However, in female rats the uterus size significantly increased during spring season and no effect on litter size and corpora leutea. It was revealed from this study season has profound effect on body measurement dynamics and reproductive organs of the male and female rats.

LENGTH-WEIGHT RELATION OF MALE POPULATION OF WILD *NOTOPTEROUS NOTOPTEROUS* IN RELATION TO BODY SIZE AND CONDITION FACTOR

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This research was conducted in order to investigate the allometric growth of wild male population of *Notopterous notopterous* in Ghazi Ghat, river Indus. Forty-eight specimens of different body size ranging from 19.2 to 29.5 cm total length and 47.80 to 227.49 g body weight were used for the analysis of morphometric variable of head length, dorsal fin length, dorsal fin base, pectoral fin length, pectoral fin base, anal fin length, anal fin base, gap of mouth, body depth, body girth, eye diameter, inter orbital width, preorbital length, post orbital length, pre dorsal length, post dorsal length, pre pelvic distance and caudal fin length in relation to total length and body weight. Slopes of the log transfered data were used to compare with an isometric slope and it was observed that all relations showed very high correlations. Regression parameters were found to be highly significant.

EFFECT OF SALINITY AND TEMPERATURE ON ANOMURAN CRABS {DECAPODA: ANOMURA) FROMKARACHI COAST

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The three hermit crab species, *Clibanarius signatus, Diogenes diogenes* and *Pagurus* species were tested for the effects of temperature, salinity and interaction of

temperature-salinity on the survival rate and behaviour. Four temperatures (5°C, 10°C, 20°C, 30°C) and four salinities (10%0, 20%0, 30%0, 40%0) were tested. When crabs were acclimated for 6 days and their temperature was changed, the primary effect was a decreased survival at low temperature. Mortality usually occurred by 48 h) followed by increased tolerance. Crabs were susceptible to extreme temperatures and no mortality was observed at 30°C and most mortality occurred at 5°C. In other similar experiment salinity levels were changed. Survival rate was higher at higher salinities while most of the mortality was observed at $l0^{o}/_{oo}$ S. Mortality took place due to salinity change is lower than that of temperature, which shows that these crabs can tolerance wide changes in salinities. Like temperature tolerance mortality usually occur with in 48 hours followed by increased tolerance. For temperature-sal inity interaction tolerance al! three species of crabs were acclimated either 15°C or 25°C and subjected to various test combinations of temperature and salinity. The 48 hour cumulative percent mortalities were noted. The temperature-salinity interaction effect was most important variable for all species. In *Clibanarius signatus* it accounts for 32-37% of the total mortality. 'femperature accounts for 17.77% and salinity alone contributes approximately 14.44%) of the mortality. For *Diogenes diogenes* temperature contribt.ltes 19% and salinity contributes around 14% mortality, while interaction of temperature-salinity causes 31-37'% of the total mortalities. Similar results observed in *Pagurus* species that interaction of temperature and salinity accounts for most of the mortalities 29 35<%. However, the effect of temperature and salinity is relatively even (17-19%). The acclimation temperature clearly had very little effect upon temperature and salinity tolerance for any of the three species. Among the three species *Diogenes diogenes* was much more tolerant to desiccation than either *Clibanarius signatus* and *Pagurus* species.

REPRODUCTIVE BIOLOGY OF THE SANDY SHORE CRAB *MATUTA LUNARIS* (BRACHYURA: CALAPPIDAE) FROM KORANGI CREEK, SIND COAST, PAKISTAN

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Reproduction of an intertidal calappid crab. *Matuta lunaris.* Was studied histologically based on monthly gonad samples were collected from Korangi Creek (Sindh coast, Pakistan)at weekly intervalsduring the period,population bctween April 2009 and May 2010. Males and females became sexually mature at carapace widths of 43 and 37 mm respectively.Reproductive activity, as determined from gamete production, mating behaviour and brood incubation, is continuous throughout the year. There was marked asynchrony betV\cen individual crabs and no apparent seasonality. Adult females could produce more than one egg batch each egg batch comprised about 65 000 eggs.

LENGTH -WEIGHT RELATIONSHIP AND RELATIVE CONDITIONS FACTOR FOR *HYPORAMPHUS DUSSEMERI* (FAMILY HEMIRAMPHIDAE) FROM KARACHI COAST

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In the present study, length - weight relationship and condition factors were estimated for the samples of 236 specimens of *Hyporamphus dussemeri* caught from the Karachi coast,Karachi Fish Harbour, during the"'TIonthly sampling from August 2009 - November 2010. In fishery research, length and weight measurements are standard tasks and data . obtained are the backbone of many models used in fish population. Log transformed regression was used to test the growth of the specimens. Estimation of length-weight relationship in *Hyporamphus* species justifies the equation for each month as, August, (male): Log W = 14.51 + 1.85 log L; (female): Log W = - 13.35 +- 1.51 log L; September, (male): Log W = - 39.12 + 3.12 log L; (female): Log W = - 32.35 + 2.71 log L; October, male): Log W ,= - 36.96 + 2.92 log L; (female): Log W = - 24.51 +2.33 log L; November, (male): Log W = - 49.32 + 3.55 log L; (female): Log W = - 28.47 + 2.58 log L.The parameters 'a' and 'b' of the length weight relationship of *Hyporamphus* in the form of W = aLb was determined.It was observed that the growth is allometric as the value of coefficient 'b' is greater in males as compare to females. Their value of"b" varied between 1.51 and 3.55. The overall mean value of "b" was 2.50 for the complete set of data. The condition factor (K) was ranged from 0.26 - 0.40 (mean of 0.30) in males and 0.26 - 0.28 (mean of 0.27) in females. The relationships (linear regression) was estimated as 0.97 - 0.99. Also concluded that the growth offish or body proportions of fishes changed with their growth.

DEVELOPMENTAL STAGES OF *PALAEOMON PACIFICUS* (STIMPSON, 1860) (CRUSTACEA, DECAPODA, PALAEMONIDAE) REARED UNDER LABORATORY CONDITIONS

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An ovigerous female of *Palaeomon pacific us* (Stimpson, 1860), captured from Buleji coast (Karachi, Pakistan) and was kept under the laboratory conditions. After 13 days larvae were hatched out as first zoea and survived till zoea V. The zoea I to zoea V passed within sixteen days at room temperature: 28°C– 30°C, in filtered seawater of a salinity of 35 ppt and pH 7.9. The larvae are described along with their illustrations and compared with planktonic zoea I of the same species from wild and earlier studied *Palaeomon pacificus* of other authors and shown in tabulated form.

CULTURE AND ASSESSMENT OF MICROALGAE FOR AQUACULTURE FEEDS AND PRODUCTION OF BIOFUEL

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Aquaculture-produced animals reared using microalgae as a feed source during one or more stages of their life cycle. The increasing worldwide interest in aquaculture production demands intensification and greater control over total nutritional inputs. Algal production for feeds is divided into intensive mono culture for larval stages of bivalves, shrimp, and certain fish species, andextensive culture for growout of bivalves, carp, and shrimp. The culture of microalgae is one of the modern biotechnologies. Microalgae are able to produce a wide range of commercially interesting byproducts such as fats, oils, sugars, pigments and functional bioactive compounds. Certain micro algae are effective in the production of hydrogen and oxygen through the process of bio-photolysis while others naturally manufacture hydrocarbons, which are suitable for direct use as high-energy liquid fuels. Microalgae are also used for conditioning of culture waters. To date more than 40 different species of micro algae isolated in different parts of the world are cultured as pure strains. Microalgae have the potential to become even more important resource which can spawn various new industries in addition to the existing ones for aqua feeds, pigment extraction, cosmetics, and pharmaceutical uses. The present study involves the collection, identification, isolation, culture, maintenance, flocculation, harvesting and processing of some indigenous species of micro algae to assess their growth parameters under local climatic conditions. Lipid content was'also determined to assess the suitability of micro algae for the production of biofuels. Total 31 species were collected, 21 identified, 7 were isolated, cultured and studied in detail.

OBSERVATION ON THE WATERFOWLS OF JIWANI WETLAND COMPLEX

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The total areas of Pakistan coastline nearly 1050 km extends from Indus delta and Sir Creek in the South East to the Gawater Bay and Dasht river estuary in the South West Jiwani coastal areas. During the study 30 species of water fowls have been studied from areas during the period of 2009 to 2010, which include some rare and threatened species. The areas were regularly visited in all season during the period from 2009-2010.The species were observed and identified by using spotting scopes and binoculars and field guide by Grimmett (1998), Sonobe and Usui (1993). The areas are important for water fowls practically Flamingo, grey heron, Waders and Pelican.

FOOD HABITS OF MOLE RAT *(NESOKIA* SP.) IN DATE-PALM ORCHARDS OF NOK- KUNDI, DISTT CHAGHAI BALOCHISTAN

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In this study, the mole rat, *Nesokia* sp. faecal pallets, were collected month wise (June2006- July2007) from the rodent infested date-palm orchards of Nok- Kundi, District Chaghai, Balochistan. It was revealed from the study that mole rat has a narrow feeding niche. The summer diet (Fruiting season of date- palm) df the rat was relatively least diversified than "those of the winter diet (Non fruiting season' of date- palm). In fruiting season the mole rat fed mainly on date-palm fruits 32.35% and date palm stem 25.08%, while in non fruiting season date palm - fruit was fed 12.99% and date palm stem 41.87%. About 30.5% fragments of faecal pallets were not identified. Grass leaves and tubers were. consumed much less. Insects were eaten sparingly.

OPTIMUM PROTEIN REQUIREMENTS FOR THE INTENSIVE CULTURE OF *LABEO ROHITA* (HAMILTON) IN GLASS AQUARIA

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To study the optimum protein requirements for the intensive culture of *Labeo rohita* (Hamilton) reared in glass aquaria during April to July 2009. Three iso-caloric pellet feeds were prepared from the locally available feed stuffs (rice protein, rice bran and wheat bran) of different protein levels such as 35%, 38% and 40% (dietary protein levels) of 2 mm dia. These feed stuffs were tested for proximate (bio-chemical composition) analysis according to the methods given in AOAC (1980) and found 13%, 12% and 40% protein respectively. Each feed was supplied at a rate of 8% of the body weight of fish twice a day. The results of the various growth parameters like suitability of protein level requirement, specific growth rate, mean total weight gain, percentage weight gain, feed conversion ratio, survival rate and production. The experimental fish showed significantly ($p<0.05$) highest growth and production observed in feed B (38% gross protein) followed by feed C (40% gross protein) while significantly ($p<0.05$) lowest growth and production was recorded from feed A (35% gross protein). The water quality parameters were recorded throughout the study period and were found with in the suitable ranges of fish culture. It is therefore concluded that the pellet feed with 38% (gross protein) found to be optimum protein level for the better growth and production of major carp, *Labeo rohita.*

MORPHOMETRIC, MERISTIC CHARACTERS AND THEIR RELATIONSHIPS IN *CHANNA PUNCTATUS* (BLOH) FROM RIVER INDUS, NEAR JAMSHORO SINDH, PAKISTAN

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The present study on the morphometric measurements and their relationship in *Channa punctatus* (Bloch) was based on 200 fish specimen collected from river Indus during March to August 2010. The total length, standard length, lateral line length, tail fin length, dorsal fin length, ventral fin length, pectoral fin length, pelvic fin length, girth, head length of fish were found to be highly correlated with the total length, the relationships may be expressed as linear. Whereas eye diameter, gape of mouth were observed highly correlated with the head length of fish. The six meristic characters have been counted, dorsal fin rays, ventral fin rays, pectoral fin rays, pelvic fin rays, tail fin rays, and lateral line scales count. The morphometric measurements and meristic counts did not show any significant difference between male and female except in girth measurement. The female is more pot-bellied and broader than male; male was found to be slim body. It was also inferred that there is a single homogeneous stock of this species is available in river Indus

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COMPARATIVE MORPHOLOGY OF MALE REPRODUCTIVE SYSTEM IN FOUR GENERA OF PENAEID SHRIMPS

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The morphological variations in testis, vas deferes and spermatophores~ have been studied in four genera of penaeid shrimps viz, *Fenneropenaeus, Penaeus, Metapenaeus* and *Parapenaeopsis.* The male reproductive system consists of two symmetrical halves; each half bears testis, vas deferens and an ejaculatory duct. Each testis comprised of variable number of short stout or long finger like lobes; vas deferens is divisible into proximal, medial and distal parts, with variable length, thickness and with or without internal partitioning. The ejaculatory duct varied from a simple tubular dilation to a complicated 2-chambered glandular structure. The shape and structure of the testicular lobes, vas deferens, ejaculatory duct and spermatophore was round to be greatly varied among studied genera of penaeid shrimps. Among species of genera *Fenneropenaeus, Penaeus,* and *Metapenaeus,* one large spermatophore is found from each ejaculatory duct, which is associated with a non spermatic accessory structure whereas, in *Parapenaeopsis* spp. large number of spermatophores with out any~ accessory structure were found from each ejaculatory duct. The variations in vas deferens and ejaculatory duct are mainly associated with the structure of spermatophores.

DISTRIBUTION AND POPULATION STATUS OF GORAL, *NAEMORHEDUS GORAL* (HARDWICK) IN PATTAN AND KEYAL VALLEYS OF DISTRICT KOHISTAN

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Distribution and population status of grey goral *(Naemorhedus goral,* order: Artiodactyla, sub-order: Ruminantia, family: Bovidae) was conducted from 24th May to 10th July 2010 in the Pattan and Keyal valleys of District Kohistan, Khyber Pakhtunkhwa, Pakistan. Field survey, direct sighting, informal discussion, interviews, discussion with community and use of questionnaire were the major tools of data collection. In Pattan valley, 90 questionnaires were distributed in 05 different sites (Sighayoon Khaas, Janchil, Amba Jaak, Bohil and Nabaz) among the local people and hunters. In Keyal valley, 75 questionnaires w~re distributed in 07 different sites (Keyal Nala, Fagaiel, Bhapobanda, Keero, Balkhun,Rodair and Lotos). In Pattan valley, the population status of goral was decreased during last five years due to over hunting and unawareness, while in Keyal valley the population status of goral was increased due to stop hunting and awareness by Wildlife department. In Pattan Valley, 08 vantage points (Tankor Janchil, Rasta Dong Janchil, Barho Kandogay, Landai Sar Bohil, Hawery Kamar Bohil, Barho Bohil, Barho Gulkand and Nabaz) were selected, where gorals were directly sighted and data was entered into survey form. In these vantage points 21 gorals were found. Out of 21 gorals 13 were adults, 6 sub adults and 2 fawns. While in Keyal valley, 06 Vantage point (Baroon Nala Fagaiel, Galto Fagaiel, Shaig Bhapobanda, Keero Keyal, Balkhun and Rodair) were visited and total 40 gorals were found. Among them 22 were adults, 14 were sub adults and 4 were fawns. Physical, geological and biodiverstical characteristics of both vaJleys were not more different from one another. Due to lack of education most of the hunters do not know about the importance of goral and wild life. Education and to aware the people by wildlife department is still needed.

SEVERITY OF ASCARIS LUMBRICOIDES IN DIFFERENT AREAS OF KARACHI CITY

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Present research work was conducted during a period of two years *i.e.* January 2009 to December 2010. An attempt was made to study the patients having Ascariasis in different areas of Karachi, reported in different hospital *i.e.* Baqai hospital, MAP hospital, Nimra hospital, Sindh government hospital, Sindh heart and care hospital, Al-Haider memorial medical center and Shaan hospital. The stool examination with stain

and unstained microscopy method of slide preparation was made. The patients under study were mostly children; however, large number of male and female was also recorded who suffered from Ascariasis. Month wise data of two year from January 2009 to December 2010 was recorded. The total number of patients who were infected with Ascariasis infection in Karachi was 933 cases in which 458 cases (49.08) were positive.

POPULATION STATUS AND DISTRIBUTION OF ASIATIC BLACK BEAR *(URSUS THIBETANUS)* IN PALAS VALLEY, KOBISTAN

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The Asiatic black bear *(Ursus thibetanus)* was widely distributed throughout Pakistan Few decades ago, but now it is only limited to moist temperate forests of the Himalayan mountains of Hazara forest division, Kohistan and Azad Jammu Kashmir. To study the present distribution of *U. thibetanous* in Palas valley, district Kohistan was conducted from May to July, 2010. During the survey two black bears were seen directly, fecal material was found at 5 different places and footprints were also found at 6 places in the valley. The signs of crop damaging and attacks on animals were also observed. Findings showed that Asiatic black bear is distributed throughout Palas valley. The major population is present in Karoser, Kunsher and Sharaid areas of Palas valley. Population of. *U thibetanus* is recorded 29 animals in Palas valley. The major reasons for decrease in- population of black bear are urbanization and human-bear conflict which arises when humans and bears comes in contact with each other. The reason for human-bear conflict is lake of knowledge and awareness about black bear. There is urgent need required to monitor the black bear population, assessment of human-bear conflict in its habitat in the valley on pure scientific methods. To conserve the declining population of black bear concerned department should focus on awareness about importance of wildlife in the area and also to correct the knowledge about behavior and ecology of black bear.

AN EVALUATION OF COUMATETRALYL FOR THE CONTORL OF WILD BOAR, *SUS SCROFA CRISTATUS*

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Evaluated the field efficacy of an anticoagulant (coumatetralyl) maize grain bait (0.0375%) against wild boar population in Fatima Jinnah Park, Islamabad. A total of 1838 kg bait was applied at 32 bait points, out of which 1627 kg was consumed by wild boars during 17 nights. The consumption of bait increased asymptotically and attained peak on the 7th night. The bait in-take, external and internal pathological symptoms were

similar as has been described for warfarin and brodifacoum. The bleeding period ranged from 8.06 to 12.94 nights with 13% variation in time. Based upon the total consumption of coumatetralyl bait, 400-450 wild boars were estimated as killed during the operation. The results of the study showed that coumatetralyl is a promising and potential alternate to sodium flouroacetate (compound 1080) for managing wild boar populations in diverse .environments

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A NEW 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 contributed a lot 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 and 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, racumin and brodifacoum) and acute poisons such as (1080 or 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/reduce these problems a new bait delivery method, using acute poisons, has been evaluated by conducting preliminary This method involves the use of poultry bird heads (broiler & layer), and sodium mono or flouroacetate (1081 & 1 080) as toxicants. The toxicants are mixed with peanut butter at 0.03 and 2 percent w/w, respectively. This paste like mixture is inserted (2-3 g) into the buckle cavity of the head. Surface and sub-surface baiting is conducted by using these treated heads. The baiting strategy includes the use of heads as piles (10-15 or more) or placing (surface or sub-surface) 1 or 2 heads at equal intervals of distance along the wild boar trails. To test this wild boar control method a large farm near Mirjal (Distt. Attock) was selected where high infestations of ~ild b.oar were reported. Three nights baiting using 1081 showed very high consumption (90-100%) of the bait Dead bodies of wild boar, although very difficult to locate, were also observed in the treated area. No non-target protected wildlife species were affected during this environment friendly baiting method. Similar field studies were conducted at NARC, Islamabad and highly effective results were achieved.

EXPLORING THE FISH FAUNA OF UPPER SWAT RIVER, NORTHERN PAKISTAN

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The Swat river is a historical river. Swat river with a peculiar combination of fish fauna. A survey of fish fauna of river Swat at Khwaza Id1ela to Qandil was conducted during April to September (2010). In this collection 9 species of fishes were identified, that" belong to three orders, Cypriniformes, Salmcniformes, Silurifomis and 4 families, Cyprinidae, Sisoridae, Balitcridae and Salmonidae. Among the families, the family Cyprinidae was the richest and represented by 4 species vlz.: *Crm/mcheilus diplocheilus, Schizothora richardsonii, Cyprinus carpio* and *Carassius auratus.* Family Balitoridae was repre~ented by 3 species viz.: *Schistora alipidota, Triplophysa choprai* and *TriplophJ'sa naziri,* where as Family Salmonidae by' *Oncorhyilcus mykiss* and Family Sisoridae by *Glyptosternum retieulatum* were represented by single species each. Comparison of size of the collected fish species revealed that *Oncorhyncus mykiss* was the hirgest with 20 em, whereas *Triplophysa choprai* was the smallest with 8.2 cm. Comparison of fin rays revealed that *Oncorhyncus mykiss* has greater number of caudal fin rays with 27, whereas *Triphlophysa choprai* has smaller number with 10. Comparison of body depth of fish species revealed that *Carassius auratus* has the largest body depth with 3.2 cm, whereas *Triphlophysa choprai* has the smallest body depth with 1.4 cm. It is, concluded that the great diversity of fish species is found in Swat river with great variatio~ in their morphology. More research is requited to explore the fish fa:una of Swat river.

DIVERSITY AND ABUNDANCE OF MACROFAUNA AT SANDY BEACH DURING SW MONSOON SEASON

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Sandy beaches provide the dynamic benthic environment and substrate which is composed of fine grained sediments that have a capacity of retaining water and detritus. Thus, sandy beaches support the settlement of benthic organisms. The abundance and variation in species composition in sandy beaches are influenced by various environmental and physical factors such as sediment texture and by the availability of food resources. The aim of this study is to explore the variation in the sandy beach macrobenthic community structure during South-West monsoon period. A total of 31 macrobenthic species were recorded during pre-monsoon and SW-monsoon period. Molluscs, polycheates and crustaceans were the most dominating macrobenthic groups

throughout the study. The results obtained during the study showed variation in macrobenthic diversity and abundance during the studied monsoon season. The diversity indices and abundance were high during pre-monsoon period and lowest during mid monsoon period. The variation in salinity, dissolved oxygen concentration and wave action might be the possible cause affecting the macrobenthic abundance during monsoon season at this site. This study is an approach towards the understanding of sandy beach structure and benthic fauna which will be helpful in the management of the sandy beaches in future.

POPULATION DENSITY OF BENGAL MONITOR (*VARANUS BENGALENSIS*) AT DIFFERENT HABITATS OF DISTRICT SWABI, KHYBER PAKHTUNKHWA, PAKISTAN

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Bengal Monitor (*Varanus bengalensis*) is a widespread and well known large-sized lizard species in Pakistan. The present study was carried out at three selected sites viz. Zahir Shah Aratt, Palosi Khwar and Gangodher Kaly of District Swabi, Khyber PakhtunKhwa to record density (number of individuals and burrows) of Bengal Monitor at different habitats viz. agriculture fields, bank streams and muddy houses near human habitations from March to November, 2010. The Line Transect Method was used. Population densities (per square kilometer) recorded from agriculture fields, bank streams and muddy houses were 0.13 ± 0.01, 0.16 ± 0.01 and 0.50 ± 0.43 respectively. Densities of active burrows (per square kilometer) were 3.62 ± 0.13 and 4.89 ± 0.79 while of passive burrows were 3.63 ± 0.47 and 4.96 ± 0.32 in agriculture fields and bank streams respectively. Higher density of lizard and burrows along bank streams reflects lizard's habitat preference. Trapping of lizards and persecution by locals were recorded as main threats to the species.

SOME OBSERVATIONS ON POPULATION DENSITY AND BREEDING OF RANID FROGS (*EUPHLYCTIS CYANOPHLYCTIS* AND *HOPLOBATRACHUS TIGERINUS*) AT RAWAL LAKE, ISLAMABAD

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The population density and breeding of two selected species of Ranid frogs viz. *Euphlyctis cyanophlyctis* (Skittering Frog) and *Hoplobatrachus tigerinus* (Bull Frog)

were studied at Rawal Lake, Islamabad, Pakistan, by using Visual Encounter Method from September, 2009 to August, 2010. Average population density of selected Ranid frogs was 0.77 per hectare, of Skittering Frog was 1.00 per hectare and of Bull Frog was 6.75 per hectare respectively. It is concluded that both species showed explosive breeding i.e. breeding activities confined during the first showers of the monsoon season. The mean weight of spawn size of Skittering Frog was 1.5 grams with above 1000 eggs in the spawn while the mean weight of spawn size of Bull Frog was 0.26 grams with eggs below 1000 eggs. Average air and water temperatures of breeding sites were 28 °C to 26.6 °C respectively. Spawn sites were characterized by grasses such as *Paspalidium flavidum*. No threat to the populations was observed. However, opening of the spillway of Rawal Dam following the torrential rain destroyed breeding sites of the frogs. It is maintained that the event did not produce any significant impact on the populations and breeding as the two species were quite common.

POPULATION DENSITY AND SOME ASPECTS OF BREEDING OF RANID FROGS (*EUPHLYCTIS CYANOPHLYCTIS* AND *HOPLOBATRACHUS TIGERINUS*) AT RAWAL LAKE, ISLAMABAD

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The population density and breeding (time of breeding, spawn size and sex ratio) of two selected species of Ranid frogs viz. *Euphlyctis cyanophlyctis* (Skittering Frog) and *Hoplobatrachus tigerinus* (Bull Frog) were studied at Rawal Lake, Islamabad, Pakistan, by using Visual Encounter Method from September, 2009 to August, 2010. As many as 84 individuals of selected Ranid frog species with 0.77 Ranid frogs per hectare were recorded. A total of 115 individuals of Skittering Frog (1.00 frogs per hectare) and 50.93 individuals of Bull Frog (6.57 frogs per hectare) were recorded. Both species showed explosive breeding i.e. breeding activities confined during the first showers of the monsoon season. The mean weight of spawn size of Skittering Frog was 1.5 grams with above 1000 eggs in the spawn while the mean weight of spawn size of Bull Frog was 0.26 grams with eggs below 1000 eggs. Mean air and water temperatures of breeding sites were 28°C to 26.6°C respectively. Spawn sites were characterized by grasses such as *Paspalidium flavidum*. No threat to the populations was observed. However, opening of the spillway of Rawal Dam following the torrential rain destroyed breeding sites of the frogs. It is maintained that the event did not produce any significant impact on the populations and breeding as the two species were quite common.

BASELINE STUDY ON WILDLIFE DIVERSITY OF KALLAR KAHAR LAKE, CHAKWAL, PUNJAB

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The present study was carried out to gather baseline data on species occurrence of mammals, birds, reptiles and amphibians and to explore future research arenas at Kallar Kahar Lake and its environs from October, 2008 to October, 2010. Species presence/ absence was ascertained through direct and indirect signs by surveying four selected sampling units using standard methods. A total of one hundred and forty six wildlife species belonging to twenty six orders and sixty two families were recorded from the study site. The recorded species included twenty seven (18.49 %) species of mammals belonging to thirteen families and seven orders with Family Muridae (n=10; 37.03. %) as the most abundant family; ninety (61.64 %) birds belonging to thirty six families and sixteen orders with Family Ardeidae (n=8; 8.88 %) as the most abundant family; twenty four (16.43%) reptiles belonging to nine families and a single order with Family Colubridae (n=7; 29.16 %) as the most abundant family and five (3.42 %) amphibians belonging to three families and a single order. Potential future research areas identified include breeding ecology of resident waterfowls species especially of moorhens, grebes, egrets, cormorants and summer breeders such as night heron; wetland ecological health assessment using macrophytes, algae and invertebrates as indicator organisms; management of invasive species such as crows and common myna, tourism as a basis of wetland valuation and detailed studies on population dynamics of small mammals, water birds, passerine birds, birds of prey and snakes of the area.

EFFECTS OF NITROGEN FERTILIZER ON TADPOLE SURVIVAL OF BULL FROG (*HOPLOBATRACHUS TIGERINUS*) UNDER LABORATORY CONDITIONS

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Decline in amphibian populations is a global phenomenon which is attributed to several factors such as use of fertilizers and other chemicals on agriculture lands. Effects of selected nitrogen fertilizer (ammonium nitrate) were investigated on tadpole survival of Bull Frog during a twenty days (30th July to 17th August, 2010) experiment. Eggs and tadpoles were collected from seasonal shallow water ponds during rainy season from the

university campus. Four groups of tadpoles, each consisting of 20 tadpoles, were subjected to four different sub-lethal concentrations of nitrate i.e. T1=1000, T2=1500, T3=2000 and T4=2500 µg NO3-N L^{1}. Each group was compared with a control (untreated), and survival of tadpoles upto froglet stage was observed. Mean tadpole survival was recorded as 93.66%, 93.00% , 80.58% and 79.50%, for 1000, 1500, 2000 and 2500 µg NO3-N L^{1} respectively compared to 97.83% for untreated tadpoles. It is concluded that nitrates in higher concentration have severe impact on the survival of tadpoles of Bull Frog. Mean tadpole survivals for different treatments and control were significantly different ($p = < 0.05$).

MANGROVE – A DELICATE ECOSYSTEM: THREATS AND oppertunities

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Mangrove are botanical amphibians occupying a zone of desiccating heat, chocking mud and salt levels that will kill ordinary plant within hours. Forest mangrove alongwith its associated fauna form among the most productive, biologically complex ecosystem on earth. Pakistan has worlds' largest mangrove forest in arid zone. Providing livelihood to 100,00 coastal community of coastal areas is source of shell and finfish, fuel and fodder. Food source for many vertebrates and invertebrates. Protect sea port from sittation. Reduce intensity of cyclone and assimilate sewage water from domestic and industrial area. Due to many anthropogenic activities and lack of flow of freshwater mangrove forest hae been reduced from 280,000 ha to 160,000 ha. Many attempts are being made to conserve, regenerate mangrove forests.

WATERFOWL DIVERSITY OF MANGLA WATER RESERVIR, AZAD JAMMU AND KASHMIR

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The present study was conducted to study waterfowl species diversity of Mangla Water Reservoir, Azad Jammu and Kashmir from Novemeber, 2009 to July, 2010. Total count of waterfowls was undertaken from vantage point of the four selected sampling units. Checklist of waterfowl species of the reservoir along with data on population density and relative abundance are given. As many as 39 waterfowl species belonging to

eight families and four orders (viz. Ciconiformes, Anseriformes, Gruiformes and Charadriformes) were recorded. These included four egret species (10.25%), three herons (7.69%), ten ducks (25.64%), three water hens/moorhens (7.69 %), one coot (2.56 %), nine shorebirds (23.07%), four gulls (10.25%) and five tern species (12.82%). Order Charadriformes had highest number of waterfowl species (n=18; 46.15%) while order Gruiformes had lowest (n=04; 10.25%). Of the recorded 39 waterfowl species, 69.23% were winter visitor, 20.51% resident, 7.69% summer visitor and 2.56% passage migrant. As many as 4774 individuals with a population density of 23.86 waterfowls per hectare were recorded. Common gull (10.91%) was recorded as the most dominant species. Fluctuations in the water level, hunting and construction activities at the reservoir were recorded as main threats. Further detailed avifaunal studies are required to establish the status of the reservoir as Ramsar Site.

POPULATION STATUS OF SNOW LEOPARD AND ITS CONFLICT WITH LOCAL PEOPLE IN LASPUR VALLEY, CHITRAL, PAKISTAN

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Snow leopard (*Uncia uncia*) is a solitary felid of cryptic nature and occupies a habitat that makes very difficult to enumerate it. Though poorly studied, the species is found in northern parts of Pakistan. The present study assessed population status of snow leopard and human-carnivore conflicts in Laspur Valley (about 1400 km^2 area), district Chitral (Khyber Pakhtunkhwa). The study employed the use of questionnaires, interviews from local informants, sign surveys and camera trapping. Twenty out of 59 households confirmed the sighting of snow leopard in the study area. Community perception about snow leopard was favorable as most of the people either wanted to increase (58%) or stabilize (36%) the population of snow leopard. Nobody ranked snow leopard as a very dangerous (Rank I) animal, majority (58%) ranked it as low danger (Rank III) to community as compared to wolf (Rank I= 58%) and lynx (Rank I= 42%). The length of 41 sign survey transects carried out in three study blocks (Bashqar Gol, Phargram Gol and Shandur) was 27.1 km, and we observed 2.55 signs (pug mark, feces, scrape) per km on average. Following SLIMS (Snow Leopard Information Management System), this sign density equates to 1-2 individuals in the area. Furthermore, the findings of questionnaires and SLIMS sign surveys were also supported by the camera trapping results which confirmed at least one snow leopard in the area by three photos. Population of wild ungulates consisted of 174 ibex in the area, revealed by the Point Vantage Counts method. Economic burden of predation was found to be significant for the poor communities. Total number of livestock in study area was estimated at 48000 animals while nearly 6800 animals were lost to depredation in five years (2005-2010). An annual

loss to predation per household was estimated to be 0.68 animals, which is equivalent to an economic loss of PKR 5,800 (US$ 70) per household in the valley. Wolf (44% predation cases) dominated the predation followed by snow leopard (25%) and lynx (21%).

PLANKTONIC STUDIES OF KEENJHAR LAKE, DISTRICT THATTA, SINDH

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Keenjhar Lake is an artificial and tropical lake. It is located almost 120 km from Karachi; Keenjhar Lake is essential for diverse aspects such as, supply of drinking water, irrigation and wild life intention. The aim of present research work was planned to resolve the physicochemical properties and primary productivity of Keenjhar Lake. Planktons and physicochemical properties of Keenjhar Lake were determined from January to December 2009. A total of 142 phytoplanktonic species, representing 68 genera and 7 classes. There were 21 species of *Bacillariophyceae,* 57 species of *Cyanophyceae,* 55 species of *Chlorophyceae,* 1 species of *Chrysophyceae,* 2 species of *Dinophyceae,* 5 species of *Euglenophyceae,* and 1 species of *Xanthophyceae.* A total of 37 species of Zooplankton comprising 15 species of *Cladocera,* 3 species of *Copepoda,* 4 species of *Ostracoda* and 15 species of *Rolf/era* were determined during present studies. The physicochemical properties such as temperature, alkalinity, dissolved oxygen, salinity, conductivity, total dissolved solids, chlorides, turbidity, pH, and hardness were determined on monthly basis. The physicochemical properties of Keenjhar Lake were suitable for growth of aquatic biota.

FOOD HABITS OF DEMOISELLE CRANE *(ANTHROPOIDES VIRGO)* MIGRATING THROUGH LAKKI MARWAT AREA OF PAKISTAN

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[2]*The Pakistan Wetlands Programme*

A study was designed to investigate diet composition of the demoiselle crane *(Anthropoides virgo)* migrating through Lakki Marwat district of Khyber Pakhtoonkhawa Province, Pakistan. In total, 16 gizzards were collected in spring 2010 while 35 fecal samples were available during both fall 2009 and spring 2010 migrations. The analysis of gizzard contents was carried out by simple mechanical segregation while the fecal samples were processed for micro-histological technique. The results revealed that this crane species was predominantly vegetarian and it derived the plant based food

component from agricultural fields falling in the premises of its migratory stopover. The analysis of gizzard contents revealed the presence of three cultivated plant species *viz:* wheat (43.75%), sorghum (43.75%) and chickpea (12.5%). However, analysis of feces samples collected during the same season revealed occurrence of five cultivated plant species *viz:* wheat (50 %), sorghum (25 %) and chickpea (18.75 %), Egyptian clover (18.75%), garlic (10.5 %) and three wild plant species *viz:* wild onion (31.25 %), beard grass (25 %) and bur clover (18.75 %). Analysis of fecal samples collected during the fall migration season showed the presence of cultivated crops, namely chickpea (47.37 %), mustard (42.11%) and spinach (21.05 %). The wild species prevailing in these samples included wild onion (42.11%), bur clover (21.05 %), salt cedar (15.79 %), beard grass (10.5 %) and alkali seep weed (10.53%). The demoiselle cranes supplemented its diet with animal based food including invertebrates belonging to Phylum Arthropoda and vertebrate species i.e. tadpoles, *Mus* spp. and nestlings of birds. The invertebrates (arthopoda) were present in 12.5 % gizzards and 8.57% feces samples while the remains *Muss* spp. were available in 18.75 % and 2.86 % gizzards and feces samples, respectively. However, tadpoles and nestlings of birds were identified in 12.5 % and 6.25 % gizzards, respectively. On tbe wbole, 87.5 % gizzard samples ihd roo %4'eeal samples were positive for plant based food while the animal based food was found in 50 % gizzard samples and 11.4 % fecal samples. Frequency of animal based food was more in the gut materials of this crane collected during spring migration, prior to breeding season. In addition, large quantities of grit composed of small stones and quartz crystals were also found in the gizzards and fecal samples of this migratory crane, the latter could even help to construct migration pathways of this species through Pakistan. T~ study provided information on important flora and fauna contributing towards the diet of migratory demoiselle cranes that ultimately could be useful for the conservation of this game bird species in Pakistan.

POPULATION DYNAMICS OF CRUSTACEAN ZOOPLANKTON OF HALEJI LAKE

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Seasonal abundance of 32 genera of crustacean zooplankton belonging to 3 groups (Cladocera, Copepoda and Ostracoda) observed on monthly basis in Haleji Lake during 2007.0ut of 32 genera 15 belonged to Cladocera, 8 from Copepod "and 8 belonged to Ostracoda. Among all zooplankters, the most abundant group was Copepoda (38.88%) quantitatively .The least frequently found group was Cladocera (25.09%).Results revealed that maximum numbers of all zooplankters were present in May (34.79%) while minimum quantity was noted in Febru¥)' (02.84%).The genera which observed throughout the year were *Cyclops* sp., *Mesocyclops* sp., *Eucyclops* sp., *Ectocyclops* sp., *Eucypris* sp. and *Cyprinotus* sp. There was no genus of Cladoeera that showed

continuous population during the whole study year. Among Cladocera , *Alona* sp. (03.57%) , in Copepods, *Cyclops* sp. (14.08%) and in Ostracoda, *Cyprinotus* sp. (23.49%) were most abundant genera. Each group showed one distinct maxima and minima during January to December 2007.

ROOSTING BEHAVIOR OF BIRDS AROUND SOME AIRFIELDS OF KARACHI

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The problem of bird strike hazard to aviation has assumed alarming proportions resulting .in the large scale damage to aircrafts. In order to develop a risk analysis and wildlife control management plan for airfields, an ecological study has been conducted. The present work is related with roosting behavior of birds including bird's population estimation; birds strike hazards, their preventive measures and their effects on aircrafts. In this respect three major airfields of Karachi have been selected to monitor the number of species and movement of birds around these airfields. Many methods were used for avoidance and repellence of birds near Runways including Trapping, Surfactants, water sprays, live ammunition and scare crow techniques. Remains of birds have been identified by treating them through HMDS. This study resulted that in Karachi region the highest threatening and hazardous species to air crafts is *Milvus migrans* (Black kite).The population of this species has been spreaded so rapidly in Karachi because they are well adapted to live in cities and regularly attracted in large numbers to uncovered garbage bins in airfields. Apart from soaring on thermals at high elevations, it is also quarters very low using any other available air currents and frequently settles on runways. Other species which were found as major threats to aircrafts in airfields of Karachi are *Butaster teesa, Corvus splendesis, Psitlacula kremeri, Acridotheres tristis, Bubulcus ibis, Columba livia, Passer domesticus and Appus affinis.*

ICHTHYODIVERSITY OF RIVER INDUS AT JAMSHORO DISTRICT, SINDH

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The River Indus is key resource of fisheries potential and diversity as it covers the whole length of Pakistan. Present study deals with ichthyodiversity of River Indus and reports 52 fish species belonging to 35 genera, 12 sub families, 16 families and 8 orders. Among these two species were exotic ofteleostean fishes from Jamshoro district, Sindh. During present studies, nineteen species belonged to Cyprinidae, six species to Bagridae, four species to Channidae, three species each to Clupeidae and Mastacembelidae, two

species each to Notopteridae, Siliuridae, Schilbeilidae, Chandidae, Nandidae and Belontidae, one specie each to Clariidae, Heteropneustidae, Cichlidae, Gobiidae and Belonidae. Physico-chemical parameters were within the range.

DISTRIBUTION, POPULATION AND FOOD HABITS OF INDIAN PANGOLIN *(MANIS CRASSICAUDATA)* IN DISTRICT CHAKWAL, PAKISTAN

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Indian pangolin *(Manis crassicaudata)*, is a "Near Threatened" solitary mammal which faces a high risk of endangerment due to various threats in its wild habitat. The current study aimed to investigate its distribution, population status and food habits in District Chakwal, Potohar Plateau, Punjab from March 2010 to February 2011. Detailed surveys were conducted throughout the district and the animal species was found distributed around Chakwal city (Jabair pur), Sehgalabad, Mureed village, Khokhar Zer dam (around Bhaun area), Chumbi Surla Wildlife Santuary, and Choa Saiden Shah (especially around Basharat hills). For its population estimation of the species, both direct (visual observations) and indirect methods (burrow counts, foot patches)were employed by using area searches in seven selected study sites and its average population density of 0.30/km2 was recorded. Four individuals of the animal species were also captured (two live and two dead) from the study area and their morphometrical parameters were recorded. The food habits of the species were investigated by fecal analysis which revealed 40% ants (35 % heads and 65% other body parts), and 60% soil particles and some unknown materials.

STUDY OF BIO-ECOLOGICAL STATUS, MANAGEMENT AND CONSERVATION OF MARSH CROCODILES, *CROCODYLUS PALUSTRIS* IN SINDH-PAKISTAN

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The order Crocodilia (Crocodiles, Alligators and Gavials) is well-known which are living in the tropical and subtropical regions. There are 23 species of Crocodiles in the world today. Order Crocodylia comprised of three families viz: (1). Alligatoridae (the Caimans /Alligators), (2). Crocodylidea: *Crocodylus palustris* (the Mugger Crocodile)

and (3). Gavilidae: the Gavial. Marsh Crocodile (*Crocodylus palustris*) is the only species that has been recorded in Sindh Pakistan, occurring in Mangho Pir, Karachi Zoological Garden and Khar Center (Karachi), Haleji Lake (Thatta), Chotiari Reservoir (Sanghar), Deh Akro 2 (Nawabshah), Nara Game Reserve (Khairpur mirus) and New Jatoi (Noshehroferroze). Present study on the Bio-ecological status and distribution of Marsh Crocodile or Mugger (*Crocodylus palustris*) was carried out from January 2006 to June 2009. The ecological factors were analyzed to identify the impacts of turn down in Marsh Crocodile population due to indiscriminate habitat destruction, environmental problems, low water quality and hunting demands. The physico-chemical parameters study was carried out several sites to know the ecological status and water quality of species.

STUDY OF AVIAN DIVERSITY IN AND AROUND CHINARI, DISTRICT HATTIAN, AZAD JAMMU AND KASHMIR, PAKISTAN

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A survey was carried out from March, 2009 to October, 2009 to estimate avian diversity in and around the Chinari Jehlum Valley, District Hattian Azad Jammu and Kashmir. The area was surveyed at different times, especially in the morning and evening when the birds were more active. Birds were recorded by using Fixed Count Method at different predominant points in each site of the study area. For the identification of birds binocular, digital camera and field guides were used. During the study a total of 72 bird species, belonging to 13 orders and 39 families were recorded. Species richness was recorded highest in summer (n=54) followed by autumn (n=46) and spring (n=40). Seasonal Shannon Wiener index of these species were recorded highest in summer season (4.95) followed by spring (4.2) and autumn (3.26). Of the total, 8 (11.1%) species were resident, 9 (12.5%) were resident confined to Himalayan ecosystem, 7 (9.72%) were summer visitors, 5 (6.94%) were winter visitor, 12 (16.6%) species showed seasonal altitudinal migration while remaining 30 (41.6%) species were confined to Himalayan ecosystem. According to the calculated population status, 50 (69%) species were common, 4 (5.55%) were abundant, and 9 (1.25%) were rare while the remaining 9 (1.25%) species were frequent. The most common birds were House sparrow, Common myna, House crow, Red vented bulbul, Koel, Spotted dove, Rose ringed parakeet. The children and women, during wood cutting, destroy the nest and eggs of the birds that can cause population decline of bird species. There is a strong need to educate the local people about the importance of the bird species to enhance the bird's diversity of the study area and their role in the ecosystem management.

COMPETITION FOR FOOD RESOURCES AMONG *CANIS* SPP. IN CHITRAL AREA

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The Grey Wolf exists in a wide range of habitats fronl cold tundra to the warm deserts. and Jack:lls occupy all types of habitats including extreme d~serts and dense forests. Wolf is classified as Endangered globally as well as in Pakistan whl1c Asiatic jackal is listed as near Threatened in Pakistan. The present study determined the extent of food overlap between wolf and jackal in Chitral area. Scat analysis of both the species (25 scat samples each) was carried out. The medullary and scale patterns of hairs were used to identify the prey items consumed by wolf and jackal and matching with reference photographic key of 17 prey species of the study area. Wolf consumed 14 prey species while jackal consumed 13, among which 11 species were common in the diet of both species including domestic sheep, Cape Hare, Royal Pika, Royal Mountain Vole, Flying Squirrel, Golden Marmot, Palm Civet, Wood Mouse, House Mouse, Hamster and birds. Sheep (10.63%) and Palm Civet (10.63%) Were the dominant prey species in the diet of wolf while Palm Civet (8.51 %), Golden MarnlOt (8.51 %), Cape Hare (8.51 %) and Wood Mouse (8.51 %) were dominant prey species in the diet of jackal. The study revealed that competition between Wolf and Jackal for food resources was very high, especially for small mammals. Generally, Wolf preys on large mammals; however, in areas where human hunting pressure is high, it seems that wolf has been forced to switch to smaller prey, which leads to competition with jackal. It is suggested that the potential habitat of Grey wolf and Asiatic Jackal along with their natural prey species should be protected for the conservation of these two species, especially the endangered wolf. Compensation to the owndS of livestock killed by Wolf also needs to be paid in order to save it from retaliating killing.

COMPETITION FOR FOOD RESOURCES BETWEEN MARKHOR *(CAPRA FALCONERI)* AND DOMESTIC UNGULATES IN CHITRAL AREA

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Markhor is typically associated with steppic mountain regions between 600m and 3600m elevations and Chitral Gol National Park (CGNP) supports the largest population of Kashmir Markhor *(Capra falconeri cashmiriensis)* in Pakistan. Markhor is c1assifieded as endangered globally as per IUCN Red List. The study determined the food overlap between Markhor and domestic goat in Chitral area through microscopic analysis of faecal material of both the species. Different characteristics of plant cells were

used to identify the plant species consumed by Markhor and domestic goat. During the spring, eight plant species were consumed by Markhor and five by domestic goat among which four were common in the diet of both animals i.e. *Salix iliensis, Juniperus excelsa, Prangos pabularia and Linum perenne.* Durimg the summer, nine plant species were consumed both by Markhor and domestic goat i.e. *Artemisia maritime, Pistacia integerrima, Origanum vulgare, Rheum emodi, Hypsicum perforatum, Quercus baloot, Prangos pabularia, Linum perenne, Bergenia ciliate).* During the spring season, Markhor preferred *Linum perenne* and domestic goat preferred *Linum perenne* and *Salix iliensis.* The value of diet overlap factor during the spring was 0.69 indicating that the diets of Markhor and domestic goat were not completely overlapped. During summer season, both Markhor and domestic goat strongly preferred *Linum perenne* with diet overlap factor of 0.99 indicating very high diet overlap between Markhor and domestic goat. The results revealed that food competition between Markhor and domestic goat was very high during the summer season which leads to competition for food during this season between the two ungulates.

PREVALENCE OF TREE CAVITIES AND THEIR USE BY VERTEBRATE FAUNA IN FOREST ECOSYSTEM OF DHIRKOT, AJ&K

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Population of tree cavities is considered an important element of forest ecosystem for providing foraging, nesting, roosting and breeding sites to its animal diversity, but little is known about their prevalence, characteristics and the associated vertebrate species in forest ecologies of Pakistan. The present study was carried out during September 2009 to August 2010 to explore cavity bearing tree species, distribution oftree cavities and associated vertebrate species in a forest area of Dhirkot, Azad Jammu & Kashmir (34°3°N; 73° 34" E). The study area was comprised of two forest types, situated at an elevation ranging from 1066 m to 2042 m. The upper plantation was predominantly a moist temperate forest having blue pine *(Pinus wallichiana)* and rein or oak *(Quercus incana)* with some mixed deciduous broad leaved species. The forest on lower elevation was typically sub-tropical pine forest with dry mixed deciduous speyies, dominated by *Pinus roxburgii, Quercus incana* and. *Olea ferruginea.* In total 1140 trees were surveyed, out of which only 83 trees were recorded to have 211 cavities and among these 83 trees, 34 (41 %) were live and 49 (59 %) dead. Highest population of tree cavities 60 (28.4%) was associated with *Olea ferruginea* which was followed by *Pinus wallichiana* with 34 (16.1 %) cavities, *Melia azedarach* with 32 (15.2%) cavities, *Quercus incana* with 26 (12.3%) cavities and the remaining 5,9 (28%) cavities were present on rest of the 8 plant species. There were 136 (64 %) natural cavities and 75 (36 %) cavities were developed by primary excavators. Position of 156 (74 %) cavities was recorded in trunk region and

of 55 (26%) cavities in the branches. Only 60 (28.4%) were found . positive for presence of any vertebrate species including 11 (l8.3%, 41 (68.3 %) and 8 (13.3%) occupied by mammals, birds and reptiles, respectively. Entrance diameters (mean±SEM) of tree cavities used by mammals, birds' and reptiles were 8.78±1.79 cm, 5.56±0.23 cm and 6.38±1.24 cm, respectively. Similarly, the mean depths of tree cavities used by mammals, birds and reptiles were recorded >90 cm, 42.43±1.54 cm and 30±5.35 cm, respectively. Cavity entrance height from ground level used by mammals was 9.9±1.54 m, by birds was7.05±1.13 m and by reptiles was 3.13±0.44 m. In totaL19 vertebrate species were recorded comprising of 2 mammals, 13 birds and 4 reptiles. The mammals included giant red flying squirrel *(Petaurista petaurista)* and long fingered bat *(Myotis longipes).* The birds were; common myna *(Acridotheres lristis),* great tit *(Parus major),* Himalayan tree creeper *(Certhia himalayana),* house sparrow *(Passer domesticus),* chestnut-bellied Nuthatch *(Sitta castaea),* red-head longtailed tit *(Aegithalos concinnus),* white-cheeked longtailed tit *(Aegithalos leucogenys),* yellow-fronted woodpecker *(Mt;lanerpes flavifrons),* Himalayan pied woodpecker *(Dendrocopos himalayensis),* Scaly-bellied woodpecker *(Picus squamatus),* Rufous-bellied woodpecker *(Dendrocopos hyperythrus),* golden-backed woodpecker *(Dinopium benghalense)* and blue throated barbet *(Megalaima asiatica).* The record of reptiles showed two snakes; Kashmir cliff racer *(Coluber rhodorachis)* and common cat snake *(Boiga trigonata)* and two lizards; spotted barn gecko *(Hemidactylus brookii)* and Kumaon mountain lizard *(Japalura kumaonensis).* This preliminary study suggests that availability of dead trees has significant role in providing tree cavities and should not be completely removed during silvicultural practices. Similar .studies should be extended to all major forest ecologies because such information are important for providing ecological insight that can be applied to the conservation of cavity-using fauna of a forest ecosystem.

POPULATION DENSITY AND HABITAT PREFERENCE OF PUNJAB URIAL *(OVIS VIGNEI PUNJABIENSIS)* IN DILJABBA-DOMELI GAME RESERVE, DISTRICT JHELUM

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Punjab Urial *(Ovis vignei punjabiensis)* was studied for its habitat preference, distribution range, population status and threats in Diljabba-Domeli Game Reserve district Jhelum during 2009-2010. The data on population density was collected through direct observations using line transect method at fcur study sites. For habitat analysis, vegetation sampling of Urial habitat was done by taking 92 quadrates of 4 x 4 m^2 each. A t0tal of 51 Urial (37 females, 14 males) were observed between 332 m and 600 m elevati0n. Overall population density of Punjab Urial in the study area was 2.68 animals/km^2; however, it varied among study sites depending upon the habitat quality

and extent of disturbance to the animals by different sources. Dominant plant species in Urial habitat included; trees: *Acacia modesta, Capparis aphylla, Ziziphus nwnmularia* and *Prosopis glandulosa,* shrubs: *Adhaloda zeyfanica. Sagerctia theezans, May tenus royfeana, Dodonea viscose, Grewia tenax* and *Lantana camara,* grasses: *Cynodon dactylon, Saccharum bengalenses, Eulaliopsis binata* and *Eragrostis cywJsuroides* and herbs: *Pergulari.a tomentos, Sonchus arvensis* and *Suaeda jruticosa.* Urial preferfed grasses whenever available even though browse was more abundant. In dry season \",hen domestic livestock had consumed most available grasses, they shifted to browsing on trees/shrubs. Major threats to Urial in the study area included; loss/degradation of habitat, illegal killing, competition with livestock, habitat fragmentation, scarcity of water during summer months and capturing the Uriallambs to keep *as* pet, which need to be addressed to save this threatened endemic species.

HABITAT PREFERENCE OF DESERT HARE *(LEPUS NIGRICOLLIS)* IN DILJABBA-DOMELI GAME RESERVE, DISTRICT JHELUM

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The study on habitat preference of Desert or Black-naped Hare *(Lepus nigricollis)* was conducted in Dilijabba-Domeli Game Reserve located in Jhelum district. Total area of the game reserve is 118, 116 ha and it is part of the Salt Rang in the northern Punjab. Four study sites i.e. forest land, shrub land, grass land and open land within the potential habitat of Desert Hare were selected which differed with respect to terrain, elevation, soil and vegetation. A total of 72 quadrates were laid down in four study sites for analysis of habitat in addition to recording other features of habitat such as elevation, aspects, water availability, etc. Overall density of trees, shrubs, herbs and grasses was 3.07%, 19.97%, 16.84%, and 49.7%, respectively in Desert Hare habitat. *Cynodon dactylon* was the most dominant species followed by *Adhatoda zeylanica, Gymnosporia royleana* and *Dodonea viscosa.* Among trees, *Acacia modesta* was dominant followed by *Prosopis glandulosa* and *Zizphus nummularia.* Major shurab included *Adhatoda zeylanica, Gymnosporea royleana* and *Dodonea viscosa.* Herb layer was dominated by *Malvestrumcoromendalianum, Parthenium hysterophorous* and *Tribulus terrestris.* Dominant grass species were *Cyndon dactylon, Saccharum bengalenses* and *Eleusine indica.* However, vegetation differed among four study sites in terms of dominance. Pellet count method was used to determine habitat preference of desert hare by taking 67 quadrats of 4 x 4 m each at four study sites. Average density of faecal pellets was 36.20, 34.77, 24.4 and 24.06 per quadrat at four study sites, respectively. Desert hare showed preference for plain sandy areas with scattered vegetation, gentle slopes and small rocks. However, preference of habitat was not significantly different for four sites. Habitat fragmentation, accidental killing, and cutting of grasses were the major threats to Desert

hare in the game reserve which need to be addressed for its conservation. Particularly, cutting of tall grasses such as *Saccharum bengalenses* which provide shelter to Desert hare should be avoided by providing awareness to the people.

DEINOTHERIUM FROM THE MIOCENE LOCALITY OF LAVA, CHAKWAL, PAKISTAN

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An inimitable discovery of the fossilized lower dentition of *Deinotherium* was made during the excavation at Dhok Rehmatay Aali near village Lava, district Chakwal. Stratigraphically the locality belongs to the Upper Chinji Formation characterized by interbedded sandstone, siltstone, and bright red clays. The sample (PUPC 09/116) comprises IP3- M3; rP3-4 and rM2-3. The whole material belongs to the same individual. Owing to the early stage of wear the animal seems to be a young individual. Morphologically the sample shows similarities with the genus *Deinotherium* which is evident by the occlusal outline of p3, where the protoconid and hypoconid form an almost continuous longitudinal ridge and a conic-shaped metaconid. On the basis of comparative morphology and measurements the present material is ascribed to *Deinotherium indicum.Deinotherium indicum* is known to survive in the Siwaliks of Pakistan in Miocene epoch.

FIRST REPORT OF *PUNJABITHERIUM PLATYRHINUS* FROM JARI KAS, AZAD KASHMIR, PAKISTAN

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The collection of fossils discussed here in this paper verifies the presence of a unique extinct rhinocerotid species in the Siwaliks of J ari Kas, Mirpur, Azad Jammu and Kashmir. The sample comprises an isolated premolar and molar belonging to the upper dentition of *Punjabitherium platyrhinus* (Falconer et Cautley). The specimens resemble fairly well morphologically and metrically to those described earlier for this species from the Siwaliks of India.. The discovery of *Punjabitherium platyrhinus* from the upper Siwaliks of Pakistan extends its geographic range and rejects the idea of limited

distribution of this species only in the typical Siwaliks of India near the Ganga and Jamuna River.

ISOLATION OF SOME PATHOGENIC FUNGI FROM FRESHWATER FISHES

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Six freshwater fish species, *Labeo rohita* (Hamilton); *Catla catla* (Hamilton); *Ctenopharyngodon idella* (Valenciennes, 1844); *Hypophthalmichthys molitrix* (Richardsons, 1845) and *Carassius auratus* (L.) were studied for fungal infection. *Labeo rohita* and C. *auratus* showed fungal infection with typical cotton wool like appearance at gills, head, fins, skin and bloody spots at the sites of infection. Grossly, the skin colour was faded and fins were eroded in infected fishes. The fungi isolated from the infected areas of the fish, was cultured on Maltose extract agar medium. The isolated fungi were incubated for a week at room temperature (28°C) and the growth of fungi was observed. The control plates showed no growth. Different fungal colonies were labeled. The slides were prepared by taking the material from each colony and stained with Trypanblue in Lactophenol. The stained slides were observed under microscope and photographed. *Aspergillus* spp; *Mucor* sp and *Penicillium* spp were isolated from gills, caudal fin, pelvic fins and skin of C. *auratus.* In *L.rohita* only *Aspergillus* sp was isolated from caudal fin. The probable causes of fungal infection in these fishes are discussed.

ANANCUS SIVALENSIS FROM THE UPPER SIWALIKS OF ROHTAS FORT, DISTRICT JHELUM, PUNJAB, PAKISTAN.

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The present material PUPC 10/75 has been unearthed from an Upper Siwalik locality exposed at the bank of Kahan River near Rohtas Fort, district Jhelum. The sample comprises right and left mandibular ramii with m^3. The specimens are partly damaged both at the crown as well as the dentary. The overall preservation is good and shows enough dental morphology for comparison with the already known material of similar proboscidean forms. Morphologically the molars represent weak 'anancoidy' which refers to the establishment of an alternating arrangement of the pre trite and post trite half-Iophids (ridge plates) which is the basic diagnostic feature of the genus *Anancus.* The lower molars have five tuberculated lophids. Enamel is smooth, non-

lustress and non ptychoid, and lophids have a central conule. The crown is brachydont. A prominent post talon is also present. Based upon the comparison for above mentioned diagnostic features with already known material of similar anancine forms, the present material has been ascribed to the genus *Anancus* and species *Anancus sivalensis.*

ELEPHAS PLANIFRONS FROM THE UPPER SIWALIKS OF KUNDAL (CHASMA) DISTRICT MIANWALI, PAKISTAN

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Elephas planifrons has been recovered from Tatrot Stage of the Soan Formation of Kundal district Mianwali. The specimen PUPC 10/76 is right and left mandibular rami of the same individual having m^3. They are in late stage of wear and damaged. The preservation is good and enough diagnostic features are preserved to show the dental morphology. All the material was thoroughly studied and compared with previously published data. The lowers molars show diagnostic features of the species like m3 with broad and widely spaced ridge plates with thick enamel. Devoid of placations, ridge plates have reduced median expansion, well developed cement in the valleys; plates are transversely arranged and progressively compressed. Based upon the morphological aijd metrical similarities studied specimens are ascribed to genus *Elephas* and species *Elephas planifrons.*

RHINOCEROS SIVALENSIS FROM THE DHOK PATHAN FORMATION OF THE MIDDLE SIWALIKS, PAKISTAN

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Rhinoceros sivalensis has been recovered from Dhok Pathan Formation near the Dhok Pathan rest house. The specimen PUPC 09/115 is a right maxillary fragment with P2-Ml. The specimen is in late wear and damaged. The preservation is good and enough diagnostic features are preserved in dental morphology. Some already misidentified and published material (PUPC 68/843a and 68/843b) present in Dr. Abu Baker Fossil Display and Research Center (FDRC) is also included. All the material was thoroughly examined and compared with previously published data. Both premolars and molars show

diagnostic characteristics of the species, like molars with parastyle buttress, distinct crochet that may unite with protoloph to enclose a fossette and without a crista. Molars have strong parastyle, metaloph somewhat short, very backwardly directed on M2 and there is a great projection of protoloph, no mesostyle and protocone constricted by posterior and anterior grooves. Based upon comparative study the present material has been identified as *Rhinoceros sivalensis.*

A PIONEER STUDY ON CONSERVATION STATUS OF INDIAN ROCK PYTHON *(PYTHON MOLURUS MOLURUS)* IN DEVA VATALA NATIONAL PARK, AZAD JAMMU AND KASHMIR, PAKISTAN

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Conservation status of Indian Rock Python (Python molurus molurus) was studied in Deva Vatala National Park Azad Jammu and Kashmir during April to September 2009. Study area was divided in four localities (Chumb, Deva, Barmala and Vatala). Visual Encounter Survey Method was used to explore the distribution, population status and general habitat preference, while human-python conflicts and past sighting record were collected through interviewing the local community. Indian rock python was randomly distributed in all localities of the study area with an overall population density of 3.06/km2. Population density was highest in Deva (4.37/km2) followed by Chumb (3.75/km2) and Barmala (2.5/km2) while minimum density was estimated at Vatala (2.18/km2). Most sightings were recorded in the months of July (26%) and August (15%) during day time (45%), near the water sources (86%) in the forest area (86%). Pythons preferred the sub-tropical zone in the park. Human-python conflict was the main conservation concern. A total of 91 python attacks were reported which resulted 74 livestock deaths and other 17 injured. Maximum depredation were reported in the month of July (22%), August (19%) and September (15%) in which goats were depredated in majority (84%). Maximum livestock was depredated at adult stage (70%), in the forest area (84%) during day time (54%). Livestock depredation enhanced rage in human that resulted the killing of 92% python during their attacks in study area. Livestock depredation and the python killings by the locals were significantly correlated. Herding practices has increased habitat disturbance and declining in the natural prey species of python which in turn has increased livestock losses, increasing a negative perception of the local community. Habitat destruction due to the forest cutting, overgrazing, fodder and fuel wood collection and illegal python trade were the other major threats to the species.

STEGODON BOMBIFRONS FROM THE PINJOR STAGE, SOAN FORMATION OF JARI KAS, AZAD KASHMIR, PAKISTAN

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The specimen (PUPC 2010/16) presented here is a fragmentary cranium with right and left maxillary molars. The specimen was thoroughly examined and compared with already known fossils of similar proboscidian forms. The diagnostic features observed in the present collection include, vertically high cranium compressed antero-posteriorly with convex skull vertex. The teeth are broad and large; ridge-plates intermediate between *Stegolophodon* and *Elephas planifrons* types, progressively multiplying from 6-11 in the intermediate molars and 9-15.5 in the posterior molars. The 'Cones rapidly subdivide by binary or tertiary fission into multiple conelets. Ridge-plates elevating from brachyodont to subhypsodont. Cement developing in the valleys and gradually increases in the successive teeth. Enamel is very thick. Usually a trace of median longitudinal cleft is present in anterior ridges. Inner columns of ridge-plates occasionally show accessory tubercles near the longitudinal cleft. Due to the morphological similarities with the published *stegodon bombifrons* specimens the present material has been identified as such species. The earliest chronological record of the genus is from the lower Pliocene of Pakistan. The genus flourished towards Middle and late Pliocene of Java, China and Japan. It survived till late Pleistocene and chronologically the latest record is from China.

INTESTINAL DERANGEMENTS OF *LABEO ROHITA* SAMPLED FROM THE DOWNSTREAM LOGATION, HEAD BOLLOKI, RIVER RAVI

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Freshwater fish Rohu, *Labeo rohita* were collected from head Bolloki during low flow season and -Punjab University research farm. Following dissection, intestine of the pond as well as riverine fish were obtained, cut into less than one cm long pieces, washed with water and immediately fixed in Bouin's fixative. The tissue were routinely processed for dehydration, paraffin embedding, cross sectioning and H & E staining. The intestine of control fish had elaborative glands comprising of vivid goblet cells. The glands measure upto 490 /lm in length starting from basement membrane to their mouth in the lumen. Goblet cells, oval in shape appeared filled with mucus ranging up to 17.5 x 7.5 µm. The massive infolding of the villi was found to occupy most of the area within the

intestine, while lumen was represented by a narrow irregular shaped area outlined by the villi which spanned maximally up to 392 µm. Epithelium of the lumen of the intestine comprised of a layer of columnar cells separated by the rest of the tissue by earmarked uniform distinct connective tissue basement. The cells were characterized by prominent nuclei measuring up to 3.96 x 2.57 µm. While the cells diameter ranged up to 4.95 µm and height up to 6.43 µm. The open border of the gut epithelium had, in general, regular look with occasional wavy margin defining the brush striated border. This structure was vivid at the areas where largest cells were located. The fish sampled from head Bolloki had, much prominent muscularis externa which were not comparable with the structure from the control fish. Extensive mucosal folds reduced the area of lumen while the epithelium of the lumen was found distorted and not delineately lined by the recognizable columnar cells. Similarly, the goblet glands were not, in general, characterized by mucous filled cells. In some fish, central lacteals were identifieable but in a highly condense form. The recognizable lumen expand maximally up to 313.6 µm. The present study indicates the effects of sewage and industrial pollutions. Elevated coliform bacterial contact of the fish of the study area as compared to the control pond fish (Unpublished data), heavy metals discharge and organic loads appear to deteriorate gut normal histological structures. The histological intestinal appearance was highly pathological. This might be the probable reason for diminishing level of digestion and absorption which resulted significantly low growth factors of the sampled fish as compared to those collected from an upstream locations (Unpublished data). This study provides a model for assessment of pollutant of varied nature on fish development and growth under natural ecosystems. This model even permits to visualize chronic effects of pollutant in the system.

FECUNDITY OF BACHWA *(CLUPISOMA NAZIRI)* FROM THE RIVER INDUS AT ATTOCK KHURD

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The fecundity of *Clupisoma naziri* was studied by gravimetric method. Ovaries were weighed to nearest 0.001 gm. Then a part (52-74mg) was cut, weighed and processed for separating and counting the eggs. Absolute fecundity varied from 1223 to 31373 eggs for a fish of 21.5cm to 32.2cm total length and 55gm to 278gm body weight. The relative fecundity varied considerably. Relation of fecundity to size revealed that the larger fish were more fecund than the smaller ones. Eggs' diameters were measured with the help of a calibrated ocular fitted electric microscope at 40X. The diameter ranged from 1215 µm to 1509 µm. Further up and low stream data may identify best spawning locations of the fish.

APPARENT DIGESTIBILITY OF FISH MEAL, BLOOD MEAL AND MEAT MEAL FOR *LABEO ROHITA* FINGERLINGS

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Apparent nutrient (dry matter, crude protein, crude fat & gross energy) digestibility of three feed ingredients fish meal, blood meal and meat meal was determined for evaluating the nutrients potential for *Labeo rohita* fingerlings. Reference diet was mixed with test ingredients in 70:30 ratios to formulate test diets. Chromic oxide was added as an indigestible marker. Apparent nutrient digestibility coefficient of dry matter was highest for blood meal (56.85±2.39) followed by fish meal (50.15±0.94) and meat meal (42.35±1.28). The apparent crude protein digestibility for fish meal was higher (80.20±1.64) and this was followed by meat meal (79.90±4.31) and blood meal (71.90±0.93). Apparent crude fat digestibility for fish meal was higher (78.30±3.84) as compared to blood meal (75.30±4.89) and meat meal (67.40±4.56). The apparent gross energy digestibility of fish meal was better (69.30±3.71) than meat meal (61.55±4.63) and blood meal (59.55±5.20). The results of present study indicated that the fish meal was most digestible ingredient so fish meal can preferably be included in the formulation of diets for *Labeo rohita* fingerlings.

DORCATHERIUM FROM THE HASNOT OF THE MIDDLE SIWALIKS, NORTHERN PAKISTAN

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The late Miocene-early Pliocene Hasnot, northern Pakistan, has yielded remains of the Siwalik tragulid *Dorcatherium.* The same unit has also yielded *Selenoportax, Pachyportax, Gazella* and *Bramatherium.* The known tragulid teeth are well worn. In this paper new remains of *Dorcatherium* have been identified, described and discussed in detail. This discovery adds weight to the suggestion that the late Miocene tragulids inhabited well wooded to forested regions rather than open country.

COMMUNITIES DEPENDENCE AND NATURAL RESOURCES USE AT TAUNSA BARRAGE WILDLIFE SANCTUARY, PAKISTAN

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The study has been conducted in the agricultural and fishing communities of the Taunsa barrage wildlife sanctuary, Muzarfargarh District. These communities are selected due to their immediate dependence on the sanctuary area. The major objective of the study was to document the resilience of rural communities to climate change. That has been achieved by developing an elaborate questionnaire to define the socio-economic status of the different communities and their resilience to the climate change patterns in the country and in the area. The questionnaire has included the health issues faced by the women on a larger scale. The results obtained would be used to identify resilience among rural communities to climate change and also to calculate the percentage of kitchen particulate matter to commend on use of fuel in rural communities and adaptability for change.

HABITAT SELECTION BY BROWN BEARS IN DEOSAI NATIONAL PARK, PAKISTAN, AND IMPLICATIONS FOR PARK MANAGMENT

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The Himalayan brown bear is threatened and has a fragmented range in the Himalayas, yet its habit requirements are not known. We investigated habitat selection of brown bears and the impact of human disturbance factors in Deosai National Park, Pakistan. An Ecological Niche Factor Analysis indicated that bears avoided higher elevations and steeper slopes and selected more productive parts of the park (marshy, grassy, and stony vegetation types). Only 65% of the park area was vegetatively productive, with a standing crop of about 900 kg dry matter/km^2. The marshy vegetation was the most preferred habitat, probably because it had the highest forage production and density of golden marmots. Brown bears tolerated human infrastructures, like roads and camps, but strongly avoided grazing areaswith high livestock density. The habitat

suitability map generally followed the biomass productivity patterns of the park. It indicated the central part as suitable, and classified half of the park, mainly peripheral areas, as not suitable for brown bears. The vegetation and habitat suitability maps provide an objective criterion for evaluating present and future developments in the park. Until recently, communities seem to have used the park's resources without significantly affecting the brown bear population. However in recent years a large influx of livestock by nomad grazers has become a major challenge, which needs urgent attention to continue the present brown bear population recovery and to secure its habitat. We recommend monitoring the livestock and a detailed inventory of the rangeland to understand grazing dynamics in the park and to maintain sustainable stocking rates.

CORRELATIVE STUDY OF AVIAN DIVERSITY WITH THE VARYING URBAN CONGESTIONS OF LAHORE

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A correlative study testing the impact of different urban congestions in Lahore upon the bird abundance and diversity was conducted in the monsoon and winter season. A platform of 6 feet diameter and 4 feet height, with a variety of seeds, was offered to attract the grainivorous birds. The number and variety of birds visiting the feeding station was noted, from Dawn to dusk after a period of 6 day installation, which depicted the bird's urbanization in the area. A significant increase in the abundance of birds was observed in the winter season. Densely populated areas had the highest number of sparrow and pigeon population whereas areas with large sized pockets of vegetation supported the largest diversity of birds. Grain preference of the birds was also recorded in the different areas studied which showed a relationship between the age long practices of feeding birds with the habituation of the birds.

PREVALENCE OF *LERNAEA CYPRINACEA* IN VARIOUS DISTRICTS OF PUNJAB AND ITS CONTROL WITH VARIOUS COMMERCIALLY AVAILABLE INSECTICIDES

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Fish farming is rapidly flourishing in Punjab. However, per acre fish yield is severely affected by the infestation of *Lernaea cyprinacea*. Highest proliferation of Lerneasis was observed in district Jhang and *Catla catla* was found to be the most affected species. To solve this problem an experiment was designed to check the effect of

various commercially available insecticides on proliferation of *L. cyprinacea* at finger lings of rohu, mori, thala, silver carp and grass carp. 240 finger lings of each species (W=31±9.5g; L= 13.3±2.6cm) were stocked @15 finger lings / 50L aquaria in each trial. Various concentrations (0.1, 0.2, 0.3, 0.4 and 0.5mg/l) of Triazophose, Deptrix, Tender and Lambda were used in triplicate/concentration/species. Temperature, pH and DO were monitored on daily basis. Tender was found to be most effective for the control of lernea @ 0.3mg/l. Result of this experiment has been successfully used in field at various fish farms.

STATUS OF COMMON LEOPARD (*PANTHERA PARDUS*) IN AND AROUND MACHIARA NATIONAL PARK AZAD JAMMU AND KASHMIR, PAKISTAN

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Machiara National Park (MNP) is one of the potential areas in Azad Jammu and Kashmir for the existence of common leopard which covers an area of about 135 km² (13532 ha), having an altitude of 1790 m to 4733 m above the sea level. Common Leopard (*Panthera pardus*) is listed by IUCN as critically endangered in Pakistan. The present study was conducted to evaluate the status of common leopard in the MNP between May 2007 and July 2008 by counting the number of pug marks on transect lines and by sighting. People and livestock activities along the transects were recorded as an index of disturbance. Fifteen fixed monitoring transects were surveyed to get the mean encounter rate for leopard scats, footprints, livestock and people seen in the area. Mean encounter rate calculated for the leopard pugmarks was1.928, for scat 2.022, for livestock 32.94, and for people 30.77. This information showed that the park is highly disturbed because of the human activity (grazing, fuel wood and fodder collection). Adult leopards were sighted at 23 locations while cubs with mother were sighted at four different locations in the study area. About 30% sightings happened near the water sources. Of the total sightings, 35 % were recorded in the morning, 15% at the day time, 29% in the evening time and 21% during the night. The geographical coordinates of all the sighting places were recorded and plotted on the GIS map. Maximum sightings were recorded between 15629.2m to 31599.5m elevation consisting of moist temperate zone with *pinus wallichiana* as a dominant species. Due to constant interaction, the leopard has lost his shyness from the human beings, and hence was seen frequently in the area. It was estimated that 11 leopards existed in the study area. The information generated from the study will be helpful for the conservation and management of this threatened species in Azad Jammu and Kashmir.

ECOLOGICAL INTERACTION OF MUSHROOMS WITH TROPICAL FOREST OF MURREE (KALDANNA) AS FIRST ATTEMPT

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19 samples of mushrooms belonging to 18 genera were recorded from Kaldanna (Murree). The soil of this area is soft because of plenty rainfall, the decomposition rate is high, that's why the soil is fertile also. The upper 2-3 inches layer of the soil was soft which was derived from the leaves or needles of these trees and herbs growing under them. Soil colors are brown to dark brown. The mushrooms that were collected grown as saprophyte are also mentioned previous pages. Most of the area is covered with thick vegetation consisting of conifers, cedrus deodar and other broad leaved trees, bushes etc.The soil is rich in all the required elements phosphorus, potassium, favour able PH, nitrogen and organic matter. The mychorizal element was also associated with conifers and cedrus species. The monsoon rainfalls favour their growth. This locality is good for fungal growth and it is at its peak during September and October for all types of mushrooms. Some small and tiny mushrooms were growing among under stones during September to October. The bare rocks were covered with lichens. The area was green covered with herb; dominant plants were belonging to conifers. These mushrooms appear after light rainfall, Michorizal mushroom were associated with pine-trees. The wood inhabiting (bracket) fungi were also collected from the area (kaldanna). . Present on small twigs, grass leaves and soil etc. distributed throughout this locality. These were enlisted systematically with silent characters of Genus and species also autoecological notes, habitat, distribution and edibility etc. Carpophores of fleshy fungi usually consist of for more of then 90% water because they do not possess effective structure to restrict evaporation in mostly irreversible and lead to the death of the concerned carpophores. During September to October this area have good rainfall and the moisture content of the soil and humidity of atmosphere is much high which promote the growth of mushroom in this area. Macro fungi with drought resistant carpophores of wood and leathery consistent such as polyporoid fungi. Fleshy carpophores also very sensitive to freezing so they are not observed in winter. So the process of fructification must depend mainly on the course of precipitation and temperature. Other factors such as exposure to wind, sun, water capacity, ground water level the minimum and maximum temperature effect their growth adversely. All collected mushroom were enlisted systematically with silent character of genus and species, autoecological notes, habitat, distribution and edibility etc. The environment of some mushroom was supported with photograph. About 3 species were medically important. This report will be beneficial to then who want to study the mushroom taxonomically or ecologically .biologist, chemist, biochemist and industrialist will come to know about the mushroom flora of this area. They can arrange the required material to extract the value able compound like anti tumor, anti bacterial medicines.

INDOOR REARING OF FRY OF *CIRRHINUS MRIGALA* USING LOCALLY AVAILABLE FEED INGRDIENTS AND ITS COMPARISON WITH THE CONVENTIONAL METHOD OF REARING OF FISH FRY

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Pond fish culture occupies a significant position in aquaculture sector which employees the methods of artificial propagation of fish and fish seed and supplementary feeding to meet their nutritional requirements for fast growth and optimum fish production. Conventionally, the fish seed is reared under semi-intensive conditions in ponds where it depends mainly on the natural feed produced through the use of organic and inorganic fertilizers. This experiment was designed for indoor rearing of fish seed under hatchery conditions on supplementary feeding using various feed ingredients on *Cirrhinus mrigala* to compare the results with that of rearing seed reared using conventional method. Better survival and growth rate was observed during indoor rearing.

AN INVESTIGATION INTO THE TRADE OF WILDLIFE SPECIES AT PET SHOPS OF RAWALPINDI AND MULTAN CITIES

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In Pakistan, several wildlife species are legally protected through provincial wildlife acts and rules in order to maintain their sustainable populations. However, various wildlife species are being illegally traded in different parts of the country. The current study aimed to collect information on the trade of wildlife species in selected markets; “Goodri" market situated near Shah Rukn-e-Alum tomb Multan and “College road" market situated near Raja Bazaar in Rawalpindi city during September, 2009 to July 2010. Data were collected by using primary and secondary methods. The results revealed that three major groups of wildlife (reptiles, birds and mammals) were on sale in Multan market; birds constituted 76% (maximum) by numbers, mammals 7.8% while reptiles 4% (least). Similarly, in Rawalpindi market, birds constituted maximum number (85%), followed by fish (6%), mammals (5%) and reptiles (4%). A comparison of the two markets revealed major differences between numbers of wild animals being traded; 625 birds, 64 mammals, and 36 reptiles in Multan market versus 371 birds, 22 mammals, and 18 reptiles in Rawalpindi market. Noticeable differences were found in the average prices of various animal species in the two markets; generally lower sale prices were

recorded in Multan than in Rawalpindi market. Regression analysis between volume of trade (VOT) and other variables showed that an increase in average sale price of animal species caused a decrease in Volume of Trade (VOT) of the market.

EFFECT OF SNAIL POPULATION ON THE FISH GROWTH AND PRODUCTION IN FISH FARMING SYSTEM

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In recent years environmental, social and economic conditions of riverine fisheries has became more critical. Therefore fish culture system is evolving with more significance to fill the gap of protein demand. The production through this system depends upon proper management, feeding and harmonizing all the factors related to optimum fish growth and production per unit area. Present study was designed to study the effects of snail population on the fish growth & production in pond. Control of snail population enhance the fish production per unit area.

CHROMIUM, LEAD, MERCURY AND COPPER RESISTANT BACTERIA FROM GUT CONTENTS OF MORI FROM UP AND DOWN STREAM LOCALITIES OF RIVER RAVI

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Gut contents of fish, Mori were used for isolation of the metals resistant bacteria. Fish specimen were caught from different localities of river Ravi and transported immediately to the lab. Isolates were purified by spreading on the metal containing nutrient agar media. In the present study, twenty one heavy metals resistant bacteria were isolated. Of these isolates, 6, 8, 3 and 4 were found to resist Cr, Pb, Hg, and Cu up to 375 µg/ml, 425 µg/ml, 10 µg/ml and 250 µg/ml, respectively. These bacteria also expressed multiple metal resistance to varying levels. From presence of such heavy metal resistance bacteria it may be concluded that the fish had been exposed to these and other metal pollutants in the river water. The isolates may be good candidates for remediation of the heavy metals polluted sites.

GROWTH FACTOR OF THE RIVERINE FISH, MORI FOLLOWING EXPOSURE TO URBAN POLLUTANTS

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Length weight relationship was derived to study the growth of Mori, *Cirrhina mrigala.* Fish specimen of various sizes were collected from four localities viz., Siphon, Shahdera, Sunder and head Bolloki during high and low flow of the river Ravi and transported to the lab. For the study of growth pattern, weight and length of each specimen were measured. Log transformed regression was used to assess the growth profile. The growth factor (b=3.01) indicated the isometric growth of specimen caught from the first upstream sampling point (Siphon) and negative allometric growth for the specimens collected from second (b=2.96), third (b=2.71) and forth (b=2.31) localities (downstream) of the river Ravi during low flow season. Varied growth pattern of Mori clearly indicates negative impact of urban pollutants of the river Ravi on the fish fauna.

EFFECTS OF URBAN POLLUTANTS ON GROWTH OF *CATLA CATLA* IN THE RIVER RAVI

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This study was undertaken to assess growth profile of freshwater fish Thaila (*Catla catla),* collected from different sampling sites (Siphon (A), Shahdera (B), Sunder (C) and head Bolloki (D) of the river Ravi during low and high flow of the river. The specimen were transported immediately to the lab. Length and weight of each specimen were measured accurately to calculate the growth factor (b). Log transformed regression was used for this purpose. The value of growth factor (b=3.24) indicated allometric growth at the first (A) upstream sampling site which decreased by 7 % and 13 % at the second (B) and third (C) sampling points, respectively. The parameter decreased by 5 % for the last (D) downstream sampling site showing rather more or less recovery as compared to second and third study areas. These information reveals stresses of river Ravi pollutants for the inhabitant thaila fish. Further downstream studies may are imperative for verifying the recovery of the fish growth stress due to urban pollutants load in the river Ravi.

ASSESSMENT OF SELECTED PERSISTANT ORGANIC POLLUTANTS (POPS) IN WATER FROM STREAM OF SIALKOT DISTRICT, PAKISTAN

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Samples of stream water were collected from the Nullah Aik and Palkhu, southern tributaries of the River Chanab, Sialkot and were analyzed for eleven Persistent Organic Pollutants (POPs) viz., Beta-HCH, Lindane, Heptachlor, HeptachlorExoepoxide, Heptachlor-Endoepoxide, Dieldrin, DDD, DDE, Endrin, 2, 4-DDT and 4, 4DDT. Residue of Beta -HCH, Lindane, Heptachlor, DDD, DDE, Endrin, 2, 4-DDT and 4, 4-DDT were detected in surface water. Increasing trend in concentration of POPs was observed from upstream to downstream in water samples. The results were compared with international guidelines values for pollutant concentration in streamwater. Concentration levels of studied POPs were found higher than the recommended limits set by World Health. Organization (WHO) and United States Environmental Protection Agency (US, EP A) for stramwater. The results of this study highlighted its usefulness in the management of POPs in study area and its catchments.

INTESTINAL DERANGEMENTS OF *LABEO ROHITA* SAMPLED FROM THE DOWNSTREAM LOCATION, HEAD BOLLOKI, RIVER RAVI

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Freshwater fish Rohu, *Labeo rohita* were collected from head Bolloki during low flow season and Punjab University research farm. Following dissection, intestine of the pond as well as riverine fish were obtained, cut into less than one cm long pieces, washed with water and immediately fixed in Bouin's fixative. The tissue were routinely processed for dehydration, paraffin embedding, cross sectioning and H & E staining. The intestine of control fish had elaborative glands comprising of vivid goblet cells. The glands measure upto 490 µm in length starting from basement membrane to their mouth in the lumen. Goblet cells, oval in shape appeared filled with mucus ranging up to 17.5 x 7.5 µm. The massive infolding of the villi was found to occupy most of the area within the intestine, while lumen was represented by a narrow irregular shaped area outlined by the villi which spanned maximally up to 392 µm. Epithelium of the lumen of the intestine comprised of a layer of columnar cells separated by the rest of the tissue by earmarked uniform distinct connective tissue basement. The cells were characterized by prominent nuclei measuring up to 3.96 x 2.57 µm. While the cells diameter ranged up to 4.95 µm

and height up to 6.43 µm. The open border of the gut epithelium had, in general, regular look with occasional wavy margin defining the brush striated border. This structure was vivid at the areas where largest cells were located. The fish sampled from head Bolloki had, much prominent muscularis externa which were not comparable with the structure from the control fish. Extensive mucosal folds reduced the area of lumen while the epithelium of the lumen was found distorted and not delineately lined by the recognizable columnar cells. Similarly, the goblet glands were not, in general, characterized by mucous filled cells. In some fish, central lacteals were identifieable but in a highly condense form. The recognizable lumen expand maximally up to 313.6 µm. The present study indicates the effects of sewage and industrial pollutions. Elevated coliform bacterial contact of the fish of the study area as compared to the control pond fish (Unpublished data), heavy metals discharge and organic loads appear to deteriorate gut normal histological structures. The histological intestinal appearance was highly pathological. This might be the probable reason for diminishing level of digestion and absorption which resulted significantly low growth factors of the sampled fish as compared to those collected from an upstream locations (Unpublished data). This study provides a model for assessment of pollutant of varied nature on fish development and growth under natural ecosystems. This model even permits to visualize chronic effects of pollutant in the system.

APPLICATION OF WATER QUALITY INDEX TO DETERMINE THE STREAM HEALTH OF NULLAH PALKHU; A TRIBUTARY OF RIVER CHENAB, PAKISTAN

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Present study was aimed to investigate the water quality of Nullah Palkhu, which is severely affected from industrial effluents, municipal sewage and other non point sources. Water quality indices have been developed all over the world to assess the pollution load on water resources and decide the managerial mitigations for water treatment for human, domestic, agricultural and industrial uses. For this study, water samples were collected from Nullah Palkhu for 19 physico- chemical parameters on spatial and seasonal variations. To determine the quality of water, six classes viz, excellent, good, fair, poor, very poor and critical were designated on basis of physico chemical parameters. On spatial scale, no site showed excellent water quality, however, it was good in upstream but it started to deteriorate severely in middle reaches of stream after receiving industrial effluents and municipal sewage and placed in very poor and critical classes. In downstream, water quality gradually was improved, which could be attributed to the influence of many small freshwater stream join the Nullah Pallkhu. On seasonal basis, good water quality was recorded, while rest of the seasons showed poor and critical water quality. Overall results indicated that water quality of Nullah Palkhu

was ranged between poor and critical. The water of Nullah Palkhu should not be used for human, domestic and v irrigation purposes. Water quality index gives an overall view of stream health and helpful in monitoring the changes in water quality and making managerial decisions about stream restoration.

SEASONAL VARIATION IN THE ABUNDANCE AND LENGTH-WEIGHT RELATIONSHIP IN PENAEID SHRIMP SPECIES FROM THE COASTAL AREA OF SONMIANI BAY LAGOON, BALOCHISTAN

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The fishery of the penaeid shrimps is highly commercial, marketed in the cooked and blanched form and export as canned, frozen, dried, peeled, fried and curry forms. The significant seasonal variations were observed in the abundance and length-weight relationships of penaeid shrimps among species and between sexes, from Sonmiani Bay Lagoon in the coastal area of Balochistan from July 2006 to June 2007. *Penaeus* species were dominated over the *Metapenaeus* and *Parapeniopsis* species. Length-weight relationships are considered as an important piece of information for fisheries research. During this study, the total body length was used to determine functional relationship between size and the weight of penaeid shrimps. The total body length has been generally considered an independent variable in penaeid morphometric studies. The results of regression showed higher value of the slope 'b' for the females as compared to males.

DISTRIBUTION OF SOME OCYPODOID CRABS FROM MANGROVE AREAS ALONG THE COAST OF PAKISTAN

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The Ocypodoid are known to form the most important taxa with regard to species diversity and total biomass among all the brachyuran crabs inhabiting in the mangrove swamps. The diversity and distribution of Ocypodoid crab species were studied by transect and quadrate method. The Ocypodoid crabs were collected at different tidal

levels of ten mangrove sites ranging from Keti bunder to Bhaira in Sonmiani along the Pakistan coast. A total of 20 species of Ocypodoid crabs were recorded belonging to the families: Ocypodidae, Macrophthalmidae, Camptandriidae and Dotillidae. Our studies show the species diversity and density distribution of crabs was significantly different among the sites and tidal height.

DISTRIBUTION AND DIVERSITY OF SWIMMING CRABS IN THE LAGOON WATERS OF SONMIANI, BALOCHISTAN, PAKISTAN

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Swimming crabs are common in various coastal habitats along the coast of Pakistan. These crabs considered as a key resource in local fisheries and are important in trophic relations of fish and organisms of sandy and sandy-mud bottoms. These crabs support the recreational fishery as an important component of the food web and also form a significant portion of our shell fisheries; they are available in the local market and exported as fresh, canned and frozen products. A large number of swimming crabs comes as by-catch and form the part of trash fishery. To estimate the diversity and abundance of swimming crabs in the lagoon, fortnightly experimental net (Gill net) was operated by the commercial fishing boat from the February 2005 to April 2007. Total nine species of Brachyuran crabs were identified from the sample, mainly belongs with Portunidae. The species diversity and catch sizes varied with the months and the biomass distribution of crab catch as well as crab species showed a seasonal pattern in lagoon water.

EFFECT OF ARTIFICIAL DIET ON SENSORY QUALITY OF FISH FLESH OF INDIAN MAJOR CARPS (*LABEO ROHITA, CATLA CATLA* AND *CIRRHINUS MRIGALA*)

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To study the effect of artificial feed (35% protein) on fish flesh two experimental trials were conducted on sensory quality of yearlings of Indian major carps (*Labeo rohita, Cirrhinus mrigala and Catla catla*). Fish was reared in earthen ponds having an area of 0.03 ha. Experimental diet was given @ 3 % of fish wet body weight per day. After

three months trial organoleptic study of fried fish was carried out using hedonic scale for each species from treated and control ponds. In monoculture, C*atla catla* showed significantly higher score for flavour in treated ponds than control. *Labeo rohita* was found to be significantly different for juiciness and tenderness scores in treated ponds versus control however; sensory scores of fried *Cirrhinus mrigala* did not exhibit any difference b/w treatments. It can be concluded from the present investigation that artificial diet can improve the sensory quality in monoculture system.

MARINE FAUNAL ASSEMBLAGE IN THE INTERTIDAL ZONE OF BHAIRA (SONMIANI BAY), BALOCHISTAN

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Knowledge of abundance and distributional ecology of macro fauna from our mangrove habitat will help us to understand the extent of utilization of mangroves and their specific role in the ecosystem they inhabit. These organisms are likely playing a major role in channelling energy within the system and also exporting it out from the system for example predation of birds (resident and migratory). The Miani Hor lagoon, Sonmiani is the significant area that contains extensive mangroves stands, which is situated some 90 km away from Karachi on the eastern most part of Balochistan coast. Of four villages, Bhaira is also an active fishing site of Sonmiani. A great variety of benthic assemblages are found within mangroves. The distribution and composition of macrofauna were studied by using beach seine. Seasonal samples were collected over a year from selected area. Different faunal groups identified mainly comprised of juveniles of several species of finfishes, crabs, and shrimps. The significant variations were observed in the diversity, distribution and abundance of identified species.

MEIOFAUNAL ASSEMBLAGE IN THE MANGROVE AREAS OF KARACHI COAST

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The meiofaunal assemblages and their relationship with environmental and

biological parameters were studied. Monthly meiofaunal samples were collected at low tide from two sites Sandspit backwater and Korangi creek, from January to December 1999. Nematodes contributed up to 90% of the total biomass followed by harpacticoid copepods, polychaetes, ostracods, turbellarians, and other meiofaunal taxa. Foramineferans and benthic diatoms (pinnate) were also very abundant in most of the samples. Completely randomized design ANOVA with nested treatment arrangement was used to study the distribution and abundance of meiofauna. Significant variations were observed between both sites and stations. Total meiofauna densities were the lowest during post monsoon and southwest monsoon periods and the highest during northeast monsoon period. The average meiofauna abundance or densities from the Sandspit backwater mangrove area ranged from $4.3 * 10^2$ to $5.5 * 10^5$ individual m^{-3} and $6.3 * 10^4$ to $1.8 * 10^7$ individuals m^{-3} in > 0.5 mm and > 63 □m size fractions, respectively. Meiofaunal abundance was correlated with environmental parameters and reveals that sediment structure, organic content, and some biologically produced structures like burrows, roots are the important variable in determining the distribution of meiobenthos.

STUDIES ON AGE AND GROWTH OF FRESHWATER SHARK *WALLAGO ATTU* (BLOCH & SCHNEIDER) USING OTOLITH, IN THE MANGLA DAM, PAKISTAN

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Age data are a common and important component of population studies of commercial fishes. Otoliths preserve a continuous record of the life cycle from the natal through the adult stage and the analysis of age and growth information contained in the otoliths of fish has become a standard technique in fisheries science. Sagittae otoliths of *Wallago attu* (Bloch & Schneider) inhabiting Mangla Dam, Pakistan were measured, weighed and aged. General relationships were explored among these parameters in relation to fish length and fish weight to both document variability in these objective parameters of otolith growth, and to compare them to subjective otolith age estimates. Symmetry between left and right otoliths within each specimen for otolith length, weight, and height, sorted by management area and gender, was estimated using the paired t-test with level of significance set at 0.05. No statistical size difference between left and right sagittae otoliths was observed. Age at length key and regressions equation for otolith size (length and width) and fish length was also developed.

AVIAN DIVERSITY OF JIWANI RAMSAR SITE ALONG MAKRAN COASTAL WETLANDS COMPLEX PAKISTAN

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Wetlands are valuable ecosystems that occupy about 6% of the world's land surface while in Pakistan covering approximately 10% of the total land area of the country. This study was undertaken to identify and analyzed the population of avifauna of Jiwani Coastal Wetlands Complex. From December, 2007 to December, 2008 study was conducted to observe the population of birds in the study area. During one year, number of species recorded was 112; representing 16 orders and 38 families recorded from the 4600 hectare area. Status of birds was also studied, total count was 15,469 that comprised 53 species as common, 10 rare, 31 scarce, 17 abundant and status of one species was unknown. The 79 species were exclusively as visitor and 33 were resident. Out of 79, 54 species were winter visitor, 11 summer visitor, 10 were irregular year round visitor, and three species were vagrant. Maximum number of species recorded from order Chadriiformes were 34 while in Passeriformes were 30; number of species belongs to order Phoenicopteriformes, Falconiformes, Piciformes, Psittaciformes and Upupiformes recorded equal in numbers; one species in each order. Relatively abundant species were Eurasian Coot *Fulica atra* (0.1577), Common Teal *Anas crecca* (0.0645), Herring Gull *Larus cachinnans* (0.0615), Eurasian Oyster Catcher *Haematopus ostralegus* (0.0606) and Shoveler *Anas clypeata* (0.0511) respectively. Density of birds also recorded in this study which was 3.36 per bird per hectare; Shannon Weiner Diversity Index value of the 112 species of recorded birds was 0.253.

DIET COMPOSITION AND FEEDING HABITS OF *MUGIL CEPHALUS* (FAMILY MUGILIDAE) FROM THE KORANGI-PHITTI CREEK SYSTEM

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Mugil cephalus (Linnaeus, 1758) (family Mugilidae) is a commercially important marine coastal species; their juveniles and adults enter into the estuaries and rivers for feeding. The food and feeding habits of *M cephalus* were studied from the Korangi Creeks System during the years 2000-200 I. Samples for this study were collected monthly by gill nets and their general diet composition was examined. The analysis of stomach . contents were done by a combination of parameters which include relative fullness of stomach; state of digestion; prey frequency of occurrence; feeding frequency;

size of prey; etc. The analysis of the gut contents of this species showed different percentages of sand, mud, detritus, plant materials, diatoms, zooplanktons, annelids, crustacean parts, and miscellaneous items. The diversity of prey items found in the stomachs of *M. cephalus* suggests that mangroves in the Korangi Creek System provide a nutritionally rich environment for the existence of marine fauna and flora. The mangroves soil creates an ideal habitat for the variety of organisms and plays a significant role in the food chain.

ORNAMENTAL CRUSTACEANS OF PAKISTAN—PRICEY AND POORLY STUDIED

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Marine Ornamental industry is a growing and economically important industry. Experts address current issues from a global perspective, covering the full–range of topics from world economics and product demand to aquatic animal health to ethnic and social/cultural concerns. The value of and the interest in marine ornamental species underline the critical need for research in this line involving scientists involved in marine biology and conservation organizations, aquarists at public and private aquaria, fish farmers, advanced hobbyists, fishery biologists, importers and exporters of marine ornamentals, commercial collectors, veterinarians who specialize in fish disease, and businesses that manufacture or sell aquarium media, equipment, and feed. At Pakistan we are aware of crustaceans academic and food values but ignore another value of this important component of invertebrate fauna -trade of its ornamental members .Ornamental fishes have gained much attraction but the crustaceans, marine or fresh water both having some of the most attractive members of animal kingdom in Pakistani waters have not yet gained popularity .These can be sold as indoor pets in aquaria and exported. All commonly traded ornamental shrimps belong to group of decapods crustaceans that incubate their embryos under the abdomen :the order Pleocymata. In this paper this pricey group is discussed in world's perspective and some good Pakistani species are nominated for the purpose. They belong to the families Lysiosquillidae, Squillidae, Epialtidae, Matutidea, Calappidae, Atiydae, Spongicolidae, Hippolytide, Rhynchocinetidae, Hymenoceridae, Gnathophyllidae and Alpheidae. Other potential invertebrates are also highlighted and comments on their sustainable use are also made. Since little or no information is generated on the aquatic ornamental invertebrates of Pakistan, this presentation is a preliminary attempt to assemble back ground information for future workers on aquatic ornamental invertebrates our waters harbour. This paper will contribute to the creation of an economically and environmentally viable future for this dynamic industry worldwide and for its diverse clientele encouraging outreach activities in the conservation and husbandry of marine ornamental species.

NEW RECORDS OF AORID AMPHIPODS (CRUSTACEA) FROM PAKISTAN COAST

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The family Aoridae Stebbing, 1899 is not previously reported from Pakistan, as no serious attempt has been made to study marine amphipods of Pakistan. Five aorid species belonging to two genera are reported, and described for the first time from Pakistani waters. A key for the identification of Pakistani genera and species is also included. A key for the identification of Pakistani genera and species is also included.

BIODIVERSITY AND THE TRADITIONAL THINKING IN THE SUBCONTINENT

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The concept of biodiversity is not clear to most of the people living in this part of the world. The gravity of the situation regarding the loss of precious species is the least understood. The dominant ideology here is that every natural resource is infinite and this Earth is too big, and is created to be exploited by man. None of man's action can significantly affect the composition of Earth's living resources. We are hurt only by sins against traditional social taboos. We miss too much the loss of old cultural traits but have no regard for the ruthless extermination of precious species which are the products of millions of years of evolution. We pollute the streams with effluents from the industries and throw garbage outside our homes, considering that the 'outside' capacity to absorb and dilute is limitless. This also shows that we are introvert, and selfish; our thinking and care limited to our homes and places of economic interest only. This in my opinion is the root cause of apathy to the environmental degradation and the consequent loss of biodiversity. An average person will never let anyone litter his home or office but will not stop any one from polluting the earth and thus reducing its capacity for sustaining the wonderful diversity of plants and animals which is the collective heritage of human beings and a sacred trust that we have borrowed from our children. The protection of trees and animals located in the shrines is very impressive in the subcontinent. They have a spiritual importance. I wish our general view about precious diversity of organisms should have the same spiritual. We also need to change the traditional concept that earth and its resources are infinite.

EXTENSION OF RANGE OF *MACROBRACHIUM SUPERBUM* (HELLER,1862) (CRUSTACEA,PALAEOMONIDAE) –A SOUTH-EAST ASIAN SPECIES INTO PAKISTANI WATERS

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Macrobrachium is a notoriously difficult genus taxonomically, as the morphological plasticity of taxonomically important features for example, the rostrum and/or the 2nd pair of legs change so much and so gradually during their growth (Holthius, 1950) and are influenced by environmental parameters (Dimmock et al., 2004). So far fifteen species of the genus *Macrobrachium* have been reported from brackish and fresh waters of Pakistan (Kazmi&Kazmi, 2010*). Macrobrachium superbum* could not be included in this monograph as the present specimens were sent to Dr X.Li in China for verification. We are grateful to him now for confirming the species which is presently the sixteenth species of the genus from here. *Macrobrachium superbum* previously has been reported from China, Indonesia and Thailand (Li et al, 2003, Bisby et al, 2005).The specimens at hand are first time being reported from the Indian subcontinent and their presence in Pakistan extends the distributional range of the species considerably from southeast Asia to westward .The species is described as an addition to the aquatic fauna of Pakistan.

POPULATION SIZE, DISTRIBUTION AND POPULATION STRUCTURE OF HIMALAYAN IBEX IN KHUNJERAB NATIONAL PARK

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Khunjerab National Park is one of the fourteen national parks of Pakistan. It is located in Gojal valley between the coordinates Longitude 74° 55’ E to 75° 57’ E and Latitude 36° 01’ N to 37° 02’ N. The present study was conducted in KNP from Pamerchi Bridge to Khunjerab top taking road as transect to study population size, distribution and population structure of Himalayan Ibex. Three field surveys were conducted in May, July and September, 2009. The 52 km study area was divided into to 10 vantage points. Here we made observation with the help of binoculars and spotting scope. Digital camera was used to take photographs of Habitat and Ibexes viewed. All the data was tabulated and made respective graphs. A Total of 246 Ibexes were counted during spring season. Out of which 81 were adult males, 93 females, 32 yearlings and 40 were trophy size animals, no kids were found during spring survey, because Ibex gave birth to their young ones during summer season (Roberts 1997). During the survey in

July, we counted about 100 Ibexes at Khunjerab Top, out of which 31 were males, 45 females, 11 yearling and 13 were kids. No trophy size Ibexes were sighted during this survey due to movement of trophies to the higher elevations, and their preferences towards higher elevation due to temperature sensitivity (Aublet, 2009). In September only 3 Ibexes were sighted, out of which 2 were females and 1 was male. This study shows that presently there is an abundant population of Ibex but the results indicate that most of the population is of reproductive and post reproductive stage while Ibexes in pre reproductive stages are very few indicating non violability of population in future. The decreasing population of Ibexes may be due to overgrazing, predation by snow leopard, fox and wolf, migration due to increasing human disturbances and some diseases.

MEAN HAEMOGLOBIN LEVEL DURING PREGNANCY IN THE HUMAN POPULATION OF GILGIT BALTISTAN

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Anemia is regarded as a major risk factor for unfavorable pregnancy outcomes, but there have been no previous studies describing the pattern of hemoglobin concentration during pregnancy in Gilgit-Baltistan and the relationship between altitude and Hemoglobin concentration in the pregnant women living in the different areas of Gilgit- Baltistan. The main objective of this study was to study the hemoglobin levels and prevalence of anemia in the human population of Gilgit-Baltistan and to evaluate potential associations of hemoglobin and anemia with women's characteristics. Out of 361 blood specimens 167 (46.26%) were found anemic. Out of 167(46.26%), 97(58.08%) were anemic with Vitamin B12, 39 (23.35%) were anemic with Iron deficiency and 31(18.56%) were found anemic with megaloblastic anemia. In age wise investigation during pregnancy, out of 361 blood specimen 213 (59.00%) cases were clinically investigated in the age group of (15- 25years), 120 (33.24%), in the age group (26-35), 18 (4.98%) were in the age group of (36-45years), 10 (2.77%) in the age group of (46-55). The anemic cases were found high in the age group of 15- 25years i.e. 96 (45%), 53 (44.16%) in the age group of (26-35years), 13 (72.22%) in the age group of (36-45years) and 05 (50%) in the age group of (46-55). In area-wise distribution out of 361 the highest cases were clinically investigated in district Gilgit i.e. 231 (63.98%), of which 100 (43.2%) were anemic,66 (18.28%) cases were recorded in district Ghizer, out of which 24 (36.36%) cases were anemic, 36 (9.97%)cases were recorded in district Diamer, out of which 28 (77.77%) were anemic and only 28 (7.75%) cases were recorded in district Hunza Nagar and out of which 15 (53.57%) cases were anemic. The highest anemic cases were found in district Gilgit and district Ghizer respectively. In month-wise distribution the highest cases were clinically investigated in the month of July i.e. 106 (29.36%), and lowest cases were investigated in June which were only 30 (8.31%). In July out of 106

clinical investigations 48 (45.28%) were found anemic, and in June out of 30 clinical investigations 18 (60%) were found anemic.

SOCIO-ECONOMIC IMPACT OF TROPHY HUNTING ON COMMUNITIES IN GILGIT BALTISTAN

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The study was conducted to find out the socio economic impact of trophy hunting on communities of the Northern Area from November 2008 to February 2009. The main objective of the study was to find out the benefits, socio economic conditions of the community and impact on ecosystem and conservation of endangered species. For that purpose the data was collected from ten communities randomly from the entire trophy hunting area. Interviews were conducted on the basis of specially designed questionnaires. The questions were asked and filled in by an interviewer in face to face interview with the respondent. Almost all respondent had same perception about general effect of the conservation of wildlife and trophy hunting projects upon the area. Majority (90%) of the local community member were of the view that the project had brought positive change in the income and technical skills of the people living in the project area. Only (10%) people opined that the project changes were not visible. The study concludes that through the conservation of wildlife and trophy hunting projects, now the people are well aware about the wildlife and environment. The people want to protect the wild life for recreational value and preservation of natural environment and their sustainability as well.

THE IMPACT OF PSEUDO-INTELLECTUALISM AND POLITICIANS' SHORT VISION ON THE INITIATIVES AND FOLLOW-UP ACTIONS FOR THE CONSERVATION OF BIODIVERSITY IN PAKISTAN

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Biodiversity has been high on the agenda and there is a high level national and regional interest in the issues related to the initiatives and establishment of biodiversity centers/departments in the institutes ofhigher education in the country. Various educational spots have been designated as places for biodiversity work and numerous biodiversity related projects have been proposed, initiated, poorly implemented or prematurely terminated for known reasons. Most of these projects are of undergraduate

level and are centered on identifying, counting and classifying in order to make species inventories. There might be a limited number of projects dealing with specific issues related to conservation and ecosystem management, and we hardly see projects related to local community involvement in conserving and sustainability. The major goal is to believe after seeing that biodiversity is being conserved in the best interest of human welfare in terms of their socioeconomic status in the long run. This article mentions various factors/obstacle in the way forward. These include lack of pluralism of knowledge, lack of coordination, missing links, degree-oriented research, besides pseudo-intellectualism, and short vision of politicians in the context of Pakistani system. In the end possible measures are suggested to overcome these obstacles

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EARLY EVIDENCES OF FAMILY CERVIDAE FROM THE SIWALIKS OF PAKISTAN

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The early history of family Cervidae is obscure and very little known. Although they have been described from Asia, mainly from the Upper Siwaliks but their record is fragmentary and scanty especially during the Early/Middle Pliocene of Siwalik continental deposits of IndoPakistan. The present paper is an effort to describe this rare fauna from the Early/Middle Pliocene and to evaluate the Cervinae material collected from the Siwalik continental deposits Hitherto.

SOIL MACRO INVERTEBRATES OF BAGROT VALLEY IN CENTRAL KARAKORAM NATIONAL PARK, GILGIT BALTISTAN

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The study was carried out in the Bagrot Valley which is a glacial valley located in the Gilgit district in Gilgit Baltistan. The aim of study was to identify the Biodiversity of soil macro-invertebrates of Bagrot valley. Six sites were selected with in CKNP region

Site 1; near the Hinarche glacier snout;
Site 2; 250 meters below the Hinarche glacier snout;

Site 3; in the lateral moraine;
Site 4; Lateral moraines near a stream;
Site 5; under deciduous trees;
Site 6; under deciduous tree near a stream

Three field surveys were carried out.i.e.17 - 29 June 2008, 25 October – 3 November 2008 and July 2009. Pitfall traps, Malaise traps and Manual collection used to trap the invertebrates in the field. Total 8604 invertebrates collected in the field. Among them highest number of individuals and taxa was recorded in the forest region (site 6) 2470,, less number of individuals were found near glacier (site 1 # and percentage). Among the community of invertebrate's coleopteran (4356 in number and 51.8%) were the dominant community and Chilopoda (78 in number and 0.9%) were lees in number. The other community of invertebrates like Araneae (279 in number and 3.3 %), Hymoptera (2151 in number and 25.6 %), Acari (255 in number and 3 %) and Neuroptera (150 in number and 1.8 %) were moderate at each collection site.

DIVERSITY OF CETACEAN IN PAKISTAN STUDIED THROUGH REMAINS COLLECTED DURING BEACH SURVEYS ALONG SINDH AND BALOCHISTAN COAST

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Over 150 km of sandy beaches and rocky shores have been surveyed along Sindh and Balochistan coast of Pakistan. Over 20 species of cetaceans are known to exist in Pakistani EEZ. Little is known about their diversity in this region especially through remains. A practical research regarding cetaceans has been introduced for last five years in Pakistan. Recently, surveys conducted along the coast of Sindh and Balochistan have given a bright picture of diversity, threats and distribution of cetaceans. By confirming through stranding and skeletal materials found on beaches, a valuable data have been compiled which explains the need of conservation of cetacean in Pakistan. Cuvier's beaked whale (*Ziphius cavirostris*), Sperm whale (*Physeter macrocephalus*), Humpback whale (*Megaptera novaeangliae*), Common dolphin (*Delphinus delphis*), spinner dolphin (*Stenella longirostris*), humpback dolphin (*Sousa chinensis*), Bottlenose dolphin (*Tursiops truncates*) and finless porpoise (*Neophocaena phocaenoides*) have been confirmed through the remains collected during beach surveys. The main objective of these surveys focused on recording strandings, threats, and remains (skeletons) of cetacean.

DORCATHERIUM MAJUS FROM THE CHINJI ZONE, SALT RANGE, PUNJAB, PAKISTAN

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Several new fossil specimens of *Dorcatherium majus* (Lydekker, 1876) have been found and described from the Chinji Formation near Dhok Bun Ameer Khatoon (32° 47' 26.4" N, 72° 55' 35.7" E). The material comprises upper and lower dentitions. This study presents the new data and gives additional information about the distribution of *Dorcatherium majus* from the Lower Silwaliks of Pakistan.

SECTION - VI

POSTER SESSION

EPIDEMIOLOGICAL STUDY AND MOLECULAR DETECTION OF *TRYPANOSOME* INFECTION IN SEMI-CAPTIVE BEARS

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The present study was conducted during the years 2009-2010 when a sudden change was noticed in the physiological conditions of some bears causing few unfortunate deaths. The symptoms of disease included prolonged sleeping periods, raised heartbeat and reduction in appetite. Microscopic examination of blood samples of such apparently sick bears revealed haemoflagellates which were suspected to be trypanosomes. Subsequently, the PCR technique was exploited to detect and hence confirm the presence of trypanosomal species in all bears' blood samples. A total of 28 blood samples from 20 individual bears were screened using three sets of primers specific to the *Trypanosoma evansi* species. The results revealed five positive carriers of the parasite. Two out of these five died prior to any medication while the rest were given an administered dose of Immiticide. The treated bears survived and were assured to be aparasitemic on post-treatment examination.

DIVERSITY OF FECAL MICROFLORA FOR THE SPATIALLY DISTRIBUTED BEAR SPECIES OF PAKISTAN

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The present study was aimed for the analysis of microbial diversity among bear species of Pakistan. Fecal samples of bears were collected from different bear habitats and evaluated for the presence of microbial diversity on the basis of carbon/nitrogen utilization and their biochemical pattern. Microbes identified from fecal samples of 12 bear individuals were: *Acinetobacter baumannii, Burkholderia Cepa (Ps. Cepaci) Citrobacter freundii, Escheria coli, Escheria vulneris, Hafnia alvei, Pantoea-2 (Ent. Agglumerans), Plesiomonas shiglloides, Proteus mirabilis, Pseudomonas aerogenosa, P.fluorescens / putida/ aerognosa, Pseudomonas picketti, Pseudomonas sp, Salmonella para typhi B, Salmonella typhi, Shewenella putrifaciens, Stenotrophomonas maltophilia and Yersinia pseudotuberculosis.* The relative distribution of these microbes among

different fecal samples were: *Pseudomonas* sp > *Acinetobacter baumannii, Citrobacter freundii, E.coli, Pseudomonas sp > Hafnia alvei, P. fluorescens / putida / aerognosa, Pseudomonas picketti, Shewenella putrifacienspresent > Pseudomonas aerogenosa, Salmonella typhi Burkholderia Cepa (Ps. Cepacia) Stenotrophomonas maltophilia, Yersinia pseudotuberculosis >Escheria vulneris, Pantoea-2 (Ent. Agglumerans), Plesiomonas shiglloides, Proteus mirabilisand Salmonella para typhi B.* Colony Forming Unit (CFU) determined for fecal samples of 12 bear individuals were found higher for *P.fluorescens / putida/ aerognosa* (6×10^8) where as where as it was found lower for *Salmonella typhi* (1.5×10^5). It was found that complex ecological interaction was exhibited among the microflora of different bear species which varied from one locality to other locality depending upon the nutritional requirements and habitat conditions of the bear species.

GENETIC DIVERSITY OF MUSK DEER (*MOSCHUS CHRYSOGASTER*) POPULATION IN PAKISTAN BY RAPD ANALYSIS

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The Musk deer (Moschus chrysogaster) is one of the most endangered Himalayan populations that have been drastically reduced to small and isolated ones, mainly because of poaching and its habitat destruction. It is difficult to estimation of population of such little size and nocturnal habitat species. Therefore, molecular techniques were applied for genetic analysis of population. Random amplified polymorphic DNA (RAPD) markers were optimized and used to analyze population diversity and genetic variation within and between different region populations of M. chrysogaster. The results of ten RAPD markers showed substantial genetic variation by amplifying of 141 polymorphic bands with mean 95.5% polymorphism. The estimated mean numbers of loci were 14 per RAPD-PCR markers in genotype samples of musk deer. Analysis of RAPD suggested that Nei's genetic diversity index varied from (0.05 to 0.24) with mean of (0.15± 0.19). The mean value of Gst was 0.45 with the estimated mean gene flow Nm (0.62) for all populations of the studied area. AMOVA and UPMGA cluster analysis was applied to assess levels of genetic differentiation between populations. AMOVA analysis results revealed the variation of 32% within and 68% among the populations. Genetic distance data of RAPD indicated that the samples of musk deer populations are constructed into three main clusters, which having fewer genetic distances than similarity distances. However, this preliminary data obtained on genetic diversity will be useful further monitoring of genome study within the population of such a endangered species and for future conservation.

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

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Antioxidant activity of methanolic leaves extract of the hill toon, *Cedrela serrata* (Royle) was determined *in vitro* by DPPH free radical scavenging assay (using Ascrobic acid as standard) and DNA protection assay. All the analysis was made with the use of UV-Visible Spectrophotometer (DAD 8453, Agilent). The antioxidant activity of *C. serrata* leaves extracts was evaluated using 1,1-diphenyl-2-plerylhydrazyl (DPPH) radical scavenging assay in which all of the leaf extracts showed significant activities compared t standard. Inhibitory concentration (IC_{50}) of Ascorbic acid 5.54 ppm, whereas, IC_{50} for methanolic extract was 4.39 ppm. For n-butanol fraction IC_{50} was <1 ppm and for ethyl acetate fraction and aqueous fraction IC50 was 70 and 5.60 ppm, respectively. DNA protection against hydroxyl radical from hydrogen peroxide was assayed by agarose gell electrophoresis. Plant has no damaging effects on DNA and was able to reduce the hydroxyl radical-induced DNA damage. The results concluded that the extracts have a potential source of antioxidants of natural origin. These results suggest that *C. serrata* may act as a chemo-preventative agent, providing antioxidant properties and offering effective protection from free radicals.

OPTIMIZATION OF THE CONDITIONS FOR THE ASSESSMENT OF GENETIC DIVERSITY IN ASIATIC BEARS

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Extending human activities in wild is a major factor in rapid decline of flora and fauna in Pakistan. Many species are facing the threat of decline in which bear is of serious concern. The present study was designed on the hypothesis that various DNA extraction techniques will produce distinctive yields and will work differently with PCR. DNA was extracted from hair samples, although the quality of DNA extracted was much lower than blood and tissue. Hair samples of different species were collected by hair snare method from twelve locations. Six various reagents, i.e. Chelex, SDS, Triton X-100, SDS+Triton X-100, SDS+NP 40 NP 40+ Triton X-100 were separately used to achieve cell lysis. DNA was extracted using a standard organic extraction protocol. The quality and quantity of the extracted DNA were then evaluated to assess the efficiency of each reagent. After the optimization of the lysis buffer recipes, a comparison among the five different DNA extraction procedures was made. Four SSR markers, including

MSUT-3,MSUT-6, G10H and G10J were used to amplify four distinct loci of the bear genome. The amplified fragment of each SSR locus was assessed based on electrophoretic mobility, and SSR profiles were scored for the presence or absence of each band. The genetic distance was used to reveal the association between populations based on un- weighed pair group with arithmetic averages (UPGMA) in statistical program "Statistica" version 7. Based upon the limited data the number of alleles, allelic frequency and gene diversity of four SSR markers suggest total 10 0f alleles present in these markers. Four alleles were identified for MUST-6 (4), when two alleles each could be separated MSUT-3, G 10-J, and G 10-H. The analysis of the genetic distances between individual bears across using mapped SSR markers revealed that these ranged between 0.40 and 1.00. No previous comparable study is available on the bear population of Pakistan. The present result does not reflect the level of genetic diversity, expected for bear population of the area and required further studies. As no previous study is available on such parameters in bears or my other group. Therefore, these results cannot be viewed as complete deriving inferences. However, this work may hint towards adequate optimization of the DNA study techniques and can be pressed into service or a larger scale for arriving at definite inferences on population isolation.

COMPARISON OF THE GENETIC DIVERSITY BETWEEN MALE AND FEMALE INDIVIDUALS OF PHEASANTS OF NORTHERN PAKISTAN

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Present study is concerned on the genetic distance of six male and female species i.e., Koklass Pheasant, Himalayan Monal, Western Tragopan, White Crested Kalij Pheasant, Cheer Pheasant and Blue Peacock. DNA was isolated from blood samples, separately from both the male and female representatives of each species. DNA was amplified through using 10 pheasant's specific Simple Sequence Repeat Primers (SSRs). Genetic distance (GD) among the genotypes was estimated using unweighted pair of group arithmetic mean (UPGAM) procedure and bivariate data was also used to construct a dendrogram using Computer program POPGENE Ver. 3.2. The results revealed low to medium of genetic distance (GD) 3,4, 8, 9, 28 and 46% were obtained between males and females of Koklas Pheasant, White Crested Kalij Pheasant, Western Tragopan, Blue Peacock, Himalayan Monal and Cheer Pheasant, respectively. Genetic distance. among used pheasant's genotypes supported their ancestral development. Phylogeny of the six species of pheasants was constructed using cluster analysis. Phylogenetics of the species was analyzed through constructing a dendrogram on PCR based amplification of DNA data showed that the pheasant genotype sorted into three main clusters i.e. A, B and C. All the male and females of each species clustered into their own groups, e.g., Koklas

male and female clustered into group A, HimalY9P Monal, Western Tragopan and Blue Peacock clustered into group B. The White Crested Kalij male and female were clustered in group C. Unexpectedly the male and female Cheer Pheasant were sorted into different clusters i.e. groups C and B, respectively, which need further scientific elaborations.

ON THE INDIVIDUAL, SEX AND AGE DIFFERENTIATION OF THE INDIAN HOUSE CROW (*CORVUS SPLENDENS*) "CAW" CALL

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Considering the increasing importance of acoustics studies in population ecology recordings of 500 (275 ♂♂, 145 ♀♀, 80 juveniles) calls of the Indian House Crow (*Corvus splendous*), carried out in the morning - mid - afternoon hours in January, 2009 from different urban areas of Potohar, Pakistan using Sony CFS 1030 S sound records (sampling rate = 48 KHz) were edited using Sound Analysis Pro (Version 1.02). software using FFT method rate 50%, data window 9.27 ms, advanced window 1.36 ms to select 60 (37 ♂♂, 17 ♀♀, 6 Juvenile ♂♂) good quality spectrograms. The spectrograms were characterized by rapid frequency modulations using six (call pitch, mean pitch goodness, mean frequency of the calls, frequency of modulations, mean amplitude modulation, mean wiener entropy) acoustic parameters and the significance of different of difference analysis using Multivariate Analysis of Variance and Discriminate Function Analysis. Calls could be assigned to correct individual in 10.8% males, 21.0% female and 42.9% juvenile, which was significantly higher than the percentage of the correct classification per chance. The calls could be correctly attributes to correct sex in 88.5% and to correct age group in 80.6% of cases.

STUDY OF MALIGNANT CANCER (*IN VITRO*) USING LASER LIGHT

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The cytotoxic and phototoxic effect have been investigated on the Hep2c (human larynx squamous cell carcinoma) cell line as an experimental model using photofrin® mediated photodynamic therapy (PDT). The current study emphasized the apoptotic effect on the mentioned cell line by using diode laser (635 nm) as a source of illumination and initiation of photodynamic action. The optimal uptake time of Photofrin® for Hep2c cell line was investigated by means of spectrophotometric measurement. Quantification of the live cell population was determined by means of neutral red assay (NRA). The

spectrometric measurement showed that after 46 h incubation, the maximal cellular uptake of photofrin® was achieved. No significant cytotoxic effects on Hep2c cells were observed due to light doses or photosensitizer, when studied independently of each other but the combine action of both gives good result and photofrin® showed good anti-tumor effects. In the second experiment 24 well plate has been used, each column (consists of 4 wells) has been irradiatd for different cell lines samples e.g. (50 µL of ZnO, 50 µL of photofrin®, 25+25 µL of ZnO and PHotofrin ®, 50 µL of ALA). After 24 h of incubation, the above mentioned plate has been examined under confocal microscope. The experimental results shows that ZnO itself does not produces toxicity into liver cancer cells but whenevr it is exposed in the presence of a photosensitizer, photochemical reaction occur, therefore, different values of cellular viability at different concentration has been obtained. In the third experiment, cytotoxicity of HepG2 was experimentally determined by using cell cultured into 96 well plates. The cytotoxicity values at different exposure times were studied using NRA technique. This result shows that ALA owing significant cytotoxicity as compared to the other drugs (ZnO and Photofrin).

POPULATION SIZE, STRUCTURE, AND CONSERVATION STATUS OF HIMALAYAN GREY GORAL IN PAKISTAN

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The Himalayan grey goral, *Naemorhedus goral bedfordi* (Artiodactyla: Bovidae) is listed as "lower risk: near threatened" on the IUCN Red List, but was last assessed in 1996. Between 2002–04, we conducted surveys of goral distribution and population structure in the western limit of its range, in northern Pakistan and Kashmir (Northern Areas and Azad Kashmir). Since the 1996 assessment, human and livestock populations have grown in the region, potentially impacting grey goral populations. We established 98 stands and counted the number of gorals observed from a prominent peak over area falling within visual range of telescope. Gorals were present between 994 m and 2,461 m, with animals moving to lower altitudes during winter. We find 817 goral distributed among 7 subpopulations, with the largest subpopulation of 200 goral in Azad Kashmir. Across all subpopulations, sex ratios were skewed with nearly 2 females per male observed. We also observed 0.31 sub-adults per adult female. These subpopulations are politically and spatially disjunct from the main portion of the range further east, and warrant a vulnerable status under IUCN criteria. Population status can be improved by creating and managing habitat corridors to link subpopulations. Furthermore, the Azad Kashmir subpopulation should serve as a focal point for future management. It is the largest subpopulation and its long-term viability will be linked to international conservation agreements between Pakistan and adjacent India.

STUDIES ON THE CHANGES IN AVIAN COMMUNITY STRUCTURE OF THE RIVER RAVI, LAHORE

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The study was conducted to analyze the effect of food energy infused into ecosystem in the form of anthropogenic organic solid waste and ritual of offering meat in the peri-urban Lahore (Pakistan) between November (2007) and October (2008) at three disturbance level including low (Baboosab), medium(Shahdara) and high (Sagian). There were 20 species recorded at low and medium disturbed site and 5 species at highly disturbance site, suggested the gradual decrease in species richness depending on disturbance levels. The population densities of 15 species (Spanish Sparrow, Asian pied Starling, Common Starling, Rose Ring Parakeet, Green- Bee Eater, Crested lark, Common Myna, Oriental Magpie Robin, Grey Wagtail, Plain Prinia, Common Hoopoe, Pied Bush Chat, long Tail Shrike, Black Drongo and Yellow Wagtail) directly declined with increasing disturbance and no population of these was recorded at high disturbance site and were regarded as avoider of meat subsidy. The population densities of Rock Pigeon, House Crow and House Sparrow though declined with increasing disturbance levels yet some populations were maintained at all disturbance levels. The densities of Black Kite and Bank Myna gradually increased with the increasing energy subsidy and were regarded as exploiter species. The bird community at low disturbance site presented a normal trophic structure, while the different levels of inverse trophic pyramid appeared with increasing disturbance. The infusion of food energy at higher trophic level and exclusion of avoider and adopter species explained the appearance of inverse trophic structure at higher disturbance levels.

TO STUDY THE RELATIONSHIP BETWEEN BODY MEASURES AND WEB CHARACTERS OF *TETRAGNATHA JAVANA* (ARANEAE: TETRAGNATHIDAE)

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Present study was conducted to find the relationship between body measures and web characteristics of *Tetragnatha javana* (Araneae: Tetragnathidae). For this purpose, female spiders were collected from the agricultural fields bfUniversity of the Punjab, Lahore, Pakistan. The spiders constructed horizontal as well as inclined webs at the height that ranged from 35 cm to 97 cm above ground. The average diameter of the web was 16±4.9 cm. The web capture area of *T. javana* showed a positive correlation with carapace width and body length. Web characteristics (number of radii, number of spirals and mesh height) were not correlated with any of the body measures (i.e., carapace width,

total length and wet weight). *T. javana* construct webs of different sizes at different heights but always maintain the basic web architecture (number of radii, number of spirals and mesh height). Most of the prey items recorded from the webs belonged to orders Diptera, Homoptera and Lepidoptera.

DETECTION AND PROPAGATION OF ENTEROVIRUSES FROM SEWERAGE WATER

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Study was conducted to testify the prevalence of different types of Enteroviruses in sewerage water. Primers were designed and PCR conditions were optimized for Entero coat protein gene. Samples were collected from different areas of Rawalpindi, Islamabad and Lahore. Various enteroviuses including Reovirus, Achi virus, Schfoldvirus, Polio virus, Enterovirus, Astro virus and Torovirus were detected in the waste water. Enterovirus was further propagated in Hela cell line and a 450 base pair region of the viral genome was cloned in the TA vector for phylogenetic analysis.

STABILITY OF BIOCORROSION PROTECTING EXOPRODUCTS OF *BACILLUS THURINGIENSIS*-SN8.

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Microorganisms and their exoproducts also play a role in inhibiting the corrosion process. Stability of corrosion inhibiting exoproduct(s) of *Bacillus thuringiensis*-SN8 was evaluated at different pH and temperatures. Cell free cultural fluid (CFCF) of *Bacillus thuringiensis*- SN8 was stable in the pH range of 3-7 and maximum stability was observed at pH 7. Bacterial exoproduct(s) were found unstable at pH 9 and 11 and at above 100°C. Protective effect of CFCF of *Bacillus thuringiensis*-SN8 on metal corrosion was investigated by employing mild steel coupons (MSCs). MSCs exposed to corrosion causative bacteria *Bacillus cereus*-SNB4 showed maximum corrosion rate and average percent weight loss (APWL) of 0.03 $mgdm^{-2}d^{-1}$ and 3.44%, respectively after 70 days. Coupons exposed to the *B.cereus*-SNB4 in the presence of CFCF of the antagonistic bacterium *B.thuringiensis*-SN8 showed least values of corrosion rate and APWL i.e., 0.0047 $mgdm^{-2}d^{-1}$ and 0.397%, respectively. When experimental fluids were processed for the estimation of iron, protein and carbohydrate contents, iron concentration appeared more in control and *B.cereus*-SNB4 inoculated culture having respective values of 46.93ppm and 30.11ppm. at the end of experiment. Carbohydrate

and protein contents were found higher in CFCF of *B.thuringiensis*-SN8. Conclusively, antagonistic microorganisms or their exoproducts thereof have a potential for controlling MIC.

POTENTIAL OF CHITINOLYTIC BACTERIA FOR ENVIRONMENTALLY COMPATIBLE TERMITE CONTROL

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Seventy two strains of chitinase producing bacteria were isolated from termite influenced soils on a selective medium containing g/l: Chitin, 1.5; $(NH_4)_2SO_4$, 1.0; KH_2PO_4, 0.2; K_2HPO_4, 1.6; $MgSO_4.7H_2O$, 0.2; NaCl, 0.1; $FeSO_4.7H2O$, 0.01; $CaCl_2.2H_2O$, 0.02. Majority of the isolates grew well at 30ºC and agitation at 120 rpm. The isolates were selected for further study on the basis of higher ratio of their zones of clearance to colony size following growth on the select medium. Two bacterial isolates designated as JF17 and JF38 were cultivated in the select medium broth and the 5-days old cultures were employed in biological assays for assessing their termiticidal potential. Filter paper soaked with the cultures caused 100% mortalities of the termites within 24 hrs. The enzymes as well as the bacteria and their endospores' diversity covering a range of pH and temperature stabilities can be employed for making termite resistant artificial wood, cardboard and other termite sensitive materials. Application of this biological control would strengthen environmental concern addressing improvement of the indoor environments and improve health of workers of the wood industry.

TERMITICIDAL EFFECTS OF FOUR DIFFERENT PLANT ESSENTIAL OILS AGAINST *MICROCEROTERMES CHAMPIONI* (SNYDER)

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The effect of four plant essential oils was studied on *Microcerotermes championi* (Snyder). The termiticidal activity of Clove oil *(Eugenia caryophyllata),* Eucalyptus oil *(Eucalptus globulus),* Orange oil *(Citrus sinensis)* and Ajwain oil *(Trachy~permum copticum)* was observed at the rate of 15-114 gram of soil for two weeks. 300 healthy termites worker were exposed. Three replicates were maintained for each setup. 100% mortality was recorded within one day after exposure to Ajwain oil. However in case of Eucalyptus oil, Orange oil and Clove oil 100% mortality was obtained within three days of laboratory trial. Hence all oils proved their toxic effects in causing 100% mortality of *M. championi* as compared to control setup.

FATE OF THREE YEARS SOIL BURIED MILD STEEL EXPOSED TO CORROSION CAUSING *PAENIBACILLUS DENDRITIFORMIS* MB-14 AND ITS ANTAGONIST *BACILLUS SUBTILIS* MB-6

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Process of corrosion is known to be influenced by abiotic and biotic components of environment. For the latter aspect, recent studies have identified bacteria, responsible for initiating and escalating the metal corrosion process, while microbes inhibiting growth and deteriorative activities of corrosion causing ones are also known. In the present study, mild steel coupons (MSCs) lost more weight in the presence of *Paenibacillus dendritiformis* MB-14 as compared to the control coupons. While those exposed to *Bacillus subtilis* MB-6 had much lesser metal loss as compared to those exposed to *Paenibacillus dendritiformis* MB-14. MSCs exposed to the co-culture indicated corrosion protective role by bacterium *Bacillus subtilis* MB-6. Regarding the effects of nutrient substances; pH, conductivity, moisture contents, and C.F.U. of nutrient added soils increased as compared to the nutrient non added soils. The results indicate the importance of physiochemical and bacterial content of soils, through which the transporting metallic pipelines are to be passed, for protecting and controlling of the process of corrosion.

EFFECT OF DIFFERENT DIETS ON THE PYRETHROID DEGRADING ENZYMES OF *LYCOSA TERRESTRIS*

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Spiders are among the most adverse groups of general predators in agroecosystems and can suppress the population of various insect pests. Present study was designed to assess the effect of diet quality on detoxifying enzymes of wolf spider *(Lycosa terrestris)* exposed to sublethal doses of pyrethroids. For this purpose two insecticides (Cypermethrin and Deltamethrin) and two diets i.e., aphid or plant hopper (low quality) and larvae of *Drosophila* (high quality) were selected. Biochemical analysis were performed to determine the effect of insecticides on the the detoxifying enzymes viz., acetylcholinesterase (AChE) carboxylesterase (CarE) and glutathione S-transferase (GST). Result indicated that both insecticides were highly toxic to *L. terrestris,* however, data analysis showed that in starved and low . quality diet spider AChE activity was inhibited, while in high quality diet spiders the activity of AChE was induced by insecticides. Whereas CarE showed a strong inhibition in both diet groups and this inhibition is significantly increased with increasing doses of each insecticide. While the

results demonstrated that role of GST was unclear on the toxicity of pyrethroid insecticides. The detoxification abilities of *L. terrestrist* increased with the improvement in the diet quality. It is hereby concluded that resistance to insecticides depends on the quality of food available to the spiders.

DETECTION AND CHARACTERIZATION OF CHITINASE IN GRAM-POSITIVE BACTERIA

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Chitinase is an enzyme which is of significant importance in paper and textile production, bioremediation of heavy metal contamination, as additive in animal feed and as consumer products such as cosmetics. In this study 12 bacterial isolates were screened for chitinase activity and 3 were further selected based on enzyme activity (%). All bacterial isolates showed optimum growth at 37°C and at pH 7. All isolates were gram positive and two bacterial isolates on the basis of biochemical tests were identified as *Bacillus cereus* and one was not identified yet. Growth curves in the presence of chitin were also determined. Enzyme was characterized for its optimum temperature and pH activity. Enzyme activity in the presence of various metal ions was also determined.

ISOLATION, CHARACTERIZATION AND OPTIMIZATION OF LIPASE FROM BACTERIA INHABITING OIL CONTAMINATED ENVIRONMENT

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Lipases are an important group of biotechnologically relevant enzymes and they find immense applications in food, dairy, detergent and pharmaceutical industries. Lipases are hydrolases, which are able to act under aqueous conditions on the carboxyl ester bonds present in triacylglycerols to liberate fatty acids and glycerol. Three bacterial isolates were obtained from oil contaminated effluents of different industries on Sheikhupura Road and showed high production of lipase by the formation of zones of hydrolysis on PYA olive oil agar medium. On the basis of biochemical and molecular characterization the isolates have been identified as *Pseudomonas aeruginosa*, *Serratia* sp. and *Bacillus megaterium*. *P. aeruginosa*, *Serratia* sp. and *B. megaterium* showed lipase activity of 800, 600 and 1800 EU/ml, respectively. Optimum growth temperature for *P. aeruginosa* and *B. megaterium* was 37°C, while for *Serratia* sp. it was 28°C. All bacterial isolates showed maximum growth at pH 7. Olive oil and Tween80 were found

to be inducers of lipase while concentration of oil greater than 2% retarded the growth of the isolates. These bacterial strains were also able to produce alkane hydroxylase and biosurfactants, which adds to their lipotytic activity and biodegradation potential. These bacterial strains have substantial potential for industrial lipase production.

CHARACTERIZATION AND OPTIMIZATION OF PHYTASE FROM YEASTS ISOLATED FROM INDUSTRIAL WASTEWATER

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Phytase is an enzyme which is of significant importance in poultry, health-care and environmental perspectives. In this study 13 yeasts isolates were screened for phytase activity and 9 were found to be phytase producing and verified by solid plate assay, turbidity assay and enzyme assay. Three yeasts isolates were further selected based on their high turbidty in broth turbidity assay, zone of hydrolysis in solid plate assay and enzyme activity (%). Yeast Isolates YR and 21A showed maximum enzyme activity of 64% and 59%, respectively. Growth curves were also determined. All yeast isolates showed less growth in phytic acid supplemented medium as sole phosphorous source as compared to KH_2PO_4 supplemented medium. Enzyme was characterized for its optimum temperature and pH activity. Enzyme activity in the presence of various metal ions was also determined.

ISOLATION AND BIOCHEMICAL CHARACTERIZATION OF CADMIUM RESISTANT BACTERIA FROM INDUSTRIAL WASTEWATER

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Heavy metal contamination due to natural and anthropogenic sources is a global environmental concern. Heavy metals are the most abundant pollutants in the sewage and in wastewater and are one of the main causes of water and soil pollution. Heavy metal pollution is badly affecting human health as it gets entry into human food chain and drinking water. It is important to establish an efficient and low cost method for the removal of toxic metal ions. Microorganisms with the ability to grow in the presence of heavy metals and with a significant metal uptake have a potential use in bioremediation of polluted waters. The present research work aims at the isolation, growth and tolerance to cadmium toxic ions of microorganism from industrial wastewaters of different industrial areas of Pakistan. Samples were collected from water reservoirs receiving

industrial effluents from different areas of Pakistan. Screening was done and three bacterial isolates that showed maximum tolerance against Cadmium were selected. Minimum inhibitory concentration (MIC) of these isolates against different metal ions was also determined. Two isolates were able to grow at 3000 µg/ml of $CdCl_2$ and one bacterial isolate was able to resist cadmium up to 2500 µg/ml. Biochemical characterization was performed to identify these Cd-resistant bacterial isolates.

ANTIOXIDATIVE RESPONSE TO CADMIUM AND COPPER IN THE YEAST CELLS

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Reactive oxygen species (ROS) are formed in biological systems as part of normal metabolism. ROS production increased in microbes under stress conditions and to avoid damage caused by these excess ROS, organisms have developed elaborate mechanisms to manage them at sustainable levels. Antioxidant enzymes play an important role in lowering the ROS levels and helping avoid oxidative stress. Three yeast isolates were isolated from industrial wastewater, Sheikhupura. Yeast isolates, 21A and BR, were able to resist cadmium up to 5mM while isolate 4S resisted copper up to 5mM. Optimum growth temperature for isolates 4S and 21A was 30ºC and for isolate BR it was 37ºC. All yeast isolates showed optimum growth at pH 6. Yeasts were characterized through 18s rDNA. The levels of antioxidants: glutathione (GSH) and non-protein thiols (NPSH) and the activities of antioxidant enzymes were influenced by cadmium treatments.

ENVIRONMENTAL POLLUTANTS IN HUMAN BODY

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The haphazard use of conventional pesticides resulted in the deposi,jon of toxic cherllicals into non target organism, including human being. These chemicals have been reported to the cause of various human diseases viz: liver and kidney diseases ete. many of these chemicals become the part of the adipose tissue, blood, milk and other biological compartments. Outside Pakistan, in many of the foreign countries the subject is taken very seriously and there are many reports available. Globally the organochlorine compounds become banned but in Pakistan such chemicals remain in use by many authorities in public sector and in health sector even by farmers. In the present investigation many of the organochlorine and PCB were tested in blood, adipose tissues, milk and umbilical cord samples obtain a clear picture of such toxic chemicals in Karachi

people. The patkrn of pest:_cidcs occurrence in the various tissues can be written in the order viz. milk < umbilical cord > adipose tissue < serum. The biological material of human covers a wide range of variation like blood serum, adipose tissues, milk and umbilical cord samples. The heaviest deposited of PCBs and Ocs was found in women matrices (milk and umbilical cord). This kind of finding was never reported before as this was the first attemptin women tissues.

SCREENING AND CHARACTERIZATION OF HIGH ACTIVITY OF PROTEASE EXHIBITING BACTERIA FROM INDIGENOUS ENVIRONMENT

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Microbial protease represents about 60% of all the industrial enzyme's sales in the world due to their applications in several industrial sectors like in the detergent, food, pharmaceuticals, chemicals, leather, paper and pulp and silk industries. All the bacterial isolates showed the ability to readily utilize complex protein substrates. Biochemical characterization showed that two isolates belong to genus *Bacillus* while two were characterized as *Lactobacillus* sp. and *Staphylococcus aureus* respectively. The optimum temperature for *Bacillus subtilis* and *Bacillus megaterium* was 45^{o}C while for *Lactobacillus* sp. and *Staphylococcus aureus* was 37^{o}C. The optimum pH for *B. subtilis, B. magaterium* and *S. aureus* was 7 while for *Lactobacillus* sp. it was 5. The growth curves of bacterial isolates showed maximum cell density at 24th hour of incubation for *B. subtilis* and *B. magaterium.* While for *Lactobacillus* sp. at 16th hour and for *Staphylococcus aureus* at 12th hour of incubation maximum cell density was obtained. Relative protease activity of *B. subtilis* and *B. magaterium* was found to 2600% and 2300%, respectively. *Lactobacillus* sp. and *S. aureus* showed maximum enzyme activity of 340% and 70%, respectively. It was revealed that proteases are active over a wide range of pH and temperature, *i.e.,* 5-9 and 30-90^{o}C which showed their alkaliphilic and thermo-tolerant nature.

DETECTION OF P53 GENE MUTATIONS IN BRONCHIAL BIOPSY SAMPLES OF PATIENTS WITH LUNG CANCER

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Lung cancer is the malignant transformation and expansion of lung tissue. It is the most lethal of all cancers worldwide, responsible for 1.2 million deaths annually. The

goal of this study was to detect the p53 gene mutations in lung cancer, in local population of Lahore, Pakistan. These mutations were screened in the bronchial biopsy lung cancer tissue samples. For this purpose microtomed tissue sections were collected. Following DNA extraction from tissue sections, the p53 mutations were detected by amplifying Exon 7 (145 bp) and Exon 8 (152 bp) of the p53 gene. PCR then followed by single-strand conformation polymorphism analysis for screening the p53 gene mutations. The results of SSCP were visualized by silver staining. The results showed different banding pattern indicating the presence of mutations. Majority of the mutations were found in Exon 7. Exon 7 of p53 gene may be the mutation hotspot in lung cancer. In lung cancer, the most prevalent mutations of p53 gene are G~T transversions; other types of insertions and deletions are also expected, however, the exact nature of mutations in presented work could be confirmed by direct sequencing.

HORMONAL AND HEMATOLOGICAL PROFILES FOLLOWING INTENSE MILITARY TRAINING

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The study was de~igned to investigate the hormonal and hematological variations in male soldiers (n=50, ages 25-35 yrs) before and after intense field training including strenuous physical training, obstacles, negotiation involving both mental and physical faculties, long marches and maneuvers during day and night, and combat practices at 45-50°C, for a period of four weeks. Blood sampling was performed at Army Installation, Lahore. Cortisol and testosterone were measured by Enzyme linked immunosorbent assay (ELISA). Hematological parameters were analyzed by automated hematology analyzer Sysmex K-1000. Cortisol increased, whereas, testosterone decreased significantly in post compared to pre exercise cohort of the study. Significant elevations were observed in red blood cells count, hemoglobin concentration and platelets count, however, white blood cells decreased significantly following intense training as compared to pre exercise cohort. These results suggest that prolonged and repeated exercise such as that encountered in a military training program induces immune impairment as is indicated by reduced leukocyte count. and elevated cortisol. Lowered testosterone reflects decreased steroidogenesis as a consequence of the physical and psychological strain. The hormonal variations suggest the need for energy provision during recovery and regeneration phases imposed by the tissue damage of repeated muscular contractions.

LINKAGE STUDIES OF PRIMARY MICROCEPHALY IN PAKISTANI POPULATION

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Microcephaly (MCPH) is a condition with architecturally normal brain but reduced head of occipito-frontal circumference below -2 or -3 standard deviation. Primary microcephaly is present at birth leading to non-progressive mental retardation, affecting 2-2.5% of the total population and is more prevalent in Asia and Arab than Europe. About 50% individuals with primary microcephaly have an *ASP M* mutation that is the most common cause of MCPH in Pakistan. In this present study exclusion mapping of three different families of primary miqrocephaly was done. DNA was isolated from three affected families (MCPl, MCP2, and MCP3) and PCR was followed by non-denaturing polyacrylamide gel electrophoresis (PAGE). One family MCPl not linked with all known seven loci (MCPHI-MCPH7), second family MCP2 linked at locus 2 on chromosome 19q13.1-13.2 and third family MCP3 showed complete linkage with locus5 (MCPH5) on chromosome 1 q31. The heterozygous pattern was found for MCPl family during screening of all known seven loci. This finding suggested the plausibility of a novel locus, so genome wide search of MCPl family should be supportive in future, which would lead to the mapping of a novel region associated with primary microcephaly.

EFFECTS OF THE SAME FATTY DIET COMPOSITIONS ON THE HEMATOLOGY AND SEROLOGY FEMALE WEANING WISTAR RATS

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Liver is termed as fatty when the fat contents in its hepatocytes exceed from 10% by weight. Non alcoholic fatty liver disease (NAFLD) is developed in the absence of alcoholism. The successive stages of NAFLD include steatosis, usually progresses to steatohepatitis, fibrosis, cirrhosis, and eventually to liver cancer. Eighty weaning Wistar rats of 30g *were* taken. Animals *were* divided into four groups 0, I, II, and III (n=20). All the groups *were* further divided into male and female groups (n=10) and designated with the prefix M for males and F for females. The two control groups *were* designated as M0 and F0 groups, Among the six experimental groups MI and FI *were* fed with diet "A" (33% fat + 33% carbohydrate + 34% rat chaw). The groups MII and FII were fed on the diet "B" (A + 5% *Nigella sativum)* and group MIII and F III with diet "c" (A + 5% *Psyllium*). Animals *were* kept in 12h dark and light cycle and had *ad libitum* access to water and feed. Animals were given anesthesia Norcuron (150 µl/100g) of body weight, and sacrificed on gaining the body weight of 300g or more, blood was drawn directly

from heart, and organs *were* excised, washed with 0.9% saline and stored in 10% formalin for preservation. About 2ml of blood was taken in EDT A coated tubes and the rest of the blood was centrifuged at 5000 rpm for 20 minutes. Complete blood cells count was performed in a regular bioclinical lab with the help of commercially available method. The serum was then used for biochemical analysis. The electrolytes K+' Mg H, and Cr *were* estimated with the commercially available kit by Centronic. Complete blood cell count was performed on all the groups under study and when compared with control groups showed a significant increase of (HCT)% in females ($P<0.001$) and males($P<0.01$) , (HOB) in females ($P<0.01$) and males, (MCV) in males ($P<0.001$) and female ($P<0.05$), whereas the concentration of RDW% ($P<0.01$), PLT ($P<0.01$) and RBC ($P<0.01$) increased significantly in males while decreased in females ($P<0.001$), ($P<0.01$) and ($P<0.01$) respectively, Whereas the concentration of (MCHC) decreased significantly in males ($P<0.001$) and females ($P<0.001$). However the changes in WBC and MCH *were* not significant in both males and females. Biochemical tests applied on serum showed a significant higher serum conc. of K+ ($P<0.05$) in both males and female groups. The serum cr concentration was significantly lower in females ($P<0.01$) and males ($P<0.001$).in all the experimental groups in comparison with control group. In case of Mg H changes *were* not significant in both males and females. Conclusion: Taken together these findings we can conclude that high fat contents induce significant changes in hematology and serology of the rats that varies gender wise.

CELLULOSE DEGRADING YEASTS ISOLATED FROM LOCAL ENVIRONMENT

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Cellulose degradation with the help of microbial enzyme is significant in environment rich in plant organic matter. This study was carried out to isolate cellulose degrading yeasts from local environment. All isolates (designated as A2, B5, W2 and W3) showed optimum growth at 37°C and at pH range of 6-8. The dominant form of the enzyme was found to be extracellular and yeast isolates showed maximum enzyme production after 5 days of incubation. The optimum temperature for the activity of cellulase determined was 40°C for A2, B5 and W2 isolates and 90^0C for W3 isolate. The optimum pH for cellulase activity was 9 for A2 isolate, 7 for B5 and W3 isolates and 8 for W2 isolate. Cellulase activity of A2 was increased (6%) in the presence of Zn^{+2} while other metals have slight inhibitory effect on enzyme activity. Similarly enzyme activity of B5 was enhanced 1.1% and 7.5% in the presence on Mn^{+2} and K^+ and other metals have inhibitory effect. In case of W2 isolate no metal has positive effect on enzyme activity. Cellulase activity of W3 was increased 4.2%, 6.5%, 7.2% and 15.2% by Ca^{+2}, Mg^{+2}, Mn^{+2} and K^+, respectively.
