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OF

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Volume 25, 2005

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

TWENTY FIFTH PAKISTAN CONGRESS OF ZOOLOGY

held under auspices of

THE ZOOLOGICAL SOCIETY OF PAKISTAN

at

SINDH AGRICULTURE UNIVERSITY, TANDOJAM

March 1 – 3, 2005

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ACKNOWLEDGMENTS

Sindh Agricultural University, Tandojam, hosted the 25th Pakistan Congress of Zoology (International).

The Zoological Society of Pakistan expresses its deep gratitude to the Vice Chancellor, Sindh Agricultural University, Tandojam and faculty members and students of the University for extending warm hospitality.

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TWENTY FIFTH PAKISTAN CONGRESS OF ZOOLOGY (INTERNATIONAL)

SINDH AGRICULTURAL UNIVERSITY, TANDOJAM

MARCH 1 – 3, 2005

PROGRAMME

TUESDAY, MARCH 1, 2005

09:00 AM	Registration
10:00 AM	Inaugural: Recitation
10:05 AM	Welcome Address by Vice Chancellor, SAU, Tandojam
10:20 AM	Report by Secretary, Zoological Society of Pakistan.
10:35 AM	Key Note Address by President, Zoological Society of Pakistan
10:50 AM	Distribution of Medals and Awards
11:10 AM	Address by the Chief Guest
11:25 AM	Vote of Thanks by Dean, Faculty of Sciences
11:30 AM	Refreshment

12:00 AM

JOINT SESSION I: (Plenary Lectures)

Chairperson: Dr. Mazhar-ul-Haq Siddiqui
Vice-Chancellor, University of Sindh, Jamshoro

Speakers:

1. Dr. Fereidoon Owfi
   Iranian Fisheries Research Organization, Tehran, Iran.
   Species and habitat diversity of fishes in Iranian territorial waters of the Persian Gulf, Hormoz Strait and Oman sea.

2. **Dr. Rup Lal**
   Professor of Molecular Biology, Department of Zoology, University of Delhi, Delhi, India.
   Diversity and evolution of *lin* genes in hexachlorocyclohexane degrading *Sphingomonas paucimobilis* strains and their use in developing bioremediation biotechnologies.

01:00 PM Lunch and Prayer

HALL – 1

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

SESSION I

	Chairperson:	Prof. Dr. Shamsuddin Shaikh
	Co-chairperson:	Dr. M. Ismail Rind
02:00 AM	Paper reading	
04:30 PM	Tea Time	

SESSION II

	Chairperson:	Dr. N.N. Ansari
	Co-chairperson:	Dr. Tasawar H. Khan
05:00 PM	Paper reading	
06:30 PM	Prayer	

SESSION III

	Chairperson:	Prof. Dr. Afsar Mian
	Co-chairperson:	Dr. M.U. Samo
06:45 AM	Paper reading	
08:00 PM	Dinner	

HALL – 2

SECTION II: PEST AND PEST CONTROL

SESSION I

	Chairperson:	Dr. Sana Ullah Khan Khattak
	Co-chairperson:	Dr. Tajwar Sultana
02:00 PM	Paper reading	
04:30 PM	Tea Time	

SESSION II

	Chairperson:	Prof. Dr. M.K. Lohar
	Co-chairperson:	Prof. Dr. M.S. Wagan
05:00 PM	Paper reading	
06:30 PM	Prayer	

SESSION III

	Chairperson:	Prof. Dr. G.M. Rahu
	Co-chairperson:	Prof. Dr. Syed Kamaluddin
06:45 PM	Paper reading	
08:00 PM	Dinner	

WEDNESDAY, MARCH 2, 2005

JOINT SESSION II: (Plenary Lectures)

Chairman: Dr. Asadullah Kazi
Vice-Chancellor, ISRA University, Hyderabad

09:00 AM Meritorious Prof. Dr. A.R. Shakoori
School of Biological Sciences, University of the Punjab, Lahore.
Role of Biotechnology in Livestock Development.

Chairman: Dr. Bashir Ahmad Shaikh
Vice-Chancellor, Sindhg Agriculture University, Tandojam

09:30 AM Dr. Muhammad Naeem Khan
Department of Zoology, University of the Punjab, Lahore.
Promotion of Inland Recreational Fisheries in Developing Countries: A South East Asian Perspective.

HALL – 1

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

SESSION IV

	Chairperson:	Prof. Dr. M. Naeem Khan
	Co-chairperson:	Dr. Naz Abbas
10:00 AM	Paper reading	
11:00 PM	Tea Break	

SESSION V

	Chairperson:	Dr. Arif Siddiqui
	Co-chairperson:	Dr. Shahid Nadeem
11:30 AM	Paper reading	
01:00 PM	Lunch and Prayer	

SESSION VI

	Chairperson:	Prof. Maqbool A. Memon
	Co-chairperson:	Dr. N.M. Soomro
02:00 PM	Paper reading	
04:30 PM	Tea Break	

SESSION VII

	Chairperson:	Dr. M. Afzal Kazmi
	Co-chairperson:	Dr. Maqsood Rustamani
05:00 PM	Paper reading	
06:30 PM	Prayer	

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

SESSION I

	Chairperson:	Dr. Ghulam Muhammad Memon
	Co-chairperson:	Dr. Nikhat Yasmin
06:45 PM	Paper reading	
07:00 PM	Poster Session	
08:00 PM	Dinner	

HALL – 2

SECTION II: PEST AND PEST CONTROL

SESSION IV

	Chairperson:	Prof. Dr. Imtiaz Ahmad
	Co-chairperson:	Dr. Abdul Sattar Buriro
10:00 AM	Paper reading	
11:00 PM	Tea Break	

SECTION III: ENTOMOLOGY

SESSION I

	Chairperson:	Dr. Nazir Ahmad
	Co-chairperson:	Dr. Mushtaq A. Saleem
11:30 AM	Paper reading	
01:00 PM	Lunch and Prayer	

SESSION II

	Chairperson:	Prof. Dr. M. Suleman
	Co-chairperson:	Dr. Zahoor Saliha
02:00 PM	Paper reading	
04:30 PM	Tea Break	

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

SESSION II

	Chairperson:	Dr. Baz M. Junejo
	Co-chairperson:	Dr. M. Rahbbaniha
05:00 PM	Paper reading	
06:30 PM	Prayer	

SECTION IV: PARASITOLOGY

SESSION I

	Chairperson:	Prof. Dr. Fatima Mujeeb Bilquees
	Co-chairperson:	Dr. A.M. Dharejo
06:45 PM	Paper reading	
07:00 PM	Poster Session	
08:00 PM	Dinner	

THURSDAY, MARCH 3, 2005

HALL – 1

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

SESSION III

	Chairperson:	Mr. Abdul Aziz Khan
	Co-chairperson:	Dr. M. Moazzam
09:00 AM	Paper reading	
11:00 AM	Tea Break	

SESSION V

	Chairperson:	Prof. Dr. S.I.H. Jafri
	Co-chairperson:	Dr. Muhammad Ali
11:30 AM	Paper reading	
01:00 PM	Lunch and Prayer.	

HALL – 2

SECTION IV: PARASITOLOGY

SESSION II

	Chairperson:	Dr. A.G. Arijo
	Co-chairperson:	Dr. Aly Khan
09:00 AM	Paper reading	
11:00 AM	Tea break	

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

SESSION V

	Chairperson:	Prof. Dr. Q.B. Kazmi
	Co-chairperson:	Dr. Samina Kidwai
11:30 AM	Paper reading	
01:00 PM	Lunch and Prayer.	
02:30 PM	General Body Meeting	
04:00 PM	Concluding Session	
	Recitation	
	Congress Report by President ZSP	
	Award Ceremony	
	Concluding Remarks by the Chief Guest	
	Vote of Thanks	
05:00 PM	Refreshments	

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CITATIONS

RECIPIENT OF ZOOLOGIST OF THE YEAR AWARD 2005*

Prof. Dr. Mushtaq A. Saleem
Principal, University College of Agriculture, Bauahuddin Zakariya University, Multan

Prof. Dr. Mushtaq A. Saleem obtained B.Sc. (Hons.) Agriculture degree in Entomology from University of Agriculture, Faisalabad, M.Sc. (Hons.) Agriculture Entomology from University of Newcastle upon Tyne, England, Ph.D. from university of the Punjab, Lahore and Post-doctorate from University of Newcastle upon Tyne, England. He won merit scholarships and obtained high first divisions in all examinations. He served as Associate Professor of Agricultural Entomology at University of Agriculture, Faisalabad and then Professor of Agriculture (Entomology) at University College of Agriculture, Bahauddin Zakariya University, Multan since 2002. He is presently Principal of University College of Agriculture, Bahauddin Zakariya University.

*Other nominees for this award were Prof. Dr. Khalid Pervez Lone, Lahore, Prof. Dr. Syed Iftikhar Hussain Jafri, Jamshoro, Dr. Aly Khan, Karachi, Prof. Dr. Muhammad Suleman, Peshawar, Prof. Dr. Muhammad Naeem Khan, Lahore and Dr. Abdul Aleem Chaudhary, Islamabad.

Dr. Mushtaq A. Saleem has so far published 72 research papers in Journals of international repute published from Canada, USA, UK, Poland, Bangladesh and Pakistan. He has written 5 books, presented 79 research papers in various Congresses and published 90 popular articles in various newspaper and magazines. Fifty-two students have done/doing M.Sc./M.Phil/ Ph.D. under him as Supervisor/Co-supervisor/Member of Supervisory Committee. He introduced novel field of research during his Ph.D. and Post-doctorate studies. On this basis he was given Cash Prize of Rs.1.20 lac by the Ministry of Science & Technology, Government of Pakistan; Star Award in 2002; Presidential Academic Award, Izaaz-i-Fazeelat in 2003. He received the Best University Teacher Award of HEC in 2004.

RECIPIENT OF
PROF. A.R. SHAKOORI GOLD MEDAL 2005*

Dr. Anujm Perveen
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Dr. Anjum Perveen She was awarded B.Sc. (Hon's) in 1994, M.Sc. in 1985 and Ph.D. in January 1995 from Karachi University. She was awarded post doctoral fellowship by Svenska Institute Stockholm, Sweden, where she worked in the Palynological Laboratory of National Museum of Natural History on "Pollen world and spore flora family Tiliacea".

Dr. Anjum Perveen has published more than 50 papers on pollen flora of Pakistan.

Dr. Perveen has worked as Principal and Co-Principal Investigator in several projects sponsored by Pakistan Science Foundation and Higher Education Commission. She has attended many national and international conferences held in Karachi and Norway. She is member of several scientific societies such as Botkarians, Pakistan Botanical Society, German Palynological Society APP (Arbeitskreis für Palaeobotanik and Palynologie) and also member of International Federation of Palynological Society (IFPS).

*Other applicants for this award were Mr. Sikander Ali, Lahore, Mr. Zulfiqar Ali, Lahore, Dr. Dileep Kumar Rohra, Karachi, Dr. Noor-un-Nisa, Karachi, Miss Farzana Perveen, Karachi, Dr. Sajjad-ur-Rahman, Faisalabad, Dr. Muhammad Saeed Akhtar, Faisalabad and Dr. Farah Rauf Shakoori, Lahore.

RECIPIENT OF
PROF. DR. MIRZA AZHAR BEG GOLD MEDAL 2005*

Dr. Farah Rauf Shakoori
Assistant Professor
Department of Zoology, Government College University, Lahore.

Dr. Farah Rauf Shakoori, Assistant Professor in the Department of Zoology, Government College University, Lahore obtained her M.Sc. degree in Zoology from the University of the Punjab in 1990. Later she proceeded to USA take up reseach work for her Ph.D. degree in Molecular Biology from University of Massachutts Medical School under a collaborative arrangement with University of the Punjab, which she successfully completed in 1996.

Dr. Shakoori has published 30 research papers on histone gene expression in most prestigious scientific journal of the world. Her research publications appeared in Nature, PNAS, JBC, Biochemistry, Journal of Cell Physiology, and Journal of Cellular Biochemistry.

She has attended many national and international conferences. She is member of several scientific societies. She has supervised research work of several M.Sc. and M.Phil students. Presently she is supervising 3 Ph.D. students.

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*Other applicants for this award were Mr. Zulfiqar Ali, Lahore, Dr. Naeem Tariq Narejo, Jamshoro and Dr. Noor-un-Nisa, Karachi.

RECIPIENT OF
PROF. DR. NASIMA TIRMIZI GOLD MEDAL 2005

Dr. Itrat Zehra
Associate Professor
Centre of Excellence in Marine Biology University of Karachi, Karachi.

Dr. Itrat Zehra has been engaged in teaching and research since 1970. She has a distinguished academic career which culminated into a Ph.D. degree in 1981 from the Zoology Department, University of Karachi. She has the distinction of being a USAID postdoctoral scholar at Univesity of Rhode Island, USA. She has a number of publications in national and international journals to her credit. A number of students have completed their research degree under her supervision.

Dr. Zehra is a well traveled person. For her academic assignments she has visited a number of universities and institutions across the globe Universities of Hull, Reading and Glasgow in Britain; Natural History Museum London; California Academy of Sciences, San Francisco USA. She also has working experience in Africa where she worked as a Lecturer for two years in College of Natural Resources and Environmental Studies, University of Juba, Sudan.

RECIPIENTS OF GOLD MEDALS AWARDED BY THE ZOOLOGICAL SOCIETY OF PAKISTAN

1. **Mujib Memorial Gold Medal 2005**
   This Gold Medal is awarded to a student of Karachi University standing first in the recent M.Sc. Zoology examination with specialization in Parasitology. Eleven Medals have already been given. This year's Mujib Memorial Gold Medal was received by Syeda Kiran Batool.

2. **Mohd Afzal Hussain Qadri Memorial Gold Medal 2005**
   This Gold Medal is awarded to a student of Karachi University, standing first in the recent M.Sc. Zoology examination. Nine medals have already been awarded. The eighth medal was received by Mr. Muhammad Rais.

3. **Muzaffar Ahmad Gold Medal 2005**
   This Gold Medal is awarded to a student of the Punjab University, standing first in the recent M.Sc. Zoology examination. Eleven medals have already been given. Muzaffer Ahmad Gold Medal 2005 was received by Miss Erum Gul.

4. **Ahmad Mohiuddin Memorial Gold Medal 2005**
   This Gold Medal is awarded to M.Sc. Zoology student of University of Sindh, Jamshoro standing first in the recent M.Sc. Zoology examination. Four Gold Medals have already been given. This year Ahmed Mohiuddin Memorial Gold Medal 2005 was given Ms. Moonsa Pirah.

5. **Prof. Imtiaz Ahmad Gold Medal 2005**
   This Gold Medal is awarded to a student of Karachi University, standing first in the recent M.Sc. Zoology examination with specialization in Entomology. Four gold medals have already been given. This year Prof. Imtiaz Ahmed Gold Medal 2005 was given Mr. Muhammad Zeeshan.

6. **Prof. Dr. S.N.H. Naqvi Gold Medal 2005**
   This Gold Medal is awarded to a student of Karachi University, obtaining Ph.D. Zoology degree with specializing in the field of Toxicology. Three gold medals have already been given. This year Prof. Dr. S.N.H. Naqvi Gold Medal 2005 was given Mr Muhammad Zeeshan.

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EFFECT OF ENDOSULFAN, A CHLORINATED HYDROCARBON, ON THE REPRODUCTIVE ORGANS OF RABBIT

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Abstract.- An attempt was made to study the possible role of a chlorinated hydrocarbon, endosulfan EC-35, as an endocrine disruptor. In this context about 50 (male and female) rabbits, *Oryctolagus cuniculus,* were kept in the cages designed according to their environmental needs. Three groups, each of six animals (three males and three females) were used as experimental groups, whereas one group of six (3 males and 3 females) was maintained as control group. They were fed lucern daily after the administration of insecticide. Three different doses of endosulfan *i.e.* Group I, 15.9 5mg/kg body weight; Group II, 38.3 10mg/kg body weight; and Group III, 66.0 15mg/kg body weight were given for 15 days. All the animals were sacrificed at the termination of experiment and the gonads were carefully isolated and fixed for histological examination. Blood was collected from each animal of control and experimental groups for cholesterol estimation. Drastic changes were noted in both testicular and ovarian structures. The seminiferous tubules (mean diameter, 296.1±25.0 µm) in the testes of control were reduced in size, 137.37±18.7 µm in group I and 107.18±16.7µm in group II. A decrease in the number of spermatogonia, spermatocytes, spermatids, spermatozoa and leydig's cells was also noted predominantly. The ovarian follicles in the control that were in the pre-antral and antral phase became atretic in experimental groups. The granulosa cells in the follicles of experimental groups were reduced in size, lost their columnar shape and became rounded. The theca cells were also reduced in number and size. The serum cholesterol levels were insignificantly increased in the experimental group I and II *i.e.* 22.0±3.0 mg% and 23.0±2.0 mg% respectively. However, significant increase (32.0±3.2 mg%) was noted in group II, as compared with control (18.2±1.9 mg%). The drastic changes in the testicular structure, atresia in ovarian follicles and increasing serum cholesterol pattern suggest the effective role of the endosulfan as an endocrine disruptor.

Key words: Reproductive organs, endosulfan, endocrine disruptor, cholesterol, rabbit.

INTRODUCTION

No doubt, insecticides and pesticides used for protecting the crops from the harmful pests have played a positive role, but their indiscriminate use not only damages the natural integrity of the crops, they also kill or drastically affect the metabolic/ reproductive pathways of animals living around. The damage of reproductive system directly affects the population of animals like rodents,

lagomorphs etc. which are directly or indirectly useful for the crops. Endosulfan is a broad spectrum insecticide and acaricide first registered for use in United States in 1954 to control agricultural insects, mite pests on a variety of field, fruit, and vegetable crops (Environment Protection Agency, HAZDAT, 2000).

Endosulfan, amongst many insecticides/pesticides having been used indiscriminately and have caused much more damage to the animals throughout the world that is reported in the literature. Now it has been totally banned in the world but it is still being used in Pakistan indiscriminately.

Reported effects of endosulfan on the male and female reproductive system in experimental animals have been variable, depending on the species, age at exposure, dose, duration of exposure, and study end points (Murray *et al.*, 2001). Several other studies over last three decades suggest that environmental exposure to synthetic estrogenic chemicals and related endocrine active compounds might be responsible for a global decrease in sperm count, and decreased male reproductive capacity (Safe, 2000). Organochlorine pesticides have such estrogenic properties (Khan and Sinha, 1996; Olea *et al.*, 1998; Safe, 2000). Sufficient studies have been carried out on toxic effects of many compounds, like organophosphate, chlorinated hydrocarbon, pyrethroids, and PCBs. However, such studies have never or very few have been done in the area, especially to ascertain the effect of endosulfan as an endocrine disruptor on the rabbit. Convolution and salivation have been reported in both male and female shortly after the administration besides increased respiratory rate, hyperactivity and reddish nasal discharge (Ebert and Leist 1990). Testicular atrophy was reported in the rats orally administered endosulfan (Barnard, 1985). Significant increase in the ovarian weight and number of atretic follicles in ovaries of the hemicastrated albino mice exposed to lower doses of endosulfan has been reported by Hiremath and Kaliwal (2000). Organochlorine pesticides have such estrogenic properties (Olea *et al.*, 1998). These compounds have received the most attention because of their persistence in the environment and their ability to concentrate upon the food web and drinking water (Snedeker, 2001). They also seem to accumulate in organisms and then cause endocrine disruption at environmentally realistic exposure levels (Vos *et al.*, 2000). Lindane, the hexachlorocyclohexane, can have both estrogenic and anti-estrogenic effects in the rat (Cooper *et al.*, 1989). Methoxychlor has been widely used as a substitute of DDT since it was shown to have a lower mammalian toxicity and to be quickly degraded. However, it was then shown that this pesticide could also alter various reproductive functions such as ovary structure and functions (Cummings, 1997; Eroschenko *et al.*, 1995, 1997). It alters initiation and maintenance of

pregnancy in mice (Swartz and Eroschenko, 1998). Dieldrin also displays estrogenic activity and can affect ovarian function (Arnold, 1996). Sinha *et al*. (1995) postulated that endosulfan impairs testicular functions by altering the enzyme activity responsible for spermatogenesis thus influencing intratesticular spermatid count and resulting in low sperm production and increased sperm deformities. Degenerative changes in seminiferous epithelium including testicular atrophy and the occurrence of ovarian cysts were noticed in rat (Naqvi and Vaishnavi, 1993). Pickford and Morris (1999) claimed chlorinated hydrocarbon as the endocrine disruptors by having estrogenic activity. Gestational exposure to dioxin severely alters male and female reproductive function of hamster offspring by reducing ejaculated sperm count and delays puberty while reduced ovarian weight and fecundity in females (Wolf, 1999). It has been noted that monocrotophos caused the interruption in estrus cycle by observing the decrease in healthy follicles and increase in the atretic follicles probably due to the hormonal imbalance or toxic effect of the insecticide (Rao and Kaliwal, 2002). The present study was carried out to understand the role of chlorinated hydrocarbon endosulfan on male and female reproductive organs in rabbits.

MATERIALS AND METHODS

Animals

About fifty rabbits. *Oryctolagus cuniculus* (1400±75.5 g) were purchased from the market of Hyderabad and brought to the laboratory. They were kept in the animal house measuring 10 ′×14′ sq. ft. for acclimatization in the new environment for 15 days. During this period the animals were fed three times a day with the grass Alfalfa (*Medicago sativa*) which is commonly known as lucerne. After the expiry of the acclimatization period the animals were transferred to the experimental cage which was especially designed as per natural requirements of the animals. Four batches, each of six animals (three males and three females) were kept as control and three experimental groups and re-acclimatized for 3 days, before administration of insecticide.

Endosulfan EC-35

Endosulfan EC-35 purchased from the local market was diluted in 10 ml deionized water. The experimental groups of rabbits were administered the insecticide as follows for 15 days: Group I, 15.9µl / kg body weight / day; Group II, 38.3µl / kg body weight / day and Group III, 66.0µl / kg body weight / day.

Experimental procedure

The insecticide was administered daily after the feed was consumed by the animals. The control group was also maintained by giving same quantity of feed (Lucerne) and water as per requirement. At the termination of experiment the animals were anesthetized by using chloroform. All the animals (Control and Experimental) were dissected out and heart of each animal was exposed and cleaned with the help of tissue paper. Blood (1ml) was collected in vial by rupturing the ventricle for cholesterol estimation according to CHOP-PAP method of E. Merck. Testes and ovaries were removed and fixed in Bouin's fluid for histological studies. The paraffin wax embedded sections of the tissues were cut by using rotary microtome at a thickness of 6µm. The sections were stained in haemotoxylin and eosin.

RESULTS

Histological structure of testis

In the control group the seminiferous tubules (mean diameter, 296.1±25.0µm) as shown in Table I, were large rounded/oval shaped containing spermatogenic cells in different stages (Fig. 1). However, the tubules of the testis in the rabbits of group I were reduced in size (mean diameter, 137.37±18.7µm) (Fig. 2). The seminiferous tubules were found further reduced (mean diameter, 107.18±16.7µm) in group II (Fig. 3). The animals of group III did not survive.

Large number of spermatogenic cells (Table I), were present in the tubules of the control group (Fig.4). A decrease in the number of spermatogonia was noted in the testes of rabbits of group I (Fig.6) and further decrease of such cells was noted in group II (Fig. 7). Few spermatocytes were found in the seminiferous tubules of the testes of control group (Table I; Fig. 5). The decreased number of spermatocytes was noted in the animals of group I (Fig.6) which further decreased in group II (Fig. 7). The spermatids in control group were 50 in average in each tubules (Fig. 5) that decreased in group I (Fig. 8) and further decreased to 7.0 only in group II (Fig. 9). All the spermatogenic cells in the testes of the animals exposed to 38.3µl / kg body weight were hypertrophied.

The mean number of spermatozoa when counted in the seminiferous tubules of the control group were 22 (Fig. 5) (Table I). The production of the spermatozoa was affected *i.e.* 5 only in group I (Fig. 6) one or none in group II (Fig. 9).

TABLE I.- THE VARIOUS HISTOLOGICAL PARAMETERS OF TESTES AND OVARIES OF THE CONTROL AND EXPERIMENTAL RABBITS.

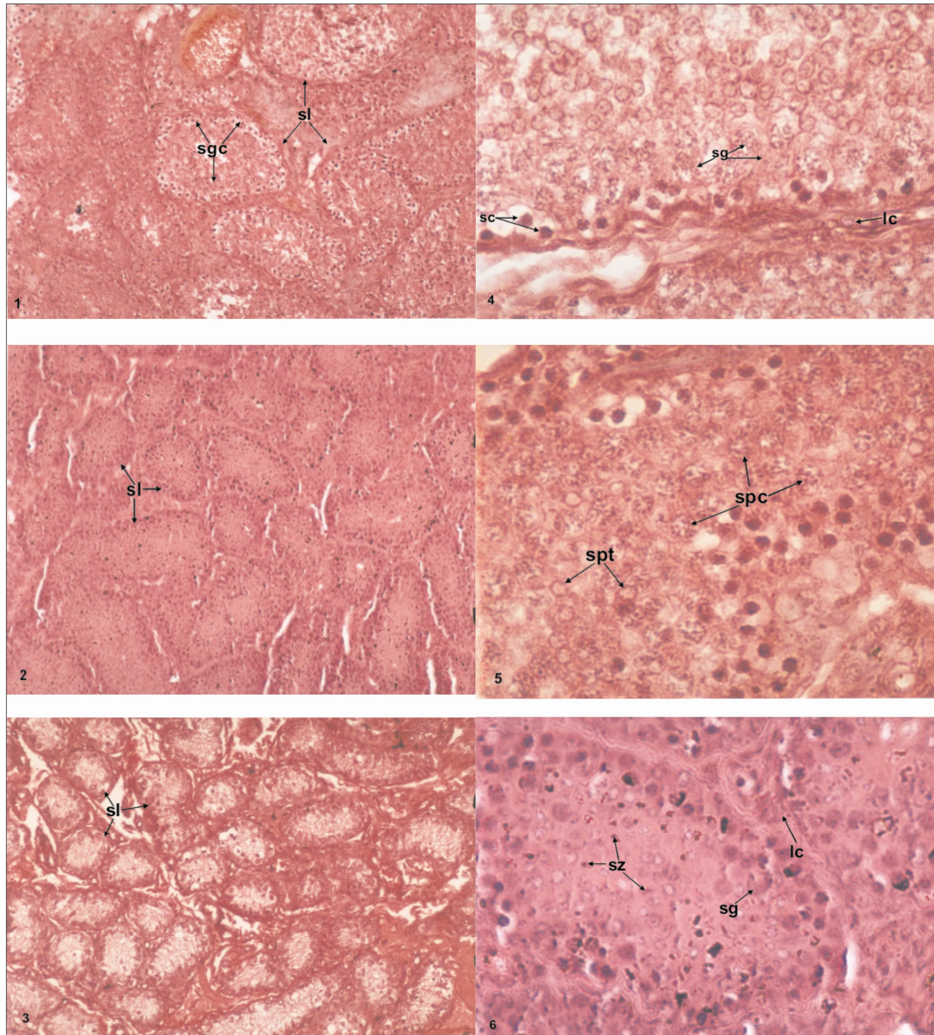
Parameters	Control	Group I	Group II	Group III
Testes				
Seminiferous tubules (µm)	296.1±25.0	137.37±18.7	107.18±16.7	
Spermatogenic cells				Male animals could not survive.
Spermatogonia	63±5.0	50±4.0	28±3.5	
Spermatocytes	60±6.0	45±3.5	20±2.65	
Spermatids	50±4.0	40±6.0	10±2.0	
Spermatozoa	25±3.5	6±2.0	2±1.0	
Cholesterol (mg%)	18.2±1.9	22.0±3.0	28.0±4.2	
Ovaries				
Follicle size				
Matured follicles (µm)	1085.87±200.38	402.5±43.5	399±53.4	990.5±102.5
Maturing follicles (µm)	89.40±5.80	122.5±0.0	297.5±76.5	115.5±10.6
Cholesterol (%)	18.2 ±1.9	22.0±3.0	23.0±2.0	32.2±3.2

The leydig's cells were present in the interstitial space of control group. The mean number was only 4.0 (Fig. 4) This number was also decreased to only 2 in group I (Fig.6) and only one or none in group II (Fig. 7).

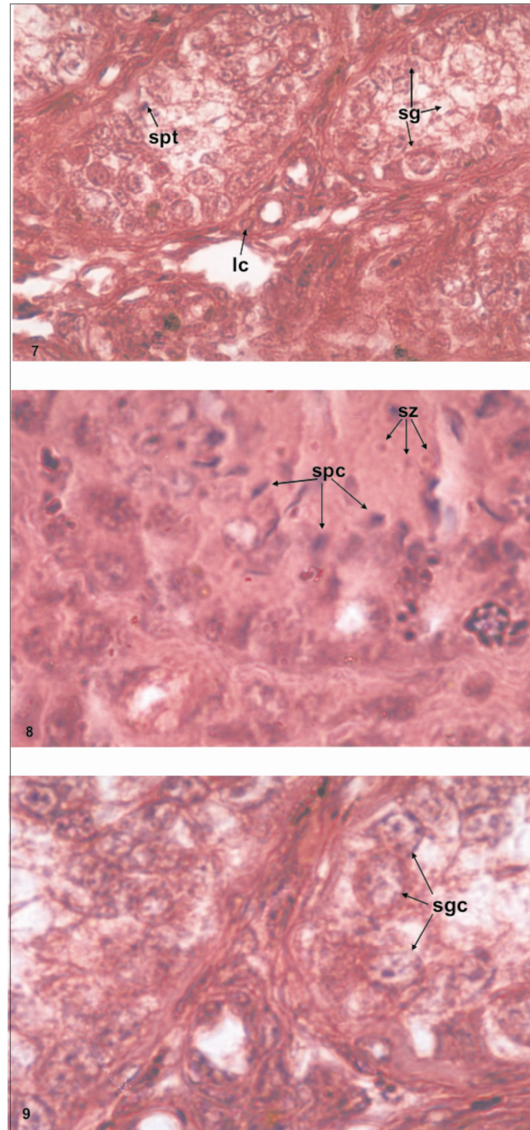
Very few oblong sertoli cells were present in the vicinity of the tubules of the testis of control group (Fig. 4). However, there was no trace of the sertoli cells in any of the experimental group.

Histological structure of ovary

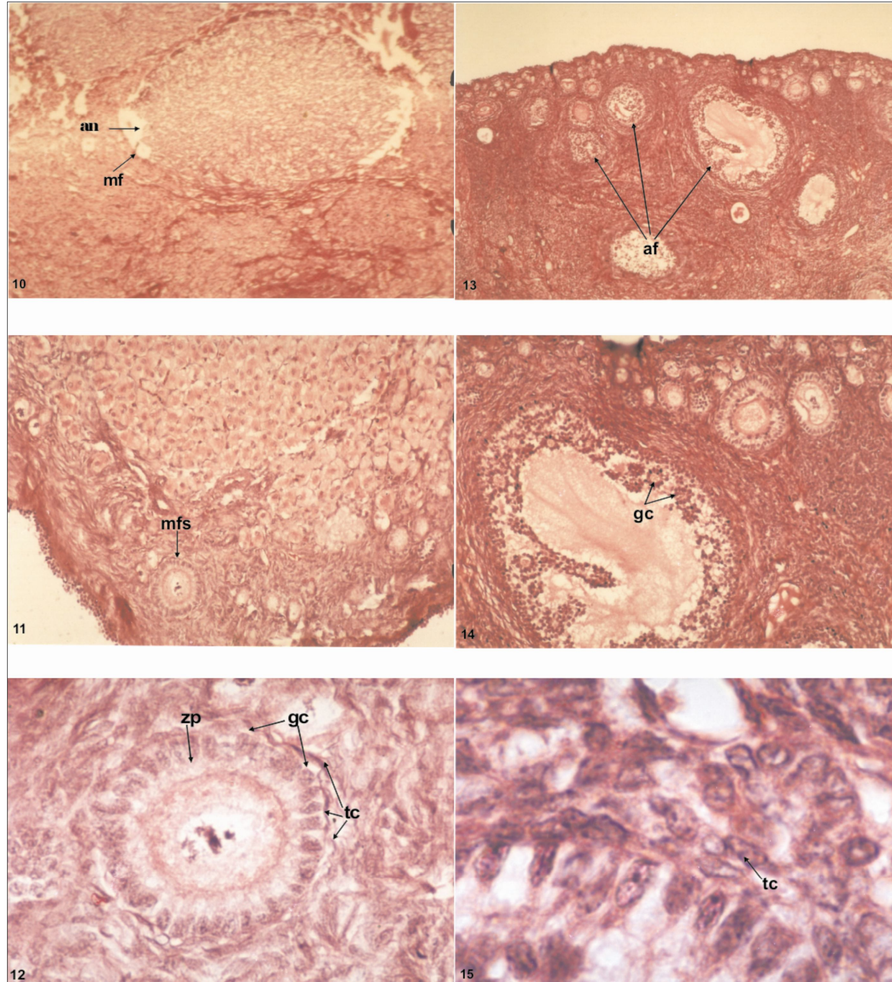
The ovary of the control rabbits reveal that these contain the follicles of two types: (1) Matured follicles (mf) and (2) Maturing follicles (mfs). The matured follicles in control group (mean diameter, 1085.87±200.38µm) were oval shaped filled with yolk and antrum formation was seen (Fig.10). The maturing follicles of different sizes were present at the cortical region of the ovary (Fig. 11) amongst which the larger one predominantly containing columnar shaped granulosa cells arranged in circular row around zona pellucida. The theca cells were also seen present outer to the granulosa cells (Fig. 12). Nuclear membrane of the oocyte was intact. The follicles were in the pre-antral phase.



Figs. 1-6. Histological sections of the testis of cortical group of rabbits showing seminiferous tubules (sl) and spermatogenic cells (agc) (1), testis of group-I of rabbits showing seminiferous tubules (sl) (2), testis of group-II rabbits showing seminiferous tubules (sl) (3), testis of cortical group of rabbits showing large number of spermatogonia (sg) present in tubules. Leydgi's cells (lc) in intestitial space, few sertoli cells (sc) in the vicinity of tubules (4), testis of cortical group of rabbits showing, spermatocytes (spc) and spermatids (spt) in tubule (5), testis of the group-I, showing significant decrease in number of spermatogonia (sg). Spermatozoa (sz). Leydig's cells (lc) are also seen (6). Magnifications: 1-3, = 100x; 4-6, = 40x.



Figs. 7-9. Histological sections of the testis of group-II of rabbits showing further decrease in spermatogonia (sg), one or no spermatozoa (sz), significant decrease in the number of spermatids (spt) and one or no testis Leydig's cell (lc) (7), of the group-II, showing decreased number of spermatocytes (spc), and spermatozoa (sz) (8), testis of the section of the group-II showing hypertrophied (hyt), spermatogenic cells (sgc). Magnifications: 7 = 40x; 8, 9 = 100x.



Figs. 10-15. Histological sections of the ovary of control group of rabbits, showing oval shaped matured follicles (mf) filled with yolk, and formation of antrum (an) (10), ovary of control group of rabbits showing oval shaped matured follicles (mf) at the cortical region of the ovary (11), ovary of control group of rabbits showing maturing follicles (mfs) predominantly containing columnar shaped granulose cells (gc) arranged in circular row around zona pellucida (zp) and theca cells (tc) outer to the granulose cells (12), ovary of group-I showing artretic pre-antral or antral phase follicles (af) (13), ovary of group-I rabbits showing largest follicles in which granulose cells (gc) have lost their columnar shape and changed into rounded form and also reduced in size (14), ovary of group-I showing reduced Theca cells (tc). Magnifications: 10-13 = 40x; 14-15 = 100x.

In rabbits exposed to 15.9 µl of insecticide/kg body weight per day, all the follicles, whether in pre-antral or antral phase, have become atretic (Fig. 13). The largest maturing follicles showed that the granulosa cells had lost their columnar shape and changed into rounded form and also reduced in size (402.5±43.5µm) (Fig. 14). The theca cells which were clearly seen in the controls had also been reduced in size and shape (Fig. 15).

The rabbits exposed to 38.3 µl insecticide/kg body weight per day revealed that ovarian follicles (399±53.4µm) in different stages had totally collapsed/disintegrated (Fig. 16). The cavities were formed inside the follicle (Fig. 17). The follicles of antral phase had regressed and converted into atretic follicles (Fig. 16).

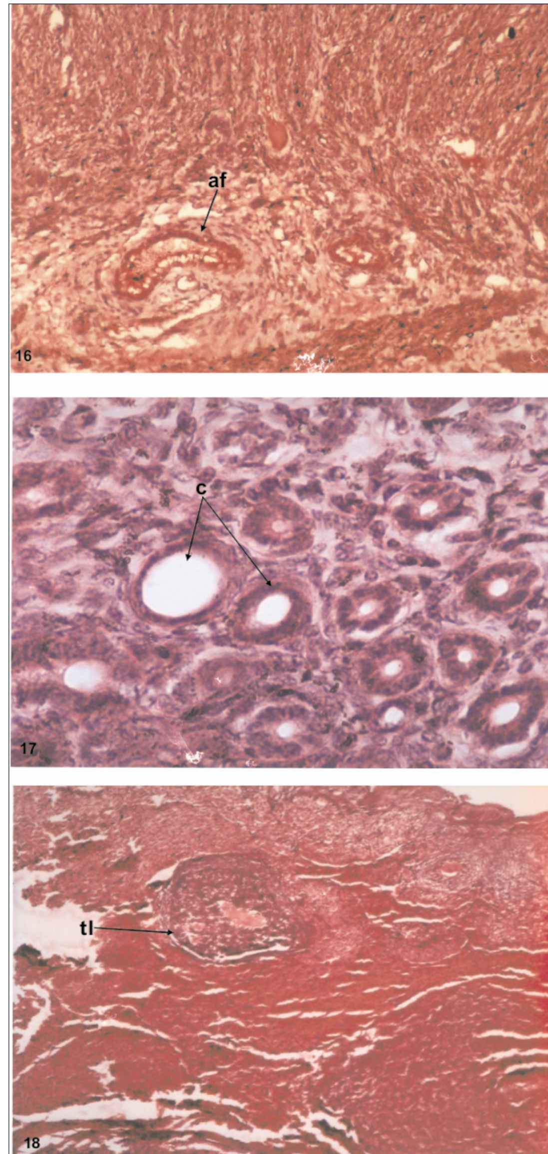
The rabbits exposed to 66.0 µl of insecticide/kg body weight per day did not survive till the termination of experiment. However, 2 out of six animals survived in which all the follicles were disintegrated, hypertrophied and converted into the tumor-like structure (Fig. 18).

Serum cholesterol

The mean serum cholesterol in the control group was 18.2±1.9 mg% that increased gradually in the group I *i.e.* 22.0±3.0 mg%. The rabbits of the experimental group II had 23.0±2.0mg%. The group III in female contained higher level *i.e.* 32.2±3.2 mg% (Table I).

DISCUSSION

The present study describes the effects of chlorinated hydrocarbon endosulfan on the reproductive organs (testes and ovaries) of rabbit. It was noted that lowest oral dosage 15.9 µl/kg body weight caused severe effect on both the tissues, whereas dose of 66.0 µl/kg body weight, caused severe damage to the animals were not died but the effect on both the testes and ovaries. Earlier reports, regarding the effect of endosulfan on the male reproductive organs are variable, depending on species, age at exposure, dose, duration of exposure and study end points. Routine gross and histopathologic examination of the reproductive organs of male mice that consumed doses of 7.3mg/kg/day for 13 weeks (Hoechst. Unpublished data) or 2.5-5.0 mg/kg/day for 2 years (Hack *et al*., 1995; Hoechst Unpublished data). National Cancer Institute (1978) revealed no toxic effects. Later on, more detailed studies on adult rats exposed to 2.5, 5 and 10 mg/kg/day endosulfan for 5 days per weeks showed reduced intratesticular



Figs. 16-18. Histological sections of ovary of group-II showing follicles of antral phase (a) (16), ovary of the group-II showing the cavities (c) inside the follicles (17), ovary of group-III showing disintegrated, hypertrophied follicles with tumor –like structures (tl) (18). Magnifications: 16-18 = 100x.

spermatid counts, sperm abnormalties and changes in the marker enzymes of testicular activities, such as lactate dehydrogenase, sorbitol dehydrogenase, γ-glutamyl transpeptidase, and glucose-6-phosphate dehydrogenase, providing further evidence of effects on spermatogenesis (Khan and Sinha, 1996; Sinha *et al*., 1995). Exposure of younger animals (3 weeks old) showed marked depletion of spermatid count as well as decreased daily sperm production at a dose of 2.5 mg/kg/day (Sinha *et al*., 1997), which was earlier seen only at 5 mg/kg/day in adult rats by the same investigators (Sinha *et al*., 1995). More recent studies have shown that exposure of pregnant rats to endosulfan at 1 mg/kg day from day 12 through parturition leads to decreased spermatogenesis in offspring's (Sinha *et al*., 2001). Dalsenter *et al*. (1999) reported similar observations at 3 mg/kg/day but not at 1.5 mg/kg/day, as they attributed this to strain variation (Dalsenter *et al.,* 2003). Thus experimental studies suggest that endosulfan can affect the male reproductive system and also that these effects are likely to be greater if exposure occurs during the developmental phase.

Our results with regard to the animals of control and exposed to different doses of the insecticides reveal that the spermatogenic cells of different categories were present in sufficient quantity in seminiferous tubules. The leydig's cells and the sertoli cells were also found in the interstitial space and at the seminiferous tubular sheeth respectively. The number of leydig's cells and sertoli cells were either reduced or suppressed in the animals that exposed to lowest dose *i.e.* 15.9 µl/kg body weight probably because of the estrogenic endocrine disrupting role of the endosulfan. This has already been reported by many workers in the last decade (Fisher, 2004; Bremmer and Leist, 1998; Sargent, 2000; Crisp *et al*., 1998; Edward *et al*., 1984). They opined that the endocrine disruptors like endosulfan can exert their effect in many ways. They can either bind to the hormone receptor and mimic the hormone or block the action of the hormone. Alternatively, they can stimulate or inhibit the enzyme responsible for the synthesis or clearance of a hormone, and thereby give rise to an increased or decreased action of the hormone. The decrease of sperm count in the testes of animals exposed to insecticide and eliminated totally in group II confims the statements already made as estrogens and production capacity. The sperm production as stated by many workors is dependent on permissive action of FSH and testosterone, and therefore LH. The sperm production capacity depends upon the number of sertoli cells in the seminiferous tubules (Orth *et al*., 1988; Russel and Peterson, 1984). It has also been reported that much of the wild life specially mammals are vulnerable to the endocrine disrupting effects of pesticides/ insecticide like organochlorines (Lyons, 1999, Singh and Pandey, 1990; Sinha *et al*., 1995, 1997). As far as our finding about the effect of

endosulfan on the ovaries of the rabbit is concerned, this reveals that the ovaries of rabbits exposed to smallest dose *i.e.* 15.9 µl/kg body weight have increased number of oocytes of pre-antral phase and total collapse was noted in the matured follicles/oocytes especially those which were mature and ready to spawn converted into atresia in the animals subjected to endosulfan even at lowest dose. The development of oocytes in the pre-antral phase and regression of the follicles in the antral phase is thought to be due to the accumulation of endosulfan that has an effect of endocrine disrupting by having an estrogenic composition (Hiremath and Kaliwal, 2002; Lyons, 1999). Naqvi and Vaishnavi (1993) have also reported testicular atrophy and the ovarian cysts followed by endosulfan application in rats.

The increase in serum cholesterol level at 66.0µl/kg body weight in the present study support the earlier reports (Wade,2002) in which it has been claimed that LDH level is increased in treated rats (Dikshith *et al*., 1988). Other reports in which reduced serum LDH level in the highest doses (Wade, 2002) suggests that it might be possible that the levels of cholesterol increase at the low doses and decrease at higher doses.

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EXPEDIENCY OF DIFFERENT BOTANICAL PRODUCTS INTENDED FOR MANAGING THE POPULATION OF RICE STEM BORERS

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Abstract.- Present endeavor has been made to evolve cheap and effective plant origin products to manage the rice stem borers. The effectiveness of available plant materials such as Neem *(Azadirachta indica* A. Juas), Hing *(Ferula foetida* Regel) and Dhatoora *(Datura metch* Linn.) were tested against the pests on 4 rice varieties (Aromatic- Basmati 370, Super Basmati; non- aromatic- IR 8, Sarshar). The shade dried plant materials were powdered and passed through 20 mesh sieve. First spray of these products was given near flowering stage and remaining two at 15 days interval subsequently. Results gave an initiative that all the extracts proved to be lethal against borers suppression than control units. The Hing extract proved to be more deleterious than Neem and Dhatoora having the intensity of 2.88, 2.54; 3.98, 3.49 and 4.34, 4.40 for % borers infestation (dead hearts, whiteheads) and yielded 4483, 4180 and 3686 Kg/Ha grains, respectively; contrary to control treatment by having 6.93, 8.40 % borers infestation and 3019.33 kg produce. Consequently, Hing *(Ferula foetida* Regel) can be well .suited for an Integrated Pest Management Programme because of a natural mortality product, absolutely non-toxic, biodegradable and environmentally friendly.

Key words: Rice stem borers, natural insecticides, botanical products, pest control.

INTRODUCTION

Rice is the primary food for half the people in the world, providing more calories than any other single food. It supplies an average of 889 calories per day per person. Rice is a nutritious food, providing about 90 percent of calories from carbohydrates and as much as 13 percent of calories from protein (Anonymous, 2004).

Rice stem borers are serious pests of rice. They infest plants from the seedling stage to maturity. Although worldwide in distribution, rice stem borers are particularly destructive in rice. On the basis of the extent and severity of the damage, the important species of stem borers, considered as major pests of rice are; Yellow stem borer *Scirpophaga incertulas* (Walker); White stem borer *S. innotata* (Walker), Striped stem borer *Chilo suppressalis* (Walker), Gold-fringed stem borer *C. auricilius* Dudgeon, Dark-headed stem borer *C. polychrysus*

(Meyrick), and Pink stem borer, *Sesamia inferens* (Walker). The yellow stem borer (monophagous to rice) is an important pest of irrigated rice in South and Southeast Asia. It causes about 1 to 19% yield loss in early-planted rice crops and 38% to 80% yield reduction in late-planted rice. The white stem borer is an important pest in rain fed wetland rice, this pest has also been observed in irrigated rice fields and causes occasional outbreaks. The white stem borer feeds primarily on rice, its secondary host includes grasses. The striped stem borer is one of the most important insect pests in temperate regions. During the vegetative stage, larval feeding causes dead heart. The rice plant can compensate by growing new tillers. At the reproductive stage, feeding causes whitehead; the damage could reach up to 100 %. The gold-fringed stem borer is a major pest of sugarcane in India and Taiwan. It is a pest of maize and upland rice. Yield losses of 30% and 20% due to this insect were reported in India and Bangladesh, respectively. Among the stem borers, the dark-headed and the pink stem borer are less important. The pink stem borer is polyphagous and prefers sugarcane to rice. In Asia, yield losses due to the two most important species, the yellow and striped stem borers range from 1-20%. However, during outbreak conditions, yield losses may range from 30 to 100% (Barrion and Litsinger, 1994; Shepard *et al.,* 1995).

Stem borers can be managed by using cultural control measures, biological control agents, the use of resistant varieties and chemical control. Such a crop of immense global importance has a certain target for control by transnational corporations, especially chemicals. Understanding chemical mediators of oviposition in the important group of insects that contains many agricultural pests could be useful in the development of environmentally sustainable pest management. The real search for environmentally sound pesticides received an impetus by Carson in 1962. Since then, the detailed investigations are being conducted under field conditions to utilize plant material in order to develop strategy for proper management of insects' pest, particularly lepidopteran. It was around this period that Pradhan *et al.* (1962) reported the feeding deterrent property of neem seed kernel suspension against desert locust. Chopra *et al.* (1965) listed poisonous plants in their areas of research. Lydon and Duke (1989), Isman (1994) and Mackinnon *et al.* (1997) surveyed several plant families that showed promise as sources of new botanical insecticides. Hummelbrunner and Isman (2001) tested 10 essential oil compounds and found them more effective as a contact insecticide, feeding deterrent and effective as inhibiting larval growth. Stadler (2002) reviewed chemical cues affecting egg deposition by herbivorous insects, and several references were made to species of Lepidoptera.

Botanicals usually have broader spectrum of activity than most biopesticides and therefore may be used against pest species. Arnason *et al.* (1993) reported essential plant products generally broad spectrum because of the presence of the several active ingredients that operate through several modes of action. Several are non neurotoxic and exert antifeedant activity, inhibit moulting and respiration, reduce growth and fecundity, and display phyto toxicity. Essential oils also may affect cuticle of the soft-bodied insects such as aphids, whitefly and thrips (Isman, 1999). The toxicity of botanical compounds often exerts differential effects, depending upon the target pest (Isman, 2000). This mode of action may prove to be safe for hard-bodied insects such as the adult stage of the Hymenoptera parasitoids (Chiasson *et al.,* 2004). On the whole, the essential botanicals should consist of compounds that are active against arthropods, while being harmless to beneficials and safe for humans and environment. Scott *et al.* (2004) tested the extracts from three species of plant, one plant family Piperaceae from 5 orders trailed against insects, revealed that formulations had a repellent activity, thus protecting the plant leaves from herbivory and oviposition.

Many commonly used plant protection products such as synthetic insecticides may affect beneficial predators and parasites to intensify further pest problem. Some studies have been taken on the effects of various plant extracts to control or repel insect pests; however since the advent of synthetic insecticides, the primary reason for their lack of use in commercial agriculture is their poor efficacy as compared with synthetic pesticides. But efficacious botanical derivatives can prove an alternative to synthetic pesticides, and agrochemical research has been initiated to focus on this area. Herein reported arguments of trials, we are reporting on the effect of plant materials for the control of rice stem borers and their effects on seed yield which could serve as an inexpensive and organic alternative to commercial pesticides, and safety to natural control. Our study and results on botanical chemicals will open new possibilities of application of chemical ecology to manage these pests. Present preliminary field test will show that application of plant material can significantly reduce the levels of leaf infestation and dead heart injury due to larvae of the stem borers, and resulted in increase in crop yield relative to the untreated controls.

MATERIALS AND METHODS

These studies were demeanored in the field site located at the experimental farm of Nuclear Institute of Agriculture, Tandojam to evaluate the effectiveness of 3 biologically based plant resources for pest management. For this purpose,

the effectiveness of plant materials such as Neem *(Azadirachta indica* A. Juss), Hing *(Ferula foetida* Regel) and Dhatoora *(Datura metch* Linn.) were tested against the rice stem borers on 4 rice varieties (Aromatic- Basmati 370, Super Basmati; non- aromatic- IR 8, Sarshar). Rice seedlings were transplanted and fertilized according to local growers practices. From Neem and Dhatoora plants that were growing in the field located at experimental farm of NIA, the leaves were obtained when the plants were at flowering stage and taken to the laboratory. These leaves were washed thoroughly to remove any dust and then shade dried and stored in paper bags until needed. The stored leaves were initially cut into pieces and then placed in a blender to break them into very small pieces. The dried leaves were then grounded to a fine powder by using a commercial grinder. Hing (dried gum like material) was procured from local market. It was also grounded to fine powder by using grinder. All the powdered plant materials were passed through 20 mesh sieve. Each material sample (20 g) was placed in container with 100 ml of water for 24 hours at room temperature with frequent stirring. The solid material remaining undissolved was removed by filtration and discarded. All extracts were stored in different sealed bottles until used for field evaluation against borers. During this trial for each application of treatments, the fresh formulations were prepared.

Within the experimental field, 3 m^2 plots were randomly designated each to be treated with one of the plant materials along with plots marked as untreated control. There was a non-treated buffer zone of 1 meter between each plot to prevent spry drift to adjacent plots. Every treatment and control was repeated three times. Each plant material was diluted by taking 100 ml already prepared solution and adding 900 ml of distilled water immediately before use to prepare 2% formulations. Fortnightly, the 2% concerned plant dilution was applied to every plant in 3 replicates with a low-pressure knap sack sprayer. In each trial spraying was repeated 3 times at fortnightly interval in 3 replicates of each treatment. First spray of these products was given near flowering stage and remaining two at 15 days interval subsequently. Observations were recorded 72 hours after every treatment, and damage resulting from larval stem feeding during tillering and flowering stages leading to dead hearts and white heads were recorded. For each observation, 16 plants from an area of 1 m^2 were randomly chosen in every replicate, from the treated and non-treated plots and examined for stem borers infestation and damage in the form of dead hearts and white heads was counted and recorded. The data for stem borers counts were terminated after 17-18 weeks when the plant had fully matured. All the plots were harvested and threshed for yield. Field observations were transformed into percentage infestation and yield data was also pooled of all replicates and their

mean values compared. Data were analyzed by analysis of variance (ANOVA) and mean values were separated. All results were checked at alpha = 0.05 level.

RESULTS AND DISCUSSION

Results clearly confirmed that all the extracts proved to be lethal against rice stem borers for their suppression than control units. The study specified that all treatments had negative possessions against pest indicating that fewer borers were present in treated elements or more were judged in the control treatment. Overall, the results obtained on percentage borers infestation from infested plants after foliar treatments with plant stuff are shown in Table I. Infestation in the control plants was significantly higher than the infestation intensity in treated plants and all the foliar applications of plant stuff were more effective than the non-treated plants. As with Hing treatment, the degree of damage (dead hearts, whiteheads) had significantly less intensity 2.88%, 2.54%, than Neem 3.98%, 3.49% and Dhatoora *i.e.*, 4.34%, 4.40% as compared with non-treated control having 6.90%, 8.40%, dead hearts and whiteheads, respectively. From overall treatments, plants from untreated control showed almost more % injury than the average of pesticides treatments.

TABLE I.- USEFULNESS OF THREE BOTANICALS FOR THE SUPPRESSION OF RICE STEM BORERS.

Treatment	**Stem borers infestation (%)**		**Yield / plot (4.5 m^2) (g)**	**Yield Kg/hectare**
	Dead hearts	**White heads**		
Neem *(Azadirachta indica)*	3.983 b	3.495 c	1254.00 b	4180.00
Dhatoora (Thorn apple) *(Datura metch)*	4.345 b	4.400 b	1106.00 c	3686.66
Hing (Asafoetida) *(Ferula foetida)*	2.880 c	2.542 d	1345.00 a	4483.33
Control	6.930 a	8.404 a	905.80 d	3019.33
	LSD = 0.421	LSD = 0.376	LSD = 17.34	

Results on yield are also shown in Table I. All the foliar applications yielded significantly heavier yields as compared with non-treated control having the lowest produce. A comparison of treatments showed that in control design, decreased average yield as 905.80 g/3 m^2 plot or 3019.33 Kg/Hectare was observed, whereas Neem and Dhatoora had produced 254.00g and 1l06.00 g (44180.00 and 3686.66 Kg), respectively, these treatments were significantly different than Hing that gave 1315.00 g/plot (3 m^2) (4483.33 Kg/Hectare).

However, Hing plunked at apex in performance and neem performed slightly better in harvesting more yield than Dhatoora treatment, on an average untreated control plants showed yield less than that of average in protected treatments. Besides increase in rice produce, these sprays were also found effective in yielding more healthy grains, as minimum bad quality seed was noted from treated plots. The quality of grain was not so superior in non-treated plots.

Our experimental results proved botanical insecticides to be appropriate alternative to the more persistent synthetic pesticides for managing the major pests of rice. These results are in concurrence to the previous investigators; Hassid *et al.* (1976) examined the insecticidal properties of plant foliage extracts toxic to lepidopterous larvae, and dried leaves when incorporated into artificial diet, also caused 100 % mortality. Subsequently, Adeyeye and Blum (1989) showed that when plant's active compounds were added to artificial diet, these disrupted growth and development in lepidopteran species. Civelek and Weintraub (2004) tested the extracts from plants for their insecticidal activity; all dilutions caused significant control of larva and maintained population below those of the non-treated control plants. The plants extract exhibited both translaminar and systemic activity and were potential candidates as organic insecticides.

During this study, plant product Hing was found to be excellent against lepidopteran species, its pesticidal activity includes repellency from treated surface thus acting as antifeedant and oviposition deterrant. A more suitable explanation of the greater sensitivity of lepidopteran larva may be greater absorption through the cuticle as compared with insects having thicker and tough cuticle. Structural differences in insect's cuticles in different species have been documented as the reason for different rates of penetration by Smagghe *et al.* (1997). Koul *et al.* (2002) reported and investigated the antifeedant and growth-inhibitory activities of natural products against insects.

The result of this study indicated that Hing was the most effective in controlling the stem bores than Neem and Dhatoora, to pursue the development of Hing as commercially acceptable product, further studies are necessary to verify whether this trend in maintained. Mode of action studies may be important in establishing its spectrum of activity and determining its full potential. Due to scanty of knowledge of the mode of action pathways of its organic mixture and lack of experimental protocol for these tests, much more work has yet to be carried out. Our results are not in line with Vijayalakshmi *et al.* (1979) where 50 indigenous plant products were screened, Dhatoora gave 100% mortality and

appeared to be the most promising of all the materials tested in the experiment. According to these researchers the neem was a potential product against the pest. Sehmutter and Rembold (1980), and Rembold *et al.* (1984) noted that seeds of *Azadirachta indica* acted as growth disrupter in a number of insect species. Azam (1991) and Dimetry *et al.* (1995), out of botanical insecticides tested against dipterans, found only neem based insecticides more effective. Applications of extracts of neem berries (seed) and pyrethrum flowers at 8% concentration resulted in 90 and 100 % mortality to I and II instar larva of *B. fusca* within three days, respectively (EARO, 1998/99).

Concludingly, the present day strategy is to use ecofriendly insecticides like plant products in a spray schedule at time when there is less pressure of borers, however at higher density of pest population, at least one round of synthetic origin insecticide may be required. It shows that depending upon unilateral use of plant or synthetic pesticide, is not economical to the growers, and maximum quality seed rice could be realized when botanicals are alternatively used with synthetic pesticides. The earlier studies have indicated that it is possible to reduce the consumption of pesticides by 50 percent by adopting IPM involving the neem based insecticides along with bio agents and biopesticides (Puri, 1997). If the borers population exploded beyond the range of control with botanical control agents owing to their higher reproductive rate, under such conditions chemical sprays become essential to combat pest problem. Gupta (1998) suggested that if a suitable IPM is formulated with botanicals and biopesticides as major supplements, in a big way, if not substituted, and then it will definitely decrease the lopsided load of synthetic insecticides on the environment. Isman (1994) was of the view that non persistent products, *viz*., botanical, may appear expensive to the growers than synthetic insecticides, because of repeated applications, except when the hidden costs of environment impacts are taken into account. Two applications of any of the botanicals were not sufficient to provide complete protection against larvae. This suggests that these botanicals have only brief persistence, and more than two applications of the extracts would be necessary to reduce pest numbers (Assefa and Ferdu, 1999). In short, it can be said that the use of plant products does not give immediate results like chemical insecticides; and some patience is required after the application of such materials.

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FEEDING HABIT AND SELECTIVE FEEDING BEHAVIOR OF PALRI, *GUDUSIA CHAPRA* (CLUPEIDAE) FROM CHILYA FISH HATCHERY, THATTA

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Abstract.- A study on selection of planktonic food organisms by palri, *Gudusia chapra* was carried out during the months of December and January 2004-2005. In total 56 specimens, size ranged between 118-160 mm were observed from Chilya fish hatchery. The selectivity of food was found in fish. The selectivity for plankton was more or less same during both of the months. *Gudusia chapra* mainly fed upon plankton *(Euglena, Chroococus, Pediastrum* and *Keratella valga).* The fish was found selective feeder on some plankton by computing the food organisms and environmental organisms through electivity indices. During this study negative electivity for some genera and positive for others was observed.

Key words: Feeding habit, electivity, *Gudusia chapra*

INTRODUCTION

Knowledge of feeding habit of fish is of prime importance to understand its ecological behavior and physiological requirement. Studies on selectivity of food mainly focus on its foraging behavior (AI-Akel *et al.*, 1987). The fish hatchery of Thatta is spread over an area of 77 acres with an inflow of water from K.B. feeder is located about 6 kilometers east form Thatta on Indus highway, near Keenjhar lake. The fish hatchery consists of two portions, nursery ponds and brooders ponds. Each polyculture brooder pond is 86 m long and 56 m wide with an average depth of 4-5 feet.

Gudusia chapra is one of the freshwater clupeid found in Pakistan. From Pakistan two freshwater clupeid are reported; one is *G. chapra* and other is *G. variegata. G. chapra* is commonly known as palri. It is abundant in rivers, lakes and ponds (Mirza and Bhatti, 1999). Different aspects of *G. chapra* have been studied from India and Bangladesh by Jhingran and Verma (1973), Bhuiyan and Hossain (1988), Quddus (1993), Rahmatullah *et al.* (1995) and Afroz *et al.* (1999). From Pakistan only one paper is so far available on the age and growth of *G. chapra* (Narejo *et al.,* 2000).

MATERIALS AND METHODS

Fish samples were collected from brooders pond of Chilya Fish Hatchery, Thatta, using a seine net during the months of December and January. The minimum and maximum length (TL) were 118 -160 mm, while minimum and maximum weight 15.9–36.0 gm. Fish were injected by 5% formalin on the spot, brought to the laboratory, sized and weighed. Afterwards fish were dissected and guts were preserved in 5% formalin. Qualitative examination of gut contents were observed by using identification keys by Ward and Whipple (1959) and Prescott (1981). For qualitative examination number method was used. Food items were counted by using Sedgwick-Rafter counting cell under binocular microscope. For environmental data each month 3 samples of water comprising 10 liter were collected from different areas of pond. Samples were filtered through fine mesh (55µ) plankton net. The collected material was poured in the bottle and preserved in 5% formalin. Qualitative study was made by using plankton identification keys and various organisms were counted by using Sedgwick-Rafter counting chamber.

Feeding selectivity analysis was made by Ivlve's electivity indices. Electivity was calculated according to,

$$E = ri - pi / ri + pi \text{ (Ivleve, 1961)}$$

Where, ri is % of food in gut, and pi is % of food in environment.

The values of electivity index ranged from (-I) to (+ I), depending upon the selectivity of food.

RESULTS AND DISCUSSIONS

Feeding habit

During the studies the gut contents of 56 specimens were analyzed. Examination of gut contents showed that fish feed on twenty seven different food items along with eggs and debris. Seven food items belonged to zooplankton and eighteen belonged to phytoplankton. All these food items were categorized in seven main groups zooplankton (rotifers), Chlorophyceae, Cyanophyceae, Bascillariophyceae, Euglenophyceae, rotifer eggs and debris. Percentage of each food item is given in Table I. Percentage composition revealed that fish has taken highest percentage of Chlorophyceae, Bascillariophyceae, Euglenophyceae.

Among phytoplankton, Cyanophyceae was taken in lesser quantity. Fish has taken only rotifers from zooplankton. Eggs and debris are also taken in lesser amount. Presence of planktonic food in the guts indicated that it is planktivorous fish.

TABLE I.- PERCENTAGE OF MAJOR FOOD GROUPS IN GUTS.

Food items	December	January
Zooplankton		
Rotifers	16.6	15.5
Phytoplankton		
Cyanophyceae	4.0	4.4
Chlorophyceae	31.3	30.9
Bascillariophyceae	23.0	23.8
Euglenophyceae	17.4	18.0
Debris	4.8	4.6
Eggs	2.9	2.7

From the analyses of food in the guts it is evident that fish is a surface feeder as it has consumed planktonic algae, rotifers and rotifer eggs etc. In the present study it was found that percentage composition of plant matter was more than that of animal matter and debris. Similar observations were made by Das and Moitra (1955) and Afroz *et al.* (1999). However, Choudhary (1960) stated that fish is mainly a zooplankton feeder. Rahmatullah *et al.* (1995) found debris as a dominant food item. These results are in contrast with the present investigation. The difference of Choudhary's results (Choudhary, 1960) from present findings might be due to lack of phytoplankton in that habitat.

Electivity

The percentage of major group composition of food items in guts as well as in environment for both the months are depicted in Figures 1 and 2. These graphs show that the percentage composition of Chlorophyceae and Euglenophyceae in the guts is greater than the environment. For other food groups percentage of rotifers, Cyanophyceae, Bascillariophyceae, eggs and debris was less than the environment.

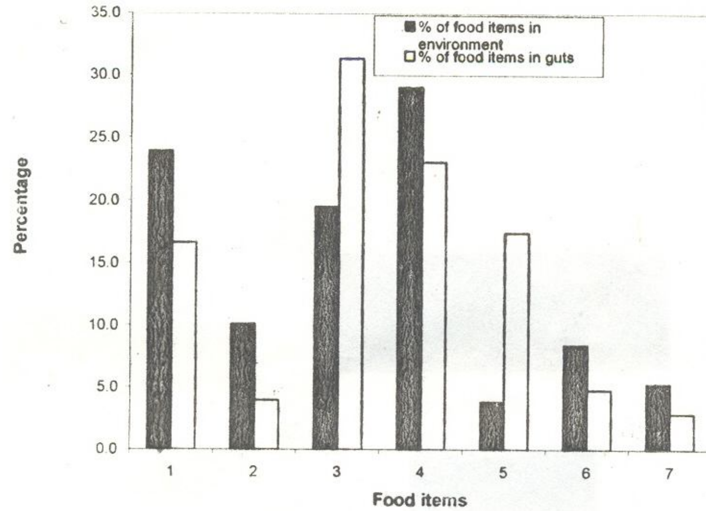

Fig. 1. Percentage composition of major groups of food items during the month of December.

1, Rotifers; 2, Cyanophyceae; 3, Chlorophyceae; 4, Bascillariophyceae; 5, Euglenophyceae; 6, debris; 7, eggs.

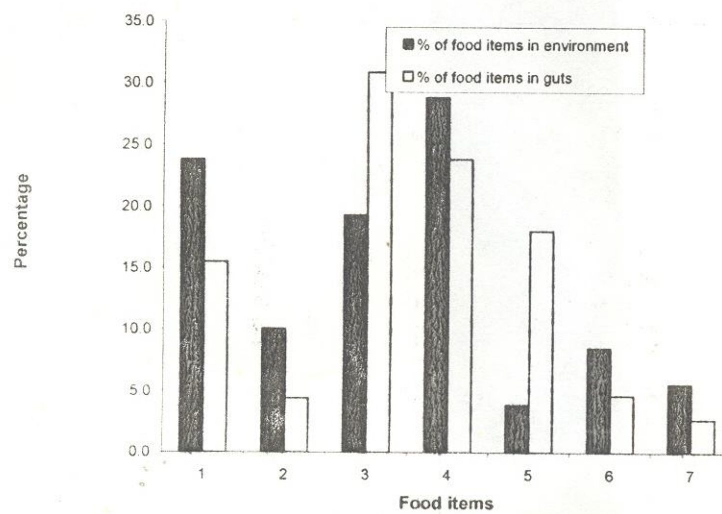

Fig. 2. Percentage composition of major groups of food items during the month of January.

1, Rotifers; 2, Cyanophyceae; 3, Chlorophyceae; 4, Bascillariophyceae; 5, Euglenophyceae; 6, debris; 7, eggs.

Electivity indices of present investigations showed that fish is a selective feeder. Results of electivity indices are given in Table II. In total seven types of food groups occurred in the guts. Among zooplankton only rotifers were found in the guts as well as in environment. Among rotifers *Keratella valga* was the only food item that was positively selected (+0.2 in December) and (+0.28 in January). The other rotifers like *Keratella cochlearis, Brachionus, Pompholynx complanta,*

TABLE II.- LIST OF FOOD ITEMS WITH ELECTIVITY INDICES.

Taxa	December	January
Zooplankton		
Brachionus	-0.82	-0.72
Keratella cochlearis	-0.04	-0.12
Monostylla bulla	-0.32	-0.42
Keratella valga	+0.20	+0.28
Notholca	-0.84	-0.84
Polyarthra trigla	-0.41	-0.45
Pompholynx complanta	-0.68	-0.67
Phytoplankton		
Cyanophyceae		
Oscillatoria	-0.44	-0.38
Nostoc	-0.50	-0.55
Chroococcus	+0.31	+0.43
Aphanocapsa	-0.44	-0.32
Microcystis	-0.86	-0.77
Marismopedia	-0.81	-0.82
Chlorophyceae		
Pediastrum	+0.24	+0.24
Spirogyra	-0.47	-0.34
Bascillariophyceae		
Gyrosigma	+0.01	+0.10
Pleurosigma	-0.53	-0.53
Navicula cryptocephala	+0.14	+0.12
Frustula	-0.12	-0.10
Synedra	-0.42	-0.45
Cymatopleura eliptica	+0.10	+0.17
Melosira	-0.80	-0.81
Euglenophyceae		
Euglena	+0.64	+0.64
Debris	-0.28	-0.29
Eggs	-0.30	-0.34

Polyarthra trigla and *Notholca* were negatively selected. Among Cyanophyceae, only *Chroococcus* was positively selected by the fish (+0.31 and +0.43) in December and January, respectively. However, *Oscillatoria, Nostoc, Aphanocapsa, Microcystis* and *Merismopedia* were negatively selected. Among green algae, fish have selected *Pediastrum* positively (+0.24) in both the months, but *Spirogyra* was negatively selected. Among Bascillariophyceae, *Gyrosigma, Navicula, Cryptocephala* and *Cymatopleura eliptica* were positively selected and *Pleurosigma, Frustula, Synedra, Melosira* were negatively selected by the fish. In Euglenophyceae, *Euglena* is the only genus found in guts, fish showed maximum positive selection for *Euglena* with electivity indices of +0.64 during both of the months.

No food item was avoided with (-1) selectivity. However, highest negative selection was observed for *Microcystis* (-0.86), *Notholca* (-0.84), *Brachionus* (-0.82), *Merismopedia* (-0.81) and *Melosira* (-0.80). A negative selection for eggs and debris was also found to be true. Results of electivity were more or less similar during both the months. It proved the fish as selective feeder, although it did not show complete avoidance, it avoided at least18 food items, might be due to distastefulness. Selective feeding can be expected from the fish when the energy gained by feeding, as preferred food items exceed the energy that has been lost during selection (Al-Akel *et al.,* 1987). Many authors (Mustafa, 1976; Wankowsky, 1979; Bartell, 1982) reported that fishes expend more energy in selecting prey of large size to get more energy. In the present investigation negative selection of zooplankton by the fish and more abundant phytoplankton may be due to the fact that the fish look for the food of more digestibility.

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EFFECT OF COTTON SOWING DATE ON THE POPULATION BUILD-UP OF SUCKING INSECT PEST COMPLEX ON NIAB-78 COTTON VARIETY

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Abstract.- The study was conducted in the field of Bukera Agriculture Farm, Taluka Tando Allahyar, Distt: Hyderabad, Sindh. The seed of cotton variety NIAB-78 was sown at fortnight intervals *i.e.* 1st May, 15th May and 29th May 2000, respectively. The population counts of jassid, thrips and white fly were made at weekly intervals starting from June, 18, 2000. Both stages *i.e.* adults and nymphs were recorded. The results showed that the population density of jassid was above economic threshold level during July in almost all three sowing dates. The over all sowing dates response to Jassid attack revealed that minimum population (4.18±0.64 jassid/plant) was in cotton sown on 15th May followed by sowing dates of 29th May(5.20±1.10 jassid/plant) and 1st May (5.21±1.17 jassid/plant). Whereas, the population densities of thrip on different sowing dates was maximum (12.4±4.58 thrips/plant) recorded in cotton sown on 15th May. The minimum population of thrips was recorded on 29th May (7.73±1.99 thrips/plant) and the moderate level of infestation was found in cotton sown on 1st May, (10.01±4.0 thrips/plant). However, the population of white flies revealed a comparatively lower trend than jassid and thrip. The result on seed cotton yield showed that the yield of cotton was significantly different at three sowing dates. The seed cotton yield (211kg/plot) was higher in cotton sown on 1st May compared to the one sown on 15th May (171kg/plot) and 29th May (192 kg/plot).

Key words: Sowing dates, *Amrasca devastance, Thrips tabaci, Bemisia tabaci.*

INTRODUCTION

Cotton is one of the most important cash crops of Pakistan. Economically it earns largest export revenues. It fulfills partial requirement for cloths and animal feed. Cotton seed is one of the main sources of edible oil and 52% oil is locally extracted from cotton seed. It is vulnerable to a wide variety of insect pests. During vegetative growth stage, sucking complex comprising of jassid, thrip, whiteflies and mites cause enormous losses to the vegetative parts of the plants (Talpur *et al*., 1993).

Cotton Jassid, *Amrasca devastans* (Dist.) is a serious pest of cotton in Sindh and Punjab. The nymph and adult suck the sap from under side of the leaves and inject toxic saliva into the plant tissue. The attacked leaves generally show turning of the edges downwards, curling, crinkling, drying in the region of the veins and

ultimately drop off. thrip, *Thrips tabaci* (Lind.) Both nymphs and adults lacerate the young leaves and suck the sap by means of pharyngeal pump. The attacked leaves develop a silvery coating on the lower surface and become saucer shaped. Whitefly *Bemisia tabaci* (Genn) suck the sap and lowers the vitality of the plant. Besides, it secretes honey dew which promotes the appearance of sooty mould and this interferes with the photosynthetic activity of the plant. The growth of plant is adversely affected and shedding off the bolls increase and yields is considerably decreased due to the attack of this insect pest (Atwal, 1978). Due to attack of these insect pests the yield is about 40% to 50% less in our country and the economy have also been adversely affected. The present studies were carried out to determine the effect of cotton sowing date on the population build-up of sucking complex and the yield of NIAB-78 cotton variety.

MATERIALS AND METHODS

Experimental design and procedure

The experiment was laid out in Randomized Complete Block Design (RCBD) with four replicates and three treatments. Each plot size measured 22 x 45 meters, distance between row to row was 90 cm and plant to plant was 23-30 cm. Before sowing about 1kg/plot seed was soaked in water about for 12-15 hrs. The seed was then rubbed with gunny bags or silty dry soil to remove the hairs for proper settlement for sowing. 1st sowing was done on 1st May, 2nd on 15th May and 3rd on 29th May on ridges by dipling method. Normal agronomic practices were followed for each plot.

Recording of cotton sucking insect pest population

Observation on the population build-up by sucking insect pests *viz*: Thrip, Jassid and Whiteflies were recorded at weekly intervals on Niab-78 variety at morning time. For recording the insect population 20 plants were selected randomly from each treatment and five leaves from each plant including top, middle and bottom portions were examined. The data thus obtained and percent infestations of sucking complex were subjected to analysis of variance.

RESULTS

Jassid Amrasca devastans *(Dist.)*

The results on the weekly mean population per leaf of jassid on cotton NIAB-

78 variety shown on three different sowing dates at fortnight intervals *i.e.* 1st May, 15th May and 29th May 2000 are presented in Table I. The data showed that jassid population varied significantly with the date of sowing and phenology of plant. The observation on the population of jassid was recorded on 18th June on all three sowing dates. Initially the population was low (3.71, 3.58 and 4.05 jassid/leaf) on cotton sown on 1st, 15th and 29th May, 2000 respectively. The population increased as the plant phenology progressed and recorded (14.37, 7.46 and 14.97 jassid/plant) on cotton sown on 1st, 15th and 29th May, 2000 respectively. There after, the population of jassid gradually decreased and towards the maturity of the crop plants the population practically disappeared in the month of September. The response of test cotton variety NIAB-78 to the attack of jassid was significantly higher at vegitative growth stage and establishment stages of the plant as compared to reproductive stage.

The overall population densities of jassid on cotton NIAB-78 variety showed that the jassid was above economic threshold level during July almost in all three sowing dates and never crossed this level during the reproductive stage of the cotton plants. The overall sowing dates response to jassid attack (Table I) revealed that minimum population (4.18±0.64 jassid/plant) was recorded on NIAB-78 sown on 15th May. It was followed by cotton sown on 29th May (5.20±1.10 jassid/leaf) and May, 1st (5.21±1.17 jassid/leaf) respectively. The statistical analysis of the data revealed that the jassid population was statistically non significant on all the sowing dates during vegetative stage of the cotton plant.

Thrip, Thrips tabaci *(Lind)*

The results on the mean per leaf population of thrips on different sowing dates are shown in Table II. The data revealed that the incidence of the thrip on NIAB-78 variety was 6.25, 5.22 and 5.17 thrip/leaf as recorded with cotton sown on 1st, 15th and 29th May 2000, respectively. The population of thrip increased with the plant phenology and reached to its peak (48.17, 58.22 and 23.15 thrip/leaf) on 9th July respectively. The thrip population was above economic threshold level during 3rd week of June to 3rd week of July and significant attack was recorded at this stage. During this stage the plants are healthy and all the leaves of the plants were tender and green. There after, the population drastically declined (6.38, 13.31 and 13.77 thrip/leaf) on 16th July due to the heavy rain shower. The population of thrip in reproductive stage of the crop plant was very low and recorded below economic threshold. This level was constant from 6th August to 10th September, 2000 on three sowing dates respectively. The statistical analysis of the data revealed significant variation amongst observation dates.

TABLE I.- WEEKLY MEAN POPULATION PER LEAF OF *A. DEVASTANS* ON COTTON SOWN ON DIFFERENT SOWING DATES DURING SUMMER 2000.

Date of observations	Weekly mean population on sowing dates		
	1ST May	**15th May**	**29th May**
June 18	3.71	3.58	4.05
June 25	5.33	4.56	4.96
July 02	14.37	6.93	10.23
July 09	8.25	5.15	14.97
July 16	12.02	6.98	8.56
July 23	7.26	7.46	7.05
July 30	5.32	6.86	4.43
August 06	3.13	3.76	2.77
August 13	2.28	2.43	2.07
August 20	2.47	2.11	2.98
August 27	1.41	1.61	2.32
September 03	1.20	1.66	1.75
September 10	1.02	1.03	1.55
Mean±S.E	5.21±1.17	4.18±0.64	5.20±1.10

Weekly data = $P<0.01$; Sowing dates = $P<0.05$

The overall mean populations of thrips on different sowing dates shown in Table II. reveal that maximum population (12.4±4.58 thrip/leaf) was recorded on NIAB-78 variety sown on 15th May, 2000, where as the minimum population of thrip was recorded on cotton sown on 29th May (7.73±1.99 thrip/leaf). The moderate level of thrip infestation was recorded on cotton sown on 1st May, 2000 (10.01±4.0 thrip/leaf). It is evident from the results that 15th May, 2000 sowing date is favourable for multiplication of population of thrip, whereas, May 29th sowing date is unfavourable for multiplication of the thrip population.

White fly, Bemisia tabaci *(Genn)*

The results on the mean number of whiteflies per leaf on cotton NIAB-78 variety sown on 1st, 15th and 29th May, 2000 are presented in Table III. A critical review of the data revealed that whiteflies incidence was 1.83, 1.97 and 3.68 whiteflies/leaf on 18th June respectively. Later the population declined and recorded in significant (0.88, 0.35 and 0.92 whiteflies/leaf) on 25th June respectively. There after, the population slightly increased in the 1st and 2nd week of July but was not in economic threshold level. It supposed to be a first peak of population during the month of July *i.e.* (1.40, 0.86 and 1.01 whiteflies/leaf) on 2nd July, 2000 respectively on different dates of survey. Whereas, during the heavy rain shower the

population of whiteflies drastically reduced and was recorded in negligible level of infestation on 16th July. However, the population of whiteflies again increased (1.51, 1.55 whiteflies/leaf) on 6th August, 2000 respectively, but it was below the economic threshold level. The statistical analysis of the data revealed non-significant variations amongst the sowing dates. This indicated that the whiteflies, population remained stable on all the dates during observation.

TABLE II.- WEEKLY MEAN POPULATION PER LEAF OF *T. TABACI* ON COTTON SOWN ON DIFFERENT SOWING DATES DURING SUMMER 2000.

Date of observations	Weekly mean population on sowing dates		
	1ST May	15th May	29th May
June 18	6.25	5.22	5.17
June 25	17.66	25.43	10.82
July 02	32.00	29.16	20.10
July 09	48.17	58.22	23.15
July 16	6.38	13.31	13.77
July 23	4.88	9.43	7.95
July 30	5.23	7.61	4.15
August 06	1.92	4.27	2.35
August 13	1.57	1.98	1.65
August 20	1.72	2.66	4.03
August 27	1.15	1.22	3.25
September 03	1.87	1.43	2.17
September 10	1.42	1.26	1.96
Mean±S.E	10.01±4.00	12.40±4.58	7.73±1.99

Weekly data = $P<0.01$; Sowing dates = $P<0.05$

The overall response (Table III) of cotton NIAB-78 variety to whitefly attack revealed a comparatively lower population as compared to Jassid and thrip. The over all sowing dates' response to whiteflies showed that minimum population was recorded (0.99±0.09 whiteflies/leaf) on cotton sown on 1st May, 2000. Whereas, on the rest two sowing dates i.e. May 15th and May 29th, the population level was identical (1.25±0.13 and 1.24±0.21 whiteflies/leaf).

Seed cotton yield

The results on the seed cotton yield (kg/plot) of NIAB-78 variety sown on different sowing dates are presented in Table IV. The yield was influenced significantly by the time of sowing and it was significantly different at different sowing dates. The cotton sown on May 1st, 2000 yielded significantly higher seed

cotton (211 kg/plot). This was followed in descending order by May 29th (192 kg/plot) and May 15th (171 kg/plot), respectively.

TABLE III.- WEEKLY MEAN POPULATION PER LEAF OF WHITE FLY, *B. TABACI* ON COTTON SOWN ON DIFFERENT SOWING DATES DURING SUMMER 2000.

Date of observations	**Weekly mean population on sowing dates**		
	1ST May	**15th May**	**29th May**
June 18	1.83	1.97	3.68
June 25	0.88	0.35	0.92
July 02	1.40	0.86	1.01
July 09	1.12	0.75	0.61
July 16	0.68	1.17	0.80
July 23	0.76	0.90	0.91
July 30	1.21	0.95	0.77
August 06	1.08	1.55	1.51
August 13	0.95	1.28	1.18
August 20	1.02	1.65	1.32
August 27	0.57	2.12	1.03
September 03	0.86	1.51	1.17
September 10	0.60	1.18	1.30
Mean±S.E	0.99 ±0.09	1.25±0.13	1.24±0.21

Weekly data = $P<0.01$; Sowing dates = $P<0.05$

TABLE IV.- SEED COTTON YIELD (KG/PLOT) OF COTTON NIAB-78 VARIETY SOWN ON DIFFERENT DATES DURING SUMMER 2000.

Sowing dates	**Yield Kg/Plot**
May, 1, 2000	211
May, 15, 2000	171
May, 29, 2000	192

DISCUSSION

The results of present study are also in agreement with those of Bughio et al (1986) who reported from Tando Jam, Pakistan in 1980-82 the population density of *Amrasca devastans, Thrips tabaci, Bemisia tabaci* on 3 mutant strains (189/72, B-60 and B-2) and commercial varieties (M-100 and Qalandri) of cotton and found

no significant differences in the responses of all the mutant strains and commercial varieties to the attack of the pest or in the variation of pest population densities on all the genotypes. The *Amrasca devastans* population remained above economic threshold (1 insect/leaf) from the 4th week of May to the end of June. *Thrips tabaci* population reaches economic threshold (8-10 thrips/leaf) on all the cotton genotypes during 3rd week of June; this trend was maintained only until the 4th week of June during 1980.

The results of present study are also in agreement with those of Dhawan *et al*. (1990) who carried out field experiments in Punjab, India, to determine effect of different sowing dates on cotton cv. LH-900 on the incidence of insect pests, compared to cotton cv. F-286. LH-900 sown on 3 dates, 13 May, 26 May and 13 June, where as F-286 was sown on 13 May only. Numbers of the cicadellid *Amrasca biguttula* damage were highest on LH-900 sown on 26 May and number of the aleyrodid *Bemisia tabaci* was highest on F.286.

The results of present study are also in agreement with those of Sewify *et al*. (1996) who reported that effect of sowing date of cotton on sucking insects and their associated predators in the Giza region, Egypt. Cotton was sown on 28 February and 1st May, 1991 while only late sowing was carried out in 1992 and 1993. Late sown plants were treated with micronutrient foliar fertilizer to improve growth and yield. The highest population of *Thrips tabaci* occurred on the early sown crop, but the population density was very low on the late sown crop.

The results of the present experiments are in partial agreement with those of May (1996) who reported that the plant height decreased and boll retention changed from a top crop to a bottom crop. Wet weather in 1996 reversed these changes. Sowing date was an important yield-forming factor, with sowing in mid-September to mid-October giving maximum yields and profits. Insecticide costs were the highest and most variable input cost in cotton production.

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POPULATION OF THRIPS, *THRIPS TABACI* (LIND) AND ITS NATURAL ENEMIES ON ONION CROP

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Abstract.- Studies on the population of thrips and its natural enemies on onion crop were carried out at the farmer's field near Tandojam during the Rabi season 2003-04 on Phulkara and Nasrpuri varieties. The results suggested that mean thrips population was relatively more on Phulkara variety (6.85/plant) as compared to Nasarpuri variety (6.48/plant). High trend of thrips population was recorded during December on both host plant. The predators' population is dependent upon the pest abundance. Among all predators the more population of spider *Scytodes sp.* (0.59) Black ant *Camponotus compressus* F. (0.49), *Syrphus sp.* (0.53) and *Chrysoperla sp.* (0.75) was recorded on Nasarpuri variety. However, the population of thrips and predators were non-significant. The statistical analysis shows strange avoidance that predators' population is correlated with the pest population.

Key words: Thrips population, natural enemies, onion.

INTRODUCTION

Onion, *Allium cepa* belonging to family Liliacea, is an important vegetable crop of Pakistan and many other countries of the world. It is used extensively as a condiment in the preparation of curries and pickles. Both the green and dry onions are used as salad and are served along with meals. The onion has medical values and is used in the preparation of tonics (Khoso, 1994).

Onion crop is attacked by a number of pests including thrips, whitefly, aphid , gram pod borer and tobacco cut worm and due to their attack of the crop yield of onion is also decreased (Atwal, 1976; Clausen, 1978; Shelton and North, 1987; Shelton and Barnard, 1988). Among others, two thrips species *i.e. Thrips tabaci* Lind and *Schizothrips dorsalis* Hood, are key pests of this crop. Some times the attack is so severe that farmers fail to get seed from their own crop (Bhardwat *et al.,* 1992; Hashmi, 1994).

Hajdu and Nagrimre (1986) reported that onion suffers from many insect pests including mites, nematodes and other diseases and due to attack approximately 30% losses occur in onion. Many natural enemies have been

reported in onion fields, which ultimately reduce the population of many insect pests and keep the balance of pest populations (Zaz and Kushwaha, 1983). Keeping in view the importance of sucking insect pests on onion crop, present studies were conducted to observe the population of thrips, *Thrips tabaci* and is natural enemies on onion crop at Tandojam, Pakistan.

MATERIALS AND METHODS

Present studies were carried out in season of 2003-2004(November to February) on farmers field near Tandojam . The experiment was laid out Randomized Block Design and plot size measured 150×18 meters having four replications. One month old seedlings of Phulkara and Nasarpuri varieties of onion were planted on ridges in two sub blocks keeping distance of 45 cm between row to row and 15cms between plant to plant . No pesticide application was applied during observation time.

The sowing was done on 20th October 2003. After 15 days of sowing the observations of thrips and their natural enemies were taken .To determined thrips population 10 plants in each replication of each variety were randomly observed at weekly intervals. The presences of thrips were observed on whole plant. The number of natural enemies present on each plant was also recorded. Finally the data was statistically analyzed.

RESULTS AND DISCUSSION

Thrips on Nasarpuri variety of onion

The data presented in Table I indicate that thrips (*Thrips tabaci*) appearance on onion variety Nasurpuri during first week of November-2003 was 2.35 per plant which started rising to 8.10/plant up to the end of November. The trend continued and the first peak of thrips population was recorded at the end of December which was 9.75/plant. In the first week of January-2004. The thrips population decreased to 4.15/plant because the pest had completed its first generation. The pest population started rising again and the second peak of thrips population was recorded during first week of February which was 10.50thrips/ plant. There after the pest population started decreasing gradually. It reached to 2.02/plant at the end of February. The overall mean population of thrips was 6.48/plant.

Table I

Population of predators on Nasarpuri variety of onion

Spider, Scytodes *sp.*

The mean population of spider (Table I) shows that it was 0.25/plant during the second week of November which increased 0.50/plant in the third week of November. The spider population fluctuated through out the crop season. First peak was recorded during last week of December (0.75) and the second peak was recorded in 4th week of January (1.50)/plant. The spider population continued until 4th week of February. The overall mean population of spider on Nasarpuri variety of onion was 0.45/ plant.

Black ant, C. compressus

The mean weekly population of ant predator presented in Table I shows that the Black ant appeared during 2nd week of November (0.25/plant) and continued until 4th week of February. The population fluctuated through out the observation period. First peak was recorded during 4th week of December (0.75/plant), the second peak was observed during 4th week of January (1.00/plant) and the third peak during 4th week of February (1.0/plant). The overall mean population of ant on Nasarpuri variety of onion was 0.42/plant.

Syrphus *sp.*

The weekly mean population of *Syrphus sp*. is predator presented in Table I. the predator appeared during 2nd week of November (0.25/plant) and continued until 4th week of February. The population of this predator fluctuated through out the crop season. First peak of population was recorded during the 4th week of December (0.75/plant), Second peak was observed during 4th week of January (1.50/plant) and the third peak was observed during the 4th week of February (1.01/plant). The overall mean population of *Syrphus* sp. on Nasaurpuri variety of onion was 0.51/plant.

Chrysoperla *sp.*

The mean weekly population of *Chrysoperla sp*. (Table I) appeared during 1st week of November (0.25/plant) and it fluctuated throughout the crop season. First peak was recorded during the 4th week of November (0.75/plant). This trend continued until the 3rd week of January. The second peak of population was recorded during 4th week of January (1.50/plant) and the 1st week of February

(1.0/plant). Among all predators the population of *Chrysoperla* sp. was more than others. The overall mean population of *Chrysoperla* sp. on Nasarpuri variety of onion was 0.60/plant. Zaz and Kushwaha (1983) mentioned that many natural enemies ultimately reduce the population of insect pests on onion and keep the balance of pest populations.

Thrips on Phulkara variety of onion

The data presented in Table II. revealed that the thrips (*Thrips tabaci*) started infestation on Phulkara variety of onion during the 1st week of November (2.45/plant). The population of thrips increased gradually and the first peak population was recorded during the third and 4th week of December (10.50 and 12.00/plant) respectively. The trend of increasing population continued up to 1st week of February when the second peak was recorded (10.35/plant). After that the pest population started decreasing gradually. It reached to 1.20/plant at the end of February. The overall mean population of thrips on Phulkara variety onion was 6.85/ plant.

Population of predators on Phulkara variety of onion

Spider, Scytodes *sp.*

The results presented in Table II indicate that the spider appeared on the crop during the 2nd week of November (0.25/ plant) and reached to 0.45/plant during 4th week of November. The first peak was recorded during 4th week of December (1.0/plant). The predator population fluctuated during January and the second highest population was recorded during 4th week of January (2.0/plant) and first week of February (1.0/ plant). There after the spider population decreased gradually and reached to 0.25/plant by the end of February. The overall mean population was 0.59/plant during crop season.

Black ant, Camponotus compressus *F.*

The data presented in Table II indicates that ants appeared during the 2nd week of November (0.25/plant). The population remained similar until 4th week of December when it was 0.75/ plant. The second peak was recorded during 4th week of January 1.50/ plant and second week of February 1.0/plant. There after ant population decreased gradually and reached to 0.10/plant during 4th week of February. The overall mean population was 0.49 during the crop season.

Table II

Syrphus *sp.*

The data presented in Table II indicates that the population of *Syrphus sp.* started building during 1st week of November (0.25/plant). It increased slowly and gradually and reached to 0.75/plant 4th week of November and 4th week of December (0.75/plant) in both in months. The maximum population was recorded during 4th week of January (2.00/plant). After January the predator population started decreasing and reached to 0.25/plant in the end of February. The overall mean population was 0.53/plant during the crop season.

Chrysoperla *sp.*

The data in Table II indicates that the *Chrysoperla sp.* appeared on Phulkara variety of onion during firsat week of November and it slowly increased to1.0/plant during 4th week of November and 4th week of December (1.0/plant). The maximum population of *Chrysoperla sp.* was recorded during 4th week of January (2.0/plant). There after the predator population started decreasing gradually and reached to 0.25 during 4th week of February. The overall mean population of the predator was 0.75 /plant during the crop season.

TABLE III.- OVERALL MEAN POPULATION OF THRIPS AND ITS NATURAL ENEMIES ON PHULKARA AND NASURPURI VARIETIES OF COTTON.

Pest and predator	**Varieties**		**Mean±SD**
	Nasurpuri	**Phulkara**	
Thrip	5.89	5.85	6.66±0.26
Spider	0.45	0.58	0.51± 0.09
Black ant	0.42	0.47	0.44±0.03
Syrphus sp.	0.50	0.52	0.51±0.01
Chrysoperla sp.	0.60	0.72	0.66±0.08

Overall population of thrips and predators on onion

Overall mean population of thrips and different predators is presented in Table III. the results revealed that comparatively similar population of thrips and four predators was recorded on both varieties of onion. However, on Phulkara variety the thrips population was relatively more (6.85/plant) than on Nasarpuri variety (6.48/plant). Similarly the predators population was also relatively more on phulkara, in case of spider (0.59/plant), Black ant (0.49/plant), *Syrphus* sp.

(0.53/plant) and *Chrysoperla* sp. (0.75/plant) as compared to Nasarupuri variety on which the spider, Black ant, *Syrphus* sp. and *Chrysoperla* sp. were 0.45, 0.42, 0.51 and 0.60/plant, respectively.

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TABLE I.- MEAN POPULATION OF THRIPS AND ITS NATURAL ENEMIES ON NASURPURI VARIETY OF ONION RECORDED IN 2003-04.

Time intervals	Thrips	Mean population per plant				Total
		Spider	Predators			
			Black ant	*Syrphus* sp.	*Chrysoperla* sp.	
Nov.5-2003	2.35	0.0	0.0	0.0	0.25	0.25
13	3.55	0.25	0.25	0.25	0.25	1.0
21	5.90	0.50	0.25	0.50	0.52	1.75
29	8.10	0.50	0.50	0.50	0.75	2.25
Dec.07-2003	8.55	0.25	0.25	0.25	0.50	1.25
14	8.27	0.50	0.25	0.50	0.75	2.00
21	8.15	0.25	0.25	0.50	0.75	1.75
29	7.75	0.75	0.75	0.75	0.75	3.00
Jan.05-2004	4.15	0.50	0.50	0.25	0.50	1.75
12	6.65	0.25	0.25	0.50	0.50	1.50
19	8.20	0.50	0.50	0.75	0.75	2.50
27	10.35	1.50	1.00	1.50	1.50	5.5
Feb.02-2004	10.50	0.75	0.75	0.75	1.00	3.25
10	5.25	0.50	0.50	0.50	0.75	2.25
17	4.60	0.50	0.25	0.25	0.50	1.50
24	3.85	0.25	1.00	1.00	0.25	2.50
28	2.02	0.00	0.00	0.00	0.00	0.00
Mean±SD	5.89±2.69	0.45±0.34	0.42±0.30	0.50±0.33	0.60±0.34	2.0±1.24

TABLE II.- MEAN POPULATION OF THRIPS AND ITS NATURAL ENEMIES ON PHULKARA VARIETY OF ONION RECORDED IN 2003-04.

Time intervals	Thrips	Mean population per plant				Total
		Spider	Predators			
			Black ant	*Syrphus* sp.	*Chrysoperla* sp.	
Nov.5-2003	2.45	0.0	0.0	0.25	0.25	0.50
13	3.82	0.25	0.25	0.25	0.50	1.25
21	6.15	0.50	0.25	0.50	0.75	2.00
29	8.10	0.75	0.50	0.75	1.00	3.00
Dec.07-2003	6.25	0.50	0.25	0.25	0.50	1.50
14	6.90	0.25	0.50	0.50	0.75	2.00
21	10.50	0.50	0.25	0.50	0.50	1.75
29	12.00	1.00	0.75	0.75	1.00	3.50
Jan.05-2004	10.15	0.50	0.25	0.25	0.50	1.50
12	9.40	0.25	0.25	0.50	0.25	1.25
19	8.30	0.75	0.75	0.50	1.00	3.00
27	7.80	2.00	1.50	2.00	2.00	7.50
Feb.02-2004	10.35	1.00	1.00	0.75	0.75	3.50
10	5.10	0.75	0.75	0.50	1.00	3.00
17	4.80	0.50	0.50	0.25	0.75	2.00
24	3.25	0.25	0.25	0.25	0.50	1.25
28	1.20	0.25	0.10	0.25	0.25	0.85
Mean±S.D	5.85±3.11	0.58±0.45	0.47±0.37	0.52±0.42	0.72±0.42	2.31±1.61

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EVALUATION OF DIFFERENT GRANULAR INSECTICIDES FOR THE SUPPRESSION OF RICE STEM BORERS

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Abstract.- Systemic granular insecticides constitute an important category of toxicants for protecting the rice plants from rice stem borers. A trial was undertaken to find out the efficacy of three systemic granular insecticides *i.e.*, Cartap 4G, Monomehypo 5G and Carbofuran 3G using recommended doses to reduce the population of rice stem borers on 2 rice varieties (aromatic Khushboo- 95; non-aromatic Shua- 92). On the basis of different parameters studied, an adverse effect was noted on pest population, while significant improvement in grain yield was observed in all the insecticide treatments as compared to control. Among the insecticides, Cartap 4G proved the best toxicant on the basis of least borers infestation, which has resulted in the maximum grain productivity. Comparatively, less borers infestation and grain yield were obtained in the plots treated with Monomehypo 5G and Carbofuran 3G as compared to Cartap 4G. Amongst these insecticides, the variations in their performance can be attributed to their different levels of efficiency. Taking the points of additional advantages of granular insecticides in outlook, these are easier to apply, less application hazards and non fatal to natural enemies.

Key words: Rice stem borers, insecticides, pest control.

INTRODUCTION

Rice *(Oryza sativa)* is the main stay of the diction of one half of the world population. It is the most important food crop of Asia including Pakistan, although rice is not the cardinal food of Pakistanis; it is the second most dominant cereal, attaining the rank of top foreign exchange earner. Despite the prime position of rice in the economy of the country, the crop's yield is low. The challenges to rice researches and farmers in Pakistan have many elements in common with those faced by scientists and rice producers in the world. Although soil and climatic conditions of Pakistan are conducive to high yields of rice, its low production is related to overall low level of plant protection practices in that the insects have deleterious effects on the growth of rice, where year round continuous cropping is practiced, there are several overlapping insect generations throughout the year.

Rice is attacked by approximately 128 species of insect pests, which substantially reduce rice yields. Out of them, 15-20 insect species are considered to be the pests of major economic importance causing about 40 -60% losses to the crop (Ahmed, 1981). Among the insect pests of rice; the stem borers are considered serious pests in rice production. Besides, there are many minor pests like white back plant hopper, rice bug, hispa, horned caterpillar and grasshoppers. There are a number of different types of stem borers; the 5 most destructive stem borers are: yellow stem borer, *Scirpophaga incertulas* (Walker); white stem borer, *Scirpophaga innotata* (Walker); striped stem borer, *Chilo suppressalis* (Walker); dark-headed stem borer, *Chilo polychysus* (Meyrick), and pink stem borer, *Sesamia inferens* (Walker). Stem borers feed on a number of crops including corn, sorghum, sugarcane, wild rice and other species of grasses, except for the yellow stem borer, which is monophagous to rice. Stem borers occur at all growth stages and are found in all types of low land, deep water floating and upland rice crop. Threshold level at vegetative stage is 5% dead hearts, and flowering stage-One moth or larva/m^2 (Harrion and Litsinger, 1994; Shepard *et al.,* 1995).

Yellow stem borer *Scirphophage incertulas* is the major insect pest, which often causes substantial yield losses. The yellow stem borer is a pest of deepwater rice. It is found in aquatic environments where there is continuous flooding (Garg *et al.,* 2002). Rice stem borers *Sciprophage incertulas* (Wlk) and S. *innotata* (Wlk) attack the crop right from seedling stage till harvest thus cause complete loss of affected tillers (Salim and Masih, 1987). These pests have also been reported to cause about 25-50% loss to rice crop every year in Pakistan (Ashraf *et al.,* 1986).

Because, it is difficult to control the stem borers without chemicals, the insecticides are only tools for their management in this region. When systemic granular insecticides are applied near the root zone of paddy plant, optimum kill of pest could be obtained due to their uptake, translocation and persistence in the plant system (Garg and Sethi, 1982). As the use of pesticides has enormously increased the yields of many crops by controlling insect pests, the farmers have to depend upon pesticides for managing pest problems in rice. However, their indiscriminate use had disturbed natural balance existing between pests and their natural enemies.

The intention of our study was to compare the effectiveness of granular insecticides which could be typically applied to rice crop in standing water because in advanced stage of crop growth, it is difficult to give thorough

covering with foliar formulations to the basal portion of the rice plant where the borers larvae are inflicting the damage. Further more, it was desired to select such granules, which have the ability to translocate from the base to the upward foliage. Some other factors for the selection of granular insecticides over the foliar spray were that they are easy to apply in water with minimum usage of equipment, have greater residual effects to kill the larvae in the stems, and the beneficial insects can be saved. The results on the efficacy of judiciously used insecticides are reported in this manuscript.

MATERIALS AND METHODS

This study was conducted at the experimental site at Nuclear Institute of Agriculture (NIA), Tando Jam to study the relative efficacy of three pesticides viz., granular formulations TI (Cartap-4G), T2 (Carbofuran 3G) and T3 (Monomehypo-4G), and control T4 were evaluated against rice stem borers, using 2 rice varieties (aromatic-Khushboo-95; non-aromatic-Shua-92). All granules were applied @ 10 kg a.i. / acre, firstly 55 days after transplanting and second application was done after 20 days of first application, in the control plot no any granular insecticide was used. The experiment was laid down according to complete randomized block design; general agronomic practices and methods of nursery sowing and transplanting were identical for whole the experiment. Control and treated plots were replicated thrice; each seedbed under every replication measured an area of $4.5m^2$, all attempts were made to evaluate the toxicity of the concerned insecticides under similar field conditions. To ensure high stem borers infestation, nursery sowing was delayed for about a fortnight from the normal sowing date during May.

Assessment of the efficacy of insecticides was done on the basis of number of dead hearts and white heads present and seed yield in each plot. Data on the infestation of borers was recorded at tillering, flowering and pre harvest times of the crop by counting the dead hearts and white heads per square meter taken randomly from each plot. Observations on borers infestation were recorded after 24, 48 and 72 hours of granules applications and mean values for different treatments determined. For this, 16 plants within an area of 1 meter square in each replicate from every set of treatment were observed. Number of infested tillers (borers infestation) in each treatment were recorded by counting damaged tillers on these randomly selected plants and the percent damage was calculated.

From the plots, the grain yield was recorded by taking the sample within the radius of 4.5 square meters (m^2) after harvesting and threshing operations.

The percent damaged tillers, and increase in grain yield over the control plot were worked out on the basis of the grain yield recorded from the whole experiment. The data recorded on different parameters was subjected to analysis of variance. The results of mean values for different treatments based on fixed parameters were compared and their performance was worked out.

RESULTS AND DISCUSSION

Results on insecticides used and grains yield are discussed in the Table I. Prior to treatments the larval infestation was uniformly distributed in all the observational plots, but after the application of chemicals, populations' reduction was found significantly variable in treated replicates. Apparently, it was evident from the results that all the granular insecticides were superior in reducing borers infestation and ultimately increasing rice yield than control. The highest borers infestation and lowest amount of grain acquiesce were in untreated plot as 7.90 % (dead hearts), 4.88 % (white heads) and 2053 grams/ 4.5 m^2, respectively. Contrary to this all the three insecticides were found to reduce percentage of damage and increase yield over untreated control. It can be seen form data that during observations, Cartap proved the best and effective among all the treatments in checking borer's infestation by exhibiting 2.82, 0.57% followed by Monomehypo 3.42, 1.67%, and Carbofuran 3.74 and 3.03% dead hearts and white heads respectively, as against 7.90, 4.88% dead hearts and white heads respectively, with control treatment. This fact was correlated with the yield, Cartap treated plots gave highest grain yield (2398.0 gm/ 4.5 m^2) (5328.88 Kg/he) followed by Monomehypo (2322.0 gm) (5160.00 Kg/he). Minimum yield was recorded in case of Carbofuran (2270.0 gm) (5044.44 Kg/he) as compared with other chemicals. But untreated replicates gave only 2053 gm/plot (4562.22 Kg/he) turn out.

TABLE I.- FIELD EVALUATION OF SOME SYSTEMIC GRANULAR CHEMICALS AGAINST RICE STEM BORERS.

Treatment	**Stem borers infestation (%)**		**Yield / plot (4.5 m^2) (g)**	**Yield Kg/hectare**
	Deadhearts	**White heads**		
Cartap 4 G	2.828 c	0.573 d	2398.00 a	5328.88
Carbofuran 3 G	3.742 b	3.037 b	2270.00 b	5044.44
Monomehypo 5 G	3.428 b	1.673 c	2322.00 b	5160.00
Control	7.907 a	4.880 a	2053.00 c	4562.22

In these trails, the data on % dead hearts, % white heads and grain yield have shown highly significant differences between insecticidal treatments and control. Relatively higher efficacy of the granular treatments can be attributed due to variably prevailing their superior toxic ingredients. Consequently, the differences in responses of both the parameters were possibly being due to differences in stem borers population control. It can also be presumed that the granules tested may have proficient active ingredients available for translocation in the seedlings.

During current findings, the mean borer's infestation in untreated replicates was ranged 2.82- 3.74 % dead hearts and 0.57- 3.07 white heads. Contrary to this according to Baloch (1975), *T. incertulas* accounted for 85 percent of the total loss due to borers, the incidence being high (8-100 percent). Beg *et al.* (1975) found that the crop had to be left un-harvested because the yield expected was too low to compensate even for the cost of harvesting. These pests are reported to be responsible for a steady annual damage of 5-10 % of the rice crop with local catastrophic outbreaks of up to 60 % damage (Catling and Islam, 1981).

Our field experiments exposed that Cartap proved superior to control borers with significant reduction in percentage of plant damage than tracked by Monomehypo and Carbofuran. These domino effects are similar to those of Anwar and Shafique (1987), and Khan and Khaliq (1989) who compared the effectiveness of granular insecticides; and Padan (Cartap) was more effective in controlling the pests and reduced the crop losses. Contradictory findings were reported by Sukhani and Jotwani (1982), the improvement in germination due to Carbofuran treatment had been reported in all the insecticides. The dead hearts and grain yield were also found to be significantly higher than other granular treatments. Waqas *et al.* (2001) as well as Sontakke and Dash (2000), also reported a maximum yield in Carbofuran treated plots as compared to other test insecticides. Afzal *et al.* (2003) evaluated the toxicity of different insecticides against rice stem borer and Carbofuran was found to the best for controlling dead hearts and white heads as well as increasing yield of super basmati followed by Cartap and Monomehypo.

The existing fallout showed that it is difficult to control stem borers without chemicals, thus the pesticides are the only tools for their management in this region. For successful control of this pest, the time of sowing and application of insecticides are more important. Our field observations revealed that as the pest infestation originated, protection of crop with recommended insecticide should be considered essential, otherwise, it can led to serious damage to crop.

Cartap proved superior to control with significant reduction in percentage of plant damage followed by Monomehypo and Carbofuran. All these insecticides proved variably effective with significant difference among themselves, but Monomehypo and Carbofuran both proved least effective insecticides. Considering over all results of the trial, the use of Cartap in areas where pest problem is chronic and acute, could be recommended. Cartap can well suit for an integrated Pest Management Programme because of the offered salient findings. It is desirable to use other test granules in rotation to break insecticidal resistance in pests. Their judicious use involving right formulation, right dosage, appropriate timing and effective application will provide an appropriate tool for stem borers control.

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BIOLOGY AND FEEDING POTENTIAL OF GREEN LACEWING, *CHRYSOPERLA CARNEA* STEPHENS (NEUROPTERA: CHRYSOPIDAE) ON MUSTARD APHID

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Abstract.- Studies were conducted during November 2002 to February 2003 at the Department of Entomology, Sindh Agriculture University, Tandojam. The culture of predator, *Chrysoperla carnea* Stephens, was obtained from IPM Laboratory, A.R.I., Tandojam and reared in the laboratory. The predator was fed with mustard aphid, *Lipaphis erysimi* Kalt and the data on its biology and feeding potential was recorded. The results revealed that mean number of eggs laid per female of *Chrysoperla carnea* were 1594.6±53.38. The mean duration of 1st, 2nd and 3rd instars were 4.0±0.31, 3.8±0.37 and 5.2±0.37 days, respectively. Total larval and pupal duration were 14.82±3.67 and 9.4±0.24 days, respectively. Mean percent emergence in males and females were 35.80±3.52 and 49.38±3.66 days, respectively. The age of predator was correlated with the feeding potential.

Key words: Biology, feeding potential, *Chrysoperla carnea*. Steph, *Lipaphis erysimi* Kalt.

INTRODUCTION

The green lacewing, *Chrysoperla spp* (Neuroptera: Chrysopidae), are considered most important insect predators on sucking insect pests of vegetables, fruits, nuts, fiber, forage and green house crops (Hoffmann and Frodsham, 1993; McEwen and Ruiz,1994; Balasubramani and Swaminappan, 1994). These are called aphid lions, because they can consume several hundred aphids during their larval stage (Henn and Weinzieral, 1990; Mani and Kirshanamoorthy, 1999). Two species of lacewing *i.e. Chrysoperla carnea* and *Chrysoperla rufilabris* have been mass reared and marketed commercially in North America and Europe since long time (Henn and Weinzieral, 1990). Milvoj (1999) reported that *Chrysoperla carnea* was potential predator and used in Integrated Pest Management to reduce the population of aphids. He also made significant new development in artificial larval diets, mechanical production methods, long term storage and commercial ecology of this predator.

Earlier, Hoffmann and Frodsham (1993) described the biology and behavior of *Chrysoperla carnea* and reported that the predator completes two to

several generations per year depending upon temperature and environmental conditions. Since the green lacewing, *Chrysoperla carnea* is a common predator on many sucking pests in Pakistan, the present preliminary studies were made to observe the biology of *Chrysoperla carnea* on mustard aphid in laboratory at Tandojam, Sindh, Pakistan.

MATERIALS AND METHODS

The adults of predator *Chrysoperla carnea* were obtained from IPM Laboratory, A.R.I., Tandojam and the stock culture was prepared under the temperature 22-25°C inside laboratory. The adults (male and female) were placed in glass jars and fed with artificial diet comprising 40 gm yeast, 70 gm sugar and 50 ml water. The diet was spread in drops on the side of glass jars. Damp cotton wool was also placed in glass jars to ensure humidity. The top of glass jars were covered with muslin cloth.

The eggs deposited by females on muslin cloth and sides of glass jars were removed daily and transferred in Petridishes (9 cm dia.) containing filter paper and mustard leaves. After hatching, the larvae were collected daily using a fine point camel hair brush and placed individually in new Petri dishes with the moist sponge. Each larval instar was provided with a known number of mustard aphid, *L. erysimi* for feeding obtained from mustard field. The number of mustard aphids was increased as the larva entered to next instar. The feeding potential was recorded by counting the number of aphids, *L. erysimi,* fed by 1st, 2nd and 3rd instars of *C. carnea* up to pupation.

RESULTS AND DISCUSSION

The female *C. carnea* laid elongated, oval green eggs on the sides and on the muslin cloth at the top of glass jars. The eggs turned grey to dark in colour before hatching. The data in Table I depicts that the mean hatching rate of eggs was 51.40±0.65 (85.67%) and the mortality in eggs was 8.6±0.65 (14.33%) in laboratory.

Saminathan *et al.* (1999) reported that hatchability of eggs of *C. Carnea* was 80% when reared on *A. craccivora* and eggs of *C. cephalinica* and *E. vittela* However, in present studies *L. erysimi* was used as prey host.

The results in Table II indicate that the mean duration days of first, second and third instars of *C. carnea* was 4.0±0.31, 3.80±0.37 and 5.20±0.37 days,

respectively. The total duration of larval instars was 13.0±0.31 days reared on *L. erysimi* in laboratory. The mean pupal duration lasted for 9.40±0.24 days and the total duration of immature stages (Larvae + pupa) averaged 22.60±0.24 days.

TABLE I.- EGG HATCHING RATE AND MORTALITY OF *C. CARNEA* UNDER LABORATORY CONDITIONS.

Replication	No. of eggs Observed	No. of eggs hatched	% hatching	Egg mortality	% egg mortality
1	60	53	88.33	7	11.66
2	60	48	80.0	12	20.0
3	60	52	86.66	8	13.33
4	60	50	83.33	10	16.66
5	60	50	83.33	10	16.66
6	60	50	83.33	10	16.66
7	60	53	88.33	7	11.66
8	60	54	90.0	6	10.0
9	60	54	90.0	6	10.0
10	60	50	83.33	10	16.66
Mean ± S.E	-	51.4±0.65	85.67±1.08	8.6±0.65	14.33±1.08

TABLE II.- DURATION (DAYS) OF LARVAL AND PUPAL STAGES OF *C. CARNEA* REARED ON *L. ERYSIMI* UNDER LABORATORY CONDITIONS.

Replication	Duration of larval period (Days)			Total larval duration period (Days)	Pupal Period (days)	Total duration Immature stage (larval+ pupal) (Days)
	1st Instar	2nd Instar	3rd Instar			
1	4	3	6	13	10	23
2	4	4	6	14	9	23
3	5	3	4	12	10	22
4	3	5	5	13	9	22
5	4	4	5	13	9	22
Mean±S.E	4.0±0.31	3.8±0.37	5.2±0.37	13.0± 0.31	9.4±0.24	22.6±0.24

Earlier, Balasubramani and Swamiappan (1994) reported that the development period of immature stages (egg to adult emergence) of *C. carnea*

was 19.15, 19.35, 20.60 and 22.50 days reared on four different aphid species. Similarly Saminathan *et al* (1999) mentioned that the duration of egg, larval and pupal period of *C. carnea* ranged between 18.59 to 22.74 days on different pest species. The results thus suggested that third instar larvae lived longer than other instars.

The data (Table III) revealed that in *C. carnea* the mean pupation development rate (%) was 87.50%, however, 12.50% pupae died during pupation. Similarly (Table IV) in adult *C. carnea*, the male emergence rate (%) was 35.80% as compared to female emergence rate (49.38%). The females emergence was comparatively more than male adults. The mean sex ratio between female and male adult emergence was 1:1.50.

TABLE III.- PUPATION RATE AND PUPAL MORTALITY OF *C. CARNEA* UNDER LABORATORY CONDITIONS.

Replication	No. of larvae observed	No. of pupae formed	Pupation %age	Pupal mortality	% Pupal mortality
1	20	14	70.0	6	30.0
2	20	19	95.0	1	5.0
3	20	18	90.0	2	10.0
4	20	17	85.0	3	15.0
5	20	18	90.0	2	10.0
6	20	17	85.0	3	15.0
7	20	17	85.0	3	15.0
8	20	17	85.0	3	15.0
9	20	19	95.0	1	5.0
10	20	19	95.0	1	5.0
Mean±S.E	-	17.5±0.47	87.5±2.38	2.5±0.47	12.50±3.51

The results in Table V reveal that fecundity in female adults of *C. carnea* ranged between 1428 to 1742 eggs with an average of 1594.60±53.28 eggs during their life time. The average number of eggs laid per female per day ranged between 26.44 to 37.06 eggs.

The perusal of data in Table VI show that the mean feeding rate of *L. erysimi* by first, second and third instar larvae of *C. carnae* was 1.62 (27.0%), 4.76 (44.07 %) and 9.21 (61.40%) aphids per day. The third instar larvae devoured more number of aphids/day followed by second and first instar larvae.

Table IV

The results thus suggested that as the age of larva increased, its feeding potential was also increased. Similarly, Balasubramani and Swamiappan (1994) reported that in all cases 3rd inster larvae of *C. carnea* consumed the major portion of the total number of *Aphis gossypii* and other pray hosts (60-80%).

TABLE V.- LONGEVITY AND FECUNDITY OF *C. CARNEA* FEMALE ON ARTIFICIAL DIET UNDER LABORATORY CONDITIONS.

Replication	**Total age of female (days)**	**Total number of eggs laid**	**Eggs laid per day (Mean ± S.E)**
1	52	1631	31.36 ± 2.25
2	49	1640	33.47 ± 2.89
3	47	1742	37.06 ± 2.89
4	52	1532	29.46 ± 2.15
5	54	1428	26.44 ± 1.97
Mean± S.E	50.8±1.24	1594.6±53.28	----

TABLE VI.- APHID CONSUMPTION AND FEEDING RATE BY LARVAL INSTARS OF *C. CARNEA* UNDER LABORATORY CONDITION DURING WINTER 2002. (MEAN TEMP. 20.81±0.337°C).

Instars	**Age (days)**	**Aphid host density**	**Aphids consumed**	**Consumption rate (%)**
First	1	5	0.5	10.0
	2	5	1.5	30.0
	3	7	2.0	28.57
	4	7	2.5	35.0
	Mean	**6.0**	**1.62**	**27.0**
Second	5	10	3.5	35.0
	6	10	3.8	38.0
	7	10	4.5	45.0
	8	12	6.0	50.0
	9	12	6.0	50
	Mean	**10.8**	**4.76**	**44.07**
Third	10	15	7.8	52.0
	11	15	8.5	56.66
	12	15	8.8	58.66
	13	15	9.0	60.00
	14	15	10.16	67.73
	15	15	11.0	73.33
	Mean	**15.0**	**9.21**	**61.40**

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TABLE IV.- ADULT EMERGENCE RATE, SEX RATIO AND MORTALITY OF MALE AND FEMALE ADULTS OF *C. CARNEA* UNDER LABORATORY CONDITIONS.

Replications	No. of pupae observed	Adult emergence				Total mortality Male + Female %	Sex ratio
		Male (No. emerged)	% male emergence	Female (No. merged)	% female emergence		
1	10	3	30.0	4	40.0	30.0	1:1.33
2	10	4	40.0	5	50.0	10.0	1:1.25
3	9	3	33.33	4	44.44	22.22	1:1.33
4	9	2	22.22	5	55.55	22.22	1:2.50
5	8	2	25.0	6	75.0	0.00	1:3.00
6	8	3	37.5	4	50.0	12.5	1:1.33
7	7	3	42.85	4	57.0	0.00	1:1.33
8	7	2	28.51	3	42.85	28.57	1:1.50
9	7	4	57.14	3	42.85	0.00	1:0.75
10	6	3	50.0	2	33.33	16.66	1:0.66
Mean± S.E	8.10±0.43	2.90±0.23	35.80±3.52	4.0±0.36	49.38±3.66	14.82±3.67	1:1.50

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HABITAT AND LIFE CYCLE OF GRASSHOPPER *ACROTYLUS HUMBERTIANUS* SAUSSURE (ORTHOPTERA:ACRIDOIDEA) IN THE DESERT AREA OF LASBELA, BALOCHISTAN

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Abstract.- The studies on habitat and life cycle of grasshopper *Acrotylus humbertianus* Saussure, were conducted in the desert area of Lasbella, Balochistan. The adults are found throughout the year. Both hoppers and adults cause damage to agricultural crops and pasture. The eggs are usually laid after summer rainfall during July and August, but eggs are also laid in winter season provided rains occur. The hoppers usually emerge after two weeks of the rainfall. They become adult in 3-4 weeks in summer season and 4 to 5 weeks in winter season. The population is in abundance in September. It usually completes one generation in a year due to rains in summer season, but second generation is also possible if rains occur in winter season. The results have been reinterpreted in the light of literature to date.

Key words: *Acrotylus humbertianus* Saussure, life cycle of grasshopper, seasonal variation, Lasbela, Balochistan.

INTRODUCTION

The grasshopper *Acrotylus humhertianus* Saussure is a serious pest of agricultural crops such as rice and bultrish millet (Ballard, 1921) and coffee and lentils (Uvarov, 1921) in India. It has also been reported to cause damage to agricultural crops like chickpea. clover, green gram. groundnut. maize, oats, pea. rice, sorghum, sugarcane. turnip and wheat in Pakistan (CIBC, 1974). Moizuddin (1994) also recorded its attack on millet. maize. sorghum and pasture in the desert area in district Lasbela, Balochistan. This species is distributed to Pakistan. India. Afghanistan, Sri Lanka. Nepal and Bangladesh, but its life cycle is not known (Haskell, 1982). It is generally distinguished by its basally yellow hindwings with a wide dark fascia. The total length of male ranges from 20.4 to 23.40 and female ranges from 25.20 to 27.50 mm (Moizuddin, 1994).

Taking into consideration its economic importance and gaps in our knowledge on habitats and life cycle the present work was undertaken.

MATERIALS AND METHODS

Regular survey was conducted in the desert area of Chachai, district Lasbela, Balochistan, for over six years. The adults and hoppers were collected at least twice a month. The average number of adults collected per man, per hour, per month was calculated from the total collection of adult insects. The collection was done between 1000 hours and 1300 hours.

RESULTS

Location

The studies on the grasshopper *Acrotylus humbertianus* Saussure were conducted in the breeding place in the desert area at Chachai, district Lasbela at Balochistan. The area is situated between 25 15° Nand 66 45°E. It is about 5 Kilometres away from the coastal beach of Gadani.

Climatic condition

The area is considered under arid/semi arid zone. During summer season from April to October the maximum temperature ranges from 26°C to 46°C and the minimum temperature ranges from 12°C to 29°C and during winter season from November to March the maximum temperature ranges from 16°C to 35 °c and the minimum temperature ranges from 1°C to 12°C. The rains occur in summer season during July to September and in winter season during December to March. The average rainfall is 150 mm in summer season and 32 mm in winter season. The average annual rainfall is 183 mm (Moizuddin, 1999). Sometimes there is no rain in one season or both the seasons.

Life cycle

The life cycle comprises egg, hopper and adult stages. The temperature and the seasonal rains appear to be the most important factors in each development stage.

Habitat

The grasshopper *Acrotylus humbertianus* is found throughout the year in the desert area. It feeds on various agricultural crops, grasses and pasture. The

hoppers and the adults both attack and damage millet *Penisetum americanum* maize *Zea mays,* sorghum *Sorghum bico!our,* castor *Ricimus communis and* water melon *Citrulus vulgaris.*

They also attack on pasture particularly to *Tribu!us terrestris. Gynandropsis gynandra, Cleome viscose, Heliotrophium ramosissium, Arnebia hispidisima, Crotolaria medicaginea, Zaleya pentendra, Peristrope bicalyculata, Cenchrus setigerus, C. biflorus, Panicum tergidium, Dactyloctinium aegyptium, etc.*

Egg laying

The eggs are usually laid after summer rainfall in July and August in the sandy soil at the depth of about one inch. The eggs are also laid in winter season during December to March provided rains occur.

Emergence of hoppers

The hoppers usually emerge on the breeding ground after two weeks of rainfall. If the rains occur in winter season, the hoppers also emerge on the breeding ground after two weeks of rainfall.

Development of hoppers

After rainfall in summer season, the desert area becomes lush green with the growth of grasses and vegetation. The newly emerged hoppers feed on fresh leaves of the plants. The young hoppers usually remain associated with the grasses and vegetation. but when they reach to 4th and 5th instars they disperse and move freely to feed on fresh leaves of the plants. The hoppers become adult in 3-4 weeks in summer season and 4-5 weeks in winter season.

Emergence of adults

The adults usually appear on the breeding ground in summer season in 2nd and 3rd week of August if rains occur in 1st week of July. The population is in abundance in September. The population of the adults decreases in the following months in October and November. The population of the adults usually remains low during winter season. If the rains occur in winter season in February. the breeding again takes place and the population of adults increase in the following month. In such case the population again reaches to peak in April. The

population of adults decreases in May and June, which is hot and dry. If the rain does not occur in both the seasons in summer and winter, the egg laying or breeding does not take place. The adults survive in low population through the year.

Annual generation

It usually completes one generation in a year depending on seasonal rains in summer. However, if the rains occur both in summer and winter seasons. it completes two generations in a year.

Adult diapause

The adults usually remain in diapause in winter season and the females lay eggs in the next summer season after rainfall. They remain in diapauses for 10-11 months. If there is no rain both in winter and the summer seasons the adults remain in diapauses for 22-23 months.

Seasonal variation

Table I shows the average number of adults collected/man/hour/month during the years 1987, 1989 and 1992. It represents the variation in adults population due to drought conditions and seasonal rains. In 1987, there was no rain during summer and winter seasons and both the seasons remained under drought conditions. As a result there was no egg laying or breeding and the adults population remained low through out the year. In 1989 there was insufficient rain during winter season. A total of 15 mm rain was recorded in February and March. Due to insufficient rains, egg laying or breeding did not take place. The population of adults remained low. During the summer season in July 175 mm rain was recorded. Due to sufficient rains in July the egg laying and breeding took place. The population of adults increased in the following month in August. The population was in peak in September. The population gradually decreased in the following months in October and November. In 1992, there was rain in both the seasons. During winter season from January to March 68.58 mm rain was recorded. Due to sufficient rains in last week of January, the egg lying and the breeding took place. The population of adults increased in February and March and reached to peak in April. The population decreased in the following months in May and June. During summer season in July and August 240 mm rain was recorded. Due to sufficient rains in 1st week of July the egg laying and the breeding took place. The population of adults increased in August and it was in

peak in September. The population decreased in the following months in October and November.

TABLE I.- AVERAGE NUMBER OF ADULTS COLLECTED/MAN/HOUR/MONTH.

Months	**Number of adults**		
	1987	**1989**	**1992**
January	1	2	3
February	1	2	4
March	2	3	8
April	2	3	15
May	3	4	8
June	2	4	5
July	3	5	5
August	5	8	13
September	4	25	30
October	3	15	20
November	2	8	12
December	2	4	5

DISCUSSION

The genus *Acrotylus* Fieber contains 20 species which are distributed over the whole Africa, S. Europe and S. W. Asia, but among these six species including *Acrotylus humbertianus* are of economic importance (Haskell, 1982). The information on their distribution, ecology, behaviour and life cycle is little known.

The grasshopper *Acrotylus humbertianus* Saussure is terricolous in habit and usually remain associated with the grasses and the vegetation which grow in the desert area of Lasbela, Balochistan, but it also attack millet, maize, sorghum, castor, gowar, water melon etc. in the near by agricultural fields. In the present study it has been observed that this species is found throughout the year. It usually completes one generation in a year due to seasonal rains in summer season, but it also completes two generations in a year if the rains occur both in summer and winter seasons. If there is no rain in both the seasons, it survives as adult. Ballard (1921) noted that in India the *Acrotylus humbertianus* is found throughout the year which is similar to the present findings, but he further noted that it may be multivoltine, which does not correlate with the present findings.

Golding (1948) in North Niger (Lake Chad Avery) and Joyce (1952) in East Central Sudan noted two generations in a year in *Acrolylus blondeli* Saussure, while Phips (1970) with reference to the above species noted that there may be more than one generation in a year in Mali and Niger. Similarly, Joyce (*op. cit.*) and Robertson and Chapman (1962) noted that there are at least two generation in a year in *Acrotylus patruelis* (Herrich - Schaeffer) in Sudan and Tanzania. It appears that one to two generations in a year is common feature in the species of the genus *Acrotylus* Fieber.

In the present species it has been observed that the incubation period of the eggs is 2 weeks and the hoppers development period is 3-4 weeks in summer season and 4-5 weeks in winter season. On the other hand Sisli (1964) in *A. insubricus* noted incubation period of eggs as 57 days and hoppers development period as 61 days and Shulov and Pener (1961) in the above species noted incubation period of eggs as 22-26 days at 27°C. The differences in incubation period of eggs and development period of hoppers in *Acrotylus humbertianus* and *Acrotylus insubricus* is due to differences in type of species.

ACKNOWLEDGEMENTS

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RECORDS OF NON-MARINE BRACHYURAN CRAB SPECIES OF PAKISTAN INCLUDING A NOTE ON THEIR ECOLOGY AND DESCRIPTION OF JUVENILES OF *SARTORIANA BLANFORDI*

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Abstract.- This is the first attempt to collect information on the taxonomy of Pakistan non-marine brachyuran crabs. In the non-marine crabs generally the semiterrestrial or semiaquatic and terrestrial crabs of the families Potamidae, Gecarcinucidae (= Gecarcinidae), Parathelphusidae are understood. The representatives of these families normally are not found in marine waters. So far some samples from Sindh, Punjab and Baluchistan provinces from diverse habitats have been obtained and examined. These crabs are important having a food value in some countries. Their utilization in our country is not documented. Earlier they were a menace in the rice fields, but not at rarely found. The literature survey reveals very little work by Pakistani workers on the group. However, pre-partition reports by Henderson (1893) and Alcock (1909, 1910) from the areas now in Pakistan are helpful to form a baseline data. After partition Hashmi (1964) listed one species of freshwater crab from Karachi and Hyderabad. Some reports prepared by Pretzman (1963-1969), a German Carcinologist are from N.W.F. province, available in German language. The only recent report is Kazmi and Perveen (2005). On compilation of data it appears that 7 species in the above 3 families are so far recorded from Pakistan. Each species is described and illustrated. The larvae of *Sartoriana blanfordi* are also described and illustrated. Biology of these crabs is also briefly described. The collection is deposited in the Zoological Survey Department and the Marin Reference Collection and Resource Centre.

Key words: Brachyuran crabs, semiaquatic, terrestrial, diversity, Pakistan.

INTRODUCTION

This is the first attempt to collect information on the taxonomy and ecology of Pakistani non-marine brachyuran crabs. In the non-marine crabs generally the semiterrestrial or semiaquatic and terrestrial crabs of the families Potamidae, Gecarcinucidae (= Gecarcinidae), Parathelphusidae, Potamonautidae and Deckeniidae are understood. The representatives of these families normally are not found in marine waters. The first four have representatives in Pakistan freshwater bodies and terrestrial ecosystem.

The literature survey reveals that hardly anything is known about the non-marine waters crabs of Pakistan. However pre-partition documents by Henderson (1893) and Alcock (1909, 1910) from the areas now in Pakistan are helpful to form a baseline data. Rathbun (1904-1906) reviewed the freshwater crab fauna of the world, but since that time, the systematics of freshwater crabs has changed considerably. After partition, Hashmi (1964) included one species of freshwater crab in a checklist from Karachi and Hyderabad (Sindh). Some reports published by Pretzmann (1965-76) are from NWFP and FATA. Ali (1973) reported a species - *Potamon fluviatile* (Herbst) from Kohat (NWFP) and Chaudri *et al*. (1978) reported another species *Potamon simulum* (Alcock) (now *Tiwaripotamon simulum*) from Rawalpindi (Punjab) and Kohat (NWFP). Presently Yeo *et al.* (in preparation) are reviewing the genus *Sartoriana* Bott basing on the Pakistani material whereas Kazmi and Perveen (in press) have added *Cardisoma carnifex* (Herbst) to the faunal list of Pakistan. This brings to total number of freshwater and terrestrial crabs reported from Pakistan to 8 species. Out of them *Himalayapotamon emphyseteum* (Alcock) in the present material is new record for the area and *Sartoriana spinigera's* range is found extended. This number is expected to rise as exploration continues.

MATERIALS AND METHODS

So far 17 samples of three families from diversified fresh water bodies of all the provinces of Pakistan (Sindh, Punjab, NWFP and Balochistan) have been obtained and examined. The collections from karezes (subterranean waterways) and gorges need special mention.

The material has been obtained from four different sources: (a) material lying unidentified in the Zoological Survey Department, Govt. of Pakistan (ZSD) and studied under an MOU between ZSD and Marine Reference Collection and Resource Centre (MRC), University of Karachi, (b) material collected from different water bodies of Baluchistan and NWFP provinces by the second author (M. Khurshid) for his M.Phil thesis during 2003-2005, (c) one sample provided by Salahuddin, Jama Millia Islamia, Karachi and (d) one specimen provided by M. Yaqoob, NARC, Islamabad.

All the material, which will be returned to relevant organizations, was sorted, identified, measured for males, females and juveniles. Berried and spent females are specially mentioned to indicate the breeding season. One species *Sartoriana blanfordi* was selected for its larvae. The present account includes all

the reported species and only those supported by the specimens are illustrated and described. One figure of *Kanpotamon simulum* is borrowed from Chaudri *et al*. (1978). Only the references from Pakistan and recent reference is given under the synonyms. A schematic diagram (Fig. 1) is given to explain the terminology used in text. Key to the identification of non-marine crab families is also included. References to authority are not explained in the 'References'. Abbreviations cl and cb denote the carapace length and maximum carapace breadth respectively and GCM stands for Government College Museum, Lahore.

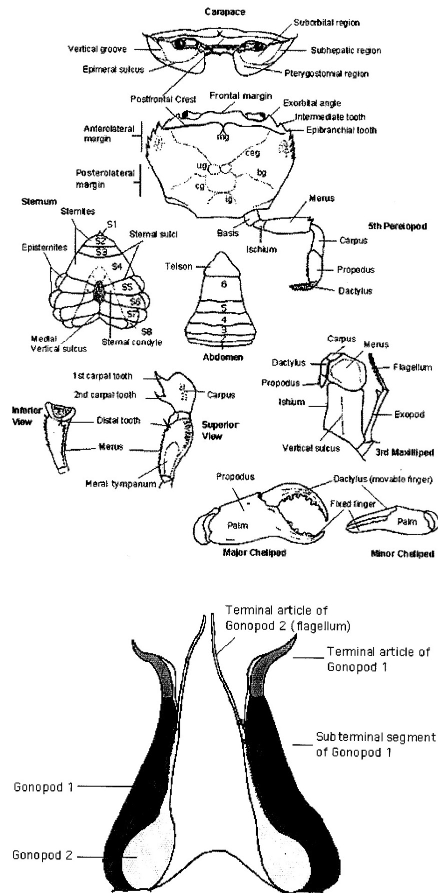

Fig. 1. Schematic diagram of non-marine crab.

SYSTEMATICS

Key to the families
(adapted from Cumberlidge, 1996 and Yule and Sen, 2004)

1. Mandibular palp 3-segmented with single terminal lobe; male abdomen distinctly triangular in shape ....Potamidae: Tropical Asia, the Middle East, Romania, Bulgaria, and ....some Mediterranean countries (Greece, Italy, Morocco, Tunisia, and Algeria).
- Mandibular palp 2-segmented with terminal part bilobed; male abdomen T-shaped in shape to varying degrees 2
2. Terminal segment of mandibular palp with a large, single posterior lobe; some species with a very small and anterior process (about 1/8 the size of the posterior lobe) present at the junction between the two segments 3
-. Terminal segment of mandibular palp clearly bilobed, with anterior lobe between ½ and 7/8 the size of the posterior lobe ....Partelphusidae, Gecarcinucidae: .... West Africa, Madagascar, India, Pakistan, Southeast Asia.
3. Mouth parts forming tube-like efferent respiratory channels............ Deckeniidae: East Africa, ...including Zanzibar.
- Efferent respiratory openings are simple holes..............Potamonautidae: Afrotropical Region.

Family PARATHELPHUSIDAE
Genus *OZIOTHEPHUSA* Muller, 1887
Oziothephusa sp.
(Fig. 2)

Fig. 2. *Oziothelphusa* sp.

Remarks

This is a lowland genus, restricted to southern India and Sri Lanka (Bahir and Yeo, 2005). Therefore presence of a female *Oziotelphusa* (cl. 28mm, cb. 37mm) in the present collection, locality given as Thatta (Sindh), leads to several

considerations, for example the possibility of a disjunct distribution of the genus or the material actually collected from southern India and deposited in the ZSD with a wrong entry. In the absence of a male, even the generic status of the specimen is not certain, because another genus *Spiralothelphusa* Bott closely resembles *Oziothelphusa*. Since the whereabout of the specimen are not certain the species is not to be included in the Pakistani faunal list.

Genus *BARYTHELPHUSA* Bott, 1970
Barythelphusa (Maydelliathelphusa) masoniana
(Henderson, 1893)

Telphusa masoniana Henderson, 1893: 381, figs.
Potamon (*Potamon*) *masonianus* - Rathbun, 1904: 299, pl. xi, fig. 10.
Paratelphusa (*Barytelphusa*) *masoniana* - Alcock, 1910: 96, fig. 59.
Barytelphusa (*Maydelliathelphusa*) *lugubris masoniana* - Bott, 1970: 36, fig.

Remarks

The species has been reported by earlier workers from River Byas (Punjab) by Alcock (1910) and North West Province by Henderson (1893) and Rathbun (1905). But it is not certain that the population still exists in the River Byas. Since the river is dry almost all the year round, only the water in flood season is available.

Family POTAMIDAE Ortmann, 1846
Subfamily POTAMINAE Ortmann, 1896
Genus *POTAMON* Savigny, 1816
Potamon gedrosianum Alcock, 1909
(Figs. 3, 4)

Potamon (*Potamon*) *fluviatile* var. *gedrosianum* Alcock, 1909: 243, 1910: 23, fig. 1.
Potamon (*Orientopotamon*) *gedrosianum* - Pretzmann, 1965: 523; 1966: 297, fig; 1967: 222.
Potamon (*Orientopotamon*) *gedrosianum waziristanis* - Pretzmann, 1965: 523, 1967: 218 (map).
Potamon gedrosianum lindbergi - Pretzmann, 1966: 298.
Potamon (*Potamon*) *gedrosianum gedrosianum* - Bott, 1967: 13, fig, 1970: 139, fig.

Fig. 3. *Potamon gedrosianum* (Alcock)

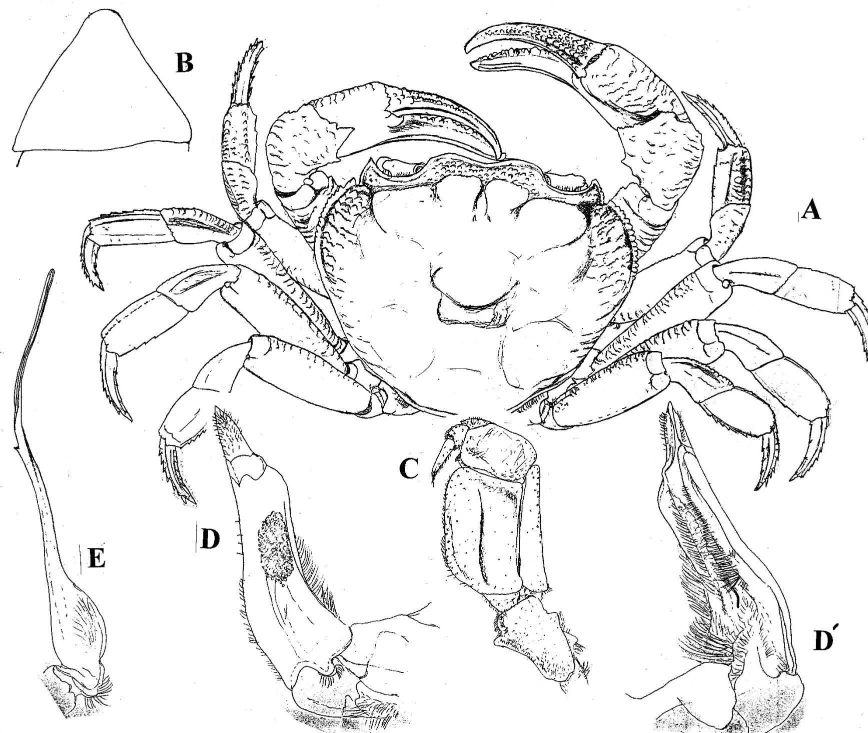

Fig. 4. *Potamon gedrosianum* Alcock (male, 45x48mm). A, Male in dorsal view; B, Telson; C, Third maxilliped; D, D’. First gonopod; E, Second gonopod.

Material examined

5 males, cl. 12-37mm, cb. 14-50mm, 1 female, cl. 32mm, cb. 40mm, Katas, Choa Said Shah (Punjab), ZSD 24 October, 1978; 14 males, cl. 19-42mm, cb. 21-50mm, 9 females, cl. 21-40mm, cb. 24-45mm, Light House, Karachi (Sindh), ZSD 15 March, 1969; 3 males, cl. 30-35mm, cb 40-45mm, 4 females, cl. 25-30mm, cb 28-30mm, Gwargo Caves, Panjgoor, coll. M. Khurshid, 25 August, 2005; 5 males, cl. 20-45mm, cb. 27-50mm; 2 females, cl. 30-35mm, karez, Quetta (Baluchistan), coll. M. Khurshid, 3 June, 2004; 4 males, cl. 30mm, cb. 40mm; 2 females, 30-45mm, cb. 40-45mm, Kohat (NWFP), coll. M. Khurshid, 11 May, 2005; 1 male, cl. 46mm, cb. 54mm, 3 females, cl. 30-33mm, cb. 30-38mm, Zhob River (Baluchistan) coll. M. Khurshid, 15 April 2005.

Description

The carapace (Fig. 4A) is broad, the broadening being due to the more marked convexity of the antero-lateral borders, gastric region is well defined except postero-laterally, the epigastric and mesogastric subregions are distinct anteriorly, cardiac region in distinguishable. The epigastric cristae are tumid and in advance of the postorbital cristae the latter have a thin, almost sharp edge, except for the small outlying lobule cut off by the cervical groove. The epibranchial regions have distinct but faint areolation. The cervical groove is deeply cut, cutting the post-orbital cristae at a point in line with the inner angle of the external orbital spine. The dorsal surface of the carapace is smooth, but the frontal and anterior portion of the gastric region are rugose. The suborbital lobes are granulated. The anterolateral margins of the carapace are longer than the posterolateral margins, clear cut, gently curved, raised and crenulate. The front is moderately declivous, very distinctly biolobed, breadth slightly more than 1/3 the greatest breadth of the carapace, edges clear cut, usually smooth. Orbits are clearly defined and somewhat averted, external orbital tooth prominent, subacute, separated from the lower orbital border by a notch.

The 6th abdominal segment of male is more than half its greatest breadth, and that of the 7th is a little less than its greatest breadth (Fig. 4B). The ischium of the third maxilliped (Fig. 4C) is longitudinally grooved in the middle, the merus is irregularly pentagonal, broader than long with external angle clearly rounded off.

The chelipeds are unequal. The three edges of the merus are bluntly crenulate, the outer surface of the carpus and propodus are fairly marked by

striae. The inner angle of the carpus is produced into a stout spine, with a cusp at its base and two or three small serrations at the anterior edge; the propodus is as long as the dactylus; the fingers are stout, curved, pointed at the tip, have small blunt teeth; dactylus has granules on the proximal part of upper edge.

The legs are stout with meri and propodi compressed, anterior borders of meri are crenulate, and both the borders of the propodi are serrate, the dactyli have four rows of spines. The first gonopod (Fig. 4D,D') is gently sinuous, penultimate segment is stout, broad, hairy, ultimate segment is pointed, pubescent and one fourth of the penultimate segment. The second gonopod (Fig. 4E) has a long distal segment, the basal segment is slightly longer than the distal segment.

Remarks

The species is already reported from several localities of Baluchistan, NWFP and Punjab provinces by Alcock (1910). All specimens from Kohat (NWFP), (coll. M. Khurshid, 11 May 2005) are of particular interest, they need to be investigated therefore are left with Dr. Yeo at the laboratories of Singapore University for further studies.

Potamon (*Potamon*) *fluviatile* (Herbst, 1785)

Potamon fluviatilis – Henderson, 1893: 385.
Potamon fluviatile – Ali, 1973: 146.

Remarks

The material (a series, coll. W.T. Blanford) of *P. fluviatilis* collected from Quetta (Baluchistan) is housed at the British Museum, London (see Henderson, 1893), so not seen by the authors, Alcock (1910) also did not mention of this material. Ali (1973) identified the species from streams of Kohat (NWFP) and Rawalpindi (Punjab) as *P. fluviatile*. Henderson's species of *fluviatilis* actually embraced two species i.e. *fluviatilis* and *ibericus,* (cf. Rathbun, 1904), the latter was downgraded into a variety of *fluviatilis* (*ibericum*) by Alcock (1910), this variety was found in the valley of Jhelum in Khewrah Gorge and *fluviatilis* being spread in Europe, African side of Mediterranean and Iran. According to him "the species (*fluviatile*) said to come from Ceylon (now Sri Lanka) depends upon a dealer's locality of more than fifty years ago, and I do not accept it". Bott (1967) included part of Alcock's material in *Potamon* (*Potamon*) *persicum* Pretzmann,

1962. It is most probable that Ali's (1973) report is the same variety which is found in the valley of Jhelum.

Genus *KANPOTAMON* Ng and Naiyanetr, 1993
Kanpotamon simulum (Alcock, 1910)
(Fig. 5)

Potamon simulum – Chaudri *et al*., 1978: 183, fig. 46-65.
Kanpotamon simulum – Ng and Yeo, 2001: 275 (discussion), 286 (key).

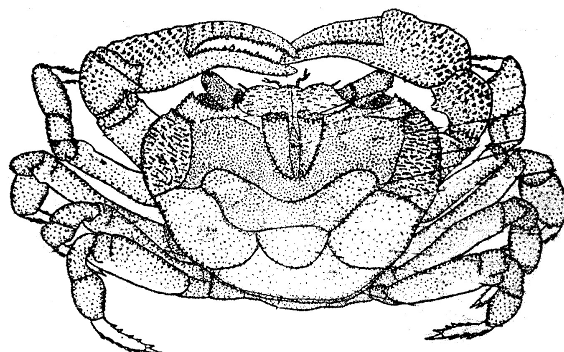

Fig. 5. *Kanpotamon simulum* (Alcock) Animal in dorsal view (modified from Chaudri *et al*. 1978).

Remarks

This species has been reported from Madyan, Swat valley (NWFP) by Chaudri *et al* (1978, coll. No. GCM (N.H) 40). Their identification needs verification, but included here to give a complete picture of the diversity.

Genus *HIMALAYAPOTAMON* Pretzmann, 1966
Himalayapotamon emphyseteum (Alcock, 1909)
(Figs. 6-9)

Himalayapotamon emphyseteum – Brandis, 2001: 95.

Material examined

2 males, cl. 40mm, cb. 43mm, ZSD No. 819; 1 male, cl. 35mm, cb. 45mm. Kurrang River, Islamabad - (Punjab), coll. M. Yaqoob, 2004.

Fig. 6. *Himalayapotamon emphyseteum* (Alcock)

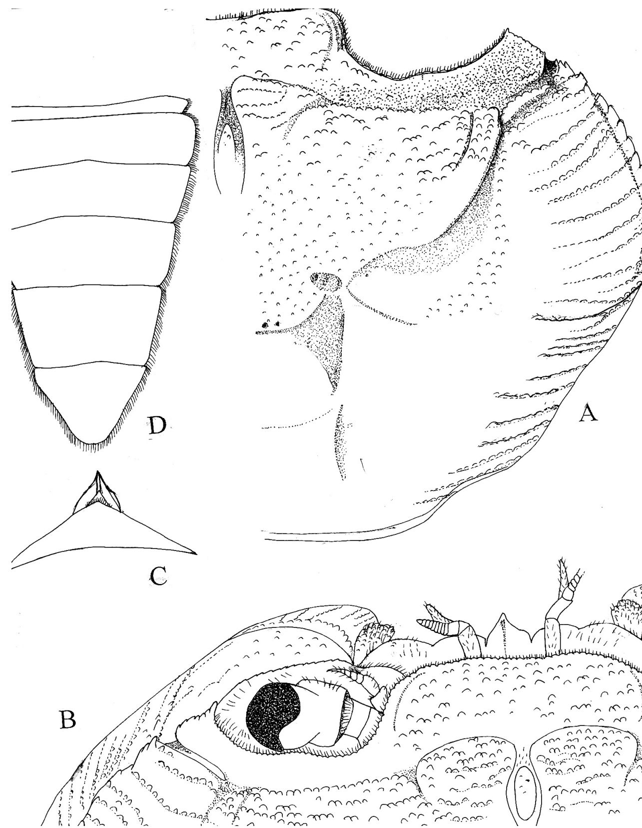

Fig. 7. *Himalayapotamon emphyseteum* Alcock (male, 35x45mm). A. Carapace, right half; B. Front; C. First sternile; D. Abdomen.

Description

The carapace is hardly three fourth its greatest breadth, the surface is quite flat behind the frontal slope. The gastric region is not well defined, only the mesogastric and epigastric subregions are clear, the other areoles outside the cervical groove are two epibranchials on either side and two smaller in the middle of cardiac region, the epibranchials are further subdivided by a broad obliquely transverse groove. The cervical groove is very deeply sunken and runs forward and cuts the postorbital cristae at a point parallel to the inner angle of external orbital spine (Fig. 7A). The frontal region is tuberculated, the anterior part of the gastric and epibranchial region is rugulose. The lateral walls of carapace is tuberculated anteriorly and obliquely striated with sparse pubescence at postero-lateral border. The epigastric cristae, though separated from the straight postorbital cristae on either side by groove do not at all overlap the postorbital cristae, but merely form the convex part of one common oblique line with them, the edge of these cristae are crenulate.

The front is moderately declivous, jointly and broadly bilobed in the dorsal view, its breadth in the adult is considerably less than one third the greatest breadth of the carapace, its edge is well-defined and very distinctly beaded (Fig. 7B). The upper edge of orbit is markedly sinuous. The external orbital tooth is prominent, subacute and separated from the lower border of the orbit by a notch. The anterolateral border of the carapace is shorter than the posterolateral border, raised, serrulate, curved, curve runs on to the dorsum of the carapace posteriorly, the epibranchial tooth being pre-eminent above the general serration of these borders.

The first sternite is dome-shaped (Fig. 7C). The 6th abdominal segment of male (Fig. 7D) is two-thirds its greatest breadth and the 7th segment is equal to its greatest breadth.

The merus of the third maxilliped (Fig. 8A) being broader than long, the outer angle is not evenly rounded. The upper and outer surface of carpus and palm and some of the inner surface of palm is granulated in cheliped (Fig. 8B). The upper border of dactylus is denticulate. The cutting edges (Fig. 8B,C) of both the chelipeds have different armature. Upper border of meri of legs (Fig. 9A) is serrulate and both borders of the propodi are spinulose, the upper borders of propodi of last three legs are double-edged, the dactyli are spinulose.

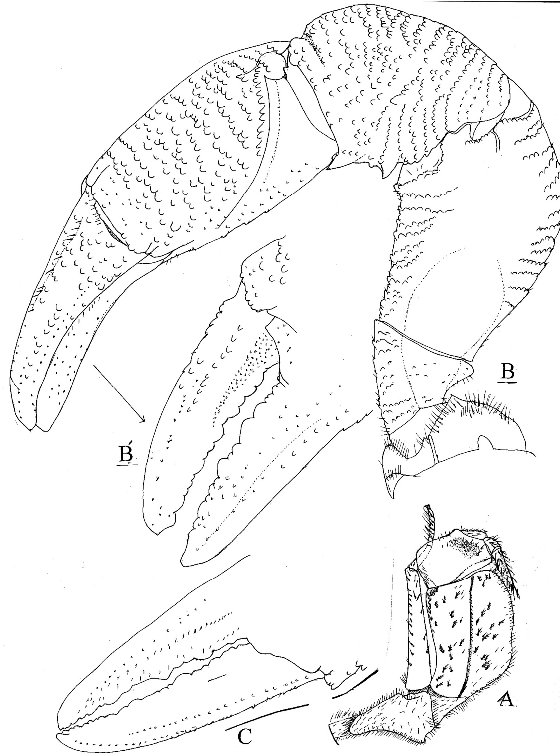

Fig. 8. *Himalayapotamon emphyseteum* Alcock (male, 35x45mm). A. Third maxilliped; B. Right cheliped (dorsal view); B'. Chela, cutting edges; C. Left chela, cutting edges.

The first gonopod (Fig. 9B,C) is stout and hairy. The distal part is leaflike, ending in a spout (Fig. 9C). The second gonopod (Fig. 9D) is slender, the distal part is much smaller than the proximal part.

Remarks

The male from Islamabad identified as *H. emphyseteum* (Alcock) was taken to the National University of Singapore, Biodiversity and Ecology Lab. by the first author where Dr. D. Yeo kindly confirmed the identification.

Himalayapotamon koolooense (Rathbun, 1904)

Potamon (*Potamon*) *koolooense* Alcock, 1910: 24, fig. 38.

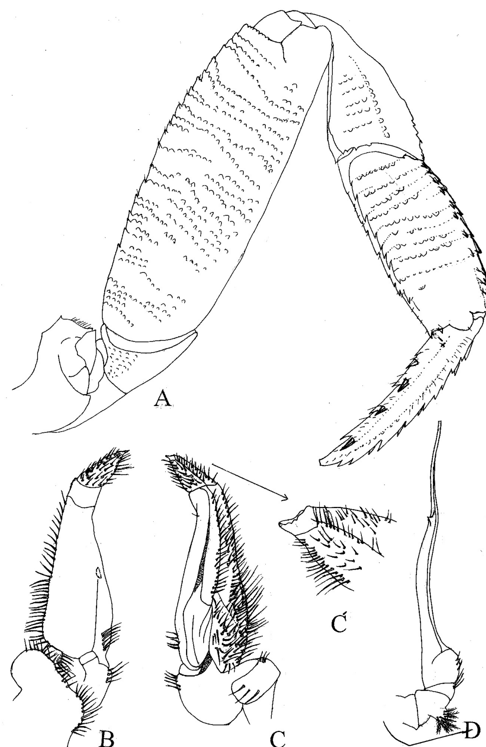

Fig. 9. *Himalayapotamon emphyseteum* Alcock (male, 35x45mm). A. Right fourth leg; B,C. First gonopod; D. Second gonopod.

Remarks

The type locality of the species is Kooloo valley, north of India (Rathbun, 1904), but Alcock (1910) reported it from Hazara now in NWFP and River Ravi (Chamba) in Punjab, however, not present in the material at hand.

Genus *ACANTHOPOTAMON* Bott, 1970
Acanthopotamon martensi (Wood-Mason, 1875)

Paratelphusa martensi Henderson, 1893: 386.
Potamon (*Parathelphusa*) *martensi* - Rathbun, 1905: 258; pl. xii, fig. 9.
Acanthopotamon martensi - Bott, 1970: 145.

Remarks

Henderson (1893) mentioned of *P. martensi* as “a comparatively small species” from North West Province of India, Rathbun (1905) also referred to Henderson’s material of north west Provinces while reviewing the species. Its present status in Pakistan is uncertain.

Family GECARCINUCIDAE Rathbun, 1905
Genus *SARTORIANA* Bott, 1970
Sartoriana spinigera (Wood-Mason, 1871)
(Fig. 10)

Fig. 10. *Sartoriana spinigera* (Wood-Mason)

Telphusa (*Parathelphusa*) *spinigera* Wood-Mason, 1871: 194.
Parathelphusa spinigera - Henderson, 1893: 386.
Potamon (*Parathelphusa*) *spinigera* - Rathbun, 1905: 231.
Paratelphusa (*Parathelphusa*) *spinigera* - Alcock, 1910: 72, fig. 53.
Sartoriana spinigera - Bott, 1970: 39, fig; Yeo, Kazmi and Ghalib, in preparation.

Material examined

9 males, cl. 18-29mm, cb. 22-36mm, 10 females, cl. 19-30mm, cb. 23-38mm, ZSD Cat No. 19. (no other data), Native Jetty, Karachi (Sindh); 3 males cl. 27-44mm, cb. 38-62mm, Thatta (Sindh) ZSD 1963, 6 males, cl. 18-22mm, cb. 26-27mm, 3 females, cl 20-21mm, cb. 25-28mm, Chato Chand (Sindh) coll. Salahuddin, dt: 2002; 1 male, cl. 36mm, cb. 52, 2 females cl. 27mm, cb. 39 mm, Keel Kaur, Panjgoor (Baluchistan) coll. M. Khurshid, 16 November, 2004.

Description

The carapace is broad, convex, its length is about two thirds its greatest breadth, its surface is smooth except for some very fine lines at the posterolateral borders. The cervical groove is deep running towards the outer ends of the postorbital cristae but becoming shallow reaching there. All the regions are distinct, a sunken oval facet is marked off in the gastrocardiac angle of each of the epibranchial regions, the postfrontal mesogastric groove is well marked. The epigastric cristae are prominent, with the anterior surface rugose, overlapping and placed slightly anterior to the postorbital cristae, the latter are thin, sinuous, and fade beyond the point where the cervical groove approaches them. The front is declivous, about one third the breadth of the carapace, distinct notch separates the external orbital and angle of lower border of the orbit.

The anterolateral border of carapace is well arched, longer than to posterolateral, the former is sharp, indistinctly crenulate or almost entire, prominent epibranchial spine is placed very far back.

The male abdomen is broad based and triangular; the 6th segment has concave sides, its length equals its proximal breadth, the 7th segment is longer than broad, broadly rounded anteriorly.

The ischium of the third maxilliped is longitudinally grooved near the inner edges, the merus is much broader than long and has an oblique anterior edge.

The chelipeds are very unequal in both sexes, larger in males than in the females, their surface is smooth; the merus has a distinct acute spine near the upper border; the carpus has a strong spine at the inner angle; the fingers are moderately broad, they are strong and sharply pointed, the tips may cross, the dactylus is longer than the palm. In the smaller cheliped the fingers are not much bent, and do not gape much when closed, one tooth on the fixed finger may be enlarged. In the larger cheliped, specially of the male dactylus is much bent and a large molariform tooth near its base and a second enlarged tooth beyond its middle is present while in the fixed finger there is a very much enlarged molariform tooth near the base.

The legs are stout, the dactyli are strong, longer than the propodi. The first gonopod is stout, ends in a pointed tip which is armed with spinules visible under high magnification.

The second gonopod is slender and the distal part is very narrow.

Remarks

The species has been reported from Karachi and Jhelum (Khewrah Gorge) by Alcock (1910), Rathbun (1905) and Henderson (1893) from Karachi (Sindh), Sutlej (Punjab) and Northwest Provinces (Present NWFP). The present material from Panjgoor (Baluchistan) extends its range to western localities of Pakistan.

Sartoriana blanfordi (Alcock, 1909)
(Figs. 11-12)

Fig. 11. *Sartoriana blanfordi* (Alcock)

Paratelphusa (*Paratelphusa*) *blanfordi* Alcock, 1910: 75, fig. 16.
Paratelphusa (*Paratelphusa*) *blanfordi blanfordi* - Pretzmann, 1966: 223.
Liotelphusa (*Sartoriana*) *blanfordi afghaniensis* - Bott, 1970: 39.
Sartoriana blanfordi - Bott, 1970: 39 (part); Yeo *et al*, in prep.

Material examined

2 males, cl 32-36mm, cb 42-48mm, temp. 25°C, salinity 10‰, Rakhshan Kaur, Panjgoor (Baluchistan), coll. M. Khurshid, 19 August 2003; 4 females, cl. 30-35mm; cb. 31-34mm, 5 males, cl. 19-30mm, cb 24-40mm, Gichki Kaur, Panjgoor (Baluchistan), coll. M. Khurshid, 18 August 2003; 5 males, cl. 25-30mm, cb. 30-40mm, 5 females, cl. 20-30mm, cb. 30-40mm, Rakhshan River,

Panjgoor (Baluchistan), coll. M. Khrushid, 25 August 2003; 5 males, cl. 30-35mm, cb. 30-38mm, 6 females, cl. 20-32mm, cb. 30-40mm, one female, larvigerous, 65 larvae, Ghichk Kaur, Panjgoor (Baluchistan), coll. M. Khurshid, 26 August 2003, 4 males, cl. 25-43mm, cb 32-48mm, 7 females, all spent, cl 27-36mm, cb 34-48mm, Panjgoor (Baluchistan), coll. M. Khurshid, 2 October 2003; 5 females, cl 25-29mm, cb 30-40mm, 2 males, cl. 30-35mm, cb. 40-45mm, Ghichk, Panjgoor (Baluchistan), coll. M. Khurshid, 21 April 2004; 4 females, cl 19-28mm, cb 03-40mm, 3 males, cl. 30-35mm, cb. 40-45mm, Nasirabad Turbat, Keech (Baluchistan), coll. M. Khurshid, 15 October 2003.

Description

The carapace is flat, its length is nearly three-fourth the greatest breadths, the surface is smooth, few fine and short oblique wrinkles are present at the posterolateral borders, the cervical groove is deep, very broad, running towards the epibranchial spine, disappears before reaching it; the regions are distinct, the epibranchial region has an irregular facet at the gastrocardiac angle; postfrontals mesogastric groove is deep. The epigastric cristae are prominent, rugulose, overlapping and a little in advance of the post-orbital cristae which are thin, sharp, crenulate more or less broken at their outer ends but clearly reaching to the epibranchial spine, or to the edge of the carapace.

The front (Fig. 12A) is nearly one-third the greatest breadth of the carapace, very slightly declivous, its edge is nearly smooth and straight in adults, crenulate and sinuous in the youngs, the external orbital angle is broad, subacute, not separated from lower border of orbit by any gap.

The anterolateral borders of carapace are convex, sharp, crenulated, more than two-fifths extend in front of the prominent and acute epibranchial spine; the posterolateral borders are markedly convergent, striated, the striae are finely granulated in young specimens.

The male abdomen (Fig. 12B) is not very broad at the base, the 6th segment has convergent and concave sides; its length equals its distal breadth, 7th segment is as long as broad, broadly rounded at tip.

The merus of third maxilliped (Fig. 12C), is broader than long has no groove running longitudinally.

Fig. 12. *Sartoriana blanfordi* (Alcock) (male, 23x27mm). A. Carapace, left part; B. Right third maxilliped; C. Abdomen; D. Left first gonopod; E. Second gonopod.

The chelipeds are enequal in both sexes, more so in the males; carpus and palm are armed with granules in short oblique rows, more definite in smaller specimens (cl 19mm), upper edge of merus is crenulate, but without any subterminal spine; the carpus has a coarse spine at inner angle, the movable finger is stout and have broad spooned tips, the proximal teeth are enlarged and molar-like and the distal teeth are small, regular, transluscent and incisor-like; the fixed finger is very broad, the dactylus is curved, and the closed fingers gape to a certain extent, at the base of the fixed finger of larger chelae one tooth is enormously enlarged.

The legs are stout, the segments are broad, the dactyli are almost equal to the propodi. The first gonopod (Fig. 12D) is almost straight, the distal portion is

short as compared to the proximal part, both lined with small setae. The second gonopod (Fig. 12E) is also divided in two parts, the basal part is broad at the base, but tapers abruptly and becomes slender, the distal segment gradually tapers into a point.

Remarks

Bott (1969) while establishing the genus *Sartoriana* included *blanfordi* and *spinigera* in this genus. But these two species differ significantly and strongly indicate that the two species are not congeneric, therefore necessitating the establishment of a separate genus (Yeo *et al*. in prep). It appears that the *Sartoriana blanfordi* is endemic to the province of Baluchistan since it is exclusively and consistently collected from that area (Alcock, 1910; Pretzmann, 1967). Some specimens in the present collection are nearer to Pretzmann's (1971) varieties: *afghaniensis* (Fig. 13) and *rokitansyki*.

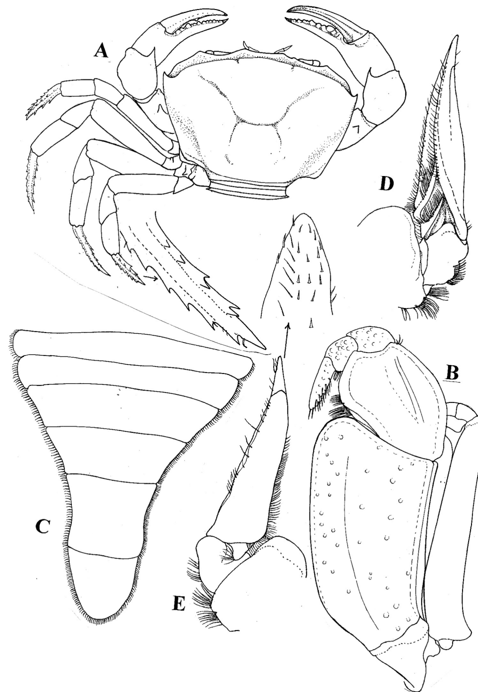

Fig. 13. *Sartoriana afghaniensis* (Wood-Mason) (male, 47x57mm). A. Male, entire; B. Left third maxilliped; C. Abdomen; D & E. First gonopod; E. Second gonopod.

Genus *CARDISOMA* Latreille, 1828
Cardisoma carnifex (Herbst, 1794)
(Fig. 14)

Fig. 14. *Cardisoma carnifex* (Herbs)

Cardisoma carnifex - Kazmi and Perveen, 2005, in press; Turkey, 1974: 224 (for complete synonyms).

Material examined

2 males, cl. 42-57mm, cb 51-56mm; 4 females (all spent) cl. 42-52mm, cb. 50-57mm, Korangi Creek, Karachi (Sindh), ZSD, 7 June, 1975.

Remarks

The Pakistani material has been described in detail by Kazmi and Perveen (in press).

The species burrows near brackish areas and estuaries but it needs water flow for dispersal of larvae, therefore it can be grouped within semiaquatic or amphibious crabs.

Family POTAMONAUTIDAE Bott, 1967
Genus *POTAMONAUTES* McLeay, 1838
Potamonautes perlatum (Milne Edwards, 1837)

Potamonautes sidneyi - Rathbun, 1905: 165.
Potaman (Potamonates) sidneyi - Hashmi, 1964: 453 (erroneous).

Remarks

The only report of the species from Pakistan (Karachi and Hyderabad) is by Hashmi (1964) as *Potamon sidneyi,* Rathbun's (1905) *Potamon sidneyi* is synonymized with *Potamon* (*Potamonautes*) *perlatum* by Colosi (1924). This species is said to be widely distributed in south African low lying streams and rivers. Therefore Hashmi's identification is questionable.

Discussion

Alcock (1910) opined that freshwater crabs of the subcontinent India appear to hang together in six territories. Out of these six parts, the three, the Western Frontier Territory, Western Himalayan Territory and the Indo-Gangetic Plain lied in the areas now located in Pakistan. *Potamon* s. str occurs only as far as Alcocks (1910) "Western Frontier Territory" (Yeo and Ng, 2004). They revalidated and redefined the subfamilies Potaminae and Potamiscinae of the family Potamidae, this in their own words has implications for the taxonomy of Indo-Chinese potamid crabs. So the genera placements are being revised by them (Yeo and Ng, 2004).

Conclusion

Alcock reported four species (one unnamed) from the Western Frontier Territory, three of them have been also rediscovered from here (present study). *Himalayapotamon emphyseteun* is a new record from the locality. It was reported from Western Himalayan Territory by Alcock (1910). According to Brandis (2001), the genus *Himalayapotamon* shows zoogeographical affinities to the Near East and the Mediterranean and *H. emphyseteum* is distributed in northwestern India, Punjab, Himachal Pradesh, western to central Nepal. *Sartoriana spinigera* is rediscovered from Indo-Gangetic Plain. *Liotelphusa* (*Sartoriana*) *blanfordi afghaniensis* synonymized with *Sartoriana blanfordi* by Bott (1970) is being revalidated and upgraded (Yeo *et al.* in prep.). Part of the present material is collected by one of two authors (M. Khurshid) for his M.Phil thesis, new localities have been visited since it was evident that the two previous workers - Alcock (1909, 1910) and Pretzmann (1962-1976) did not visit many areas of the subcontinent.

Clearly, Pakistani non-marine crabs remain insufficiently well explored and urgent measures are necessary to assess and document this fauna so that

conservation actions can be planned. Unfortunately several questions raised, normally in similar situations remain unanswered for lack of data like that for Sri Lanka by Bahir *et al*. (2005). Are the crab species that are known from exceedingly small populations naturally rare or cryptic or are they vanishing? Is it due to habitat loss or their distribution is naturally restricted? In our case *Sartoriana blanfordi* which appears restricted in Baluchistan area needs solid scientific data to support to declare it endemic. The restricted range of this species is cause for concern with regard to its conservation. Target sampling of habitats, both disturbed and relatively pristine will certainly lead to the discovery of hitherto undescribed taxa within this until neglected fauna.

Ecology of non-marine crabs

The non-marine crabs have taken different routes while migrating and colonizing from the sea. The non-marine crabs are burrowers, hence a menace for farmers as they dig in fields, bunds, internal lining of wells and even irrigation canals. They live mostly in paddy fields, lakes, ponds, karezes, gorges, caves and riverbanks. They are amphibious, gregarious and nocturnal, it is difficult to catch them. Their breathing chamber is modified to hold the maximum quantity of moisture for the gill's function. They are well adapted to live in poorly oxygenated water. This is obvious by smoother carapace, which carries air down into the burrow. The moisture is occasionally replenished by dips in nearby water body. In summer they hibernate in their intricate burrows, usually near some source of water. Some groups have pseudolungs in the dorsal part of the gill chamber. These crabs usually feed on insects, worms, fish spawn, young shoots of vegetation, organic debris, moss, slimy algae etc.

In these crabs hatching of young ones is of two types; (i) Direct hatching into juveniles, also called abbreviated larval development; (ii) Hatching as zoeae to carry diapausing eggs to enhance the larval survival by broadcasting them when food is abundantly available and to have relatively few but large eggs is a strategy adopted by these crabs. In the first group the rainwater and temperature appear to influence breeding periodicity. The hatchlings are for several weeks retained in the mothers' abdomen until the onset of favourite conditions. In the second group the zoeae are released in marine water, synchronized with full moon and high tide. They have food and medicinal value; could act as intermediate host of helminthes and are suitable for aquaculture. The non-marine or semiterrestrial crabs are good indicators of biogeography (Ng & Rodriquez, 1995) and being conservative in their patterns are a good indicators for paleographic relationships (Brandis, 2001).

Juveniles of Sartoriana blanfordi *(Figs. 15-16)*

Material examined

1 breeding female, cl. 70mm, cb 80mm, 65 hatchlings, (all males 5x6 mm-7x8 mm), Ghichk stream, Panjgoor (Baluchistan), coll. M. Khurshid, 26 August, 2003; 1 breeding female, cl 60mm, cb 70mm, 122 hatchlings, (121 males, 1 female 3x5mm-6x7mm) Rakhshan River, Panjgoor, coll. M. Khurshid, 19 August, 2003.

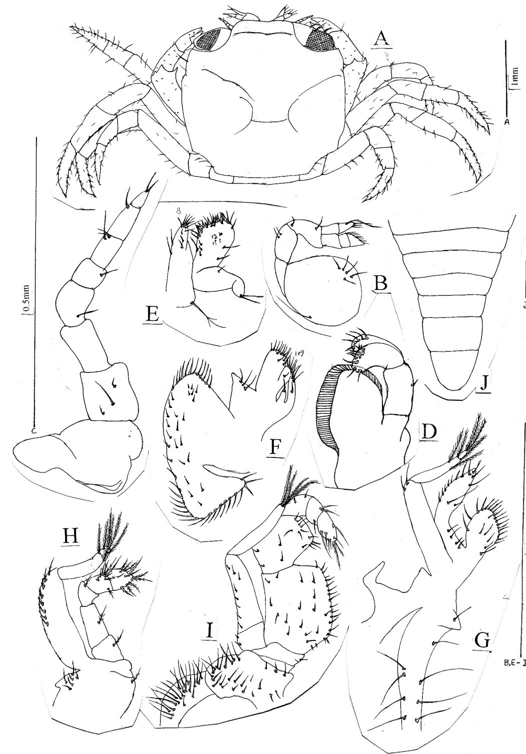

Fig. 15. *Sartoriana blanfordi* (Alcock) (juvenile, 2.5x2.9mm). A. Dorsal view; B. Antennule; C. Antenna; D. Mandible; E. Maxillule; F. Maxilla; G-I. First to third maxilliped; J. Abdomen.

Description

Carapace quadrangular, epigastric spine not prominent, regions not defined

(Fig. 15A). Abdomen as in adult. Antennule (Fig. 15B-C) small, consist of a broad precoxa, coxa and basipodite, coxa elongated and equal in length, endopod of two segments, exopod of three segments. Antenna (Fig. 15D) uniramous with a broad and flat coxa, coalesced with carapace to form an operculum, basipodite small, wedged between coxa and endopod, endopod 7 segmented. Mandible (Fig. 15E) incisor process flat, baselike, molar not developed, terminal segment of palp bilobed. Maxillule (Fig. 15F) proximal endite straight, gnathobase setose, distal endite a large blade with spines and setae, endopod proximal segment large, distal segment with two setae at the tip. Maxillary sympod proximal endite (Fig. 15G) with two unequal gnathobases, distal endite also with two gnathobases, endopod a triangular lobe with few setae, scaphognathite large fan like. First maxilliped (Fig. 15H) epipod long, coxal gnathobase sout and stumpy with dense growth of spines and setae, basipodite endite elongate, endopod three segmented. Second maxilliped (Fig. 15I) exopod with a flagellum, endopod with distal three segments turned laterally. Third maxilliped (Fig. 15J) exopod with a flagellum and endopod broad, adult like. Chelipeds (Fig. 16A), weakly developed. Legs (Fig. 16 B-E) almost like those of the adults.

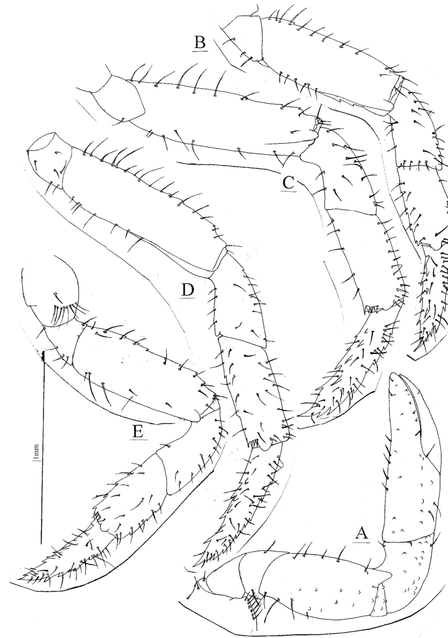

Fig. 16. *Sartoriana blanfordi* (Alcock) (juvenile, 2.5x2.9mm). Cheliped; B-E. First to fourth pereopod.

Remarks

The mother crabs were collected in the second half of the moon. As stated earlier the liberation of juveniles is said to synchronize with lunar cycle.

ACKNOWLEDGMENTS

The first author is grateful to Prof. Dr. L.B. Holthuis of National Museum of Natural History, Leiden for his help regarding *Cardisoma carnifex*, to the environmental NGO IUCN-Pakistan for inviting her to the workshop on these crabs in December 2004, to Dr. M. Yaqoob, NARC, Islamabad and Mr. Salahuddin, Jama Millia Karachi, for kindly collecting important fresh material for her. Dr. P. Ng, Director, Raffles Museum of Biodiversity Research, Singapore and Dr. D.L. Yeo, Department of Biological Sciences are specially thanked by her for certifying the identification and for facilitating access for her to the literature and material at their laboratory, Department of Biological Sciences, National University of Singapore, Singapore.

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A, *Sartoriana spinigera* (Wood-Mason) B, *Himalayapotamon emphyseteum* (Alcock)

Fig. 3. C, *Potamon gedrosianum* (Alcock) D, *Sartoriana blanfordi* (Alcock)

E, *Oziothelphusa* sp. F, *Cardisoma carnifex* (Herbs)

Proc. Pakistan Congr. Zool., Vol. 25, pp. 99-112, 2005.

EFFECT OF DIABETES ON THE BLOOD SUGAR, CHOLESTEROL, TRIGLYCERIDE AND CREATININE LEVEL OF NORMAL, OVER-WEIGHT AND OBESE SUBJECTS

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Abstract.- In this study, the population of 166 individuals was studied in two different ways. Obese non-diabetic subjects of different age groups were compared with obese diabetic subjects of respective age groups for seven parameters viz. body mass index, fasting blood sugar, triglyceride, cholesterol, HDL-cholesterol, LDL cholesterol and creatinine. FBS of non-diabetic patients remained normal in all the age groups. Their total cholesterol, HDL-cholesterol, and LDL cholesterol levels increased with the age, and in contrast their triglyceride level decreased with the age. Effects of diabetes on the various biochemical parameters of normal, over-weight and obese subjects was studied. FBS concentration of normal, over-weight and obese diabetic subjects was more than the normal limits, whereas all non-diabetic (normal, over-weight, and obese) subjects had FBS values without normal limits. The three groups (normal, over-weight, and obese) of diabetic subjects showed risk level concentrations of triglyceride and total cholesterol, whereas over-weight and obese groups of non-diabetic subjects had risk level concentration of triglyceride. The present study has concluded like others have also shown that there are differences in the subpopulation distribution of plasma lipoproteins that may predispose patients with diabetes to a high risk for CVD. Patients with type 2 diabetes have a higher proportion of small and dense LDL and a lower proportion of large HDL subtractions than non-diabetic controls. Thus, changes in the subpopulation distribution of plasma lipoproteins may contribute to the increased risk and incidence of CVD in patients with diabetes.

Key words: Obesity, body mass index, high density lipoproteins, low density lipoproteins, type 2 diabetes.

INTRODUCTION

Adipose tissue excess or obesity, particularly in the viscera compartment is associated with insulin resistance, hyperglycemia, dyslipidemia, hypertension, and prothrombotic and proinflammatory states (Grundy *et al.,* 2004). The prevalence of obesity and these associated morbidities, known as the metabolic syndrome, has reached epidemic proportions (Grundy *et al.,* 2004). It is now clear that adipose tissue is a complex and highly active metabolic and endocrine

organ (Ahima and Flier, 2000; Fruhbeck *et al.,* 2001). Besides adipocytes, adipose tissue contains connective tissue rnatrix, nerve tissue, stromovascular cells and immune cells (Frayn *et al.,* 2003). Adipocytes express and secrete several endocrine hormones such as leptin and adiponectin, many secreted proteins are derived from the nonadipocyte fraction of adipose tissue (Fain *et al.,* 2004).

The relationship between normal ageing and the clinical expression of obesity is likely to be complex. There is a general increase in body weight and BMI until approximately 60 years of age, when the amount of non-visceral fat begins to decline, whereas the population of visceral fat progressively increases. The reasons for this change are not completely understood. It is interesting to speculate that hormonal changes, reduced fatty acid utilization, resistance to leptin and modification of the body's ability to use dietary protein are partial explanation of this phenomenon (Rosmond, 2004). Advancing age involves specific biological alterations in which basal metabolic rate declines, visceral fat increases, muscle mass decreases and blood pressure increases (Evans and Compbell, 1993; Ferrannini *et al.,* 1997). The increase of visceral fat with ageing brings about significant decrease in peripheral tissue sensitivity to insulin (Elahi and Muller, 2000). Weight loss in elderly can reduce morbidity from arthritis, diabetes and other conditions, reduce cardiovascular risk factors, and improve well-being. Body mass index (BMI) also predicts morbidity in those without disease. Morbid obesity is associated with depression, where the behavioural aspect may be part of the pathogenesis rather than a result of the obese state. There seems to be a differential response to depression, where leaner subjects lose weight during depression, whereas more obese gain weight. Furthermore, increased physical activity in the elderly, which is an important component of weight management, can produce beneficial effects on muscle strength, endurance and well-being (Elia, 2001).

In an understanding of etiology of obesity the interaction and influence of environmental factors has taken an increasing importance. Many psychosocial characteristics of individuals are associated with body weight. Traits of anxiety and depression have a predictive association with visceral obesity in both men and women (Rosmond, 2001). Morbid obesity is associated with depression, where the behavioral aspect may be part of the pathogenesis rather than a result of the obese state. There seems to be a differential response to depression, where leaner subjects lose weight during depression whereas more obese gain weight. Furthermore, alcohol consumption and smoking are common among subjects with visceral body fat distribution. In addition, many socioeconomic factors have

been proposed to explain variations in obesity between individuals and groups. The most prominent factors are divorce, solitude, poor economy, low education, unemployment and problem at work when employed (Rosmond, 2001).

Obesity is increasing in prevalence among Punjabis and is associated with several adverse health problems, including type 2 diabetes, hyperlipidemia, and hypertension. The influence of obesity on the development of type 2 diabetes is complex and is likely due to an interaction of genetic, nutritional, and metabolic factors (Brownell *et al.,* 1992). Much attention has been focused on the identification of molecular pathways that contribute to the development of obesity and type 2 diabetes (Leibel *et al.,* 1995; Walston *et al.,* 1995; Silver *et al.,* 1997; Fernandez-Real *et al.,* 1997).

The aim of this study was to investigate the influence of obesity on the concentration of different type of lipids *viz.,* triglycerides, total cholesterol, high density lipoproteins, low density lipoproteins, and fasting blood sugar concentration in Punjabis. One hundred sixty six subjects, 49 men and 117 women, were taken as subjects and their fasting blood glucose, total cholesterol, triglycerides, high density lipoprotein, low density lipoprotein and creatinine were estimated for the purpose.

MATERIALS AND METHODS

Subjects

Patients, male and female of all age groups, visiting Diabetes Clinic in Shaikh Zaid Hospital, Lahore, Fatima Hospital in Sargodha and Irfan Clinic in Sargodha were included in the study. The patients/subjects which were overweight (BMI 25.0-29.9 kg/m^2/(obese ($\geq$30kg/m^2), had history of diabetes and hypertension, and have been fasting for 8 t012 hours were included, whereas there who were pregnant, under medication and had more than 5 years of history of diabetes and hypertension were excluded.

A total of 18 control (BMI 18.5-24.9kg/m^2), 60 overweight (BMI 25.0-29.9 kg/m^2), and 88 obese patients comprising 49 males and 117 females were included. The subjects with BMI between 20-24.9 kg/m^2 were used as control. These individuals ranged between 20-60 years. They had no history of diabetes and were under no medication. The subjects with BMI between 24-29 kg/m^2, who ranged between 20-60 years, had diabetes for not more than 5 years, and were not under medication. They were designated as over-weight subjects. The

patients 20-60 years of ages with BMI $\geq$30 and diabetes for not more than 5 years, and were not under medication, were labeled as obese subjects.

Blood samples

About 5ml blood was drawn from above subjects with the help of 5ml syringe and divided into two different types of vaccutainers for separation of serum and plasma. Five ml blood was added in a tube contained K_3 EDTA for separation of buffy coat; 3ml blood was added in another tube for separation of blood serum; and 2ml in the third tube containing potassium oxalate and sodium fluoride for separation of plasma. The tubes were centrifuged at 4,000 rpm (806xg) for five minutes. The blood serum and plasma were separated gently with the help of Pasteur pipette. The isolated serum was used for estimation of triglycerides according to Trinder (1969), cholesterol according to Richamond (1973), Roeschlau *et al.* (1974) and Trinder (1969), low density lipoprotein (LDL) according to Wieland and Slidel (1983), high density lipoprotein (HDL) according to Assmann (1979) and creatinine according to Bartels *et al.* (1972), whereas plasma was used for the estimation of fasting blood sugar (FBS) according to Barham and Triender (1972).

RESULTS

Blood serum profile of diabetic and non-diabetic patients

The blood samples of diabetic and non-diabetic patients of age groups 21-30 years (Group A), 31-40 years (Group B), 41-50 years (Group C), and 51-60 years (Group D) were analyzed for different parameters of obesity. Table I shows comparison of body-mass index (BMI), FBS, triglycerides, cholesterol, HDL, LDL, and creatinine, in diabetic and non-diabetic patients of different age groups. All these parameters have been discussed individually below.

Body mass index (BMI)

The average BMI of diabetic patients is 31.98±0.87 kg/m^2, whereas that of non-diabetic patients is 30.96±0.81. The non-diabetic patients have their BMIs varying between 28.95±1.32 to 32.2±1.3 kg/m^2, and diabetic patients have their BMIs varying between 27.2±0.82 to 38±7.25 kg/m^2, irrespective of the age. In the diabetic patients, however, the BMI of the age group A of diabetic patients is 38, which gradually decreased with age. It is reduced to 27.2±0.82 kg/m^2 in the age group D. The BMI decreased 12%, 22%, and 28% in the age groups B, C,

and D; when compared with the age group A. There is significant difference in BMI of diabetic compared to non-diabetic patients in the age groups A and D. The diabetic patients have significantly higher BMI in the age group 21-30 years, but significantly lower in the age group 51-60 years, compared to non-diabetic patients.

Fasting blood sugar (FBS)

The average FBS of non-diabetic patient is 97.01±3.1 mg/100 ml whereas that of diabetic patients is 177.87±11.26mg/100ml. The diabetic patients have their FBS varying between 152±22.9 to 197±63.3 mg/100ml and non-diabetic patients their F8S varying between 90.5±5.07 to 104±15.7 rrig/100ml, irrespective of the age. The FBS of diabetic patients of age group 31-40 years was significantly decreased (22%) as compared with that of age group 21-30 years. And the FBS of non-diabetic patients decreased 13%, 2.8% and 11% in the age groups 8, C, and D, when compared with the age group A of non-diabetic patients. The diabetic groups A, B, C, and D had their FBS 47%, 40%, 45%, and 48%, respectively, higher than their respective non-diabetic groups.

Triglyceride

The average blood serum triglyceride concentration of non-diabetic patients was 192.31±8.48 mg/dl, whereas that of diabetic patients was 174.68±12.52 mg/dl. The non-diabetic patients had their triglyceride concentration varying between 141.2±25.79 mg/dl to 200±47.49 mg/dl, and diabetic patients had their triglycerides varying between 138.3±29.9 mg/dl to 204.05±27.575 mg/dl, irrespective of the age. In the non-diabetic group the age group Band C showed 26 and 10% more triglyceride as compared with that of age group A, whereas it decreased 11% in age group D. The triglyceride concentration of diabetic patients increased 18%, 47%, and 39%, in age groups B, C, and D, when compared with the age group A. The diabetic patients in the age groups A and B had 15% and 23% lower triglyceride contents when compared with their respective non-diabetic patients. In the age group C and D the diabetic patients showed 14% and 26% higher triglyceride content, respectively, when compared with their respective age groups of non-diabetic patients.

Cholesterol

The average of cholesterol concentration of non-diabetic patients is

165.125±4.5 mg/dl, whereas that of diabetic patients is 166.78±6.39 mg/dl. The non-diabetic patients had their cholesterol concentration varying between 147.1±19.55 – 174.9±8.25 mg/dl, and the diabetic patients had them varying between 160.3±14.1 – 174.35±24.175 mg/dl, irrespective of ages. The cholesterol concentration of non-diabetic patients increased 13%, 18% and 17% in age groups 8, C, and D when compared with the age group A, whereas diabetic patients did not show any significant difference between its various age groups. In the diabetic groups, the cholesterol concentrations in different age groups were not significantly different from those of non-diabetic group.

High density lipoprotein (HDL)

The average of HDL concentration of non-diabetic patients is 34.52±0.94 mg/dl, whereas that of diabetic patients is 36.21±2.24 mg/dl. The non-diabetic patients have their HDL concentration varying between 31.1±3.342 mg/dl to 37.53±2.0665 mg/dl, and the diabetic patients had their concentrations varying between 29.15±2.876 - 44.16±13.085 mg/dl, irrespective of the age. The HDL concentration in diabetic patients was 21% and 11% lower in the age groups C and D, respectively, when compared with that of the age group A, whereas its concentration was 17% higher in the age group B. The diabetic patients in the age group A and B had 39% and 38% higher HDL concentration than in the respective age groups of non-diabetic patients whereas in the subsequent two age groups C and D the diabetic patient had no statistically significant different values.

Low density lipoprotein (LDL)

The average LDL concentration in non-diabetic patients is 97.63±3.8 mg/dl, whereas that of diabetic patients was 102.43±5.01 mg/dl. The non-diabetic patients had their values varying between 82.4±13.62 mg/dl - 106.75±12.25 mg/dl and diabetic patients between 95:120.4 mg/dl to 111±13.65 mg/dl, irrespective of the age. The LDL concentration of non-diabetic patients when compared with that of age group A showed 20%, 23% and 29% higher values in age group B, C, and D, respectively. The LDL concentration of diabetic patients was also 10%, 5% and 17% higher in age group B, C, and D, respectively, when compared with the age group A. The diabetic patients in the age groups A, B and C had LDL concentration 13%, 5% and 4% higher than non-diabetic age groups, whereas it showed 3% lower LDL content in the diabetic patients of age group C, when compared with the respective non-diabetic patients age groups C.

Creatinine

The average of creatinine concentration of non-diabetic patients is 1.02±0.019 mg/dl, whereas that of diabetic patients is 0.88%0.03 mg/dl. The non. diabetic patients have their creatinine concentration varying between 0.969±0.09 to 1.063±0.07 mg/dl, and diabetic patients have their creatinine concentrations varying between 0.87±0.07 to 0.89±0.13 mg/dl, irrespective of ages. The creatinine concentration of diabetic as well as non-diabetic group did not show any significant difference among different age groups. The diabetic patients show 15%, 11%, 20% and 19% more creatinine values in the age groups A, B, C and D, respectively, when compared with their respective non-diabetic age groups.

Effects of diabetes on the blood serum profile of normal, over weight and obese subjects

Blood samples of diabetic and non-diabetic subjects were divided into three BMI groups *viz.*, control group (BMI ranged 18.5-24.9kg/m^2), over weight group (BMI ranged 25-29.9 kg/m^2), obese group (BMI more than 30kg/m^2). The different biochemical parameters used in this study were then compared and the differences statistically analyzed. Table II shows comparison of FBS, triglycerides, cholesterol, HDL, LDL and creatinine in diabetic and non-diabetic patients of different BMI groups. All these parameters have been discussed individually below.

The BMI increased 17% and 55% in overweight and obese group, when compared with the normal group. The BMI of non-diabetic patients decreased 5% and 0.9% in control and overweight groups but, it increased 3% in obese group, when compared with their respective diabetic normal over weight and obese groups.

In the case of non-diabetic subject the BMI showed 21% and 67% increase respectively in overweight and obese groups, when compared with its respective non-diabetic normal group.

Fasting blood sugar (FBS)

The normal group had an average of 144.9±24087 mg/dl FBS, whereas the overweight group had 133±7.9 mg/dl, and obese group 141.15±6.54 mg/dl. In diabetic patients the FBS varied between 180±15.10 – 196±49.35 mg/dl and in the non-diabetic it varied between 93.8±7.4 – 101.9±4.10 mg/dl, irrespective of

TABLE II.- EFFECTS OF DIABETES ON THE VARIOUS BIOCHEMICAL PARAMETERS OF NORMAL, OVER WEIGHT AND OBESE SUBJECTS.

	Non-diabetic	Diabetic	Mean
Fasting blood sugar (mg/100 ml)			
Normal[1]	93.8±7.4[2] (n=6)	196±49.3* (n=4)	144.9±24.87 (n=4
Over wt.	99±5.24 (n=38)	167±19.0* (n=15)	
Obese	101.9±4.10 (n=60)	184.4±15.1* (n=10)	141.15±6.5 (n=85)
Triglycerides (mg/100 ml)			
Normal	130±27.59 (n=6)	168±30* (n=10)	149±21.4 (n=16)
Over wt.	161±16.03 (n=38)	182±18.16 (n=21)	171.5±12.16 (n=59)
Obese	162±10.17 (n=60)	203±20.5* (n=28)	182.5±9.68 (n=88)
Cholesterol (mg/100 ml)			
Normal	146.2±17.22 (n=6)	162.9±13.1 (n=10)	154.55±10.28 (n=16)
Over wt.	164.2±17.22 (n=38)	171.2±11.327 (n=21)	167.7±6.57 (n=59)
Obese	166.1±5.63 (n=60)	168.9±9.89 (n=28)	167.5±4.9 (n=88)
HLD-cholesterol (mg/100 ml)			
Normal	34.83±2.34 (n=6)	30.1±3.4* (n=10)	32.46±2.3 (n=16)
Over wt.	34.32±1.92 (n=6)	34.9±3.9 (n=21)	34.61±1.85 (n=59)
Obese	36.5±1.04 (n=60)	35.39±3.64 (n=28)	35.95±1.37 (n=88)
LDL-cholesterol (mg/100 ml)			
Normal	85.83±11.87 (n=60)	102.1±14.5* (n=10)	93.96±10.4 (n=16)
Over wt.	98.16±6.8 (n=38)	108.5±8.47 (n=21)	103.3±5.3 (n=59)
Obese	100.9±4.8 (n=60)	103±7.18 (n=28)	102±3.97 (n=88)
Creatinine (mg/100 ml)			
Normal	0.9667±0.086 (n=6)	0.877±0.05 (n=10)	0.92±0.047 (n=16)
Over wt.	1.01842±0.03 (n=38)	0.89619±0.0* (n=21)	0.9±0.03 (n=59)
Obese	0.95±0.1 (n=6)	0.82±0.0 (n=2)	0.8±0.0 (n=8)

[1]Subjects with BMI 22.85±0.25 kg/m2 were grouped as normal, those with average BMI of 27.17±0.17 kg/m2 were group as over weight those with an average BMI of 36.73± kg/m2 were considered obese subjects.
[2]Mean±SEM, Student's `t' test used to compare non-diabetic with diabetic patients; *P<0.05.
Normal, overweight and obese patients compared within each row. Normal subjects has been compared with overweight and obese patients [a,b]P<0.05.
Normal Values: Total cholesterol 200 mg/dl; Triglycerides: 150 mg/dl; HDL-high density lipid: Men, 41.0-58.7 mg/dl, Women, 48.5-75.0 mg/dl; LDL-cholesterol: >150 mg/dl; creatinine: Men, 0.6-1.1 mg/dl; Women, 0.5-0.9 mg/dl; Glucose: Plasma/Serum (Fasting) 95-105 mg/dl.

the BMI. In the diabetic group the FBS of the normal subject was 196±49.35 mg/dl. It was 8% low in obese group and 15% low in the over weight when compared with the normal group. In the case of non-diabetic subjects the FBS in the normal group was 93.8±7.4 mg/dl and it showed 6% and 9% increase in the over weight and obese when compared with the control group. The FBS is significantly higher in the diabetic patients when compared with their counterparts in the non-diabetic patients. This increase in diabetic patients is respectively, 52%, 41 % and 44% in the normal, overweight and obese groups.

Triglycerides

The normal group had an average of 149±21.35mg/dl triglycerides, whereas the overweight group had 171.5±12.16mg/dl and obese groups had 182.5±9.68 rng/dl triglycerides. Within the diabetic group the normal group had triglycerides 168±30 mg/dl whereas overweight and obese patients had 8% and 21% higher content, respectively as compared with normal group. In the case of non-diabetic patients the normal group had 130±27.59 mg/dl triglycerides. It increased 24% and 25% higher in the overweight and obese groups, respectively, when compared with the normal group. The diabetic patients had 22%, 12% and 20% more triglyceride in the blood serum of normal, overweight and obese patients, respectively, when compared with their respective non-diabetic groups.

Cholesterol

The normal group had an average of 154.55±10.28 mg/dl cholesterol, whereas the overweight and obese had 167.5±6.57 mg/dl. In diabetic patients the cholesterol concentration in the normal group is 162.9±13.1 mg/dl, which is increased 5% and 4% in the overweight and obese groups, respectively. In the non-diabetic patients the cholesterol concentration is 12% and 13% more in the overweight and the obese groups, when compared with the non-diabetic control group. The total cholesterol concentration of non-diabetic patients decreased 10%, 4% and 2% in normal, overweight and obese groups, when compared with their respective diabetic groups.

High density lipoprotein (HDL)

The average of control, over-weight and obese groups are 32.46±2.3 mg/dl, 34.61±1.85 mg/dl, and 35.95±1.37 mg/dl, respectively. In diabetic patients, the HDL of the normal group is 30.1±3.4 mg/dl, which increased 16% and 18% in the overweight and obese groups compared with the normal diabetics, while it did not differ significantly in non-diabetic patients. The HDL concentration of normal diabetic showed 16% decrease, whereas there was no significant difference between the various groups.

Low density lipoprotein (LDL)

The average LDL in normal, overweight and obese is 94±10.039 mg/dl, 103.3±5.3 mg/dl and 102±3.97 mg/dl, respectively. In diabetic patients the LDL concentration of the normal group is 102.1±14.5 mg/dl, which showed non-

significant increase of 6% and 1% in diabetic overweight and obese groups, respectively. In the case of non-diabetic patients the LDL concentration showed 14% and 18% increase in the overweight and obese groups, when compared with the normal non-diabetics. The LDL concentration of diabetic patients increased 16%, 10% and 2% in the normal, overweight and obese groups; when compared with their respective non-diabetics.

Creatinine

The average concentration of creatinine of normal, overweight, and obese groups ranged between 0.89±0.02 – 0.92±0.047 mg/dl. There is significant difference in the blood serum creatinine concentration within the diabetic and non-diabetic groups. The creatinine of diabetic patients showed 10%, 14% and 15% lower values in the normal, overweight and obese diabetic groups respectively, when compared with their respective non-diabetic groups.

DISCUSSION

In this study, the population of 166 individuals was studied in two different ways. Obese non-diabetic subjects of different age groups were compared with obese diabetic subjects of respective age groups for seven parameters *viz*. body mass index, fasting blood sugar, triglyceride, cholesterol, HDL-cholesterol, LDL cholesterol and creatinine. FBG of non-diabetic patients remained normal in all the age groups. Their total cholesterol, HDL-cholesterol, and LDL cholesterol levels increased with the age, and in contrast their triglyceride level decreased with the age. It is well established that the risk and incidence of cardiovascular disease (CVD) is related to alterations in the concentrations of plasma lipids and lipoproteins (MacLean *et al.,* 2000). In addition to the changes in plasma lipid concentrations, alterations in the chemical and physical characteristics of the lipid proteins, which cause shifts in the subpopulation distribution of these lipoproteins have been shown to contribute to CVD. Small and dense LDL and large very low density lipoprotein (VLDL) particles have been shown to be more prevalent in the plasma of patients with heart diseases (Austin *et al.,* 1988; Campos *et al.,* 1992; Gardner *et al.,* 1996; Griffin *et al.,* 1994; Stampfer *et al.,* 1996; Freedman *et al.,* 1998). Patients with cardiovascular disease tend to have less of the larger HDL particles and more of the smaller HDL particles (Freedman *et al.,* 1998; Wilson *et al.,* 1993; Johasson *et al.,* 1991; Laakso *et al.,* 1985). One recent study has indicated that analysis of the subpopulation distribution of the plasma lipoproteins may be more predictive of occlusive disease than determination of the lipid concentrations (Freedman *et al.,* 1998).

Effects of diabetes on the various biochemical parameters of normal, over-weight and obese subjects was studied. FBS concentration of normal, over-weight and obese diabetic subjects was more than the normal limits, whereas all non-diabetic (normal, over-weight, and obese) subjects had FBS values without normal limits. The three groups (normal, over-weight, and obese) of diabetic subjects showed risk level concentrations of triglyceride and total cholesterol, whereas over-weight and obese groups of non-diabetic subjects had risk level concentration of triglyceride. The risk and incidence of CVD is higher in obese patients with or without diabetic patients (Manson *et al.,* 1990; Abbott e*t al.,* 1988). The patients with type 2 diabetes have a 4-fold higher risk of CVD than obese non-diabetics. This increase in CVD in patients with diabetes could be explained, in part, by changes in plasma lipid concentrations (Kannel and McGee, 1979; Barakat *et al.,* 1990, 1992). The present study has concluded like others have also shown that there are differences in the subpopulation distribution of plasma lipoproteins that may predispose patients with diabetes to a high risk for CVD. Patients with type 2 diabetes have a higher proportion of small and dense LDL and a lower proportion of large HDL subtractions than non-diabetic controls (Barakat *et al.,* 1990, 1992). Thus, changes in the subpopulation distribution of plasma lipoproteins may contribute to the increased risk and incidence of CVD in patients with diabetes.

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TABLE I.- BLOOD SERUM BIOCHEMICAL COMPONENTS OF DIFFERENT AGE GROUPS OF DIABETIC AND NON-DIABETIC PATIENTS.

Age groups (Years)	**Body mass index (kg/m²)**		**Fasting blood sugar (mg/100 ml)**		**Triglyceride (mg/100ml)**		**Cholesterol (mg/100ml)**		**HDL-cholesterol (mg/100ml)**		**LDL-cholesterol (mg/100ml)**		**Creatinine (mg/100 ml)**	
	ND	**D**	**ND**	**D**	**ND**	**D**	**ND**	**D**	**ND**	**D**	**ND**	**D**	**ND**	**D**
Group A (21-30)	30.8± 1.54 (n=2)	38.0± 7.25 (n=4)	104.0± 15.7 (n=20)	197.0± 63.3** (n=4)	158.4± 42.13 (n=20)	138.3± 29.9 (n=4)	147.1± 19.55 (n=20)	160.3± 14.1 (n=4)	32.15± 2.77 (n=2)	37.75± 2.05** (n=4)	82.4± 13.62 (n=20)	95.0± 20.0 (n=4)	1.005± 0.04 (n=20)	0.87± 0.07** (n=4)
Group B (31-40)	32.2± 1.3 (n=3)	33.2± 1.73[a] (n=15)	90.5± 5.07 (n=34)	152.0± 22.9** (n=15)	200.65± 47.49 (n=34)	163.4± 22.7** (n=15)	166.6± 18.34 (n=34)	169.45± 29.01 (n=15)	31.1± 3.34 (n=3)	44.16± 13.1** (n=15)	99.0± 15.09 (n=34)	104.4± 25.80 (n=15)	0.96± 0.099 (n=34)	0.87± 0.03** (n=15)
Group C (41-50)	28.95± 1.32 (n=3)	29.55± 1.34[a] (n=24)	101.85± 5.00 (n=32)	186.0± 24.65** (n=24)	174.95± 21.29 (n=32)	204.1± 27.57** (n=24)	174.9± 8.25 (n=32)	163.05± 13.15 (n=24)	37.53± 2.06 (n=3)	29.15± 2.87** (n=24)	102.4± 7.75** (n=32)	99.30± 11.85 (n=24)	1.05± 0.03 (n=32)	0.87± 0.07** (n=24)
Group D (51-60)	31.9± 1.75 (n=1)	27.2a± 0.82** (n=17)	91.7± 5.06 (n=16)	176.5± 34.75** (n=17)	141.2± 25.79 (n=16)	193.0± 35.96** (n=17)	171.9± 15.33 (n=16)	174.35± 24.17 (n=17)	37.3± 3.45 (n=1)	33.9± 6.53 (n=17)	106.8± 12.25 (n=16)	111.0± 13.65 (n=17)	1.06± 0.073 (n=16)	0.89± 0.13** (n=17)
Mean	30.96± 0.81 (n=102)	31.98± 0.87 (n=60)	97.01± 3.1 (n=102)	177.87± 11.26 (n=60)	192.31± 8.48 (n=102)	174.68± 12.52 (n=60)	165.12± 4.5 (n=102)	166.78± 6.39 (n=1)	34.52± 0.94 (n=1)	36.2± 12.24 (n=60)	97.63± 3.8 (n=102)	102.43± 5.01 (n=60)	1.02± 0.01 (n=102)	0.88± 0.03 (n=60)

*Abbreviations used: ND, non-diabetic patients; D, diabetic patients.
**Mean±SEM, Student's `t' test used to compare ND with D groups; **P<0.05.
Different age groups compared within each column. Age group A has been compared with other age groups B, C and D; [a]P<0.05.
Normal Values: Total cholesterol 200 mg/dl; Triglycerides: 150 mg/dl; HDL-high density lipid: Men, 41.0-58.7 mg/dl, Women, 48.5-75.0 mg/dl; LDL-cholesterol: >150 mg/dl; creatinine: Men, 0.6-1.1 mg/dl; Women, 0.5-0.9 mg/dl; Glucose: Plasma / Serum (Fasting) 95-105 mg/dl.

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PCR BASED IDENTIFICATION OF *PASTEURELLA MULTOCIDA* SEROTYPE B THAT CAUSES HEMORRHAGIC SEPTICEMIA IN CATTLE AND BUFFALOES OF PAKISTAN

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Abstract.- *Pasteurella multocida* strains can be separated into serogroups A, B, D, E and F based on the antigenicity of their capsule and serotypes 1 to 16 based on the lipopolysaccharide antigens. Hemorrhagic septicemia (HS) caused by the infection with *P. multocida* serotypes B2 and E2 is a commonly fatal systemic disease of cattle and buffaloes in most parts of the tropical Asia. The definitive diagnosis of *P. multocida* depends on identifying the serotype B2 or closely related serotype E2. Conventional method for identification of *P. multocida* involves variety of biochemical tests. An alternative method is PCR based detection of unique DNA sequence in *P. multocida* serotype B. In this study HS specific product was amplified in different strains of *P. multocida*. The sequence analysis was carried out to determine the percentage homology of PCR products with HS specific region. The study showed that the PCR specific for B serotype can be a very rapid diagnostic tool that can be helpful in controlling disease outbreaks in the endemic areas.

Keywords: Diagnosis of hemorrhagic septicemia, *Pasteurella multocida*

INTRODUCTION

Hemorrhagic septicemia (HS) is a commonly fatal systemic disease of cattle and buffaloes. It is considered to be the most economically important disease of livestock in South East Asia (Sheikh *et al*., 1994). Pakistan ranks HS as a disease of considerable importance with approximately Rs 7.0 million loss attributed to HS (Khan *et al,*. 1994). Despite improved management practices, regular vaccination of cattle and buffalo population and enhanced diagnostic facilities, effective control of HS has not yet been achieved (Sheikh *et al.,* 1996) The disease is peracute, having a short clinical course involving severe depression, pyrexia, submandibular edema, and dyspnea, followed by recumbency and death (De Alwis, 1995)**.**A presumptive diagnosis of disease is based on the isolation of *Pasteurella multocida* from the blood and vital organs of an animal with typical signs (Merck Veterinary Manual, 2003).

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P. multocida usually forms coccoid cells or short rods on solid media. Many strains produce capsules. Growth on solid media is best on blood containing agar like sheep blood or chocolate agar. *P. multocida* causing HS produces oxidase, catalase and indole, and reduces nitrates. They do not produce hydrogen sulphide or urease, and fail to use citrate or liquefy gelatin. Glucose and sucrose are always fermented with the production of acid only. Most strains also ferment sorbitol. Some strains ferment arabinose, xylose and maltose, whereas salicin and lactose are almost invariably not fermented (Anonymous, 2004).

P. multocida strains can be separated into serogroups A, B, D, E, and F based on the antigenicity of their capsule (Rimler, 1987) and serotypes 1 to 16 based on lipopolysaccharide antigens (Heddleston *et al.,* 1972). The capsular serogroup is generally related to disease predilection. *P. multocida* serotypes B2 and E2 are the principal cause of HS. In South Asia, serotype B2 predominates (Wijewardana, 1992). Serogroups B and E of *P. multocida* are distinguished by indirect haemagglutination tests and by their inability to be affected by certain mucopolysaccharidases. The virulence factors of *P. multocida* responsible for HS have not been defined, and the antigens responsible for natural immunity are unknown**.** Of the serogroup B strains, only the B2 serotype produces hyaluronidase and chondroitinase (Richard, 2000).

Definitive diagnosis depends on identifying the serotype as B2 (or closely related serotypes) or E2. The conventional method for the identification of a suspect isolate as *P. multocida* involves subjecting the isolate to a range of biochemical tests (Rimler *et al.*, 1989). The complexity associated with conventional biochemical identification makes alternative approaches attractive. One such alternative is the use of the PCR to detect a sequence of DNA unique to *P. multocida* (Townsend *et al.,* 1998). In this study HS specific product was amplified in different strains of *P. multocida*. The sequence analysis was carried out to determine the percentage homology of PCR products with HS specific region. The present study suggests that PCR is a suitable technique for rapid initial identification of *P. multocida* serotype B associated with HS. Rapid identification of causal agent may aid in control of disease outbreaks in endemic areas.

MATERIALS AND METHODS

Pasteurella multocida *isolates*

A total of 13 isolates of *Pasteurella multocida* serotype B were used in this

study (Table I). The strains were rejuvenated using mouse inoculation (Wijewardana *et al.,* 1986).

TABLE I.- *P. MULTOCIDA* ISOLATED FROM DIFFERENT LOCALITIES IN PAKISTAN.

Sr. No	Name of isolate	Year of collection	Animal	Place of collection
1	Lahore W	1991	Buffalo	Hadiara, Lahore
2.	Strain B	--	Buffalo	Lahore
3.	Strain C	--	Buffalo	Sheikhupura
4.	Bahadarnagar	1995	Buffalo	Okara
5.	Military Dairy Farm	2001	Buffalo	Lahore
6.	Hyderabad	1992	Buffalo	Hyderabad
7.	Badeen Sind	1992	Buffalo	Badeen, Sind
8.	Daska	1993	Cattle	Daska
9.	LES Bhunkey	2001	Cattle	Pattoky
10.	Strain D	--	Buffalo	Burewala
11.	Lahore W_1	1991	Buffalo	Burkey Lahore
12.	LES Qadirabad	1991	Buffalo	Qadirabad
13.	Khar livestock Farm	1996	Buffalo	Khalakactai

Isolation of genomic DNA

Genomic DNA was isolated from the cell pellet by modified method of Rodgriguez and Tait (1983).Log phase bacterial culture was pelleted down at 9,000 rpm (1,2406Xg), suspended in TEN buffer (EDTA, 0.14g; Tris, 0.6 g; 5M NaCl, 1 ml; distilled water, 500 ml; pH 7.6) and centrifuged at 6,500 rpm (6,471Xg). The pellet was suspended in SET buffer (EDTA, 7.30g; Tris, 3.02g; distilled water, 300ml; pH 7.6) to which lysozyme and 1% SDS was added followed by freezing and thawing of the samples. Later 5M NaCl and buffered phenol was added. The samples were centrifuged at 6,500 rpm (6,471Xg). To aqueous layer buffered phenol: chloroform was added and tubes were centrifuged at 6,500 rpm (6,471Xg). Chloroform was then added in the aqueous layer. Finally ethanol was used to precipitate the DNA. The isolated genomic DNA was quantified and checked in 1 % agarose gel to determine the quality of DNA.

PCR amplification of type B specific fragment of P. multocida

The following primer pair KTSP61 and KTT72 were used which specifically amplify a product of approximately 560 bp in all type B *P. multocida* isolates possessing either type 2 or 5 as the dominant somatic antigen (Townsend *et al*., 1998).

KTSP61 5’- ATCCGCTAACACACTCTC – 3’
KTT72 5’ – AGGCTCGTTTGGATTATGAAG – 3’

The 50 µl PCR reaction mixture contained: 15µl template DNA, 1µM of each primer, 2.5 mM dNTPs, 10X PCR buffer, 25mM $MgCl_2$ and 1 unit of Taq DNA polymerase. The thermal cycling parameters were as follows: initial denaturation at 94°C for 5 minutes; followed by 35 cycles of 94°C for 1 minute, 50°C for 1 minute, and 72°C for 2 minutes, and a final extension of 72°C for 7 minutes. Amplified products were separated by electrophoresis in 1% agarose in TAE buffer and the bands observed on UV transilluminator.

Cloning of serogroup B specific fragment

The required DNA bands were extracted from the gel using DNA Extraction Kit by Fermentas (#K0513). Later the “InsT/Aclone™ PCR Product Cloning Kit (#K1214)” was used for cloning of cleaned Taq amplified PCR products (Sambrook *et al*., 2001). The high efficiency of kit is based on use of a specially designed cloning vector, pTZ57R/T.

Bacterial transformation (Maniatis *et al.,* 1989) was carried out by using competent cells of *E. coli* DH5α. Transformants were selected on LB plate having X-*gal* (5-bromo-4-Chloro-3-Indonyl ß-D–galactopyranoside) + IPTG (Isopropanol 1-b-D thiogalactopyranoside) + Ampicillin. The cells were spread on medium and growth of transformed cells was observed after 24 hours. The white transformants were selected and isolated on LB-Amp agar plates. Colony PCR was carried out by dissolving loopful of each colony in 15 µl of autoclaved distilled water in PCR tubes and kept on ice immediately. The PCR reaction mixture was prepared for 15 reactions as described previously. The products were observed by electrophoresis in 1% agarose gel in 1X TAE buffer.

Plasmid isolation and sequencing

Plasmid was isolated by alkaline lysis method (Sambrook *et al*., 2001). The results were observed by gel electrophoresis. The isolated plasmid was then digested with restriction enzymes *EcoR1* and *HindIII* to confirm the presence of insert. After confirmation of insert sequence was determined using Beckmancoulter automated sequencer CEQ 8000.

RESULTS AND DISCUSSION

A total of 13 different strains were obtained after screening mice for *P. multocida*. All the mice died within 24-48 hours of inoculation (Table II).

TABLE II.- RESULTS OF MICE INOCULATION OF *P. MULTOCIDA* AND SEROTYPE B SPECIFIC PCR FOR DIFFERENT STRAINS OF *P. MULTOCIDA*.

Sr. No	Name of isolate	Mice died within (hours)	Serotype B specific PCR
1	Lahore W	24	+
2.	Strain B	24	+
3.	Strain C	24	+
4.	Bahadarnagar	24	+
5.	Military Dairy Farm	24	+
6.	Hyderabad	24	+
7.	Badeen Sind	24	+
8.	Daska	24	+
9.	LES Bhunkey	24	+
10.	Strain D	24	-
11.	Lahore W_1	24	-
12.	LES Qadirabad	24	-
13.	Khar livestock Farm	24	-

Figure 1 shows genomic DNA isolated from the 13 strains of *P. multocida,* whereas Figure 2 shows 560 bp PCR product of *P. multocida* serotype B amplified in nine out of 13 strains of *P. multocida.* Figure 3 shows the colony PCR products specific for the serotype B of *P. multocida.* The plasmids were isolated (Fig. 4) and then restricted with *Eco*R1 and *Hind*III to confirm the presence of the serotype B specific fragment.

The sequencing of the clones was carried out with the help of automated sequencer. The sequence was compared with the serotype B specific sequence in the NCBI site. This comparison was carried out with the help of BLAST-n (Basic local alignment search tool) at NCBI site. The Lahore W, B and C strains of *P. multocida* showed 96% homology with the *P. multocida* serotype B specific region. Bahadarnagar strain showed 95% homology and the Military Dairy Farm strain showed only 91 % homology with the serotype B specific region. All these strains were isolated from buffaloes.

Serological identification of *P. multocida* is based on the detection of the capsular and somatic antigens. Serological tests like rapid slide agglutination test,

indirect haemagglutination test and agar gel precipitation test are currently used in the diagnostic laboratories (Namioka and Murata, 1961). Molecular tests used for identification of *P. multocida* include PCR, ribotyping and restriction enzyme analysis (Wilson *et al.,* 1992).

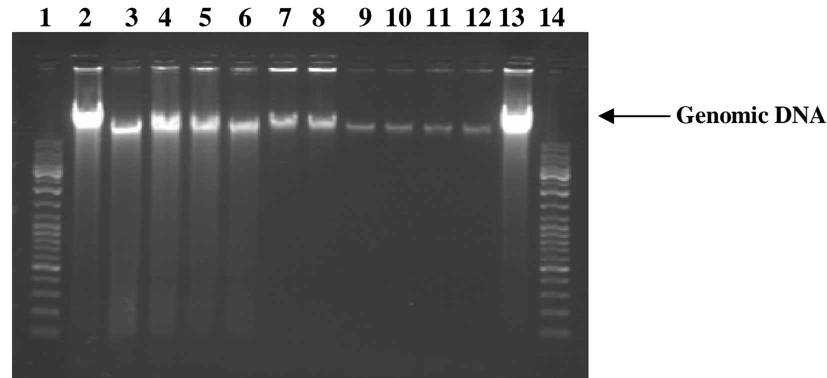

Fig. 1. Agarose gel electrophoresis of DNA isolated from different strains of *P. multocida.* Lane 1 and 14 showing 10kb ladder, Lanes 2 to 13 showing isolated DNA bands of Lahore W (3), Strain B (4), Strain C (5), Bahadarnagar (6), Military Dairy Farm (7), Hyderabad (8), Badeen Sind (9), Daska (10) and LES Bhunkey (11).

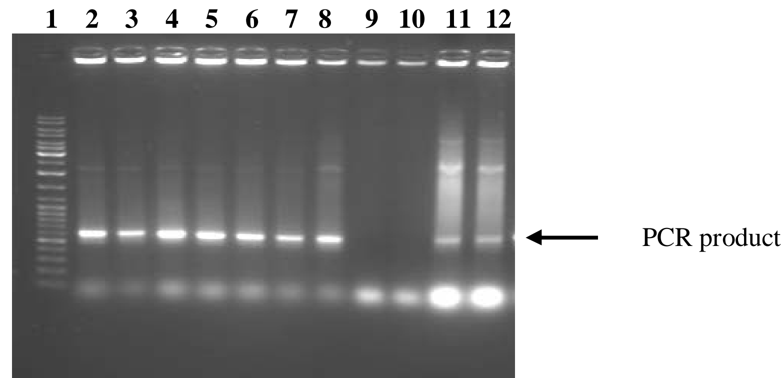

Fig. 2. Gel electrophoresis showing the serotype B specific PCR products (560 bp) in different strains of *P. multocida*. Lane 1: 10kb ladder. Lanes 2-8, 11-12: PCR products of isolates, Lahore W (2), Strain B (3), Strain C (4), Bahadarnagar (5), Military Dairy Farm (6), Hyderabad (7), Badeen Sind (8), Daska (11), LES Bhunkey (12).

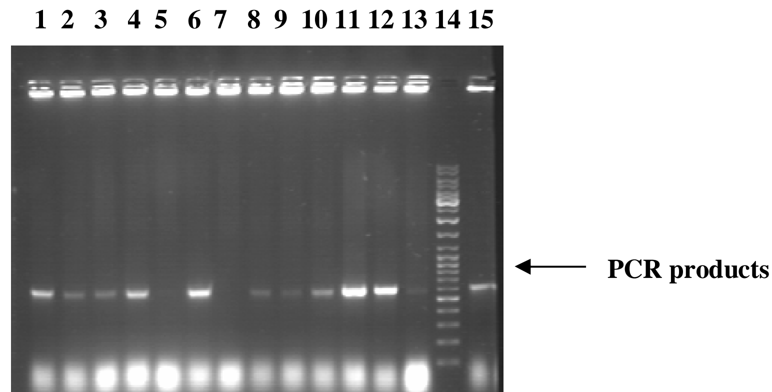

Fig. 3. Agarose gel electrophoresis showing the colony PCR of the different clones of *P. multocida.* Lane 14: 10 kb ladder, Lane 1 to 4, 6, 10 to 12 and 15: Colony PCR products. Lahore W (1), Strain B (2), Strain C (3), Bahadarnagar (4), Military Dairy Farm (6), Hyderabad (10), Badeen Sind (11), Daska (12), LES Bhunkey (15).

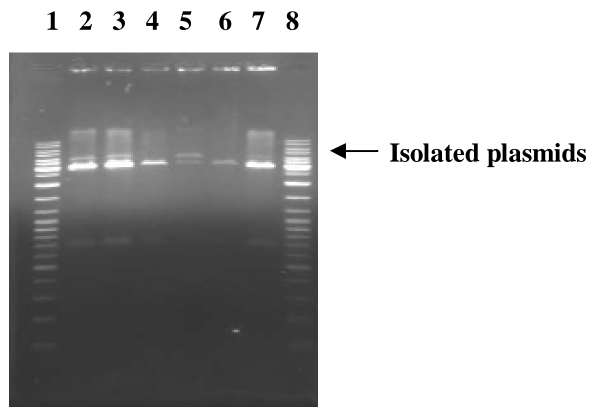

Fig. 4. Agarose gel electrophoresis showing the isolated plasmids. Lane 1 and 8: 10 kb Ladder DNA, Lane 2 to 7: Isolated plasmids. Lahore W (2), Strain B (3), Strain C (4), Daska (5), Bahadarnagar (6), Military Dairy Farm (7).

These molecular tests besides PCR are useful for identifying strains within the serotypes so are useful for epidemiological studies rather than as routine diagnostic tests. PCR methods amplify even minute quantities of DNA and allow accurate detection of specific genetic sequences. PCR tests have been developed

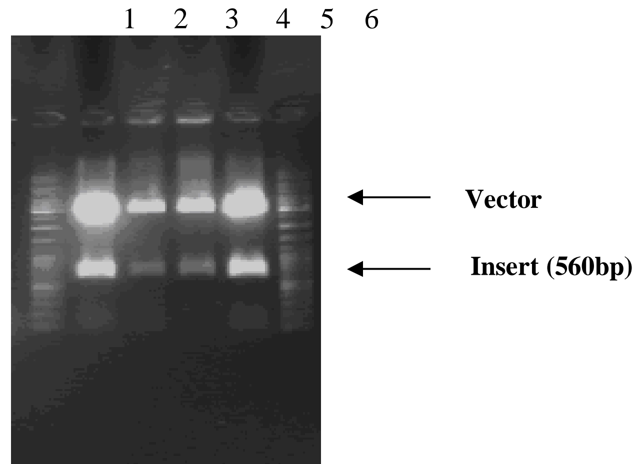

Fig. 5. Agarose gel electrophoresis showing the restriction enzyme analysis of the isolated plasmids. Lane 1 and 6: 10 kb ladder DNA, Lane 2 to 5: Restriction products. Lahore W (2), Strain B (3), Strain C (4), Bahadarnagar (5).

for the diagnosis of HS and used by many workers (Brickell *et al.,* 1998; Townsend *et al.,* 1998). The choice of diagnostic test must take into account the nature of sample available, facilities available and proximity of laboratory to the endemic area. Based on this study it can be concluded that phenotypic identification of *P. multocida* based on biochemical reactions is often limited and usually only done on a species level. While the PCR assays provide superior sensitivity, are highly specific, require less time, and are amenable to the processing of multiple samples. The rapid diagnosis can therefore be helpful in controlling disease spread in the endemic areas.

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PHYSIOCHEMICAL ANALYSIS OF PAKISTANI UNIFLORAL HONEY FROM DIFFERENT NECTAR SOURCES*

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Abstract.- Fresh honey samples from the nectar source of phulai, sunflower and sidder/ber were collected from various localities and were analyzed for moisture content, pH, free lactone, total acidity, electrical conductivity, HMF, proline content, and diastase and invertase activities for quality evaluation of Pakistani honeys. The samples were found to meet all international honey specifications, low moisture content, pH between 5.1-7.4 and proline more than 450 mg/kg in all samples. Physicochemical characteristics of Karak and Bannu sidder/ber were closer to each other than Chunian sidder honey.

Keywords: Pakistani sidder honey, HMF content, diastase activity, invertase number, proline content of honey, hydroxymethylfurfural content.

INTRODUCTION

Honey is a natural food produced by honeybees from nectars of plants. It is used as an ingredient in a number of manufactured foods, medicines and cosmetics as sweetner and moisturizer (LaGrange and Sanders, 1988; Abu-Tarboush *et al.*, 1993). The physical properties and chemical composition of honey have been investigated by many workers (White Jr. *et al.,* 1962; Sporns *et al.,* 1992; Lichtenberg-Kraag *et al.,* 2002; Iglesias *et al.,* 2004). The composition of honey depends mainly on the type of plant source utilized by the bees as well as regional and climatic conditions (Abu-Tarboush *et al.,* 1993; Iglesias *et al.,* 2004; Bonod *et al.,* 2003; Ferreres *et al.,* 1994; Sanz *et al.,* 1994; Russo-Almeida, 1997).

Acacia modesta commonly known as "Phulai" is a native forest tree in the foothills and plains of Punjab and Sind in Pakistan. There are many species of *Acacia* but none of them except *Acacia modesta* is a major source of nectar and pollen for *Apis mellifera.* It flowers during April to August. The honey produced by this plant is light brown with pleasant odor and is available at a cheaper rate to the consumers (Ahmad *et al.,* 1978).

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*Part of Ph.D. thesis of first author, University of the Punjab, Lahore.

Ziziphus jojoba is another important nectar source for honeybees in Pakistan. It is indigenous to Pakistan and is commonly known as `sidder' or `ber'. It is a bush like commonly found tree in Karak, Kohat and Bannu districts of N.W.F.P, Attock, Chakwal and Mianwali districts of Punjab and Karachi, Hyderabad and Nawabshah districts of Sind province. Although flowering season is only from September to October and it produces lesser pollens, honeybees collect a fairly good amount of nectar from this plant. The colour of fresh "ber" honey is dark brown with characteristic floral smell (Ahmad *et al.;* 1984). The "sidder honey", considered as one of the best quality honeys in Pakistan, is an exportable item of the country. In 1966, its export was more than 210 tons. It increases the net income of beekeepers by 5-10 times and sold at US $ 9-10 per kig in the country side (Muzaffar, 1999). The sunflower crops, *Helianthus annuus* is another nectar source. The areas, where sunflower is grown, usually have honeybee keeping activity. The present report aims at evaluating the quality of honey produced by *Apis mullifera* from nectars of different floral sources in Pakistan.

MATERIALS AND METHODS

Fresh Pakistani honey samples were collected from Punjab University's, New Campus area, Bannu, NWFP, Karak and Chunian (Punjab) and stored under ordinary room temperature (25°C – 29°C) in the laboratory until completely analyzed. There was no sign of granulation in honey samples. No preservative or any heating was applied at any stage.

All physicochemical determinations were essentially carried out according to the European Honey Commission methods (Bogdanov *et al.,* 1999). pH and electrical conductivity were determined in a 10g/75ml solution of honey in deionized water. Free, lactonic and total acidities were titrated in the same solution used for pH measurement (AOAC, 1975). Water content was determined by refractive index and correlation with Chataway Chart (Chataway, 1932). Proline content of honey was determined according to Cough (1969). Winkler's method was used for the determination of HMF content (Winkler, 1955). The diastase and Invertase activity of honey samples was determined according to Schade *et al.* (1958) and Siegenthaler (1977), respectively.

RESULTS AND DISCUSSION

Table I shows the results of physicochemical analysis of Pakistani honeys compared with that of International Honey Standards.

pH

The pH of Phulai, sunflower and sidder honey from Chunian had pH varying between 5.1 – 5.6, whereas sidder honey from Karak and Bannu varied between 7.1 – 7.4.

The Pakistani honey samples had pH range (5.1-7.4) as described by white *et al.* (1962) and Cervantes *et al.* (2000). Iglesias *et al.* (2004) reported 3.9 as a mean pH from 46 honey samples of Central Spain. This difference of pH in honey obtained from various floral sources verify the statement of Abu - Tarboush *et al.* (1993) that variations in pH of different honeys is due to their plant source.

Electrical conductivity (EC)

EC values are 0.1 mS/cm and 0.25 ms/cm for both phulai and sunflower honey, respectively. As expected EC values of Bannu (0.53ms/cm) and Karak (0.50ms/cm) ber honeys are close to each other, whereas low EC value was recorded for Chunian ber honey. The EC in Karak sample was 2.27 and in Bannu sample 2.41 fold as compared with that of Chunian honey sample. EC values of Pak honeys meet international EC values for blossom honey.

The EC values of Pakistani honey samples agreed with the EC values reported by Tsigouri and Passaloglou-Katrali *et al.* (2000), for thyme honey of Greek and EC values of ≤700/µS/cm for pure floral honeys (Bogdanov *et al.,* 1997).

Free acidity, lactones and total acidity

Free acidity is 11.5meq/kg in phulai honey and 6.5meq/kg in sunflower honey. Lactone is 1 meq/kg and zero, respectively. Resultantly total acidity is 12.5meq/kg in phulai honey and 6.5meq/kg in sunflower honey. Free acidity values has been calculated as 8.5meq/kg in Bunnu, 5.5meq/kg in karak and 16meq/kg in Chunian sidder honeys, whereas lactone is zero in Bunnu, 0.5meq/kg in Karak and 1meq/kg in Chunian ber honey. Consequently total acidity is 8.5meq/kg in Bunnu, 6 meq/kg in Karak and 17meq/kg in Chunian ber honey.

Chunian sample has 2.91 fold more free acidity, two fold more lactone and 2.83 fold more total acidity than Karak sidder honey. All types of acidity values

in three ber honeys from different localities satisfy the defined international standards.

The free, lactonic and total acidities in Pakistani honey samples seem to be similar to those reported by Sporns *et al.* (1992) and Gomez *et al.* (1993) in Canadian and Spanish honeys, respectively. Iglesias *et al.* (2004) reported mean free acidity (28.14meq/kg), lactone (5.08 meq/kg) and total acidity (33.23mg/kg) in 46 honey samples from central Spain higher than Pakistani honeys.

Moisture content

Content of water is 16.2% in phulai and 17.3% in the sunflower honey. The three ber samples of the honey have the same moisture content. The moisture content in all honey samples achieved the same level as recommended by the international standard.

Moisture content in all Pakistani honeys were within the standard range and were found similar to those reported by White *et al.* (1962), Laude *et al.* (1991), Joshi *et al.* (2000), Tsigouri and Passaloglou-Katrali (2000) and Iglesias *et al.* (2004) in *A. mellifera* honey.

Diastase activity

The honeys obtained from nectar source of sunflower exhibited highest diastase number (48) followed by Chunian ber honey (42) and phulai honey sample (38), whereas comparatively low diastase activity was found in Bunnu (33DN) and Karak (30DN) sidder honey. Despite the fact that there is a variation in enzyme activity, the above mentioned types of honey qualify for quality honey of international level.

Invertase activity (IN)

Invertase number has recently been proposed as another quality assessing constituent of honey. Following the international honey standard, all Pakistani honeys possessed invertase activity within the permissible limit. Invertase number is 68.8 in phulai, 62.9 in sunflower, 72.2 in Bunnu, 68.1 in Karak and 90.6 in Chunian ber honey. Chunian sample has 25.4% and 33% higher invertase number as compared with Bannu and Karak honey samples.

HMF content

The estimated HMF content in honey of phulai and sunflower are 6.5 and 0.385 mg/kg, respectively. The HMF content in Phulai honey is 19.1 fold higher than in sunflower honey. The concentration of HMF is high in Chunian (4.5mg/kg) as compared with Bunnu (2mg/kg) and Karak (1.7 mg/kg) sidder honey. Chunian sample had 125% and 153% higher HMF content as compared with Bunnu and Karak sidder honey, respectively. HMF values of Pakistani honey were well within the recommended limits for fresh honey.

Proline content

All the types of honeys have diversity in their content of proline. Karak sidder honey exhibited highest (2800 mg/kg) Proline content, whereas the sunflower honey had the lowest (466.2 mg/kg) proline content. The phulai honey had 38% more proline than the honey sample from sunflower. It was 608mg/kg in Bunnu and 521.2mg/kg in Chunian honey.

The diastase and invertase activities, proline content and HMF content in *A. mellifera* honey samples from Pakistan, were well within the DN range recommended as quality criteria by International Honey Standard and seem to be similar to those reported by Sporns *et al.* (1992) in Canadian honeys, Gomez *et al.* (1993), Iglesias *et al.* (2004) in Spanish honey samples.

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TABLE I.- PHYSICOCHEMICAL ANALYSIS OF *A. MELLIFERA* HONEY FROM DIFFERENT LOCALITIES OF PAKISTAN.

Parameters	**University of the Punjab**		**Sidder samples**			**Codex draft**	**EU draft**	**Directive 2001/EC**
	Phulai (n=3)	**Sunflower (n=3)**	**Bunnu (n=3)**	**Karak (n=3)**	**Chunia (n=3)**			
pH	5.2	5.1	7.4	7.1	5.6			
Free acidity (meq/kg)	11.5	6.5	8.5	5.5	16	≤50	≤40	≤50
Lactone (meq/kg)	I	0	0	0.5	1			
Total acidity (meq/kg)	12.5	6.5	8.5	6	17			
Moisture content (%)	16.2	17.3	17	17.3	16.2	≤21	≤21	≤21
Electrical conductivity (mS/cm)	0.10	0.25	0.53	0.50	0.22	≤0.8		
Proline content (mg/kg)	644.5	466.2	608	2800	521.2	≥180		
Diastase number (DN)	38	48	33	30	42	≥8	≥8	≥8
Invertase number (IN)	68.8	62.9	72.2	68.1	90.6	≥10		
HMF content (mg/kg)	6.5	0.34	2.1	1.7	4.6	≤60	≤40	≤40

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SIGNIFICANCE OF SEX RATIO IN COLONY MAINTENANCE OF A PREDATOR, *CHRYSOPERLA CARNEA* (STEPHENS) (NEUROPTERA: CHRYSOPIDAE)

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Abstract.- Green lacewing, *Chrysoperla carnea* (Stephens), is a broad spectrum predator devouring the eggs and neonate larvae of several insect pests. Its use as a biological control agent is restricted due to difficulty in the production of sufficient numbers at low cost. Experiments were conducted to study the effect of different sex ratios 1: 1 up to 1: 10 male and females ratios and vice versa, on the progeny production of *C. carnea.* Results showed significant effects of sex ratio on fecundity and fertility of the predator. Higher fecundity and fertility was observed when the adults were confirmed in 1:2 followed by 1:3 (male and females) ratios, while it was the lowest in 1:5 (male and females) ratios. In vice versa, confining ratio 2:1 (males and female) also proved better, but the fecundity and fertility was significantly low as compared to the 1:3 (male and females) ratio. Very few eggs were obtained from the crosses of 4:1 up to 10:1 (males and female) ratios. The studies manifested that 1:2 (male and females) ratio should be maintained for economical and successful mass production of this predator.

Key words: *Chrysoperla,* Green lacewing, sex ratio, mass production.

INTRODUCTION

The common green lacewing, *Chrysoperla carnea* (Stephens), (order Neuroptera), is a useful predator of crop insect pests. Several species of lacewings have been mass produced and released for biological control (Bellows, 2001). Among other stages, larval stage is predatory stage, while in some species adults were also predator, which are very active (Duelli, 2001). Considerable progress has been made in the manipulation of *C. carnea* colonies for augmentative purposes (Nordlund and Correa, 1995). It is reported that *Chrysoperla* spp. suppress populations of aphids, lepidopteran eggs and larval and variety of other slow or nonmoving soft-bodied arthropods (Cohen, 1995). *C. carnea,* mass multiplied from a commercial point of view, is an ideal biological control agent because it can be effective against a wide variety of insect pests in so many different cropping systems (Bansod and Sarode, 2000). The lacewings are an entirely beneficial group of insects; they undergo complete metamorphosis

with egg, larval, pupal and adult stages (Thite and Shivpune, 1999). The adults can be easily cultured on relatively simple diets, which can be manipulated for improved pest control (Roy and Nguyen, 2000).

Hence, present studies were taken up to study the biology and sex potential of *C. carnea* in the laboratory in order to develop eco-friendly mass production technology with special emphasis of sex-ratio.

MATERIALS AND METHODS

The response of different sex ratios was studied in Bio-control laboratory at Nuclear Institute of Agriculture (N.I.A.), Tando Jam. This experiment had three replications and ten treatments *viz*:- 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9 and 1:10 male : females and vice versa combinations. The experiment was conducted at controlled temperature 26±2°C and relative humidity of 60f:2 %. Four-day old virgin adults of *C. carnea* were placed in glass chimneys (7cm x 4cm) in above mentioned ratios and covered with black colour muslin cloth. Diet was provided immediately after the adults inserted in chimneys on paper cards and 50% honey-water solution with cotton impregnated was placed in vials. To study the hatchability, the laid eggs by individual female of each treatment were checked and eggs laid on cover or on the inner side of the rearing chimneys were collected and recorded daily. Observations on the fecundity, fertility, pupation and emergence percentage were recorded. The period of survival of each replication was recorded regularly in order to note pupation and emergence. The data collected on all above parameters, were subjected to analysis of variance and all the treatment means were compared using Duncan's Multiple Range Test (Gomez and Gomez, 1984) with the help of MASTATC computer soft-ware.

RESULTS AND DISCUSSION

The results on all the parameters studied have been presented in Table I. Study indicated that the ratio of 1:3 (male: females) gave better results followed by 1:2 and 1:1, and in vice versa, 2:1 showed best ratio for colony maintenance under laboratory conditions. Fecundity, fertility and pupation ratio was higher in 1:3 (male: females); there was no effect on pupation, emergence in any ratio. While, in vice versa Table II, 2:1 (males:female) ratio showed good results followed by 1:1 and 3:1 ratios. Study demonstrated that the highest egg laying is recorded in the ratio of 1:5 (male: females) followed by 1:4 and 1:6 but hatching percentage with larval survival were lowest, while in case of vice versa 3:1

(males: female) ratio yielded the best results, on the other hand among other ratios 4:1 and 5:1 (males:female) produced the poor results for all the parameters studied. The ability of natural enemies to reproduce rapidly and to search out their hosts and survive relatively at low cost makes outstanding advantages possible (Stelzel and Devetak, 1999). Michaud (2001) reported that for biological control agents, the species must have high reproductive potential, ease of rearing that makes it a fine candidate for mass-rearing and use in augmentative biological control.

The effective use of *C. carnea* in biological control either through mass-raring, colonization or conservation and manipulation of proper sex ratio should be considered (Hunter *et al.,* 1993). Therefore, for mass production of this beneficial predator the optimal use of sex ratios of 1:1, 1:2 and 1:3 (male and females) and in vice versa 2:1 (males and female) is recommended. Henry (1979, 1983) reported that during mass-rearing sustained high levels of ovipositor require good sex ratio to ensure colonization. Optimal sex ratio manipulate natural population of *C. carnea,* may be useful in increasing the rates of mating and oviposition (Venkatesan *et al.,* 2000).

TABLE I.- EFFECT OF'SEX RATIO (MALE: FEMALES) ON REPRODUCTION POTENTIAL OF *CHRYSOPERLA CARNEA.*

Sex ratio Male: Female	**No. of eggs/females**	**Hatching (%)**	**Pupation (%)**	**Emergence (%)**
1:1	251.75 ef	162.5 b	114.0 cd	79.25 c
1:2	330.0 d	241.8 b	206.0 b	160.5 b
1:3	728.0 a	640.5 a	497.8 a	412.0 a
1:4	478.8 b	220.8 b	149.5 bc	110.3 c
1:5	712.0 a	196.5 b	81.50 de	56.00 de
1:6	409.0 c	95.00 b	28.00 ef	15.50 ef
1:7	300.5 de	221.3 b	23.75 ef	5.500 f
1:8	430.8 bc	96.25 b	16.00 f	1.250 f
1:9	218.5 fg	55.50 b	6.750 f	0.00 f
1:10	155.8 g	33.75 b	3.500 f	0.00 f
LSD=0.05	65.63	238.4	65.03	40.86

Means followed by the same letters are not significantly different from each other (P>0.05) using LSD test.

The results on fecundity and fertility showed similar pattern as in case of pupation and emergence (Table I). The fecundity was the highest 728.0 in 1:3 ratios, while lowest in case of 155.8 and in the case of 1:10 combination. In all other treatments, the mean values for this parameter varied from 712 to 218.

The mean higher hatching 640.5 was observed in 1:3 (male: females) ratio, while the minimum 33.75 recorded in 1:10 ratio. Rest of the treatments showed intermediate (521 55.50) values for this parameter. For pupation of larvae maximum pupation 497.8 was noted in 1:3, on the other hand minimum 3.500 observed in 1:10. Similarly, in adult emergence maximum 412.0 adults were emerged in 1:3 and minimum 1.250 in 1:8, while nil in the case of 1:10 ratio.

In vice versa (Table II), the fecundity was highest 195.8 in 2:1 ratio, while the lowest 6.250 in the case of 8:1 combination. In other treatments the mean values for this varied from 195.80 to 10.25. The results on fertility showed less percentage as in the case of fecundity. The mean higher hatching 127.5 was observed in 2:1 ratio, while the minimum 1.00 in 8:1 ratio. Maximum pupation 105.0 was noted in 2:1, on the other hand minimum 0.50 in 6:1 ratio. Similarly, in adult emergence maximum 86.00 adults were emerged in the ratio of 2:1 and 0.250 minimum in the case of 5:1, and nil in remaining ratios, from 6:1 up to 10:1 ratios.

TABLE II.- EFFECT OF SEX RATIO (MALES: FEMALE) ON REPRODUCTION POTENTIAL OF *CHRYSOPERLA CARNEA*.

Sex ratio Male: Female	**No. of eggs/females**	**Hatching (%)**	**Pupation (%)**	**Emergence (%)**
1:1	251.75 a	162.5 a	114.0 a	79.25 a
2:1	195.8 b	127.5 b	105.0 a	86.00 a
3:1	107.8 c	77.25 c	55.25 b	32.75 b
4:1	82.50 c	29.25 d	11. 50 c	4.00 c
5:1	32.00 d	15.50 de	3.750 c	0.250 c
6:1	17.25 de	5.25 de	0.500 c	0.00 c
7:1	10.25 de	3.25 de	0.00 c	0.00 c
8:1	6.250 de	1. 00 de	0.00 c	0.00 d
9:1	0.00 e	0.00 e	0.00 e	0.00 e
10:1	0.00 e	0.00 e	0.00 e	0.00 e
L.S.D.=0.05	28.27	28.27	15.64	12.87

Means followed by the same letters are not significantly different from each other (P>0.05) using LSD test.

The current studies manifested that along with other factors, 1:2 (male and females) ratio should be maintained for economical and successful mass production of this predator *Chrysoperla carnea.*

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MANAGEMENT AND PRODUCTION PATTERN OF DHATTI BREED OF CAMEL IN DESERT AREA

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Abstract.- An investigation was undertaken by interviewing 22 camel farmers randomly selected from each taluka of the district Thar, in order to assess management and production patterns of Dhatti breed of camel in desert area of district Thar. Study revealed that literacy rate was extremely low, only 14.77% of the farmers were literate upto primary level while 85.23% were illiterate. Similarly 45.45% farmers were landless, 44.32% possessed less than 2 acres of land and 10.23% possessed 5 acres of land. The herd size possessed below 30, 30-40, 40-50 and above 50 camels by 44.30%, 32.95%, 17.04% and 7.57% farmers, respectively. The sex ratio 62.20% female and 37.80% male. The farmers preference of camel over other modes of transportation were; 61.37% farmers preferred camels and 18.18% preferred lorries 6.81% preferred tractors, 5.68% preferred jeeps and 7.95% farmers preferred buses. The average birth weight in male was 55.19 kgs and in female 51.63 kgs. The weaning age, age at puberty, age at first service, gestation period and calving interval averaged 281.25, 1151, 14223.75,393.25 and 724.5 days, respectively. The conception rate was 66.75% and time taken in parturition was 51.13 minutes. The daily milk averaged 7.03 liter and 1743 liters milk per lactation while the average lactation length was 340 days. The average dry period was 358.75 days. The mean age at first riding in male was 1272 days and in female 1073.25 days, age at first loading in male was 1277 days and in female 1081.25 days, speed without load was 7.89 km/hr speed with load was 6.88 km/hr, load carried 300.46 kg, depth of well averaged 157.5 feet, quantity of water drawn from well 2040 liters per day. Quantity of water supplied on back of camel 195.75 liters per trip and land ploughed 3.89 acres per day. The camel were shorn once a year during the months of March and April. Hair production averaged 1.05 kg in female and 1.25 kg in male per animal annually. The camel hair is used for making rope and Farasi (Kharar).the price per camel averaged Rs.21,375.

Key words: Dromedary, Camel breeding, camel marketing, draught camel, Thar desert.

INTRODUCTION

The dromedary is ideally suited to the desert conditions. The dromedary

are adopted very well to their environment, due to some of their physiological and anatomical characteristics that enable them to live and work in harsh surroundings. The camel is a hardy animal with unique physiological systems which allow to thrive under arid condition and to fill an important niche in desert Eco-system. The ability of the camel to live for long period with out feed and drinking water is one of the best known characteristics of this animal. The camel from times immemorial has been recognized as the "ship of the desert". The dromedary is primarily a browser and eat all types of salty bushes, shrubs and tree leaves of bitter taste and digest them.

The population of dromedary camel is 0.8 million heads in the Pakistan (Economic Survey 2003-04) which is scattered in the four provinces of the country. In the Thar district, the camel population is 103,0567 (livestock census 1996), which is scattered in the four talukas of district. The data on management practices and production patterns of camel is not available as little or no work has been conducted on this aspect in the Thar district. The present study was therefore conducted to assess the production patterns and management practices of Dhatti breed of camels in Thar district. The study involved to analyze management practice under various camel farming systems, to analyze the economics of camel farming under different management system. The study principally involved complete management and business analysis of camel farms to recommend certain measures that may help potential camel entrepreneur to commercialize their farming units to earn higher returns.

MATERIALS AND METHODS

A study was undertaken to assess the management practices and production patterns of the Dhatti breed of camel. To get information from the camel farmers in desert region of District Thar, a total of eighty eight farmers were selected from the four talukas of district Thar *viz:* Mithi, Diplo, Chhachhro, and Nangar Parkar. A comprehensive questionnaire was set up and pretested. The data on important aspects of camel were collected in the study which were exploratory and descriptive in nature. The data were edited, tabulated and analyzed using simple statistical tools such as the means and standard error.

RESULTS AND DISCUSSION

The present investigation includes the data on management practices and production patterns of Dhatti breed of camel in desert area of district Thar. The data, pertaining to general information, livestock inventory, draught power,

production systems, feeding practices, labour, meat and milk production, credit, extension services available were collected and analyzed. The results obtained are discussed as under:

Camel as a source of draught power

It was observed from the Table I that most of the farmers had agricultural lands. They cultivated the land with the help of camel, while tractors were seen occasionally in some places. Although the people use 4x4 pickup, jeep, lorries and buses for transportation, but camels were the major source of riding for short distances. During draught, more than 61.37±2.540% farmers preferred camel over lorries and 18.17±1.607% preferred lorries 6.81±1.137% farmers preferred tractors over the camel and lorries, 5.86±0.983% farmers preferred jeep over camel, lorries and tractors, 7.95±0.983% preferred buses over camel, lorries, tractors and jeeps.

TABLE I.- TRANSPORTATION PREFERENCES IN THE STUDY AREA AT DISTRICT THAR.

Taluka	Total No. of farmers	Number of farmers (%) using				
		Camel	**Lorries**	**Tractor**	**Jeep**	**Buses**
Mithi	22	12 (54.55)	4 (18.18)	2 (9.09)	2 (9.09)	2 (9.09)
Diplo	22	13 (59.10)	5 (22.72)	1 (4.54)	1 (4.54)	2 (9.09)
Nangarparkar	22	14 (63.64)	3 (13.63)	2 (9.09)	1 (4.55)	2 (9.09)
Chhachhro	22	15 (68.18)	4 (18.18)	1 (4.54)	1 (4.54)	1 (4.54)
Mean±SEM	22	13.50±0.55 (61.36±2.54	4±0.33 (18.17±1.6)	1.50±0.25 (6.81±1.13)	1.25±0.21 (5.68±0.98)	1.75±0.21 (7.95±0.98)

Birth weight and weaning age

The data on birth weight and weaning weight are summarized in Table II. The birth weight of Dhatti camel was higher in male (55.19±1.75 kg) as compared to the female (51.63±2.10 kg). The birth weight of male and female was 55.50± and 52.75; 48.44 and 45.05; 63.25 and 60.95, 53.52 and 47.78 kg at Mithi, Diplo, Nangarparkar and Chachro, respectively.

The data on weaning age indicated that male camel showed greater age at weaning *i.e.* 281.25±2.09 days as compared to 261.5±1.21 days female camel. A similar trend was observed in respect of weaning age of male compared with female at all the four talukas of district Thar. Thus, the weaning age in respect of

male to female was 292.5:268.5, 720:255, 277.5:262.5 and 285:268.5 days at Chachro, Mithi, Diplo and Nangarparkar talukas, respectively. The overall mean weaning age of male and female recorded were 281.252.09 and 261.5±1.21 days at all the four talukas of the district.

TABLE II.- MEAN VALUES OF BIRTH WEIGHT AND WEANING AGE OF MALE AND FEMALE DHATTI BREED OF CAMEL AT DISTRICT THAR.

Talukas	No. of farmers	Birth weight (kg)		Weaning age (days)	
		Male	Female	Male	Female
Mithi	22	55.50	52.75	270.0	255.0
Diplo	22	48.44	45.05	277.5	262.5
Nangarparkar	22	63.25	60.95	285.0	260.0
Chhachhro	22	53.52	47.78	292.5	268.5
Mean±SEM	22	55.19±1.75	51.63±2.10	281.75±2.09	261.25±1.21

Breeding practices

Table III shows data on age at puberty, age at first service, gestation period, and calving interval. The age at puberty averaged 1150, 1155, 1149 and 1158 days at Mithi, Diplo, Nangarparkar and Chachro talukas of district Thar, respectively. Mean value of age at puberty of female camels averaged 1153±0.58 days at district Thar. Memon (1999) reported age at maturity 1055 (days) of Kharai breed which is close to the results of present study.

TABLE III.- MEAN VALUES OF AGE AT PUBERTY, AGE AT FIRST SERVICE, GESTATION PERIOD AND CALVING INTERVALS OF DHATTI BREED OF CAMEL AT DISTRICT THAR.

Talukas	No. of farmers	Age at puberty (days)	Age at 1st service (days)	Gestation period (days)	Calving interval (days)
Mithi	22	1150	1460	395	715
Diplo	22	1155	1380	392	725
Nangarparkar	22	1149	1490	392	727
Chhachhro	22	1158	1385	394	730
Mean±SEM	22	1153±0.58	1423.75±13.26	393.25±0.32	724.25±1.40

The data revealed that age at first service in Dhatti breed of camel averaged 1460, 1380, 1490 and 1385 days at Mithi, Diplo, Nangarparkar and Chachro talukas of district Thar, respectively. The mean value of age at first service averaged 1423.75±13.26 days at all the four talukas of district Thar. Similar results published by Wilson (1984) reported that rut first appeared as early as at 3 years of age which is again close to the results of present study.

The gestation period averaged 395, 392, 392 and 394 days in Mithi, Diplo, Nangarparkar and Chachro talukas of district Thar, respectively. The overall gestation period averaged 393.25 days which are in agreement with results of Memon (1999). However, Rathore (1986) reported the gestation period in Bikaneri camels was 391.l±16.7 days, which is close to the results of present study.

Calving interval in Dhatti camel averaged 715, 725, 727 and 730 days in Mithi, Diplo, Nangarparkar and Chachro talukas of district Thar, respectively (Table III). The overall mean calving interval averaged 724.25± 1.40 days.

Aspects of camel breeding

Dhatti is the most preferred breed, with breeding period extending between November and March. The breeding life of male is 15-20 years, and that of female 10-15 years. The age at puberty is 3 years in female and 4 years in male. The oestrous cycle is for 21-25 days, with an average of 23 days. The breeding is by natural methods. The duration of mating is 15-30 minutes.

Milk production

Lactation yield, average daily yield and lactation length

It is revealed (Table IV) that the milk yield per lactation of Dhatti bred of camel recorded 1703, 1638, 1780 and 1745 litters in Mithi, Diplo, Nangarparkar and Chachro talukas, respectively. The daily milk yield recorded 7.15, 6.33, 7.30 and 7.35 litters respectively in; Mithi, Diplo, Nangarparkar and Chachro talukas of district Thar. The over all milk yield per lactation averaged 1743±32.48 litters and daily milk yield averaged 7.03±0.20 liters. However, Wilson (1984) reported lactation length and total lactation yields. The variation in results obtained from present study may have been due to environmental, nutritional and breed factors. The over all lactation length of Dhatti camel averaged 340±5.690 days (Table

IV). Among the talukas longer lactation period was observed at talukas Chachro (352 days) while lowest at taluka Mithi (335 days). Research conducted earlier by Memon (1999) reported that lactation length of Kharai breed was 330 days, where as the finding of Memon are near to results obtained from present study.

TABLE IV. MILK YIELD OF CAMELS (LITERS) AT VARIOUS LOCATIONS OF DISTRICT THAR.

Talukas	No. of farmers	Lactation yield		Av. daily yield		Lactation length	
		Range	Av.	Range	Av.	Range	Av.
Mithi	22	1615-1700	1703	6.50-7.80	7.15	315-350	333
Diplo	22	1590-1685	1638	5.70-6.95	6.33	310-340	325
Nangarparkar	22	1710-1850	1780	6.75-7.85	7.30	340-360	350
Chhachhro	22	1690-1800	1745	6.80-7.90	7.35	342-362	352
Mean±SEM	22	-	1743±32.48	-	7.03±0.20	-	340±5.69

Dry period

The average dry period in Dhatti breed of camel at all four talukas was 358.75±1.12 days. The dry period was 352.00, 357.50, 1651.00 and 360.00 days at Mithi, Diplo, Nangarparkar and Chachro talukas, respectively (Table V). Memon (1999) reported dry period of 351 days Kharai camel breeds which is in accordance with the results of present study.

TABLE V.- MEAN VALUES OF DRY PERIOD (DAYS) OF DHATTI BREED OF CAMEL AT DISTRICT THAR.

Talukas	No. of farmers	Average dry period
Mithi	22	352.5
Diplo	22	357.5
Nangar Parkar	22	365.5
Chhachhro	22	360.0
Mean±SEM	22	358.75±1.12

Age at first loading

The data regarding age at first loading are summarized in Table VI. These results disclosed that male camel took more time to reaches the age at first loading *i.e.* 1277.5±10.80 days, while female camel heifer attained the age at comparatively lesser number of days to reach, the age at first loading. This showed that female calf reaches this age earlier than male. The age at first loading recorded in order of male and female as 1210, 1080; 1330, 10990; 1290,1095; and 1280,1060 days at Mithi, Diplo, Nangarparkar and Chachro talukas, respectively. Earlier Afridi (1997) reported age at loading of Ghulamani, Marchi, Khadder, Camblepuri and maya breed was 1642.50m 1277.50, 12411.00, 1368.75 and 1204 days, respectively, which is higher than the results of present study due to breed and nutritional factors.

TABLE VI.- MEAN VALUES OF AGE AT FIRST LOADING (DAYS) AND RIDING (DAYS) OF MALE AND FEMALE OF DHATTI BREED OF CAMEL AT DISTRICT THAR.

Talukas	Age at first loading		Age at first riding	
	Male	**Female**	**Male**	**Female**
Mithi	1210	1080	1270	1060
Diplo	1330	1090	1290	1088
Nangarparkar	1290	1095	1310	1095
Chhachhro	1280	1060	1220	1050
Mean±SEM	1277.5±10.80	1081.25±3.35	1272.5±8.36	1073.25±4.68

Age at first riding

The data on age at first riding (Table VI) showed that female Dhatti camel reaches earlier age at first riding (1073 .25±4.68 days) than the male (1272.25±8.36 days). The further data shown in the table regarding the age at riding of male and female camels 1270 and 1060; 1290, and 1088; 1310 and 1095; and 1220, and 1050 days at Mithi, Diplo, Nangar \parkar and Chahcro, respectively. Afridi (1997) reported age at riding of Marchi breed of camel 1168.00 days, which is near to the results of present study.

Speed, quantity of load carried and distance covered with load

It is revealed from Table VII that the higher speed of Dhatti breed of camel

was 9.50 km/h at Nangarparkar followed by 8.20 km/h at Chachro, 6.55 km/h at Diplo and 7.34 km/h at Mithi talukas recorded. It was further observed that more load carried were 350.25 kg at taluka chachro and followed by 310.50 kg at Diplo, 290.75 kg at Mithi taluka and 250.37 kg at Nangarparkar. The pace of Dhatti breed of camel with load was higher 8; 45 km/h at Nangarparkar, followed by 7.05 km/h at Chachro, 5.55 km/h at Diplo and 6.50 km/h at Mithi talukas. The average speed with out load was 7.89±0.12 km/h, while average load carried by Dhatti breed of camel was 300.418.99 kg and speed with load was 6.88±0.21 Jan/h recorded at all four talukas of district Thar. The results are supported by the findings of Wilson (1976) and Wei (1980) which are in agreement with the results of present study.

TABLE VII.- MEAN VALUES OF SPEED, QUANTITY OF LOAD CARRIED AND DISTANCE COVERED WITH LOAD OF DHATTI BREED OF CAMEL AT DISTRICT THAR.

Talukas	Speed without load (km/hr)	Load carried (kg)	Speed with load (km/hour)
Mithi	7.34	290.75	6.50
Diplo	6.55	310.50	5.55
Nangarparkar	9.50	250.37	8.45
Chachro	8.20	350.25	7.05
Mean±SEM	7.89±0.12	300.46±8.99	6.88±0.21

Water drawing from the well, water carried on the back and land ploughing

The data (Table VIII) showed that the depth of wells, were 180, 120, 110, 220 feet and water drawn from these wells were, 2176, 1632, 1904 and 2448 litres per day and quantity of water carried on the back of camel was 204, 187.5 170, 221.5 litres per trip and land ploughed was 3.5, 4.25, 3.81, 4.01 acres per day at the Mithi, Diplo, Nangarparkar and Chachro talukas, respectively. It was further observed that over all average depth of well was 157.5±11.23 feet, quantity of water drawn per day 2040±84.81 litres, quantity of water carried on the back of camel was 195.75±4.78 litres per trip and land ploughed was 3.8910.06 acre per day. Wei (1980) reported that camel can plow an area of 0.2 h in about 9 hours. Wilson (1980) reported that in Ethiopia, a single camel was said to outperform a pair of oxen and could plow 1 ha in 20 hours.

TABLE VIII.- MEAN VALUE OF DEPTH OF WELL, QUANTITY OF WATER DRAWN / DAY, QUANTITY OF WATER SUPPLIED ON THE BACK OF CAMEL / TRIP AND LAND PLOUGHING / DAY AT FOUR LOCATIONS OF DISTRICT THAR.

Talukas	Depth of well (feet)	Quantity of water drawn/ day (litre)	Quantity of water supplied/ trip (litre)	Land ploughing/ day (acres)
Mithi	180	2176	204.0	3.52
Diplo	120	1632	187.5	4.25
Nangarparkar	110	1904	170.0	3.80
Chhachhro	220	2448	221.5	4.01
Mean±SEM	157.50±11.23	2040.00±84.81	195.75±4.78	3.89±0.06

Camel marketing

The camel farmers of district Thar mostly sale their camels at respective taluka head quarters as well as fairs of Saman Sarkar and Ghulam Shah at district Badin. In the absence of well developed market infrastructure and resources (*i.e.* high transport costs *etc.*), the herder prefer to dispose off their camels at the village level. The middlemen come from Laki Marwat and Dera Ghazi Khan to buy camel at a very low price which is disadvantageous. They earn good price of the money by selling of camels to the foreigners particularly people of Saudi Arabia and United Arab Emirates. The camels were also sold at the slaughter house market at Karachi where 5-8 animal are slaughtered daily as reported by incharge meat inspectors of Karachi Municipal Corporation.

Average price for draught camel

Table IX shows that the camel price averaged Rs. 21.375±1036.45 at four talukas of district Thar.

A family sells 8-10 camels every year to meet domestic expenditure and needs. Farmers normally do not sell the camels as they get milk and meat from their animal possession. Few camel farmers of district Thar who afford traveling a long distance to reach at the fair of Saman Sarkar and Ghulam Shah, sell their camels at the rate of Rs.20,000 and 23,500, respectively.

TABLE IX.- MEAN CAMEL PRICE (IN RUPEES) IN DIFFERENT MARKETS AND FAIRS IN DISTRICT THAR.

Name of Market/Fairs	Range (Rs.)	Average Camel Price (Rs.)
Markets		
Mithi	20,000-25,000	22,500
Diplo	22,000-25,000	23,500
Nangarparkar	20,000-23,000	21,500
Chhachhro	16,000-20,000	18,000
Mean±SEM	-	21,375±1036.445
Fairs		
Saman Sarkar	18,000 – 22,000	20,000
Ghulam Shah	22,000 – 25,000	23,500

CONCLUSIONS

On the basis of present study it may be concluded that majority of the farmers were illiterate. Extension services are provided to the camel farmers to improve the health of camel. Market facilities were not available. The Dhatti breed of camel serving in the most difficult areas as source of draught power and to some extent it serves to fulfil the need of milk and fiber. Similarly no credit facilities were available to the farmers. The products of camels by the farmers used for domestic purpose. To overcome domestic demand, the sale of camel is the only source in District Thar. It is suggested that market infrastructure be improved so that the farmers can get reasonable price from sale of their camels; and moreover credit facilities may be provided to the farmers so that they can improve camel trading.

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BUTTERFLY FISHES (FAMILY: CHAETODONTIDAE) OF PAKISTAN

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Abstract.- Butterfly fishes (Family Chaetodonidae) are among the least studied group of marine fishes of Pakistan. Fifteen species of these fishes belonging to 3 genera *i.e. Chaetodon, Heniochus* and *Parachaetodon* are reported from Pakistan. Of these, *Chaetodon jayakari* Norman, 1939, *Chaetodon collare* Bloch, 1787, *Chaetodon vagabundus* Linnaeus, 1758 and *Heniochus acuminatus* are found commonly, whereas other species are seldom collected from Pakistan. The paper also describes in detail the taxonomy and distribution of *Chaetodon jayakari* known to be endemic in the northern Indian Ocean.

Key words: *Chaetodon jayakari*, Butterfly fishes.

INTRODUCTION

A number of studies have been carried out on various important group of fishes occurring along the coast of Pakistan, however, family Chaetodontidae has not received the attention it deserves. In the checklists prepared by Jalil and Khalil (1971, 1980), Hoda (1985) and Hussain (2003) four species of this family *i.e. Chaetodon collare*, *C. lunula*, *Haniochus accuminatus* and *Parachaetodon ocellatus* were reported.

Commonly known as butterfly fishes, the members of family Chaetodontidae have bright coloration with a dark band across the eye and an 'eyespot' dorsally. These fishes are known to inhabit shallow coastal waters especially occurring in coral reefs and adjacent areas. In Pakistan, there are no coral reefs except some isolated patches of corals around islands, submerged rocks and on shipwrecks (Chaudahri, Personal communication), still a number of species of Chaetodontidae are known to exists in Pakistan. Recently a lot of interest has been developed to study corals and subtidal fauna in Pakistan. Present paper will help in understanding the faunal assemblages found around coral habitats as it describes the species of butterfly fishes found in Pakistan alongwith a key to the known species from the area.

MATERIALS AND METHODS

Specimens of butterfly-fishes were collected from shallow coastal waters using trawl net, as well as from the commercial landings at Karachi Fish Harbour. A few specimens were collected from the inter-tidal areas along Karachi coast. Photographs were taken before preservation of the samples in 5 % formalin. Detailed examination of the specimens were made in laboratory with some features studied under stereomicroscope. A review of the literature was made to list the species previously reported from Pakistan. A key of the known species was prepared mainly based on Pyle (2001).

RESULTS

Fifteen species of these fishes belonging to 3 genera *i.e. Parachaetodon Heniochus* and *Chaetodon,* are reported from Pakistan. Of these *Parachaetodon* is represented by one species, *Heniochus* is represented by two species whereas genus *Chaetodon* is represented by 12 species.

KEY TO THE SPECIES OF FAMILY CHAETODONTIDAE FOUND IN PAKISTAN

1. Lateral line incomplete, ending in vicinity of last rays of dorsal fin, dorsal fin rays not elongated 2

   Lateral line complete, ending at base of caudal fin, extremely elongated dorsal fin rays 4

2. Dorsal-fin spines VI ....*Parachaetodon ocellatus*

   Dorsal-fin spines X to XVI...3

3. Third to fifth dorsal-fin spines distinctly longer than others; body colour silvery to white with 3 broad dark vertical bars (including ocular bar), extending entire depth of body ....*Chaetodon jayakari*

   Third to fifth dorsal-fin spines not distinctly longer than others; body either lacking vertical bars, or have more than 3 vertical bars but bars not extending entire depth of body 5

4. Fourth dorsal spine prolonged filament as long as body; 2 black transverse bands, first from front of spinous dorsal to belly including ventral and second from last dorsal spines to last half of anal .... *Heniochus acuminatus*

   Fourth dorsal spine not prolonged in filament; body with three well defined transverse bands, firsts from spinous dorsal to chin, second from spinous dorsal medially to belly including ventral, third from bases of last dorsal spine to last half of soft anal .... *Heniochus monoceros*

5. Anal-fin spines IV (rarely V) ....*Chaetodon plebeius*

   Anal-fin spines III...6

6. Dorsal-fin spines XI .... *Chaetodon octofasciatus*

   Dorsal-fin spines XII or more...7

7 Dorsal-fin spines XIII, dorsal-fin rays 20 to 22 (usually 21); lateral-line scales 30 to 39; scale rows above lateral line to base of dorsal fin 4 to 6 .... *Chaetodon trifasciatus*

Dorsal-fin spines XII, dorsal-fin rays 23 to 28; lateral-line scales 45 to 55; scale rows above lateral line to base of dorsal fin 7 to 11...8

8. Thin white band on caudal peduncle ....*Chaetodon kleinii*

No thin white on caudal peduncle...9

9. Caudal peduncle black, or with black spots or markings 10

Caudal peduncle orange or yellow 11

10. Body ground colour mostly brownish yellow .... *Chaetodon lunula*

Body ground colour mostly white... *Chaetodon vagabundus*

11. Dorsal-fin spines XII ....*Chaetodon collare*

Dorsal-fin spines XIII or XIV (rarely XII) 12

12. Body white with 2 sets of diagonal black lines perpendicular to each other, ocular bar broad ventral to eye and faint or absent dorsal to eye .... *Chaetodon auriga*

Body yellow or pale yellow without diagonal lines; ocular bar narrow both dorsal and ventral to eye 13

13. Scales on entire body with black spot .... *Chaetodon nigropunctatus*

Body with distinct bars but lack black spots on all scales...14

14. Two well-defined black saddles on the back.. *Chaetodon falcula*

Body without distinct black saddle ....*Chaetodon xanthocephalus*

TAXONOMIC ENUMERATION

Chaetodon auriga Forsskal, 1775 (Threadfin butterfly fish) (Fig. 1A)

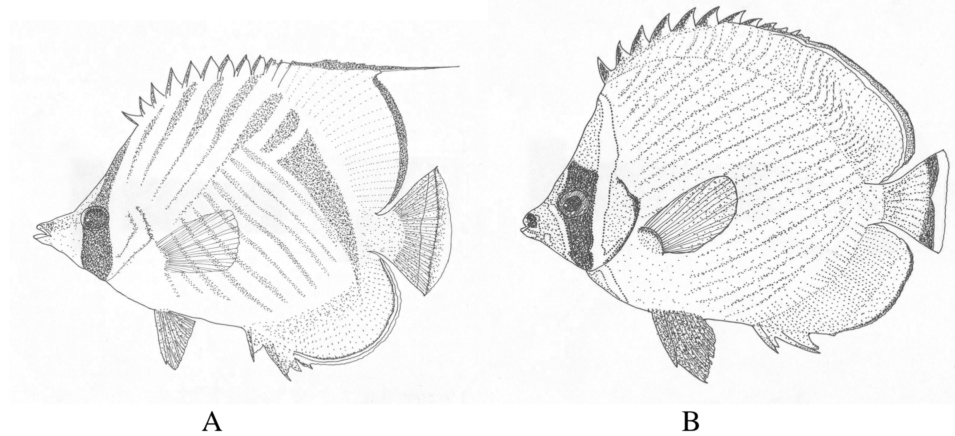


Fig. 1. A, *Chaetodon auriga; B, Chaetodon collare.*

This species is reported from waters of Sindh by Sorley (1932). This species was originally described from Jeddah, Saudi Arabia or Luhaiya, Yemen, Red Sea by Forsskal (1775). Its holotype is housed in Zoological Museum, University of Copenhagen, Denmark (Eschmeyer, 1998). Since the report of Sorely (1932), this species was not collected from Pakistan. This species is widely distributed in the Indo-Pacific areas (Burgess, 1978; Froese and Pauly, 2005) including Oman (Randall, 1995) and India (Kapoor *et al,* 2002).

Chaetodon collare Bloch, 1787 (Redtail butterfly fish)
(Fig. 1B)

This species was reported from Pakistan by Ahmed (1996), Ahmed and Wazart (1993), Edwards and Shepherd (1992), Froese and Pauly (2005), Hoda (1985, 1988) and Lieske and Myers (1994). It was also reported by Jalil and Khalil (1972, 1981) as *Chaetodon collaris* whereas Hussain (2003) reported this species as *Chaetodontops collaris*, This species was reported to occur commonly at Paradise Point, Buleji, Sonara, Pasha Bundar (Ahmed and Wazart, 1993). Originally this species was described from Japan by Bloch (1787). Its holotype is housed in Zoologisches Museum, Humboldt Universitat, Berlin (Eschmeyer, 1998). This species is widely distributed in the Indo-Pacific area including Persian Gulf and Maldives to Japan, the Philippines and Indonesia (Burgess, 1978; Froese and Pauly, 2005). Although this species can be collected from the shallow waters along rocky shore throughout the year, however, it seems to be abundant during monsoon months (June to September).

Chaetodon falcula Bloch, 1795 (Blackwedged butterfly fish)
(Fig. 2B)

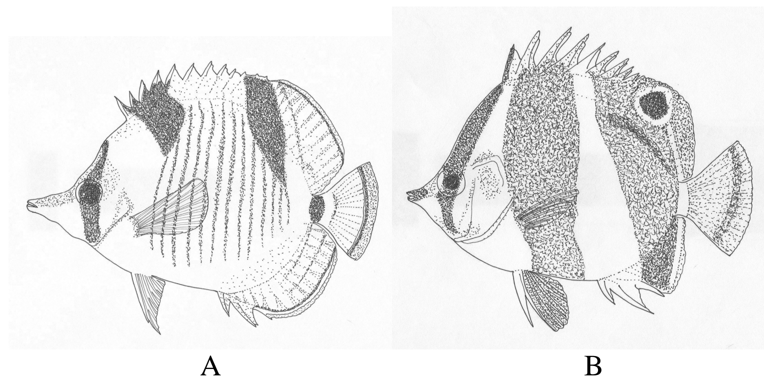


Fig. 2. A, *Chaetodon falcula;* B, *Chaetodon jayakari.*

This species was reported from Karachi by Murray (1880). It was originally described by Bloch (1795). No type locality or holotype is known, however, syntypes are housed in Zoologisches Museum, Humboldt Universitat, Berlin (Eschmeyer, 1998). This species is known from Indo-Pacific area East Africa south to 27°S and east to Indonesia. (Burgess, 1978; Froese and Pauly, 2005) including Persian Gulf and India. No subsequent record after Murray (1880) was made from Pakistan

Chaetodon jayakari Norman, 1939 (Indian golden-barred butterfly fish)
(Fig. 2B, Fig 3A-B)

This species was reported from Burgess (1978), and Moazzam *et al.* (1987) from Pakistan. This species was originally described from Muscat, Oman by Norman (1939). Its holotype is housed in British Museum of Natural History, London, U. K. (Eschmeyer, 1998). Usually this species is included in the synonymy of *Chaetodon modestes* Schlegel, 1842 (Allen, 1980). Klauswitz and Fricke (1985) have reviewed the taxonomy of this species and compared it with *C. modestes* and *Chaetodon excelsa* (Jordan, 1922) and concluded this it is valid species having characters mainly coloration which make it distinguishable from other species. They also described the ecology and distribution of the species. This species is considered to be a resident of northern Indian Ocean extending from Red Sea to west coast of India (Burgess, 1978; Klauswitz and Fricke, 1985; Randall, 1995).

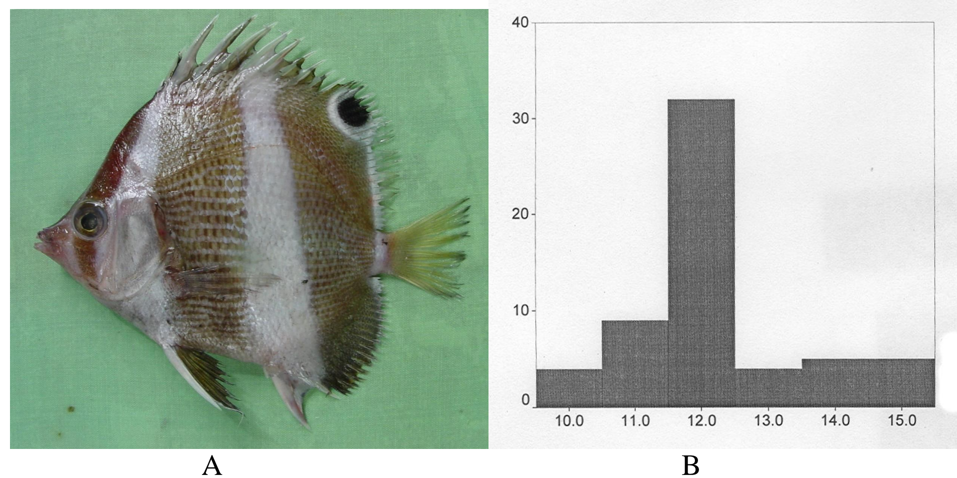


Fig. 3. A, *Chaetodon jayakari;* B, Length frequency distribution of *Chaetodon jayakari.*

Along the coast of Pakistan, this species is frequently collected from offshore waters. Although the species was collected from Balochistan coast also but its major concentration was found to be in the Sindh area. In the year 2004 a total of 59 specimens were collected from Sindh coast between a depth range from 44 to 50 m whereas only three specimens were collected from Balochistan coast. Almost all the specimens were collected during the day time. The size distribution of the collected specimens is presented in Fig. 4a which indicates that specimens of size range between 11-12 cm seems to be dominating. The smallest specimen has a size of 9.5 cm and largest has a size of 15.0 cm.

Chaetodon kleinii Bloch, 1790 (Sunburst butterfly fish)
(Fig. 4B)

This species was reported by Murray (1880) from Sindh. This species was originally described from East Indies by Bloch (1790). No holotype of this species is known, however, syntypes are housed in Zoologisches Museum, Humboldt Universitat, Berlin (Eschmeyer, 1998). This species is known from Red Sea and East Africa to the Hawaiian Islands and Samoa, north to southern Japan, south to New South Wales, Australia, New Caledonia, Micronesia and Galapagos Islands (Froese and Pauly, 2005). It is known from India by Kapoor *et al.* (2002). No subsequent record after Murray (1880) was made from Pakistan

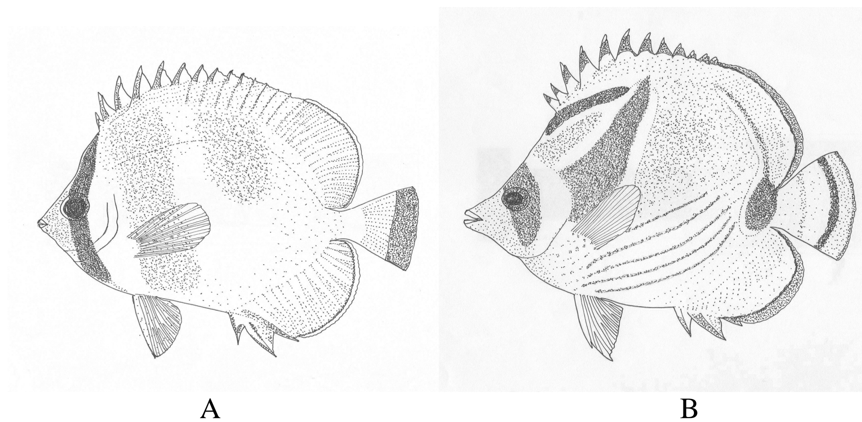


Fig. 4. A, *Chaetodon kleinii;* B, *Chaetodon lunula.*

Chaetodon lunula (Lacepede, 1802) (Raccoon butterfly fish)
(Fig. 4B)

This species was reported by Murray (1880) from Sindh. Originally this species was described as *Pomacentrus lunula* from Seas of Indes by Lacepede (1802). No holotype is known, however, syntypes are housed in Museum National d'Historie Naturelle, Paris, France (Eschmeyer, 1998). This species is known from South Africa, East Africa to the Hawaiian, Marquesan, and Ducie islands, north to southern Japan, south to Lord Howe, Rapa Islands and Micronesia (Froese and Pauly, 2005). It is known from Oman (Randall, 1995) and India (Kapoor *et al*, 2002). No subsequent record after Murray (1880) was made from Pakistan

Chaetodon nigropunctatus Sauvage, 1880 (Black-spotted butterfly fish)
(Fig. 5A)

This species was reported from Pakistan by Anonymous (1999) and Froese and Pauly (2002). It was originally described as *Chaetodon (Tetragonoptrus) nigropunctatus* from Oman by Sauvage (1880), however, no type is known (Eschmeyer, 1998). *Chaetodon obscurus* described by Boulenger (1888) from Muscat, Oman is considered to be a synonym of this species. No holotype of the latter is known, however, syntypes are housed in British Museum of Natural History, London, U. K. (Eschmeyer, 1998). This species is confined to Persian Gulf, Arabian Sea and northwestern Indian Ocean upto East Africa (Froese and Pauly, 2005).

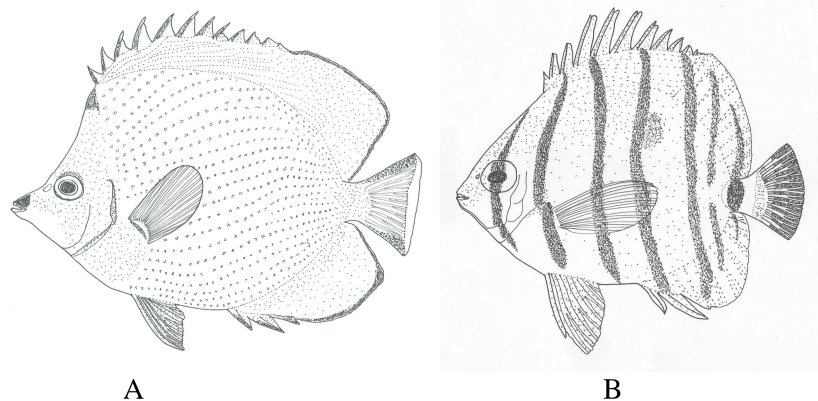


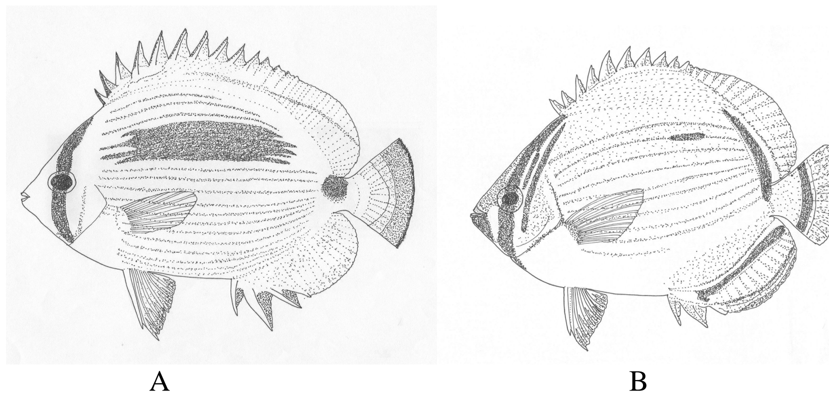
Fig. 5. A, *Chaetodon nigropunctatus;* B, *Chaetodon octofasciatus.*

Chaetodon octofasciatus Bloch, 1787 (Eightband butterfly fish)
(Fig. 5B)

This species was reported from Sindh by Murray (1880). It was originally described from Indian Ocean by Bloch (1787). No holotype is known, however, lectotype is housed in Zoologisches Museum, Humboldt Universitat, Berlin (Eschmeyer, 1998). This species is widely distributed in the Indo-Pacific area including East Indies and the Philippines, through Papua New Guinea and the Great Barrier Reef to the Solomon Island, Palau, and north to China; extends into the Indian Ocean at least to the Maldives, India and Sri Lanka (Froese and Pauly, 2005). No subsequent record after Murray (1880) was made from Pakistan

Chaetodon plebeius Cuvier, 1831 (Blueblotch butterfly fish)
(Fig. 6A)

This species was reported from Sindh waters by Sorley (1932), however, according to Allen (1984), this species is known only from Sri Lanka in the Western Indian Ocean but not from further north. It is also reported from Andaman Sea to Fiji, north to Japan, south to Australia (Steene, 1978). Originally this species was described from South Seas by Cuvier (1831). Holotype is housed in Museum National d'Historie Naturelle, Paris, France (Eschmeyer, 1998). No subsequent record after Sorely (1932) was made from Pakistan



A B

Fig. 6. A, *Chaetodon plebeius;* B, *Chaetodon trifasciatus.*

Chaetodon trifasciatus Park, 1797 (Melon butterfly fish)
(Fig. 6B)

This species is reported from Pakistan by Hoda (1985, 1988), Hussain

(2003) and Jalil and Khalil (1972, 1981) as *Rhadbophorous trifasciatus*. Originally this species was described from Sumatra, Indonesia by Park (1797). No holotype of this is known, however, syntype is housed in British Museum (Natural History) London, U. K. (Eschmeyer, 1998). This species is widely distributed in the Indo-Pacific area including from East Africa to the Hawaiian and Tuamoto islands (Froese and Pauly, 2005). The Pacific population has been recognized as a distinct subspecies (*Chaetodon trifasciatus lunulatus* Quoy & Gaimard, 1825) by Burgess (1978) while Froese and Pauly (2005) opined that *Chaetodon trifasciatus* occurs only in the Indian Ocean, while *Chaetodon lunulatus* occurs only in the Pacific.

Chaetodon vagabundus Linnaeus, 1758 (Vegabond butterfly fish)
(Fig. 7)

This species was reported by Misra (1962) and Sorley (1932) from Sindh. Originally it was described from Indies by Linnaeus (1758), however, no type is known (Eschmeyer, 1998). This species is widely distributed in the Indo Pacific area including Red Sea and East Africa to the Line and Tuamoto islands, north to southern Japan, south to the Lord Howe and the Austral islands (Froese and Pauly, 2005). It is known from Oman (Randall, 1995) and India (Kapoor *et al* , 2002). A few specimens of this species was collected from Karachi Fish Harbour in December, 2004 which according to the fishermen were caught from Sonmiani bay area.

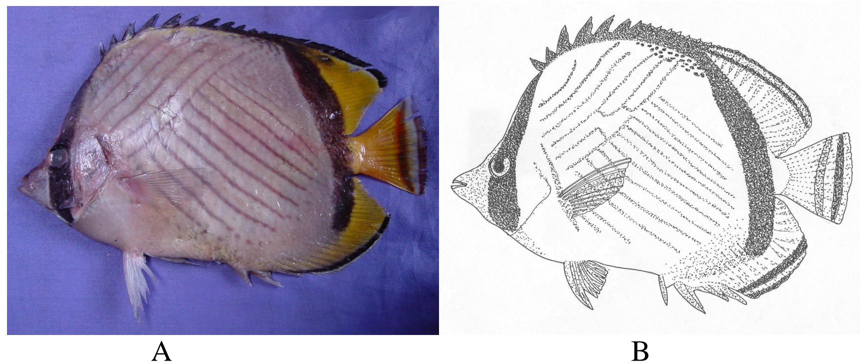
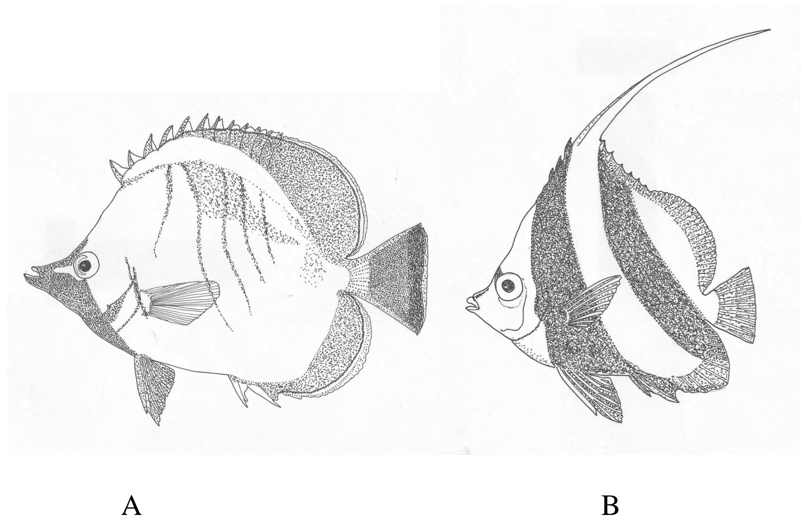


Fig. 7. *Chaetodon vagabundus*

Chaetodon xanthocephalus Bennett, 1833 (Yellowhead butterfly fish)
(Fig. 8A)

This species was reported from Sindh by Sorley (1932). It was originally described from Sri Lanka by Bennett (1833). Holotype of this species is housed in British Museum of Natural History, London, U. K. (Eschmeyer, 1998). This species is known from East Africa to Sri Lanka and the Maldives (Froese and Pauly, 2005). No subsequent record after Sorely (1932) was made from Pakistan



A B

Fig. 8. A, *Chaetodon xanthocephalus;* B, *Heniochus acuminatus.*

Heniochus acuminatus (Linnaeus, 1758) Pennant coralfish
(Fig. 8B)

This species was reported by a number of workers from Sindh including Anonymous (1955), Misra (1962) and Murray (1880). It was reported from Buleji by Ahmed and Wazarat (1993) and from Karachi and Makran coast by Anonymous (1955) and Misra (1962). Ahmed (1996), Froese and Pauly (2002), Hoda (1985, 1988), Hussain (2003), Jalil and Khalil (1972, 1981) and Steene (1978) have also reported this species from Pakistani water without specifying any particular locality or area. This species was originally described as *Chaetodon acuminatus* from Indies by Linnaeus (1758). *Chaetodon*

macrolepidotus described by Linnaeus (1758) from India is considered to be a synonym of this species. No type of latter is known (Eschmeyer, 1998). This species is widely distributed in the Indo-Pacific areas including East Africa and Persian Gulf to the Society Islands; north to southern Japan, south to Lord Howe Island and Micronesia. (Froese and Pauly, 2005; Steene, 1978). It is frequently caught by commercial fishing operations in shallow coastal waters along the coast of Pakistan especially in the areas which have rocky bottom.

Heniochus monoceros Cuvier, 1831 (Masked bannerfish)
(Fig. 9A)

This species was reported from Pakistan by Froese and Pauly (2002) and Steene (1978). It was originally described from Mauritius by Cuvier (1831). Holotype is housed in Museum National d'Historie Naturelle, Paris, France (Eschmeyer, 1998). This species is known from East Africa to the Tuamoto Islands, north to southern Japan, south to New South Wales and Tonga (Froese and Pauly, 2005). In the area it is known from Iran, Oman, Pakistan, Persian Gulf countries. This species is sometime caught by shrimp trawlers operating in shallow waters.

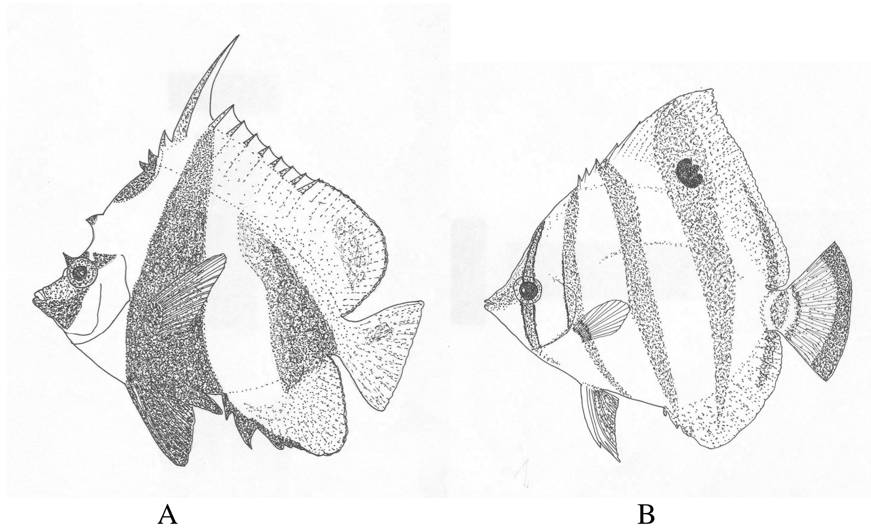


Fig. 9. A, *Heniochus monoceros;* B, *Parachaetodon ocellatus.*

Parachaetodon ocellatus (Cuvier, 1831) (Sixspine butterfly fish)
(Fig. 9B)

This species was reported from Pakistan by Hoda (1985, 1988), Hussain (2003), Jalil and Khalil (1972, 1981) and Murray (1880). This species was Originally described as *Platax ocellatus* by Cuvier (1831). No type locality is known, however, holotype is housed in British Museum of Natural History, London, U. K. (Eschmeyer, 1998). This species is known to be distributed widely in the Indo-Pacific area especially from Japan to Australia and Fiji. This species is also from southeast Asian countries including Sri Lanka, India, Malaysia, Indonesia, Singapore, Thailand, Vietnam and Cambodia (Froese and Pauly, 2005).

DISCUSSION

The study of the members of family Chaetodontidae reveals that a well diversified butterfly fish fauna inhabits coastal waters of Pakistan. This seems to interesting that majority of the members of this family are found along coral reefs (Nelson, 1994), whereas coral reefs are non existent in Pakistan. Four species *i.e. Chaetodon jayakari*, *C. collare, C. vagabundus* and *Heniochus acuminatus* seems to be the common species occurring in Pakistani waters whereas most other species are of rare occurrence. It may, however, be pointed out that adequate survey of the fish fauna along the rocky shore, submerged structure, wrecks and other sub-tidal habitats have not been properly done, therefore, there may be possibility of occurrence of a number of other butterfly fishes in Pakistan.

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TOXICITY AND UPTAKE OF CHROMIUM AND LEAD IN PROTOZOA – A REVIEW

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Abstract.- Pollution due to heavy metal toxicity is an ever-increasing problem in the developing nations. Heavy metals are major pollutants in marine, ground, industrial and even treated wastewater. These toxic metals interact with essential cellular components through covalent and ionic bonding. At high concentrations, both essential and non-essential metals can damage cell membrane, alter enzyme specificity, disrupt cellular function and damage the structure of DNA. The present review deals with uptake of chromium and lead by ciliate protozoa. Chromium is one of the major components of tanneries wastes. Several toxic effects are associated with exposure to chromium and lead compounds. Microorganisms have acquired a variety and array of mechanisms to remove or detoxify toxic metal ions. They remove toxic metal ions via adsorption to cell surfaces, complexation by exopolysaccharides, binding with bacterial cell envelopes, intracellular accumulation, biosynthesis of metallothioneins and other proteins and transformation to volatile compounds. Microorganisms have a high affinity for metals and can accumulate these toxic metals by a variety of mechanisms. These have been used to remove metals from polluted industrial and domestic effluents on a large scale. Among such microorganisms, Protozoa showed fairly high capability to uptake metals from the environment and their use is beneficial because bioremediation has advantages over other techniques as it is cheap, and non-destructive and contamination remains localized.

Key words: Chromium toxicity, chromium detoxification, lead toxicity, lead detoxification, bioremediation.

INTRODUCTION

Rapid growth of industries, exploding population and agricultural revolutions has affected greatly the man's physical environment. Besides drugs,

antibiotics and radioactive substances, industrial wastes contain heavy metals, which are mutagenic, carcinogenic and teratogenic. Many industries, discharge aqueous effluents containing relatively high levels of heavy metals, *e.g.,* uranium, cadmium, mercury, arsenic, barium, chromium, lead, nickel, zinc and copper (Blaudez *et al*., 2000; Seeber *et al.,* 2002; Akermoun *et al*., 2002). Untreated effluents from these manufacturing processes have an adverse impact on the environment (Macaskie and Dean, 1984; Nakajima and Sakaguchi, 1986; Norberg and Persson, 1984; Scott *et al*., 1986; Silver, 1992). A specific problem associated with heavy metals in the environment is accumulation in the food chain and persistence in the environment.

The presence of toxic heavy metals contaminants in aqueous streams, arising from the discharge of untreated metal containing effluents into water bodies, is one of the most important environmental issues. Heavy metals when present beyond traces are toxic to humans. Initially these may combine with proteins and may not cause any poisoning but when their concentrations exceed the threshold level, they become a real health concern (Jaffar, 1988).These toxic metals interact with essential cellular components through covalent and ionic bonding. At high levels, both essential and non-essential metals can damage cell membrane, alter enzyme specificity, disrupt cellular function and damage the structure of DNA (Bruins *et al.,* 2000; Blasiak *et al.,* 1999).

Uncontrolled discharge of heavy metal containing wastewaters to the environment can be detrimental to humans, animals and plants. Industrial wastes laden with heavy metal are posing serious problems in Pakistan where the environmental awareness is abysmally low. Waste recycling treatments and disposal of effluents is not according to world standards. In the province of Punjab, there are about 46,000 industrial units of various categories, out of which 4,600 units are considered to be the major contributors of pollution (Khalil *et al.,* 1991).

Conventional techniques for removing dissolved heavy metals include chemical precipitation, carbon adsorption, ion exchange, evaporation and membrane processes. These technologies are very costly and require either high energy or large quantities of chemicals, which means adding more chemicals to the environment. Therefore, there is a need for more practical cost effective and efficient method for environmental clean up and decontamination of the industrial wastewater of toxic contaminants including heavy metals. In recent years, bioremediation has emerged as a cost-effective and efficient alternative for the removal of heavy metals from wastewaters. Bioremediation is the use of

microorganisms for the treatment of contaminated toxic wastes. Microorganisms, including algae, bacteria, yeast, fungi, protozoa, plant leaves and root tissues can be used for detoxification and recovery of toxic or valuable metals from industrial discharges. Extensive work has been done in different laboratories on the isolation of metal resistant microorganisms (bacteria, yeast, algae and protozoa) from industrial wastes and then use them for decontamination of wastewater of different toxic metal ions (Piccinni and Albergoni, 1996; Salvado *et al*., 1997; Haq *et al*., 1997, 1997, 2000, 2001; Coppellotti, 1998; Nies, 1999; Dar and Shakoori, 1999; Fernandez-Leborans and Herrero, 1999; Shakoori *et al.*, 1999, 2000, 2001, 2002, 2004, 2005; Meagher, 2000; Rehman and Shakoori, 2001, 2003; Megharaj *et al*., 2003; Viti *et al*., 2003; Michel *et al.,* 2003; Kamaludeen *et al*., 2003; Kaszycki *et al*., 2004; Shakoori *et al*., 2004; Malik, 2004; Sannasi *et al*., 2006).

Many of the microorganisms show adaptation to the toxic materials constantly released in their environment and this adaptation is achieved through various types of genetic means. In some instances effective expression of the genes involved in biodegradation of toxic substances leads to better utilization of these substances by microorganisms (Orser *et al*., 1993; Suen and Spain, 1993). When considering features of metal ions resistance it is important to distinguish between metal resistance and metal tolerance. The former can be regarded as a genetically-encoded detoxification mechanism which is specifically induced in response to metal ions, whereas metal tolerance is a detoxification mechanism which is a by-product of normal metabolism, and is not specifically induced (Baldi, 1997).

Microorganisms have acquired a variety and array of mechanisms to remove or detoxify toxic metal ions (Silver and Phung, 2005). They remove toxic metal ions via adsorption to cell surfaces (Mullen *et al.,* 1989), complexation by exopolysaccharides (Scott and Palmer, 1988), binding with bacterial cell envelopes (Flatau *et al.,* 1987), intracellular accumulation (Laddaga and Silver, 1985), biosynthesis of metallothioneins and other proteins (Aiking *et al*., 1985) and transformation to volatile compounds (Robinson and Tuovinen, 1984).

In recent years, biosorption has emerged as a cost-effective and efficient alternative for the removal of heavy metals from low waste-waters. Adsorption which refers to the retention of solutes (metal ions) originally present in solution by the surfaces of solid material (biological cell walls/membranes) and absorption refers to the retention of the solute (metal ion) within the mass of the solid (microorganisms) rather than on its surfaces. The term sorption is used to

include both adsorption and absorption. So biosorption is the uptake of heavy metal ions and radionuclides from aqueous solution by biological materials. Microorganisms (because of negative surface charge and membrane compositions), including algae, bacteria, yeast, fungi, protozoa, plant leaves and root tissues can be used as biosorbents for detoxification and recovery of toxic or valuable metals from industrial discharges. Generally the sorption of heavy metals on the biosorbents could be described as a two-step process where the metal was initially uptaken oto the surface of the cell followed by the bioaccumulation inside the cell due to the metal uptake metabolism. Different species often had different sorption characteristics, and external factors such as pH, metal ion concentration, temperature, other metal ions, etc., were always found to influence the sorption (Kojima and Lee, 2001).

Frequent presence of protozoa in the environment containing toxic metal ions or other compounds elucidates their importance in processing of wastes; and they undoubtedly play an important role in municipal waste treatment plants (Madoni *et al*., 1994, 1996; Piccinni and Albergoni, 1996; Salvado *et al*., 1997; Coppellotti, 1998; Fernandez-Leborans *et al*., 1998, 199; Bonnet *et al*., 1999; Haq *et al*., 1998, 2000; Shakoori *et al*., 2004; Rehman *et al*., 2005, 2006). The tolerance of toxic metals by protozoa suggests the possibility of their exploitation in bioremediation.

Chromium

Chromium is a transition metal located in group VI-B of the periodic table. Cr is the seventh most abundant element on earth and 21st in the crustal rocks (McGrath and Smith, 1990). Cr abundance in Earth's crust ranges from 100 to 300 µg/g. Soils may contain between 5 and 3000 g of chromium per gram (Shewry and Peterson, 1976). The world production of Cr is in the order of 10^7 tons per year; 60-70% is used in alloys, including stainless steel, and 15% is used in chemical industrial processes, mainly leather tanning, wood processing, textiles and ceramics, pigments and electroplating (McGrath and Smith, 1990; Papp, 1985; Stern, 1982). Its widespread use has converted Cr in a serious pollutant of air, soil and water (Khasim *et al*., 1989; Armienta-Hernandez and Rodriguez-Castillo, 1995). Cr concentrations in non-polluted waters vary from 0.1 to 0.5 ppm in fresh waters and from 0.0016 to 0.05 ppm in oceanic waters (De Filippis and Pallaghy, 1994), but levels as high as 80 ppm have been observed in paper mill effluents (Sudhakar *et al.,* 1991). It is a common industrial pollutant and is discharged to the environment as industrial waste (Basu *et al*., 1997).

Chromium exists in several oxidation states, the most stable and common forms are the trivalent Cr^{3+} and the hexavalent Cr^{6+} species, which differ markedly in a number of their biological properties (Levis and Bianchi, 1982). Cr^{6+}, considered the most toxic form of Cr, is usually associated with oxygen as chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) ions (McGrath and Smith, 1990). In contrast, Cr^{3+} in the form of oxides, hydroxides or sulfates, is much less mobile and exists mostly bound to organic matter in soil and aquatic environments. Cr^{6+} is a strong oxidizing agent and in the presence of organic matter is reduced to Cr^{3+}; this transformation is faster in acid environments such as acidic soils (McGrath and Smith, 1990). However, high level of Cr^{6+} may overcome the reducing capacity of the environment and thus persist as a pollutant. In addition, Cr^{3+} may be also oxidized to Cr^{6+} in the presence of an excess of oxygen, being transformed again to the more toxic form (Kotas and Stasicka, 2000; Vajpayee *et al*., 1999).

Chromium toxicity

The environmental chemistry of chromium has been widely studied (Kotas and Stasicka, 2000; Richard and Bourg, 1991). The knowledge of the oxidation state, total Cr concentration and the reactions occurring in different environmental compartments (water, soil, plants) determines the evaluation of the actual risk of chromium contamination. The toxicity, mobility and bioavailability of Cr depend fundamentally on its chemical form. Chromium in the environment might be present mainly as Cr^{3+} and Cr^{6+}. Cr^{6+} is highly soluble and about 300 times more toxic than Cr^{3+}. On the other hand, Cr^{3+} precipitates at the average pH of natural waters. Tannery wastewaters contain mainly Cr^{3+} (Rutland, 1991). The nature and behaviour of Cr in wastewater depends on the physicochemical conditions of the effluents originating from various industrial sources (Kotas and Stasicka, 2000).

The biological effects of Cr depend on its oxidation state. Cr^{6+} is highly toxic to most organisms because it is capable of reacting with redox-active enzymes and small molecules to produce Cr^{5+}, Cr^{4+} and Cr^{3+}, as well as oxygen- and sulphur-centered radicals. All these species can damage DNA. Hence, the ability of Cr^{6+} to damage DNA depends on cellular redox systems. One such system depends on glutathione and produces Cr^{5+} and sulphur-centered glutathione-ethyl radical which may attack DNA.

Madoni *et al*. (1994) found that chromium (VI) was less toxic to the eight

ciliate species than lead. The LC_{50} values were similar and ranged from 2,177 to 3,293 µg/l for six species. The other two species had an opposite response to chromium. *Drepanomonas revolute* showed a very high sensitivity to this metal with a LC_{50} of 45.6 µg/l, while *Euplotes patella* showed a relatively low sensitivity to chromium, with a LC_{50} value of 9,472µg/l. The 24-h LC_{50} value of chromium was 8.86 mg/l for *Spirostomum teres* (Twagilimana *et al.,* 1998).

A 55% mortality in the whole protozoan community and the reduction of species richness to 11 species out of 16 was observed in the presence of 150 mg/l of soluble Cr (VI). A concentration of 293 mg/l Cr was necessary to cause a 90% reduction in the number of organisms and to reduce richness to 8 species (Madoni *et al*., 1996).The movements of *Vorticella microstoma* slowed down in the presence of $K_2Cr_2O_7$. The order of resistance on the basis of motility was Pb^{2+}>, $Zn2^{+}$>, Cr^{6+}>, Cd^{2+}>, Cu^{2+}. The number of ciliate in the presence of $K_2Cr_2O_7$ was reduced from 624 to 360 cells/ml (Shakoori *et al*., 2004).

Chromium uptake and detoxication

Madoni *et al.* (1996) found that 26.2 mg/l of Cr (VI) caused the disappearance of only 2 out of 16 species and lowered the protozoan density of the 8%, whilst 68.8 mg/l of Cr (VI) produced the disappearance of 4 out of 16 species and raised the protozoa mortality to 42%. The very low toxicity of chromium could be ascribed to the change in the valence state of Cr from the hexavalent to the less toxic and soluble trivalent form. Some authors (Moore *et al.,* 1961; Imai and Gloyna, 1990) report that this process can occur during the activated sludge treatment. This highlights the capability of the activated sludge microbiota (bacteria and protozoa) to survive and operate also when atypical concentrations of heavy metals enter the plant.

The ciliate, *Vorticella microstoma*, was found to be resistant to Cr^{6+} at a concentration of 260 µg/ml. The ciliate showed remarkable potential to remove metal ions from the culture medium. The concentration of Cr^{6+} was reduced 48% after 192 hour in a culture medium containing Cr^{6+} (100 µg/ml). Frequent occurrence of ciliates in wastewater or industrial effluents indicates that they are able to withstand the heavy metal contaminated environment. This property makes protozoa excellent candidate for exploitation in metal detoxification and bioremediation (Shakoori *et al*., 2004; Haq *et al*., 2000, 1998).

Mortuza *et al*. (2005) reported that *Paramecium bursaria* accumulated

average amount of chromium ranged from 1.72 to 15.5 pg Cr/cell in a time and concentration-dependent manner. The potential of the symbiotic *Paramecium bursaria* for the accumulation of chromium (VI) indicates its utility as a bioaccumulator and biomonitor of chromium contamination in freshwater environments.

Tachysoma could efficiently process Cr^{6+} from the medium. The ciliate culture grown in the medium containing Cr^{6+} (10 µg/ml) could reduce 77% from the medium after 48 hours, 85% after 72 hours and 92% after 96 hours, respectively (Rehman *et al*., 2006). *Stylonychia mytilus* was able to reduce 52% (700 cells/mL) chromium from the medium after 48 hours, 76% (1250 cells/mL) after 72 hours and 80% (1492 cells/mL) after 96 hours, respectively (Unpublished data).

Lead

Lead is a heavy metal with an atomic mass of 207 and belongs to the element group IVa, C, Si, Ge, Sn, Pb. Lead has been used in large amounts for 2500 years (Hong *et al.,* 1994), recently as a fuel additive, although the toxicity of lead for animals and man has been well known for a long time (Johnson, 1998). In sea water, lead is even rarer than mercury (Weast, 1984). Lead is biologically not available at high concentration due to its low solubility. Thus, lead is not extraordinarily toxic for microorganisms.

Lead is extensively used in paint industry, pesticide industry, glass and ceramic industry, formation of solder, cable covering, ammunition and storage batteries, galvanized iron pipes production and various mechanical industries (Abdul-Wahab, 2004), but lead is not used in any way in human metabolism, so there is no tolerable amount. It is considered as non-essential metal with no biological role in microorganisms, animals and plants (Bruins *et al.,* 2000).

Lead toxicity

Concentration of lead in atmosphere is of serious environmental concern (Zelikoff *et al*., 1988). The high input of the contaminants especially lead is by automobiles exhausts, stoker and fluidized bed incinerators, mining and smelting (Moore, 2004; Jung *et al*., 2004; Poikolainen *et al*., 2004). Lead contamination in surface water mainly comes from anthropogenic sources (96%), particularly from

combustion of leaded fuels, pyrometallurgical non-ferrous metal production and coal combustion. Lead in natural waters may be in the form of organic lead complexes originally from the fuel of ever growing automobile population and subsequent break down of tetraethyl lead (Andrews and Sutherland, 2004; Monterroso *et al.,* 2003; Matthai *et al*., 2002; Ashraf *et al*., 2002; Nriagu, 1989; Urban *et al*., 1987).

Madoni *et al*. (1994) found that lead was generally more toxic to ciliate populations than chromium. Little differences were appeared among the sensitivities of the eight ciliate species to lead. The LC_{50} for this metal ranged from 875 µg/l (*Drepanomonas revoluta*) to 2,323µgPb/l (*Euplotes affinis*). Parker (1979) reported a LC_{50} value of 45,000 µgPb/l to the marine ciliate *Uronema marinum*. A concentration of 6.98 mg/l of Pb killed 65% of the individuals, but caused the disappearance of only one species, *Chilodonella uncinata* (Madoni *et al.,* 1996). The 24-h LC_{50} value of lead was 10.78 mg/l for *Spirostomum teres* (Twagilimana *et al.,* 1998).

Bernal and Ruvalcaba (1996) reported that the duration of backward swimming behavior (BSB) of *Paramecium calkinsi* was partially reduced when cells were exposed to 100 µM of Ni^{2+} Cd^{2+} and Co^{2+}. In contrast, Pb^{2+} increased *Paramecium calkinsi* BSB in a dose dependent manner. Thus, 1, 10, 20, 50, and 100 µM of Pb^{2+} increased the duration of BSB by 20.4, 83.9, 143.2, 163.2 and 185.2%, respectively.

The cell density of protozoan culture was significantly decreased in the presence of lead. The profound effect was decreased cell number and arrested cell division as compared with the cells grown in Bold-basal medium without metal. This happened because of higher concentration of metals that probably poisoned essential biochemical reactions (Perego and Howell, 1997). Shakoori *et al*. (2004) found that in the presence of $Pb(NO_3)_2$ the *V. microstoma* cell number was reduced from 1540 to 935 cells/ml. The reduction in the cell population of *V. microstoma* was 43%. In the presence of Pb^{2+} (60 µg/ml) the number of *Stylonychia mytilus* cells decreased from 1.38×10^3 ±3.30 to 1.02×10^3 ±2.36 cells/ml. The reduction in the cell population was 26% (Rehman *et al.*, 2005). Janssen *et al*. (1995) also reported that the population of the ciliate *Colpoda cucculus* was decreased when the ciliate cells were exposed to metal salts.

Madoni *et al*. (2006) reported that out of four species of freshwater ciliates

(*Colpidium colpoda, Dexiotricha granulosa, Euplotes aediculatus,* and *Halteria grandinella*), tested against different heavy metals, *Halteria grandinella* showed the highest sensitivity for cadmium (0.07 mg l(-1), LC_{50}) and lead (0.12 mg l(-1), LC_{50}).

Lead uptake and detoxication

The metals assimilated by protozoa can be deposited in different organelles (Silverberg, 1975; Fujita *et al.*, 1977; Sicko-Goad, 1982). In the presence of 500 µg Pb-Cd/l, the level of accumulation in protozoa reached 147.45 µg Pb/g dw and 310.75 µg Cd/g dw more than in bacteria. In the presence of 1000 µg Pb-Cd/l the accumulation in protozoa reached 161.45 µg Pb/g dw and 140.84 µg Cd/g dw more than in bacteria (Fernandez-Leborans and Herrero, 2000).

Madoni *et al*. (1996) found that *Aspidisca cicada* showed a 70% survival in the presence of 6.98 mg/l of Pb. Shakoori *et al*. (2004) reported a very high level of metal resistance in *Vorticella microstoma*. The ciliate was found to tolerate Pb^{2+} at a concentration of 550 µg/ml and this concentration did not make any significant effect on the movement of ciliate. The order of resistance on the basis of motility was $Pb^{2+} > Zn^{2+} > Cr^{6+} > Cd^{2+} > Cu^{2+}$. Rehman *et al*. (2005) found that *Stylonychia mytilus* grown in medium containing lead (10.0 µg/ml) could reduce 80% of lead from the medium after 48 hours, 82% after 72 hours and 86% after 96 hours, respectively. The bioaccumulation of lead in protozoa has also been studied by Nilsson (1979), who described the intracellular distribution of this metal in *Tetrahymena.* Patton *et al*. (2004) reported that Pb uptake by *Tetrahymena thermophila* was increased when the ciliate preyed on Pb-exposed cells of *Pseudomonas putida*.

Rehman *et al*. (2006) reported that *Tachysoma* could efficiently remove Pb^{2+} from the medium. The ciliate culture grown in the medium containing Pb^{2+} (10 µg/ml) could decrease 68% Pb^{2+} after 48 hours, 80% after 72 hours, and 88% after 96 hours, respectively. *Stylonychia mytilus* could eliminate Pb^{2+} from the medium. The protozoan culture grown in medium containing lead (10.0µg/mL) could reduce 80% (867 cells/mL) of lead from the medium after 48 hours, 84% (1142 cells/mL) after 72 hours and 88% (1458 cells/mL) after 96 hours, respectively (Unpublished data).

Microbes display diverse interactions with Cr and Pb in the environment.

The mechanism of tolerance is based on the efflux of chromate and lead ions. Multiple metal resistant protozoa have been reported in wastewaters and metal polluted environment (Madoni *et al*., 1996; Haq *et al.,* 2000; Shakoori *et al.,* 2004; Rehman *et al*., 2005). *Halteria grandinella* and *Euplotes aediculatus* are excellent and convenient bioindicator for evaluating the toxicity of waters wastewaters polluted by heavy metals (Madoni and Romeo, 2006). Isolation of heavy metal resistant protozoa from polluted industrial wastes with the hope that protozoans may provide a tool for bioremediation of toxic metal wastes.

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INSECTICIDE TOLERANT BACTERIA AS BIOREMEDIATORS OF ENDOSULFAN AND HEPTACHLOR CONTAMINATED SOIL – A REVIEW

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Abstract.- The environmental fate of insecticide depends upon many extrinsic and intrinsic factors which determine the rate and extent of their transformation and mineralization. Soil micro-organisms may be almost entirely responsible for their disappearance from the environment. The factors involved in insecticide disappearance are biotransformation, photochemical mechanisms, physical mechanisms, chemical mechanisms, microbial degradation and bioremediation. Microbial degradation includes dehalogenation, oxidation, reduction, hydrolysis, dehydrochlorination, ring cleavage, condensation or conjugate formation and isomerization. Pesticidal degradative genes in microbes have been found to be located on plasmids, transposons, and/or on chromosomes. The studies have provided clues to the evolution of degradative pathways and the organization of catabolic genes, thus making it much easier to develop genetically engineered microbes for the purpose of decontamination. Endosulfan and Heptachlor, are the two commonly used insecticides in Pakistan, which are source of insecticidal contamination of soil and water bodies. Soil bacteria and fungi degrade endosulfan to endosulfate and endosulfandiol. Other metabolic products indentified were endoether, endohydroxyether, chlorendic acid, and endolacetate. Heptachlor epoxide, chlordane, and 1-hydroxy-2,3-epoxychlordene are the products of microbial degradation of heptachlor. Since the later two metabolites not degraded further by mixed cultures of microorganisms, this could be the reason for the occurrence of high levels of 1-hydroxychlordene and low levels of heptachlor epoxide in heptachlor-treated soils. Microbial dechlorination of heptachlor produces chlordene which undergoes microbial epoxidation to form the corresponding chlordane epoxide. Knowledge of the regulation of pollutant degrading pathways may facilitate metabolic adaptation processes, hence the use of microorganisms in environmental cleanup efforts. The microorganisms capable of degrading endosulfan and heptachlor in the soil and waste water could be used as bioremediator of contaminated environment.

Key words: Microbial degradation, insecticide resistant bacteria, bioremediation, metabolism of Endosulfan, metabolism of Heptachlor.

INTRODUCTION

Pakistan being an agriculture country has been a dumping ground for all sorts of insecticides, which are aimed at killing insects at all costs in a bid to increase agriculture production. The use of pesticides in Pakistan has increased fourteen times during the last twenty years (Fig. 1).

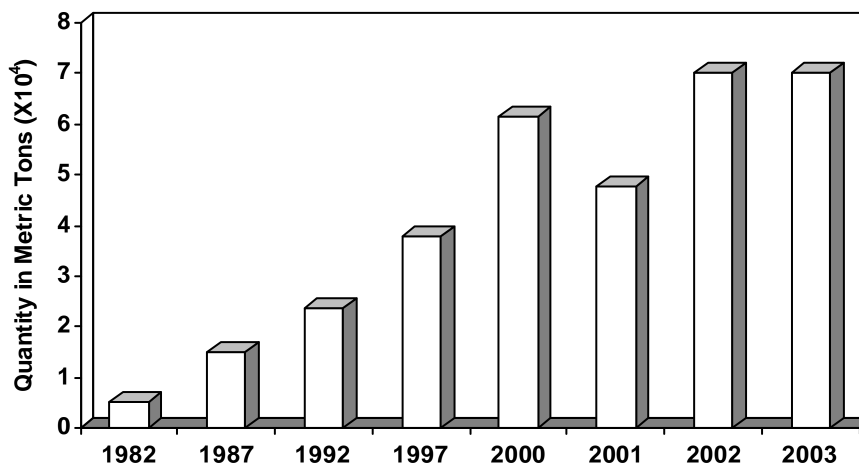

Fig. 1. Consumption of pesticides in Pakistan.

The total pesticide consumption in 1982 was five thousand metric tons, which reached upto 70,000 metric tons in 2002. Even those insecticides which are banned in the developing countries are still in use in Pakistan. The indiscriminate and unplanned use of agrochemicals have caused serious environmental problems (Kullman and Matsumura, 1996; El-Bestway *et al*., 2000). The residues of these agrochemicals directly or indirectly gain entry into the food chain and prove hazardous to animal and plant life (Hardy, 1987; Parsek *et al*., 1995). The residual effects include carcinogenicity, mutagenicity, reproductive toxicity, respiratory and circulatory problems (Sinha *et al*., 1995, 1997).

A serious concern of the agricultural community is the increase of pesticides residues (Somich *et al*., 1990), because the application of these xenobiotics in soils can cause damage to the ecosystem. They may also adversely influence microbial processes that are an essential part of the carbon, nitrogen and sulfur cycles. Many pesticides can be chemically and microbiologically transformed in

soil (Pramer and Schmidt, 1959; Tancho *et al*., 1992). Nevertheless, some pesticides are resistant to microbial attack (Somich *et al*., 1990) and many of them are affected by adsorption-desorption processes in the soil surface (Bosetto *et al*., 1992; Sposito, 1989).

Leaching of pesticide through the soil to the ground water is also a concern since these chemicals may affect the quality of drinking water supplies and surface ecosystem. Dicamba, a post emergent herbicide commonly used as insecticide has been detected in ground water (Koterba *et al*., 1993; Cox, 1994). It is therefore, imperative that the environment be decontaminated and reclaimed for human, animal and plant life.

Insecticides

The insecticides are classified into four groups on the basis of their chemical nature *i.e.* (i) organochlorines; (ii) organophosphates (iii) carbamates and (iv) pyrethroids. Organochlorine (OC) compounds have attracted attention since the discovery of DDT as an insecticide (Neilson, 1996). These are highly persistent compounds due to their lipophilicity. They contaminate the whole ecosystem and reach animal and human body directly from environment and indirectly through food chain (Fisher and Pecher, 1993; Hellou *et al*., 1993; Tripathi and Allen, 1997; Siddique *et al*., 2003).

The OC insecticides comprise dichlorodiphenylethanes, cyclodienes, hexachlorocyclohexane, *etc*. Chlorinated cyclodienes such as aldrin, dieldrin, heptachlor, chlordane and endosulfan are widely used, because of their higher toxicity and easy absorption. The use of most of these insecticide has been banned in the developed world because of their carcinogenicity. Only endosulfan remains registered for use on food in the United States of America (USA, EPA, 1996). The main target of OC is the central nervous system. Intoxication results in hyperactivity, tremors, convulsions, neuronal excitability, human parkinsonism and antagonism of the inhibitory action of GABA (Joy, 1982; Kirby and Bloomquist, 1996; Bloomquist, 1993, 1998).

Organophosphate (OP) is the second major group of insecticides. Some of the common OPs are malathian, paraoxon, primiphos methyl, methamidophos, monocrotophos, azinophos methyl and chlorpyrifos methyl etc. Cholinesterase inhibition is the most common mechanism by which OP insecticide kill insets. It is also one of the most important aspects of OP poisoning in humans.

Pyrethrum is mixture of alkaloids from the chrysanthemum plant, which has significant insecticidal properties, but is essentiality non toxic to mammals. Because of a limited supply of natural pyrethrum and rapid environmental degradation, a number of synthetic pyrethroids have been developed which enjoy widespread use today. Permethrin, cypermethrin, deltamethrin, and fenvalerate are some of the more widely used synthetic pyrethroids. The synthetic pyrethroids catagorized as type I, compounds without the ά-cyano moiety, and type II, compounds with the ά-cyano substitution. Permethrin is an example of type I pyrethroid, whereas cypermethrin, deltamethrin, and fenvalerate are examples of type II pyrethroids. The mechanisms of toxicity of the type I pyrethroids appears to be similar to that of DDT and involves interference with the axonal sodium gate resulting in a delayed repolarization of the nerve membrane. This causes a repetitive discharge of the nerve (Gamman *et al*., 1981; Viverberg *et al*., 1982).

Aldicarb, carbaryl and dialdicarb are some of the important representatives of the carbamate group of insecticides. The carbamates have a wide range of toxic effects. Almost all of the symptomology that characterizes the acute toxicities of OP compounds is the same for carbamates. The most prominent difference between the two is the duration of action.

Pesticide cycling in the environment

Pesticides can enter the atmosphere by a variety of routes, particularly from spray drift or volatization from soil or water. The pesticide movement as spray drift was much more widespread and that contamination of a whole air shed could result when large scale treatments are made. Only a portion of pesticides sprayed onto crops reach their target, the rest falls on the ground is taken up into the atmosphere by air currents or turbulence. The volatilization is obviously a major pathway for loss of applied pesticides from plant, water and soil surfaces. Other routes of entry include wind erosion and agricultural burning (Fig. 2).

Rivers, streams, lakes, ponds, oceans and bottom mud are major reservoirs for residues of persistent pesticides. The routes by which pesticides can reach the aquatic environment are (a) surface runoff and sediments transport, from treated soil (b) industrial wastes discharged into factory effluent (c) direct application as aerial sprays or granules to control water inhabiting pests (d) spray drift from normal agriculture operations (e) atmospheric transport (f) municipal waste discharge into sewage effluent (g) agriculture wastes and (h) accidents and spills. Runoff is generally considered to be the major movement into the water

environment. The pesticide may be adsorbed on eroding soil particles suspended in the runoff water.

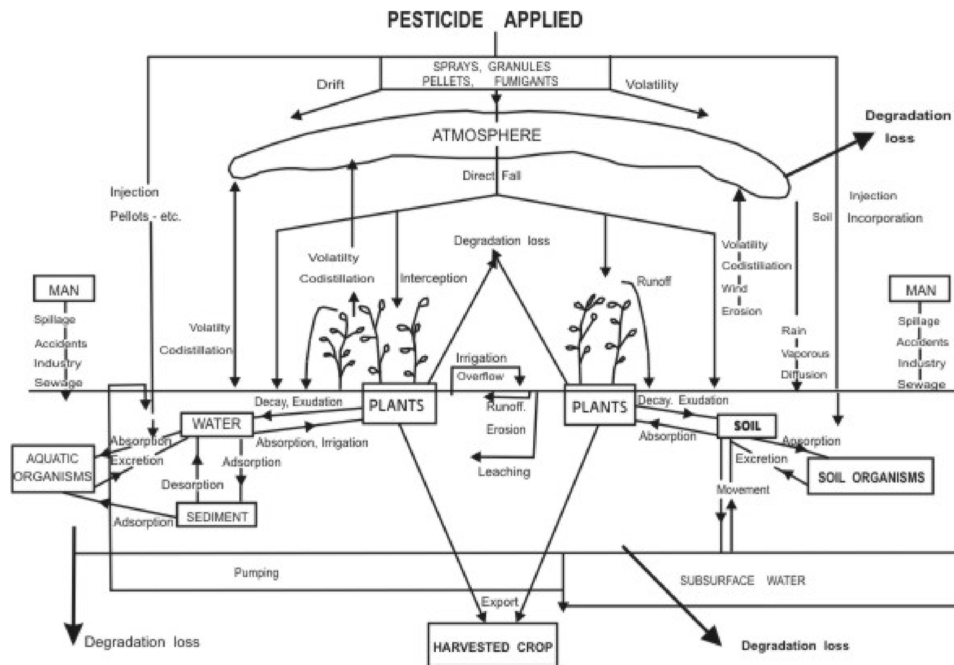

Fig. 2. Pesticide cycling in the environment.

Chlorinated hydrocarbon pesticides, because of their low water solubility are probably transported with soil particles in the adsorbed state rather than in solution. Heavy rainfall immediately after application of pesticides will have a higher potential for pesticide transport into the water environment. The waste from manufacturing and formulating plants, unless very closely controlled, contains pesticides. The effluents from industrial plants that use pesticides in their manufacturing processes may contain various amounts of pesticides. The substantial quantities of pesticides mainly chlorinated hydrocarbons, have been discharged to surface water through municipal and industrial waste discharges. Even low concentrations of pesticides in the water may result in a large emission.

The environmental fate of insecticide depends upon many extrinsic and intrinsic factors which determine the rate and extent of their transformation and

mineralization. These factors include such physiochemical properties as their structure, molecular size, nutrient status, water solubility, lipophilicity, volatility, concentration and the presence of substituents. Various environmental factors involved are the soil structure, pH, temperature, oxygen availability, salinity, light intensity, bioavailability, inorganic nutrients, and water content of soil. The indigenous microflora or the microbial ecology is also an important factor to be taken into consideration for successful biodegradation.

Factors involved in insecticide disappearance

Pesticides do not readily disappear from the environment. Soil micro-organisms may be almost entirely responsible for their disappearance from the environment. The factors involved in insecticide disappearance are biotransformation, photochemical mechanisms, physical mechanisms chemical mechanisms, microbial degradation and bioremediation.

Biotransformation

Biotransformation is the minor or complete modification in the structure of harmful compounds / toxicological pollutants through biological processes (by the involvement of enzymes). These biotic processes may result in complete conversion of the organic molecule into inorganic molecule or may result in new organic molecule or occasionally lead to minor modification.

The biotransformation consist of two phases: Phase-I reactions which are mainly hydrolytic, oxidative or reductive, may be toxicative or detoxicative and usually (but not invariably) make the parent molecules more polar. Phase-II reactions in which polar groups such as sugars, glucuronic acid and glutathione, to give water soluble conjugates which can be excreted. In mammals, the major site for biotransformation is the endoplasmic reticulum of hepatic cells, but such activity is also found in mitochondria, in the soluble fraction of cells in blood plasma Biooccumalated. Insecticides in the tissues can have important effects on biotransformation processes, by either inhibiting or stimulating the enzymes metabolizing other incoming insecticides. If the biotransformation of a particular insecticide results in toxication, its inhibition may protect the organisms, whereas its stimulation may result in increased toxicity for a given dose. Three major types of enzymes are dominat.In the non-synthetic metabolism of insecticides in animals.

1. Hydrolases: Hydrolases which split the insecticide substrates through

hyrolysis are carboxylesterases, amidases, phosphatases, and A-type esterases (O'Brien, 1960).

2. Glutathione S-transferase: Glutathione S-transferase which is characterized by dependency on reduced glutathione (GSH) for actions is DDT-dehydrochlorinase (Kearns, 1956). BHC degrading enzymes (Clark *et al*., 1969). The methyl parathion system (Fukami *et al.*, 1969) and other delakylating and perhaps dearylating systems.

3. Microsomal oxidases: Microsomal oxidases are characterized by the requirement of NADPH, microsomes and oxygen *in vitro* for degradation of their substrates. The NADPH-requiring general oxidation system, commonly referred to as the mixed function oxidase system or MFO, is located in the microsomal proportions of various tissues, particularly the liver (Brodie *et al*., 1958; Gillette *et al*., 1969). The complete mechanism by which NADPH facilitates this electron transport system has not been elucidated, but the general scheme of such a system is known (Fig. 3). In short, it involves mainly cytochrome P_{450} along with cytochrome b_5, which requires NADPH. The final process of oxidation (or hydroxylation) of drugs and pesticides involves reduced cytochrome P450 oxygen complex, which on oxidation of the substrate becomes a stable oxidized form itself.

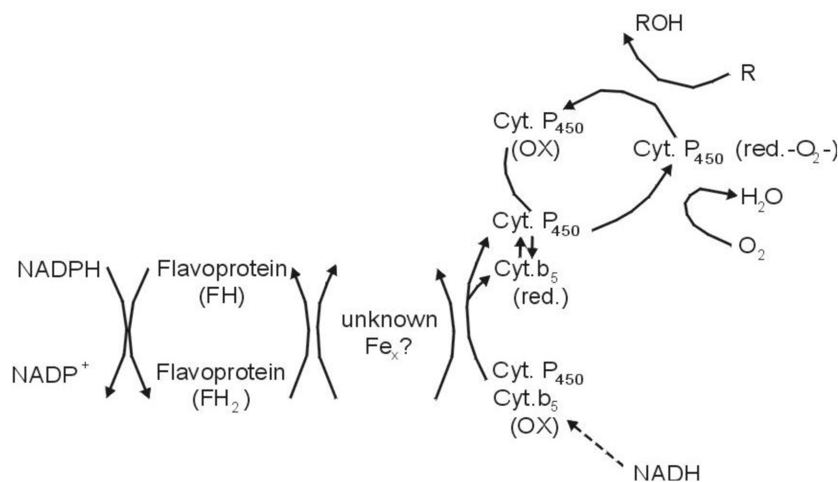

Fig. 3. Schematic diagram indicating the electron transport system involved in the NADPH-requiring general oxidation system in liver micromes (Kamin and Masters, 1968).

Microsomal epoxidation of the unchlorinated double bond is the most important metabolic reaction of certain cyclodiene insecticides such as heptachlor, aldrin, isodrin and chlordene (Brooks, 1974a,b). The epoxidation occurs rapidly both *in vivo* and *in vitro* (Davidow and Radonski, 1953; Brooks *et al*., 1963; Nakatsugawa and Dahm, 1965). Although these epoxides are often quite stable metabolically, they are subject to hydration to dihydrodiols by epoxide hydrases (Brooks and Harrison, 1969; Brooks *et al*., 1968).

Cytochrome P450 is one of the major phase I-type class of detoxification enzymes found in terrestrial and aquatic organisms ranging from bacteria to vertebrates. Insecticides are generally included in the category of non-specific inducers of cytochrome P_{450}. The OC insecticides cause mixed-function oxidase induction in mammals. Several chlorinated hydrocarbons such as chlordane, DDT, lindane and mirex have been implicated in cytochrome P_{450} induction (Hart and Fouts, 1965; Mullen *et al*., 1966; Lucier *et al*., 1972).

Insecticides can interact with cytochrome P450 in a variety of ways, either directly as substrates or inhibitors or indirectly as inducers. In many cases, insecticides or insecticide synergists can interact in more ways than one, being first inhibitors of substrates and subsequently inducers. The importance of cytochrome P_{450} cannot be seriously challenged, almost every insecticide used interacts with it in both target and non-target organisms.

Photochemical mechanisms

The light in ultraviolet (UV) region of the spectrum contains energy capable of inducing chemical transformation in organic molecules. Before a substance can undergo a photochemical reaction, it must have the ability to absorb energy from the appropriate portion of the spectrum. When energy is absorbed from UV light, electrons in the molecule are excited and the resulting disturbance may cause a breakage of existing chemical bonds or the formation of new ones; alternatively, the result may be fluorescence, or the absorbed energy may simply be lost as heat. Rosen *et al*. (1966) and Robinson *et al*. (2001) have demonstrated that the chlorinated hydrocarbon eldrin can be transformed into a substance called photoeldrin, as a result of photo-addition, appear to be less toxic than their parent compounds but some are more toxic. Heptachlor epoxide is more toxic than its parent substance heptachlor. These reactions can and do occur and they contribute, at least to some extent, to the decomposition of pesticides in the environment.

Physical mechanisms

1. *Volatilization:* It plays an important role in moving a pesticide from one place to another. This may involve transfer from a place of inactivity to one more conducive to degradation or *vice versa*. Igue (1969), found that the soil water content affected volatilization losses of organochlorine insecticides through competition for adsorption sites. The water loss as such does not increase the rate of volatilization by co-distillation but increases the pesticide concentration in the solid selection and soil, air, and therefore makes the pesticides more available for volatilization into the atmosphere (Fig. 4).

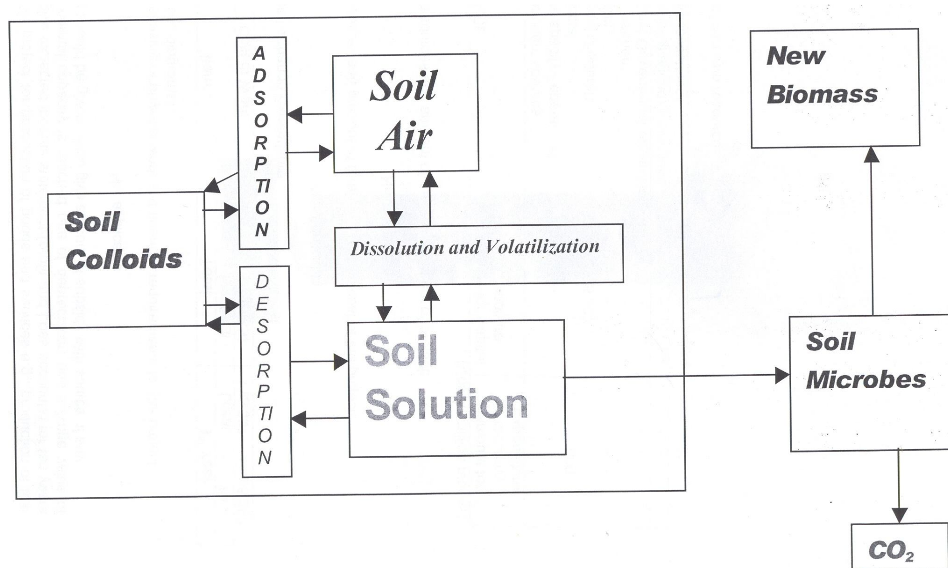

Fig. 4: Fate of insecticide among soil compartments.

2. *Adsorption to particulate matter*: Adsorption can play an important intermediate role in the decomposition of pesticides in the environment, since the process fixes the molecules to surfaces so that they may be readily available for chemical or metabolic decomposition.

The adsorption process is highly complex and may involve ion exchange, protonation, hemisalt formation, ion-dipole or coordination interaction, hydrogen bonding, vander waals forces and π bonding. Because of their physical and chemical structures, which provides relatively large surface-to-volume ratio, soil mineral, are mainly responsible for the adsorption of pesticides by clay soils.

3. Solubility in aqueous media: Despite the aqueous insolubility of many pesticides, water plays an important role in their disappearance from the environment by making them more readily available to both chemical and biological degradation. Even with the most insoluble materials, one mechanism by which this occurs is the displacement by water of pesticides to soil particles and their consequently greater availability for microbial degradation. Some pesticides, particularly herbicides, are quite soluble in water, and here water serves as a carrier medium so that the chemicals are more readily translocated in various plant species.

During recent years, concern has been expressed regarding the potential movement (leaching) of insecticidal residues in water from upper soil layers into lower uncontaminated soil strata where they could affect water sources. Similarly, lake and river waters can undoubtedly be contaminated with runoff water from adjacent agricultural land containing soil particles to which insecticidal residues are adsorbed.

4. Soil cultivation: Soil cultivation can also affect the ultimate fate of pesticides, especially those that are water insoluble and relatively volatile. Lichtenstein *et al*. (1971) studied the effects of a dense cover crop (alfalfa) repeated on same soil. The analysis indicated that alfalfa converted soil resulted in 76-82% reduction of residues derived from original applied pesticides.

Chemical mechanisms

The pesticide do undergo a variety of chemical reactions in soil and most probably in sother segments of the environment. These chemical reactions include hydrolysis, dealkylation, oxidation and dehalogenation.

Microbial degradation of insecticides

Living organisms in the biosphere are capable of obtaining energy through the degradation of organic molecules. Microorganisms are important in this process because they are capable of degrading organic substances that accumulate in the biosphere. According to Dagley (1972), “microbes are able to degrade chemical structure of many types, and it is probably true to say that every organic molecule that is synthesized by living matter may also be degraded, in turn, by microbes”.

Microorganisms play an important role in the metabolism of organochlorine

insecticides. However, the persistence of a number of organochlorine insecticides in soil and water for very long periods has been reported. This may be due either to the resistance of the insecticide to microbial degradation or to the formation of a complex with some component of the environment which is largely resistant to microbial attack (Lal and Saxena, 1982).

The chemical stability of organochlorine compounds is reflected in their resistance to microbial degradation and this fact coupled to their ubiquitous occurrence in environmental samples has aroused widespread concerns. The mechanism of microbial degradation of aliphatic and aromatic organochlorine compounds include hydrolytic, oxidative reductive and elimination mechanisms. The isolation and genetic manipulation of microbes for degradation of hexachlorohexane has been reviewed by Johri *et al*. (1997).

The importance of investigating properties of organic compounds and their interactions in soil is related to the knowledge of microbial processes and environmental contamination. Evaluation of the amount of organic compounds in soils can be obtained through estimation of the biotransformation and resistance to microbial attacks (Alexander, 1981).

Microbial degradation includes dehalogenation, oxidation, reduction, hydrolysis, dehydrochlorination, ring cleavage, condensation or conjugate formation and isomerization.

1. Dehalogenation: Soil microorganisms are capable of degrading several chlorinated pesticides by dehalogenation. DDT is slowly converted to DDE by *Aerobacter aerogenus* (Wedemeyer, 1967). The bacteria *Clostridium sporogenes* and *Bacillus coli* produce trace amount of benzene and mono-chlorobenzene from lindane (Allan, 1955) and can further metabolize it by the production of CO_2 from sub-merged soils. Cycloismerases were isolated from *Pseudomonas putida* and *Acinetobacter* play a crucial role in the dehalogenation of chloro-aromatic compounds (Vollmer *et al*., 1998).

2. Oxidation: The oxidative reactions which are important in degrading organochlorine insecticides in higher organisms are less common in microorganisms, probably due to the lack of a defined mixed-function oxidase system in microorganisms. The oxidation of aldrine to its epoxide dieldrin in microorganisms was reported by Korte *et al*. (1962). Other examples of oxidation reactions are the formation of heptachlor epoxide from heptachlor (Lichtenstein *et al*., 1963) and the formation of Kelthane from DDT (Matsumura *et al*., 1968).

Microorganisms are capable of carrying out a number of different oxidative reactions. They have the ability to convert chlorinated hydrocarbon insecticides with isolated double bonds to their corresponding epoxides *e.g.*, the conversion of aldrin to dieldrin and heptachlor to heptachlor epoxide (Bartha *et al*., 1967). They frequently dealkylate pesticides and can remove alkyl moieties attached to carbon, nitrogen, oxygen, or sulfur.

3. Reduction: Pesticides containing nitro substituents are subject to reduction to amines. Many organisms can also reduce pentachloronitrobenzene to pentachloroaniline (Chacko *et al*., 1966). Reductive dechloriation of organochlorine insecticides is an important microbial reaction. The reaction proceeds by replacing a chlorine atom on a nonaromatic carbon with a hydrogen atom. Conversion of DDT to DDD is a classic example of reductive dechlorination and has been shown to occur in yeast (Kallman and Andrews, 1963), *Proteus vulgaris* (Barker *et al*., 1965). *A. aerogenes* (Mendel *et al*., 1967), plant pathogenic and saprophytic bacteria (Johnson *et al*., 1967), and in soil samples under anaerobic conditions (Guezi and Beard, 1967). Matsumura *et al*. (1968) have reported another type of reductive reaction, which involves the reversal of epoxidation from dieldrin to aldrin.

4. Hydrolysis: Many pesticides are degraded by amide or ester hydrolysis mediated by soil microorganisms. Matsumura and Boush (1966) isolated several microorganisms capable of degrading malathion through hydrolysis of the carboxylester group and by processes involving demethylation.

5. Dehydrochlorination: Dehydrochlorination involves the simultaneous removal of hydrogen and chlorine from organochlorine insecticides. Typicaly, the reaction takes place between the saturated chlorinated carbon and the adjacent hydrogen on the neighboring carbon. The formation of DDE from DDT and the formation of γ-PCCH from γ-BHC are the most familiar examples of this reaction (Matsumura, 1985).

6. Ring cleavage: The cleavage of aromatic rings is a common reaction when soil microorganisms degrade pesticides and is one not usually encountered in higher animals. Dehalogenation to some extent is probably required prior to ring cleavage (Nakatsugawa and Morelli, 1976, Miwa and Kuwatsuka, 1991). The opening of aromatic ring of DDT in *Hydrogenomonas* sp. (Focht and Alexander, 1970) and of γ-BHC in *P. putida* (Benezet and Matsumura, 1973) also involves oxidation. Decarboxylation reactions during the oxidation of 4,4'-

dichlorobenzilic acid, a hydrolysis product of both chlorobenzilate and chloropropylate, to DBP have also been reported to occur in the yeast *R. gracilis* (Miyazaki *et al*., 1969). Thomas and Parkins (1995) have reported that atrazine degraders had two categories, one degrades the side chains and other degrade the ring. A herbicide bantazon is microbially and aerobically degraded into 6-OH-bentazone and 8-OH-bentazone (Huber and Otto, 1995).

7. Condensation or conjugation formation: Although it may not be considered by some as a metabolic pathway, certain microorganisms can condense or conjugate pesticides (Burchfield and Storrs, 1957).

8. Isomerization: Isomerization reactions in microorganisms during the metabolism of organochlorine insecticides are the conversion of γ-BHC to α-BHC (Benezet and Matsumura, 1973), dieldrin to photodieldring (Matsumura and Nelson, 1971), and δ-ketoendrin to endrin (Mendel *et al*., 1967). In each of these cases the isomer differs from the parent compound due to the difference in the position of the chlorine atom in the benzene ring.

Bioremediation and its genetic basis

Bioremediation is the selection of biological markers for the end point of remediation (Stephen *et al*., 1999). Bioremediation is being used for the destruction of chemicals in soil, ground water, sludge's, industrial water system and gases. Bioremediation may be carried out by one or more ways described as biotransformation, bioaccumulation, biomineralization, biodegradation and cometabolism.

Soil microorganisms collectively decompose various xenobiotic compounds and return elements to the mineral salts utilized by the plants. They also play important role in the dissipation of xenobiotic pesticides in the soil. Microorganisms especially bacteria, due to their continuous exposure to such environmental stresses, have developed genetically determined system against toxicants (Parsek *et al.,* 1995). These systems may enhance expression of genes on plasmids and gene transfer through plasmids (Orser *et al.,* 1993), coordinated expression of pre-existing genes (Daubaras and Chakrabarty, 1992); integrated regulation of ancestral and newly evolved genes through promotor regions of the gene (Clarke, 1984; Parsek *et al.,* 1995) and/or a continuous alteration in DNA sequences that may lead to a favourable adaptation (Van-der Meer, 1994).

The degradation of pollutants in the environment is critical, in particular, to

assess the persistence of these chemicals in the environment (Logan and Rittmann, 1998). The highly diverse microbial communities present in fresh and marine water, sewage and soils are able to transform a wide range of organic chemicals. Many synthetic organic chemicals, although biodegradeable, may persist in nature for a long time because the required catabolic capacity is not present or because the population of microorganisms bringing about their destruction is not large or active enough.

Microorganisms especially bacteria with amazing property to degrade xenobiotic contaminants have been isolated from different parts of the world. These bacteria belong to the genera *Pseudomonas, Arthrobacter, Ralstonia* and *Rhodococcus* (Noordman and Janssen, 2002; Arnett *et al*., 2000; Uragami *et al*., 2001; Margesin *et al*., 2003; Park *et al*., 2002; Arnold *et al*., 1997; Vollmer *et al*., 1998; Meckenstock *et al*., 1998; Bouchez *et al*., 1999; Thomas *et al*., 1996; Widada *et al*., 2002), *Acinetobacter* (Margesin *et al*., 2003), *Brevunidimonas* (Brown, 1980; Dumas *et al*., 1989; Horne *et al*., 2002a), *Spingomonas* (Nagata *et al*., 1993; Thomas *et al*., 1996; Arnold *et al*., 1997), *Bacillus, Planococcus, Marinococcus* and *Acetobacter* (Shakoori *et al*., 1999a, 2000a,b), *Agrobacterium* (Horne *et al*., 2002b), *Desulfitobacterium* (Tartakovsky *et al*., 1999, 2001), *Cautobacter, Flavobacterium* (Brown, 1980; Dumas *et al*., 1989), *Fusarium* (Mitra *et al*., 2001; Jirku *et al*., 2001; Yagafarova *et al*., 2001), *Methylobacterium* (Kayser *et al*., 2000; Aken *et al*., 2004), *Terrabacter* (Habe *et al*., 2002) and *Alcaligenes* (Padmanabhan *et al*., 2003).

The researchers world over have adapted various strategies to address environmental issue caused by insecticides. The use of bioaugmentation method (Habe and Omori, 2003; Dejonghe *et al*., 2000), isolation of enzymes responsible for biodegradation (Arnett *et al*., 2000; Ohshiro *et al*., 1996, 1997, 1999; Chen and Mulchandni, 1998; Sutherland *et al*., 2000, 2002; Park *et al*., 2002; Siddique *et al*., 2003; Singh *et al*., 2003; Hristova *et al*., 2003; Johri *et al*., 1997), introduction of motile chemotactic bacteria at the polluted sites (Parales *et al*., 2000), application of reverse sample genome probing (Firestone, 1998), genetically engineered microorganisms and use of particular gene encoding enzymes (Sussman *et al*., 1988; Ohshiro *et al*., 1999; Vollmer *et al*., 1998; Di-Sioudi *et al*., 1999; Commack and Mason, 1992; Kumamaru *et al*., 1998; Taguchi *et al*., 2004; Hristova *et al*., 2003) and use of anaerobic reactors (Tartakovsky *et al*., 1999, 2001) are part of those strategies.

The use of transgenic plants containing the genes of insecticide degradation have also been used (Choi *et al*., 1998). In 1994, the first microbial gene

expression factor designed to negate chemically induced gene repression and to promote rapid microbial destruction of persistent organic chemicals in treated soils (Baldwin *et al*., 2003) was employed.

Pollutants are removed from the soil by endogenous microorganisms including bacteria by their extraordinary ability to use a wide variety of xenobiotics as sole energy and carbon source (Hemmingsen, 1993; Parsek *et al*., 1995; Thomas *et al*., 1996; Nagata *et al*., 1993; Ford, 1998; Kato *et al*., 2000; Poelarends *et al*., 2000; Levanon, 1993; Parekh *et al*., 1994; Cabras *et al*., 1995; Shakoori *et al*., 1999a,b, 2000a,b, 2001a,b; Sutherland *et al*., 2002; Siddique *et al*., 2003). Microorganisms are termed as nature's biodegraders. They are scavengers in nature, responsible for recycling most natural waste materials into harmless compounds, when faced with an increasing array of synthetic compounds. Microorganisms are highly adaptive and develop the capability to degrade such recalcitrant compounds through evolution of new genes, which encode enzymes that can use these compounds as their primary substrate (Parsek *et al*., 1995; Kullman and Matsumura, 1996; Suenaga *et al*., 2001). The survival of organisms under insecticidal stress can provide efficient, cheap and environment friendly solution for bioremediation of xenobiotic contaminated soil (Arnett *et al.,* 2000; Shakoori *et al*., 2001a,b; Awasthi *et al.,* 2003; Pallud *et al*., 2004; Girvan *et al*., 2004; Aken *et al*., 2004; Hirano *et al*., 2004).

In many instances the abilities of microorganisms to utilize pesticides as their sole energy source were demonstrated using soil environment techniques (Audus, 1964). Sixteen bacterial strains were isolated by Shakoori *et al*. (2000a,b) that are capable of utilizing carbosulfan and quinalphos (a carbamate and an organophosphate respectively) as sole carbon and energy source in carbon source deficient M9-medium.

Pesticidal degradative genes in microbes have been found to be located on plasmids, transposons, and/or on chromosomes. The studies have provided clues to the evolution of degradative pathways and the organization of catabolic genes, thus making it much easier to develop genetically engineered microbes for the purpose of decontamination. Genetic manipulation offers a way of engineering microorganisms to deal with a pollutant, including pesticides that may be present in the contaminated sites (Kumar *et al*., 1996). An important aspect of earlier pesticide metabolism studies helped to develop a new approach to biological pesticide waste treatment technology. The expanding field of biotechnology helped to strengthen the idea that microbial enzymes could be used to control the fate of pesticides in the environment. There are numerous reports of the isolation

of cultures which can use pesticides, or group of pesticides, as their sole source of energy, however, it is rare indeed for this specific activity to be retained fully, or even at all, following transfer of the culture into a natural soil environment

Ohshiro *et al*. (1999) studied *Arthrobacter* sp. strain B5 which possessed OP hydrolase gene. The degrading property of the strain against different insecticides was also observed. The molecular cloning and nucleotide sequencing of OP hydrolase gene was also done. Thomas *et al*. (1996) isolated a bacterium, *P. vesicularis,* from soil that is capable of degrading xenobiotic compounds used as the sole source of carbon and energy. The bacterium could degrade γ-hexachlorocyclohexane by enzymes that appear to be extra-cellular and encoded by genes located on chromosomes. Furukawa *et al*. (1998) observed enhanced degradation of polychlorinated biphenyls by biphenyl dioxygenase and their genes (Taguchi *et al.,* 2004) responsible for biodegradation.

Endosulfan

Because of concerns about environmental persistence and resulting health effects on humans and wildlife, nearly all uses of organochlorines have been suspended. However, two compounds endosulfan and lindane are still in use. Endosulfan is currently registered for use as a foliar spray or dust on a wide range of vegetables and crops.

Technical-grade endosulfan is a mixture of two stereoisomers, α- and ß-endosulfan, in a ratio of 7:3. It is used extensively throughout the world, as a contact and stomach insecticide and as an acaricide on field crops such as cotton paddy, sorghum, oilseeds, coffee vegetables, and fruit crops (Lee *et al*., 1995; Kullman and Matsumura, 1996). Because of its abundant usage and potential transport, endosulfan contamination is frequently found in the environment a considerable distances from the point of its original applications (Mansingh and Wilson, 1995; Miles and Pfeuffer, 1997). Endosulfan has been detected in the atmosphere, soils, sediments, surface and rain waters, and food stuffs (United States Department of Health and Human Services, 1990). It is extremely toxic to fish and aquatic invertebrates (Sunderam *et al*., 1992) and has been implicated in mammalian gonadal toxicity (Singh and Pandey, 1990; Sinha *et al*., 1995, 1997; Turner *et al*., 1997), genotoxicity (Casida, 1993; Chaudhuri *et al*., 1999), and neuro-toxicity (Paul and Balasubramaniam, 1997). These health and environmental concerns have led to an interest in detoxification of endosulfan in the environment.

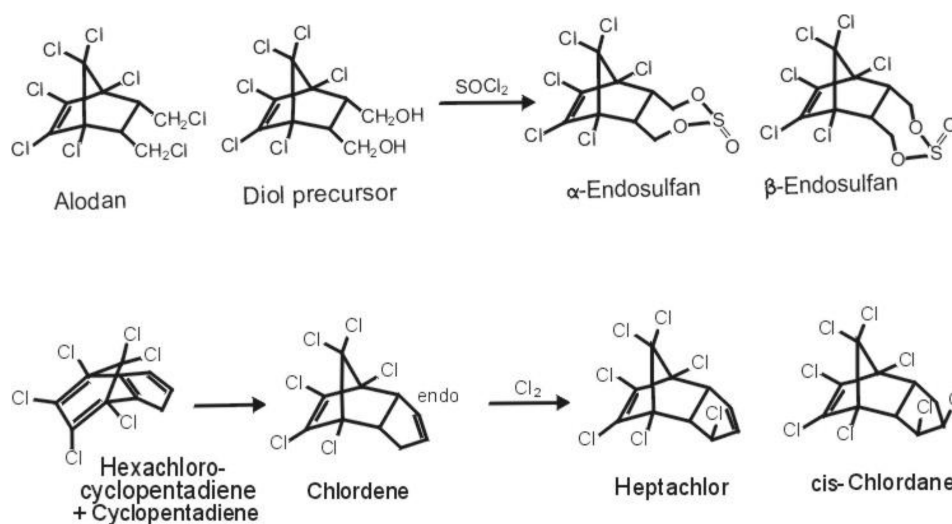

Fig. 5. Generic syntheses and chemical structures of classical polychlorocycloalkane (PCCA) insecticides (Brooks, 1974a; Turner *et al*., 1997).

Endosalfan is highly toxic via the oral route. The alpha-isomer is considered to be more toxic than the beta isomer (Smith, 1991). Stimulation of the central nervous system is the major characteristics of endosulfan poisoning (ATSDR, 1990). In human beings, the effects includ incoordination, imbalance, difficult breathing, gazing, vomiting, diarrhoea, agitation, convulsions and loss of consciousness (Smith, 1991). Endosulfan is mutagenic to bacterial and yeast cells (ATSDR, 1990, ATSDR, 2000). There is evidence that exposure to endosulfan may cause mutagenic effects in humans (USEPA, 1995, 1996).

Data from animal studies reveal the organs most likely to be affected include kidneys, liver, blood, lungs, muscles and the parathyroid gland (Smith, 1991; Wade *et al*., 2002a,b). Endosalfan residues have been detected in food stuff and human tissues (Nakata *et al*., 2002) from Bay of Bengal (Das and Das, 2004) and, from edible fish (Polder *et al*., 2003; Sarkar *et al*., 2003; deSilva *et al*., 2003). The chlordane, endosulfan and chlorinated cyclodienes strongly inhibitd the mammalian acrosome reaction which is essential for fertilization (Turner *et al*., 1997). Residues of endosulfan were also been detected in human breast milk (Cok *et al*., 2004; Burke *et al*., 2003).

Metabolism of endosulfan

Pathways of endosulfan metabolism by soil bacteria and fungi is shown in Figure 6. They degrade endosulfan to endosulfate and endosulfandiol. Endosulfate is the major metabolite in fungi, and endosulfandiol in bacteria. Degradation of endosulfan in microorganisms is dependent on pH. Microorganisms are actively dominant in endosulfan degradation. Other metabolic products indentified were endoether, endohydroxyether, chlorendic acid, and endolacetate (Martens, 1976). El-Zorgani and Omer (1974), in their study on α- and β-endosulfan degradation reported endosulfandiol as the major metabolic product, in contrast to the findings of Martens (1976), who reported endosulfate as the major metabolite.

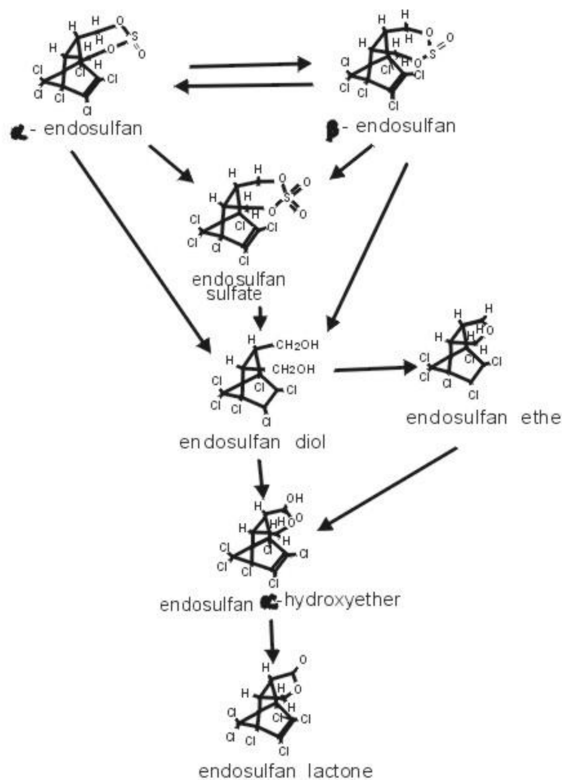

Fig. 6. Metabolism of endosulfan by microorganisms (Miles and Moy, 1979).

Miles and Moy (1979) studied the pathways of endosulfan metabolism in mixed cultures of soil microorganisms. They reported the interconversion of α- and β-endosulfan. Both α- and β-endosulfan is further converted primarly to endosulfandiol. The endosulfandiol is then converted chiefly to the α-hydroxyether with a minor pathway to endosulfanether. When incubated separately, endosulfanhydroxyether is converted almost completely to endosulfan lactone (Figs. 6-7).

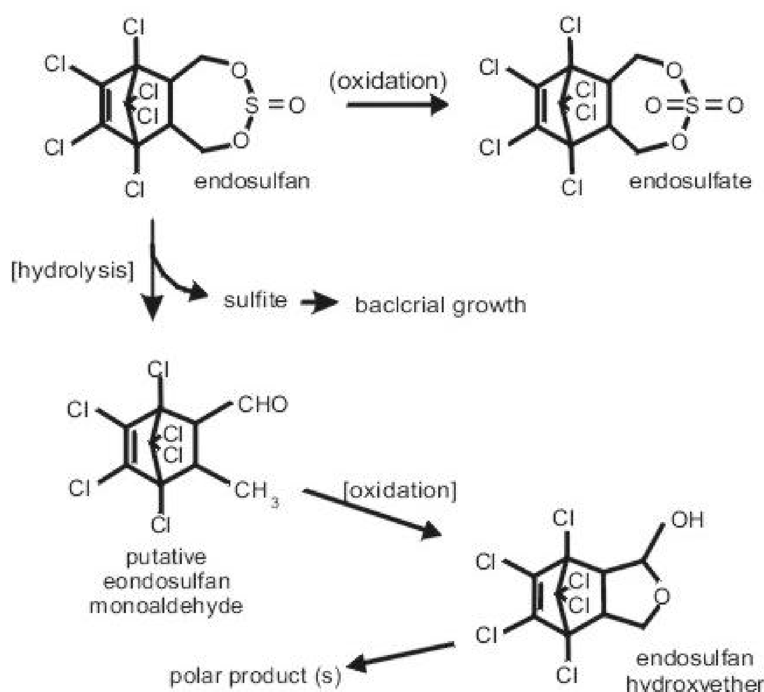

Fig. 7. Metabolism of endosulfan by mixed bacterial culture (Sutherland *et al*., 2000).

The environmental fate of endosulfan in the soil has been studied in many laboratories, and the ß-isomer has been found to persistd more than the α-isomer (Stewart and Cairns, 1974; Rao and Murty, 1980; Kaur *et al*., 1998). The difference in the persistence of the two isomers has been attributed to various factors such as their differential volatilization, photodecomposition, alkaline

hydrolysis, as well as their biotic metabolism (El-Beit *et al*., 1981; Cotham and Bidelman, 1989). When incubated with soil under aerobic conditions, endosulfan has been shown to undergo biotransformation, and endosulfan sulfate is the major metabolite formed (Stewart and Cairns, 1974; Rao and Murty, 1980). Endosulfan sulfate is nearly as toxic as the parent chemical and, as it does not undergo any further degradation, its residues tend to increase in the environment (Coleman and Dolinger, 1982). Under low oxygen or anaerobic environments, such as, sediments or sludge, endosulfan is rapidly metabolized into endosulfandiol, which is considerably less toxic than the endosulfan isomers (Guerin, 1999). Several microorganisms, both bacteria and fungi, which degrade endosulfan isomers under aerobic conditions have been isolated and characterized. Endosulfandiol, endosulfan sulfate, endosulfan ether, endosulfan hydroxyether, endosulfan lactone, and endosulfan mono- and di-aldehyde, have been reported as the major metabolites formed during the microbial metabolism of endosulfan isomers (Martens, 1976; Kullman and Matsumura, 1996; Awasthi *et al*., 1997; ATSDR, 2000; Sutherland *et al*., 2000; Awasthi *et al*., 2003).

A fungus, *Trichodermer harzianum*, has been found to degrade DDT, dieldrin, endosulfan, pentachloronitrobenzene, pentachlorophenol, but not hexachlorohexane. This fungus degrads endosulfan under various nutritional conditions through its growth stages. Endosulfan sulfate and endosulfan diol have been found to be the major fungal metabolites of endosulfan. Piperonyl butaoxide, when added to the growth medium, completely inhibits the endosulfan degradation. Di-n-prophyl malaoxon also inhibited the overall endosulfan degradation, but under such inhibitory conditions the formation of endosulfan sulfate is still observed (Katayama and Matsumura, 1993). The biodegradation kinetics of endosulfan utilized by *Fusarium ventricosum* and *Pandoraea* sp. resulted in the complete disappearance of both alpha and beta endosulfan (Siddique *et al*., 2003).

A preliminary step in the investigation of enzymatic technologies for endosulfan detoxification is the definitive identification of a biological source of endosulfan degrading activity. Microorganism have increasingly been investigated as a source of xenobiotic-degrading enzymes (Chen and Mulchandani, 1998).

Kwon *et al*. (2002) isolated 40 bacterial strains from various soil samples using endosulfan as sole carbon and energy source. Among the 40 isolated bacteria, strain KE-1, identified as *Klebsiella pneumoniae,* showed superior endosulfan degradation activity. The isolate degraded endosulfan by a non-

oxidative pathway and has potential as a biocatalyst for endosulfan bioremediation. Biodegradation of alpha and beta-isomers of endosulfan and endosulfan sulfate in Indian soil was studied in sterilized and non-sterilized soils under laboratory conditions. The results showed that alpha-endosulfan degraded more readily than beta-endosulfan and endosulfan sulfate under both sterilized and non-sterilized soil conditions (Singh *et al.*, 2000). Many studies describing degradation of endosulfan in microbial culture do not differentiate between chemical and biological hydrolysis, as culturing often leads to an increase in the pH of the growth medium (Martens, 1976; Miles and Moy, 1979). In addition to stringent pH controls the detection of metabolites is important for the confirmation of degradation, as losses of endosulfan from culture media or soils occur readily by volatilization and adsorption of surfaces (Guerin and Kennedy, 1992). As with most pesticides, the persistence and degradation of endosulfan are affected by the environmental conditions in which it is found. Endosulfan does not undergo direct photolysis but it is transformed by chemical hydrolysis under alkaline conditions, such as in seawater (Armburst, 1992). In soil, endosulfan has been shown to be degraded by a wide variety of microorganisms (Martens, 1976). However, degradation rates are usually low and metabolism often results in the formation of endosulfan sulfate, an oxidative metabolite shown to be equally as toxic and persistent as the parent compound, endosulfan. Because of its persistence and toxicity, endosulfan contamination poses a significant environmental concern. Most soil bacteria are capable of further oxidizing endosulfandiol to several other non-sulphur containing metabolites. Miles and Moy (1979) have proposed a succession of oxidation reaction converting endosulfandiol to endosulfan either, endosulfan hydroxyether and endosulfan lactone.

Awasthi *et al*. (1997) reported the isolation of bacterial and a mixed culture by Sutherland *et al*. (2000) that are capable of degrading endosulfan. Mukherjee and Gopal (1994) described the degradation of ß-endosulfan by *Aspergillus niger*. Shetty *et al*. (2000) have examined *Mucor thermohyalospora* MTCC 1384 for endosulfan degradation. These fungi were isolated for other degradative activities as well. Endosulfan is susceptible to alkaline hydrolysis (Martens, 1976), with approximately 10-fold increase in hydrolysis occurring with each increase in pH unit.

Heptachlor

It is an OC cyclodiene insecticide. Heptachlor was derived from chlordane in 1946. During 1960s and 1970s, it was used primarily to kill termites, ants and

soil insects. The two isomers of chlordane can be separated by chromatographic adsorption on aluminium oxide (Brown, 1951), and it is then possible to obtain two further derivatives of chlordane–heptachlor and hexachlor. Heptachlor has four to five times more insecticidal property than technical chlordane and is also more toxic than beta-isomer of chlordane (Brown, 1951). An important metabolite of heptachlor is heptachlor epoxide, which is an oxidation product formed from heptachlor by many plans, animals and microorganisms (US EPA, 1996; Jantunen and Bidleman *et al*., 1998). The acute toxicity of heptachlor expoxide, the main and most persistent of heptachlor's metabolits, may be greater. Effects due to heptachlor's exposure may include hyper-excitation of the central nervous system, liver damage, lethargy, incoordination, tremors, convulsions, stomach cramps and coma (Smith, 1991; ATSDR, 1989). There is evidence that heptachlor and heptachlor epoxide are associated with infertility and improper development of offsprings in animals, development of carcinoma in liver, may be stored in fatty tissues, heptachlor and heptachlor epoxide are able to cross the placenta and have been found in human milk and blood organs (Smith, 1991; WHO, 1984; Rodolico *et al*., 1999).

Many studies have shown that heptachlor, a chlorinated hydrocarbon insecticide, is a liver tumor promoter in rats and mice and induces tumour promoting – like alterations in human myeloblastic leukemia cells (Rought *et al*., 1998). Heptachlor is considered an environmentally persistent cyclodiene similar to dieldrin (Brooks, 1974a,b). There was widespread human exposure to heptachlor and heptachlor epoxide in contaminated milk products. The rural populations are at great risk of many diseases including Parkinson's disease (PD) because they drink well water leaching to exposure to herbicides and insecticides (Bloomquist *et al.,* 1999). Enrichment of (+) heptachlor exo-peroxide has been found in all regions of the world including arctic ocean waters (Jantunen and Bidleman, 1998).

Zoumis *et al*. (1998) reported that contamination caused by chlorinated organics and heavy metals can be combined, which is an enormous risk for the environment. The levels of organochlorine insecticides (chlordane, dieldrin and heptachlor epoxide), have been recorded in the river works of Georges Coops and Sydney Harbour Australia (Roach and Runcie, 1998).

Residues of persistent OC in soil and water have been detected and reported from different parts of the world (Calero *et al*., 1992; Thao *et al*., 1993). Their constant presence in the environment is a source of several physiological and biochemical abnormalities in living bodies (Ali and Shakoori, 1993; Akhtar *et*

al., 1996). They are also genotoxic, mutagenic and carcinogenic (Elespuru *et al*., 1974; Walker and Kaplan, 1992). Heptachlor effects body weight, several reproductive parameters and also induces tumour in mammals (Oduma *et al*., 1995; Crum *et al*., 1993; Wango *et al*., 1997; Rought *et al*., 1998).

Metabolism of heptachlor

Heptachlor is metabolized to heptachlor epoxide by mammals, insects, plants and soil micro-organisms. When cows graze on heptachlor treated pasture, only heptachlor epoxide is found in the body tissues and milk (Stoddard *et al*., 1954; Ely *et al*., 1955; Bache *et al*., 1960; Rusoff *et al*., 1962, 1963). Wong and Terrierer (1965) showed that in the rat expoxidation of heptachlor occurred in the microsomal part of the liver (Matsumura, 1985). Matsumura and Nelson (1971) fed heptachlor epoxide to rats and also collected a hydrophilic metabolite from feces. Miles *et al.* (1969) proposed the metabolic pathway shown in Figure 8 for the major metabolic product.

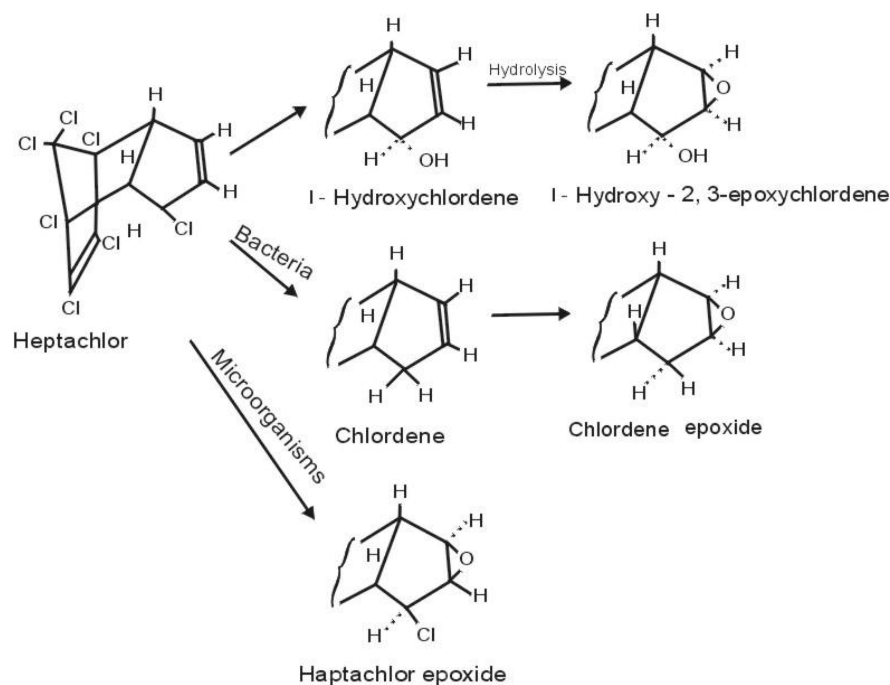

Fig. 8. Metabolism of heptachlor by microorganisms (Miles *et al*., 1969)

They show that heptachlor is metabolized by soil microorganisms to many different products by many independent metabolic pathways. In their studies they used 92 isolates of soil bacteria and fungi. Heptachlor epoxide, chlordane, and 1-hydroxy-2,3-epoxychlordene were the products of microbial degradation of heptachlor. Further studies by Miles *et al*. (1971) on mixed microbial cultures isolated from sandy loam revealed that heptachlor and its epoxide were readily converted to chlordane and 1-hydroxychlordene, respectively. These two metabolites were not degraded further by mixed cultures of microorganisms. This could be the reason for the occurrence of high levels of 1-hydroxychlordene and low levels of heptachlor epoxide in heptachlor-treated soils. Oxidation of heptachlor by microorganisms converts heptachlor to its epoxide. Heptachlor is also chemically hydrolyzed to 1-hydroxychlordence, which, upon epoxidation by microbes, produces 1-hydroxy2,3-epoxychlordene. Microbial dechlorination of heptachlor produces chlordene which undergoes microbial epoxidation to form the corresponding chlordane epoxide.

Knowledge of the regulation of pollutant degrading pathways may facilitate metabolic adaptation processes, hence the use of microorganisms in environmental cleanup efforts (Piperf and Raineke, 2000; Timmis and Pieper, 1999). Several microorganisms have been isolated which can metabolize lindane (MacRae *et al*., 1967), convert DDT to DDD (Menzie, 1969), Aldrin to Dieldrin, heptachlor to heptachlor epoxide (Bartha *et al*., 1967). Kato *et al.* (2000) reported 975 microorganisms, including 45 genera of bacteria, 11 genera of actinomyces, 22 genera of yeasts and 37 genera of fungi by monitoring the decrease of the aldoxines by high pressure liquid chromatography.

Pakistan is a thickly populated country. About 80% people obtain their livelihood through agriculture or agriculture-based industry. To meet the demands of ever growing population, the agriculture production needs to be increased, for which the use of pesticides with an object to effectively eradicate crop destroyers, becomes imperative. The insecticides, however, have been used indiscriminately resulting in severe environmental contamination. The farmers have been thus compelled to use pyrethroids, organophosphates, chlorinated compounds, fungicides and herbicides against pests in the field. The hazards of chemical pesticides have increased during the recent years. The problem has become more complicated because of extensive use of chlorinated insecticides, which were freely available in Pakistan, though banned in developed countries of the world. Because of their unusually long half life and non-biodegradable nature, these poisonous chemicals are likely to persist in the environment, gain entry into the food chain and cause serious health hazards. Besides using

chemical methods to remove these pollutants from the environment (air, soil and water), the microorganisms that survive in the environment and utilize them for obtaining energy could be the best candidates for bioremediation of insecticide contaminated soil or water. Biodegradation is a cheaper, more effective and natural way to combat the residual problem of these man made chemicals.

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