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GAGATA PAKISTANICA, A NEW CATFISH FROM PAKISTAN (PISCES : SISORIDAE)

M.R. MIRZA, A. PARVEEN AND M.N. JAVED

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Abstract: Only one species, viz., *Gagata cenia* has so far been known from Pakistan. A second species *G. pakistanica* is being described as new to science. It can be easily distinguished from *G. cenia* by its longer maxillary barbels reaching to the base of pelvies, which are shorter in *G. cenia* and only reaching to the pectoral fins.

Key words: New catfish, Pakistan: Gagata pakistanica.

INTRODUCTION

irza (1990) listed only one species of the genus Gagata (Family Sisoridae), i.e., G. cenia from Pakistan. The population of this genus from the river Indus downstream of Tarbela was found to be distinct from Gagata cenia (Hamilton) in several characters, especially the nasal and maxillary barbels. The nasal barbels in this new species extend upto the eye while the maxillary barbels almost reach to the base of the pelvic fins. In G. cenia, the nasal barbels are quite rudimentary while the maxillary barbels only reach to the base of pectoral fins. The two species are sympatric in the river Chenab near Qadirabad and might be found in the other rivers together, the specimens collected from Ghazi near Tarbela are of G. pakistanica only.

Gagata pakistanica new species Fig. 1 DI/6; PI/8; Vi/6; Ai/12; C17.

Holotype

Total length = 7 cm, standard length = 5.6 cm. Collected by Dr. William George & Khalid Abu Bakar on 28-3-1997 from river Indus near Ghazi.

Paratypes

Total length = 6.4, 7, 6, 6.5, 6.6, 6.35 cm.

Standard length = 5, 5.2, 4.6, 5.2, 5.3, 5.07 cm.

Collected by Dr. William George and Khalid Abu Bakar on 28-3-1997 from the river Indus near Ghazi.

Description

A rather small and slender species. Head compressed, its length 20-23.58% (M = 22.06, Holotype 23.2); breadth 15.09-19.23% (M = 16.95, Holotype 17.85) and height 16-19.23% (M = 17.36, Holotype 17.85) of standard length. Occipital process not reaching the basal bone of dorsal fin. The eyes present in the 1st half of the head. The eye diameter 4.46-6.73% (M = 5.57, Holotype 4.46) and the inter orbital distance 5.66-7.69% (M = 6.65, Holotype 7.14) of standard length.

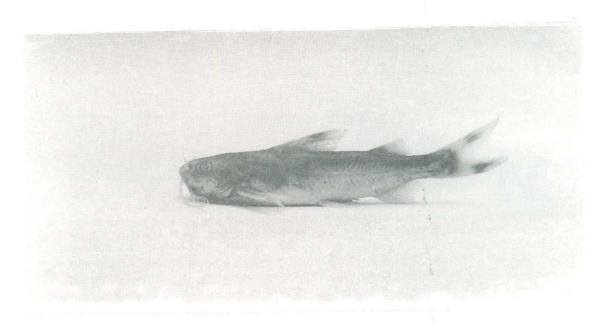


Fig. 1: Gagata pakistanica

The body smart, its breadth 10.71-17.30% (M = 13.09, Holotype 10.71) and height of the body 17.75-21.73 (M = 19.51, Holotype 17.85%) of standard length. The predorsal length 30.76-33.53% (M = 32.43, Holotype 32.14); the prepectoral length 17.75-28% (M = 22.49, Holotype 25); the prepelvic length 44-51.78% (M = 48.15, Holotype 51.78) and the preanal length 64-73.91% (M = 69.21, Holotype 73.21) of standard length.

The length of dorsal fin base 10.37-16.07% (M = 13.19, Holotype 16.07); the length of prectoral fin base 6-7.14% (M = 6.24, Holotype 7.14); the length of pelvic fin base 3.77-5.76% (M = 4.74, Holotype 5.35); the length of anal fin base

13.04-17.30% (M = 14.57, Holotype 14.28) and the length of caudal fin base 7.69 - 12.5% (M = 9.57, Holotype 12.5) of standard length.

Dorsal fin length 25.96—30.43% (M = 27.97, Holotype 28.57); the pectoral fin length 26-32.69% (M = 27.48, Holotype 26.78); the pelvic fin length 16.98 - 19.23% (M = 17.78, Holotype 17.85); the anal fin length 13.20 - 21.15% (M = 17.55, Holotype 17.85); the adipose dorsal length 12.5—19.23% (M = 15.97, Holotype 17.85) and the caudal fin length 22.64—32.69% (M = 27.44, Holotype 30.35); the length of caudal peduncle 5.76-13.20% (M = 9.70, Holotype 10.71), the least height of caudal peduncle 5.66—10.86% (M = 7.98, Holotype 7.14); the distance between the adipose and dorsal fin 18.86-23.91% (M = 20.63, Holotype 19.64) of standard length. The dorsal spine smooth and the pectoral spine with 7 serrations.

The length of maxillary barbels 41.42-49.05% (M = 44.68, Holotype 42.55); the length of outer mandibular 11.32-17.85% (M = 13.99, Holotype 17.85%); the length of inner mandibular 9.43—14.28% (M = 11.11, Holotype 14.28) and the length of nasal barbels 3.94-7.14% (M = 5.40, Holotype 7.14) of standard length.

The caudal fin is forked. The upper lobe is slightly longer than the lower lobe.

Colour

In 70% ethanol the body colour of the specimens is dirty white with copper coloured vertical bands. First band is situated toward the posterior end of the head, 2nd at the origin of the dorsal fin, 3rd behind the dorsal fin, 4th at the origin of the adipose dorsal and 5th at the caudal peduncle. There are colour bands on the dorsal fin.

A black verticle stripe at the base of the caudal fin. Anterior end of the adipose dorsal is black. The colour of the skin is black between the nostrils and between the orbits.

No. of specimens: 7

Longest specimen: 7 cm

Locality

Ghazi (river Indus).

Distribution

Pakistan.

Remarks

Specimens from Ghazi show the following differences from Gagata cenia:

Gagata pakistanica

- 1) Maxillary barbels attaining 1 length of 41.42—49.05% (M=44.68) of standard length.
- 2) Nasal barbels attaining length of 3.94—7.14% (M=5.40) of standard length.
- 3) Maxillary barbel reaching the base of the pelvic fins.

Gagata cenia

- Maxillary barbels attaining length of 16.27—22.22% (M=19.46) of standard length.
- 2) Nasal barbels attaining length of 1.13—2.43% (M=2.09) of standard length.
- 3) Maxillary barbel not reaching the base of the pelvic fins.

Roberts and Ferraris (1998) reviewed the genera *Gagata*. In more of the species described by them the maxillary barbels reach the pelvic fins.

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EFFECT OF TEMPERATURE ON CALLOSOBRUCHUS CHINENSIS (BRUCHIDAE : COLEOPTERA) REARED ON DIFFERENT STORED PRODUCTS

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Abstract: Callosobruchus chinensis was reared on Vigna radiata (green gram), Lence esculentum (Lentils) and Cicer arietinum (chickpea). Longevity, fecundity and hatchability of eggs of this pest were studied at different temperatures (30°C, 32°C, 34°C, 38°C, 42°C and 46°C) when reared on these stored grains. Fecundity and longevity were found to be significantly less at higher temperatures. Each female laid about 94 eggs at 30°C and only 21 eggs at 46°C on V. radiata. On L. esculentum, 98 eggs were laid by a single female at 32°C but only 20 eggs at 46°C. While on chickpea 72 and 22 eggs were laid at 32°C and 46°C respectively. The longevity decreased from 7.6 days at 30°C to 3.3 days at 46°C on Vigna radiata, from 7 days at 30°C to 3.3 days at 46°C on Lence esculentum and on Cicer arietinum from 6.3 days to 3.6 days at 30°C and 46°C, respectively. Maximum eggs were laid on L. esculentum at 32°C and on Vigna radiata at 30°C, but hatching was maximum on V. radiata (84%) at 32°C. Almost no eggs hatched at 38°C and above, 54°C was found to be the lethal temperature as the insects died within 20 minutes at this temperature.

Key words: Temperature, whole grains, pulses.

INTRODUCTION

ach insect species can exist only in a specifically limited range of ambient temperature, relative humidity, radiation and other physical factors of the environment. They are to a great extent temperature oriented (Wigglesworth, 1953) and utilize radiant energy from the sun as a source of heat (Duspiva and Cerny, 1928). Insects show various adaptations to control their body temperature as has been shown for various insects belonging to different orders like Orthoptera, Lepidoptera and Hymenoptera (Fraenkel, 1929; Rucker, 1934).

In nature insects may rest in spots cooled by evaporation, and are thus able to exist in environments far above lethal temperature (Mellanby, 1933). But this ability exists only in insects above a certain size. As the insect becomes smaller the volume to surface ratio decreases, and it can lower its body temperature only by evaporating more of its

body water that it could affort to lose (Mellanby, 1932). This emphasizes the importance of temperature in the life history of the insects.

Temperature affects the birth and death rates, thus the density of insects in a given population will be in the measure a reflection of its thermal history. The effect of temperature on the birth rate of insects may be considered under two main headings: its effect on the rate of egg deposition in reproducing adults; and its effect on the developmental stages, the time required for hatching and completion of metamorphosis (Bursell, 1974a; Kakuda *et al.*, 1990).

Insect tissue tolerance to different temperatures vary considerably from one species to another (Deal, 1941). Death probably results from denaturation of proteins and in balance of some metabolic processes leading to the accumulation of some toxic products more rapidly than they could be removed (Hopf, 1940; Larsen, 1943; Bursell, 1974a). Some point between the limits of tolerance range is described as the optimum temperature (Nieschulz, 1933; Uvarov, 1931), at which the greatest percentage of individuals complete their development, or the shortest time at which the development is completed (Blunck, 1914; Galliard, 1935).

Reproduction is adversely affected by temperature more readily than any other physiological functions, and the range of temperatures over which it will occur is correspondingly limited (Bursell, 1974a). The exact nature and the extent of the range varies from species to species and either sex may suffer ill effects. Tobacco budworm, Heliothis virescens will not mate or oviposit at a temperature of 10°C while the maximum egg deposition occurs at 21°C and 27°C (Henneberry and Clayton, 1991). In cotton tipworm, Crocidosema plebejana 28°C is the optimum temperature. At 34°C its development rate and survival rates were found to be greatly reduced and all eggs laid were non-viable (Graeme and Zalucki, 1991). In Kiefferulus barbitarsis also the fecundity and the hatchability rates become accelerated from 25°C to 30°C (Palavesam and Muthukrishan, 1992). Duration of the oviposition period is also affected beyond the optimum temperature (Bursell, 1974a). These few examples demonstrate the critical nature of the process of reproduction when compared with other physiological processes.

Like other insects, insects of stored grains have certain temperature, moisture and food requirements which directly effect their abundance. It is a characteristic of most of them that their generations are short, rate of reproduction is high and individuals are long lived. Within certain limits their rate of development and reproduction of increases with rising temperature causing serve damage to stored grain (Cotton, 1963). Changes in temperature or humidity (Metcalf and Flint, 1967) or exposure to radiant energy (Murdock and Shade, 1991; Begum et al., 1991) are the effective physical measure to destroy pests of stored grains. Exposure to high temperatures have been found to be effective in causing sterility (Saxena et al., 1992). Most insects including those which attack stored grains and their developmental stages, are killed within three hours of exposure to temperature ranging from 125°F to 130°F (Metcalf and Flint, 1967; Hand and An, 1990). Temperature also affects the rate of oviposition, hatching and the rate at which they reach maturity.

Rates of oviposition are sometimes sensitive to changes in humidity. At low humidities egg laying is depressed. At higher humidities there is a sharp drop in the rate of oviposition. The preferred humidity varies from species to species. Some species prefer the saturated end of the humidity gradient e.g., larvae of *Agriotes*. Some prefer

intermediate humidities like *Drosophila* and *Schistocerca*. The pests of stored products prefer relatively dry air (Bursell, 1974b; Willis and Roth, 1950).

Humidity also influences the survival of insect mainly through its effect on the water content of the body (Bursell, 1974b; Koidsumi, 1934). It also helps the insect to resist high temperature (Necheles, 1924; Mellanby, 1932; Cotton, 1963; Bursell, 1974b).

The present work was undertaken to study the effect of rising temperature accompanied by a more or less constant range of humidity on *Callosobruchus chinensis*, because it is advisable to control these insects by changing their ecological conditions rather than using insecticides.

MATERIALS AND METHODS

Collection and maintenance of beetles

The whole grain pulses infested with *Callosobruchus chinensis* were obtained from different local sources i.e., utility stores and other general stores. The infested grains were brought to the laboratory and placed in 6"x3" glass jars. The adults emerging from these grains were transferred to fresh glass jars filled 2/3 with different pulses.

Ten to fifteen pairs of insects were placed in each of them for egg laying. The jars were covered with a muslin cloth. All control rearing and maintenance was carried on at a temperature of 30°C and 60-70% relative humidity. The jars were checked daily to observe egg laying and also to remove any dead individuals. Eventually only grains bearing eggs were left in them.

Grains used: Vigna radiata (green gram), Lence esculentum (Lentils), Cicer arietinum (chickpeas).

Experimental set-up

Culture bottles 4"x3/4" were filled each with 5 gm of three different types of pulses. A newly emerged pair (a male and a female) was placed in each of them. Three replicates of each of the three different whole grains were then kept at different temperatures.

Experimental conditions

The insects were kept at 30°C, 32°C, 34°C, 38°C, 42°C and 46°C. The required temperatures were maintained by means of electric bulbs hung in wodden cabinets, in ovens and in incubators water-soaked cotton plugs for the culture bottles and petri dishes containing water were kept in ovens, cabinets and incubators to maintain the required range of humidity. Three replicates at each temperature were maintained. The number of eggs laid by each pair at different temperatures were counted after every 48 hours till death and a record was kept. Then the infested grains were trnasferred to a temperature of 30°C and 60-70% relative humidity. Hatching percentage was noted for insects maintained on different grains at each temperature.

Temperature was also increased to observe the thermal death point. All the glassware used was sterilized at 70°C to 80°C for 5 to 8 hours in an oven.

RESULTS

Mean of three replicates was taken for calculating fecundity, longevity and percentage hatch for insects reared on *Vigna radiata*, *Lence esculentum* and *Cicer arietinum* at different temperatures. The results are given below:

Temperature 30°C (Tables I-III)

Vigna radiata

Newly emerged pairs of *C. chinensis* (a male and a female) remained alive for about 7.6 days and during this period the female laid a total of 94 eggs. The eggs deposited by a single pair were kept separately to observe hatching. Out of 94 eggs laid by a single pair 38% hatched. They were kept for 24 days to observe whether they hatched or not.

Lence esculentum

Each pair lived for about 7 days and during this period the female laid 66 eggs out of which 41% hatched.

Cicer arietinum

Each pair lived for about 6.3 days and during this period the number of eggs laid by a single female was 62 out of which 44% hatched.

The longevity and number of eggs laid on *V. radiata* was found to be significantly higher than on *L. esculentum* or *C. arietinum*.

Table I: The number of eggs deposited at different temperature on different grains by a single pair of *Callosobruchus chinensis* till death (mean of 3 replicates).

Types of		Mean nur	nber of eg	gs laid at d	ifferent te	mperature	
pulses	30°C	32°C	34°C	38°C	42°C	46°C	Total No. of eggs
Vigna	94	82	80	41	26	21	344
radiata	±5.96	±9.27	±5.78	±10.59	±2.9	±0.57	
Lence	66	98	72	45	18	20	319
esculentum	±4.04	±1.15	±3.75	±7.5	±1.73	±1.44	
Cicer	62	75	62	57	20	22	298
arietinum	±1.96	±2.17	±0.28	±10.7	±0.57	±0.28	

L.S.D. for temperature = 13.6; L.S.D. for grain = 15.61.

Temperature 32°C

V. radiata

Each pair lived for about 7 days and during this period the female laid 82 eggs out of which 84% hatched.

L. esculentum

Each pair lived for about 6.6 days and during this period they laid 98 eggs out of which 48% hatched.

C. arietinum

Each pair lived for about 6 days on this grain and during this period 75 eggs were laid by a single female out of which only 14% hatched.

Fecundity on L. esculentum was significantly higher at this temper-ture while longevity was significantly lower on V. radiata only. Significant difference was observed in longevity on V. radiata and on C. arietinum when compared to 30°C .

Table II: Longevity of Callosobruchus chinensis at different temperatures on differ pulses (mean of 3 replicates).

Types of		Mean lon	gevity at di	ifferent ten	nperatures	
pulses	30°C	32°C	34°C	38°C	42°C	16°C
Vigna	7.6d	7d	6.3d	5.3d	6.6d	3.3d
radiata	±0.0	±0.28	±0.57	±0.0	±0.28	±0.0
Lence	7d	6.6d	6d	5.6d	6.3d	3.3d
esculen <mark>tum</mark>	±0.28	±0.0	±0.28	±0.28	±0.28	±0.0
Cicer	6.3d	6d	5.3d	5d	6.3d	3.6d
arietinum	±0.28	±0.0	±0.0	±0.57	±0.0	±0.28

L.S.D. for temperature = 0.420; L.S.D. for grains = 0.59.

Temperature 34°C

V. radiata

Each pair lived for about 6.3 days and during this period the female laid 80 eggs out of which 56% hatched.

L. esculentum

Each pair lived for about 6 days and during this period 72 eggs were laid but only 29% hatched.

C. arietinum

Each pair lived for about 5.3 days and during this period the female laid 62 eggs while less than 1% hatched.

Fecundity was significantly lower *V. radiata* only. However, longevity was significantly lower on all grains. Significant difference was observed in fecundity on green gram and chickpeas. Longevity was significantly higher on *V. radiata* than on *C. arietinum*.

Table III: Percentage of egg hatched at different temperatures on different pulses during 24 days.

Types of	Perce	entage of e	ggs hatche	d at differe	ent tempera	atures
pulses	30°C	32°C	34°C	,38°C	42°C	46°C
Vigna radiata	38	84	56	0.05	N.H	N.H.
Lence esculentum	41	48	29	N.H	N.H	N.H
Cicer arietinum	44	14	0.008	N.H	N.H	N.H

N.H = No hatching

Temperature 38°C

V. radiata

Each pair lived for about 5.3 days and during this period the female laid 41 eggs out of which less than 1% hatched.

L. esculentum

Each pair lived for about 5.6 days and 45 eggs were laid at this temperature by a single female but no egg hatched at this temperature.

C. arietinum

At this temperature the female lived for five days and 57 eggs were laid per female out of which none hatched.

Both fecundity and longevity were significantly lower at this temperature, but significantly higher on chickpeas than on green gram. Longevity was significantly higher on *L. esculentum* than on *C. arietinum* at this temperature.

Temperature 42°C

V. radiata

Each pair lived for about 6.6 days and during this period each female laid about 26 eggs out of which none hatched.

L. esculentum

Each pair lived for 6.3 days and during this period the number of eggs laid by a single female was 18. No hatching was observed.

C. arietinum

Each pair lived for about 6.3 days and during this period each female laid about 20 eggs but no hatching was observed.

Longevity were significantly higher at 42°C than at 34°C and 38°C. No significant difference was observed between fecundity and longevity on different grain at this temperature.

Temperature 46°C

V. radiata

Each pair lived for about 3.3 days and during this period each female laid about 21 eggs which did not hatch.

L. esculentum

Each pair survived for about 3.3 days and during this period each female laid 20 eggs with no hatching.

C. arietinum

Each pair lived for about 3.6 days and each female laid 22 eggs with no hatching.

Significantly lower fecundity and longevity was observed at this temperature but without any significant difference on different grains.

Thermal death point

At 54°C all the insects died within 20 minutes.

DISCUSSION

Species of the genus *Callosobruchus* are the major damaging pests of stored legumes (Southgate, 1964; Giga and Smith, 1987). Two best known species are *C. chinensis* (L.) and *C. maculatus* (F.).

Major biological barriers to any of the *Callosobruchus* species becoming pests are temperature variations and the inability of the insects to attack the leguminous seeds available (Howe and Currie, 1964). Some stored legumers are not attacked by Bruchidae. They show discrimination at two levels. One is the host selection, this is sensory to a greater extent. Second is the ability to complete larval development on the preferred host (Hanzen, 1977). The present research work was carried on three different types of host leguminous seeds, *Vigna radiata* (green gram), *Lence esculentum* (lentils) and *Cicer arietinum* (chickpeas). It concerned two main aspects of evaluation (i) to make a comparative study of the ability of *C. chinensis* adults to lay eggs and survival on these three common food legumes, (ii) to examine the effect of temperature on the fecundity, longevity and hatchability of the insect under study.

Closer examination on different legumes has revealed that the legumes on which the beetle breed influence the number of eggs laid (El-Sawaf, 1956). The smooth coated V. radiata was found to be the preferred host for opiposition, then L. esculentum and lastly C. arietinum. Different workers have also shown that smooth coated seeds are preferred for oviposition (Giga and Smith, 1987; Singh et al., 1977; Ahmad et al., 1993; Parr et al., 1996). The smoothness and the thickness can be an important factor which facilitates penetration into the seed by the newly hatched larvae. The attraction and repulsion by odours emitted by different chemical substances in the seeds have been given as reasons by other workers when considering egg deposition by stored grain pests (Nwanze and Horber, 1976; Giga and Smith, 1985). Vigna radiata has a smooth and a thinner coat when compared to others. It was also shown that at different temperature this beetle showed different preferences for different food legumes. The maximum eggs were laid on V. radiata seeds at 30°C while 32°C was found to be the optimum temperature for egg laying on L. esculentum and C. arietinum. It could be that at this temperature the maximum inducement, in the form of vapours, was present to deposit eggs. This odour in some way could act as a primer for the insects to mature and deposit their eggs. These seeds are rough coated containing volatile chemical having maximum evaporation at 32°C.

The other aspect that was studied concerned the effect of varying temperatures on the number of eggs laying, longevity and the rate of hatching of the eggs laid. The profound effect of temperature on living organisms is well known. Insects have evolved the means to maintain the internal temperature within tolerable limits despite wide fluctuations in the temperature of their surroundings. Literally hundreds of insect species have been tested in various temperature gradient and choice chambers (Herter, 1953; Thiele, 1977; Uvarov, 1977). The range of temperature at which the majority of individuals conduct their activities normally is defined as preferred temperature range (Bursell, 1974a). Temperature selection studies have revealed that when temperature increased above the preferred levels, profound effects on fecundity and longevity occurred. Kim and Choi (1987) have also shown that life span of *C. chinensis* decreased with increase in temperature, while the number of eggs decreased with the increase of temperature from 30°C to 32°C on *V. radiata*. On *L. esculentum* the maximum eggs were laid at 32°C, the same as on *C. arietinum*. The decrease in fecundity with

increasing temperature has been reported by various other workers like Bursell (1974a), Graeme and Zalucki (1991) and many others.

During the present study longevity was found to be significantly affected by temperature. At 30°C and 32°C the overall mean survival limits were found to be 7-7.6 days on *V. radiata*. Whereas at 34°C, 38°C and 46°C the overall mean survival time was 5-6 days, 5-5.6 days and 3.3-3.6 days respectively on this pulse. Although longevity was found to be different on different host seeds with varying temperature the trend was the same as found for *V. radiata*. It has also been reported by Yoshio (1990) that life expectation within a viable range is affected by temperature. The increase in temperature accelerates rate of metabolism thus decreasing the food reserves at a faster rate (Bursell, 1974a). At 42°C the longevity of the adults showed deviation from the usual pattern of behaviour i.e., the reduced longevity towards high temperatures had been replaced by increased duration of survival.

However, the egg laying behaviour remained almost the same. This unusual behaviour at 42°C is perhaps due to the triggering of some defence mechanism. Hence more energy is spent for increasing the survival limits than on reproduction. 54°C seemed to be the lethal temperature as 100% mortality occurred within 20 minutes at this temperature. These experiments show that like other insects this beetle also has a fairly well defined range of temperature within which it remains viable. The excessive heating causes too much evaporation leading to dessication and also resulting in denaturation of enzymes, thus disrupting the life processes which sooner or later cause death.

Reproduction and development take place within a fairly narrow range of temperature. Reproduction is adversely affected by temperature than any other physiological function and the range of temperature over which it occurs is correspondingly limited. The exact nature and extent of the range varies from species to species and either sex might suffer ill effects (Bursell, 1974a; Arlian, 1990). In the present study the preferred temperature was found to be $30\pm2^{\circ}\text{C}$. At 32°C maximum number of eggs were laid by *C. chinensis* on *L. esculentum* and minimum also on the same pulse at 46°C . This seemed to be the preferred substratum as far as the egg deposition was conserved, although survival was longest on *V. radiata*.

The eggs laid on V. radiata and L. esculentum showed maximum hatching of 84% and 48% respectively at 32°C, while in the eggs laid on C. arietinum the maximum hatching occurred at 30°C (44%). Thus optimal temperatures for survival are usually lower than those which permit faster development. The eggs laid at 34°C showed less percentage hatch on all grains up to the end of the experimental time. These observations indicate that development occurred at a faster rate on V. radiata with maximum hatching as compared to other legumes used in the experiment. Thus it was found to be the most susceptible to attack by C. chinensis, as has also been shown by previous workers like Giga and Smith (1987). Another point which needing attention is humidity. Temperature selection may be affected by humidity or state of hydration of the insects (Chapman, 1965; Thomson, 1970; Thiele, 1977). They can together effect fecundity and longevity markedly. Development and growth of insects like any other organisms is highly temperature and humidity dependent. In the present study the relative humidity was kept constant to avoid any discrepancy in the results. So, on the whole, it can be concluded that by increasing the temperature sufficiently an effective control can be achieved against this grain pest.

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INVESTIGATIONS OF LERNAEID PARASITES OF CATLA CATLA

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Abstract: Lernaea spp., belonging to the family Lernaeidae were studied from Catla catla from February 1999 to January 2000 at Government Fish Hatchery Pirowal (Punjab, Pakistan). Four species of lernaeid found were Lernaea cyprinacea Linnaeus, L. polymorpha Ya, L. oryzophila Monod and L. lophiara Harding. Lernaea cyprinacea had maximum overall prevalence (26.67%) followed by L. polymorpha (25.83%), L. oryzophila (4.17%) and L. lophiara (2.50%). The results of mixed infestation showed that L. cyprinacea was the most abundant parasite. Maximum infestation in C. catla for L. cyprinacea (36), L. polymorpha (34), L. oryzophila (3) and L. lophiara (1) was recorded in 214-413 g weight groups while minimum in highest weight groups. The infestation was lowest in minimum and maximum length ranges of host.

Key words: Lernaea spp., Catla catla, overall prevalence, mixed infestation, body weight, body length.

INTRODUCTION

ernaea spp., belong to the family Lernaeidae and are parasitic on freshwater fishes (Hoffman, 1976). Adult females attach to the exposed body surfaces of host fishes, where they cause acute hemorrhage and ulcers at the site of penetration. Fatality occurs as a result of blood loss and secondary infections (Putz and Bowen, 1964). The lernaeid can develop from mature eggs to the first copepodid or parasitic larval stage in as little as 4 to 8 days (Al-Hamed and Hermiz, 1973). Only females are found to parasitize. The parasite can be found on various parts of the host's body surface, and appear as small worm-like protrusions. Lernaea spp., seem to show no preference for a particular body area for attachment, however, the most heavily infested locations are found behind the pectoral fins, in the buccal cavity and/or the base of the caudal fin (Al-Hamed and Hermiz, 1973). Infestations in crowded breeding ponds causes serious economic problems (Al-Hamed and Hermiz, 1973).

Investigations into parasites of marine and freshwater fish have been carried out in Pakistan but there are few published reports on parasitic fauna of cultured fish (Tasawar and Hussain, 1999; Tasawar and Khurshid, 1999; Tasawar and Naseem, 1999; Tasawar et al., 1999). The present study was, therefore, designed to investigate the learnaeid parasites of *C. catla*, study their overall prevalence, study the mixed infestation of these parasites, determine the relationship between weight and body length and lernaeid

parasites of C. catla.

MATERIALS AND METHODS

Fishes for the present study (Catla catla) were collected from Pirowal Fish Hatchery, Khanewal (Punjab, Pakistan) during the period from February 1999 to January 2000. Monthly samples (10 fishes/month) were collected with the help of drag net and alive fishes were examined for the presence of ectoparasites i.e., Lernaea. Ectoparasites were removed with the help of fine forceps, and preserved in 5% formalin. The fishes were identified by Rehman (personal communication), measured and weighed and then released back into the pond water. The collected parasites were brought to the Parasitology Research Laboratory (Institute of Pure and Applied Biology), B.Z. University, Multan for further examination. The permanent mounts of the parasites (Cable, 1985) were identified with the help of keys given by Kabata (1985).

RESULTS AND DISCUSSION

Lernaeid parasites of C. catla

The occurrence of lernaeid parasites on *C. catla* was studied at Government Fish Hatchery, Pirowal (Punjab, Pakistan) from February 1999 to January 2000. Four species of parasites recovered during the present investigation were *Lernaea cyprinacea*, *L. polymorpha*, *L. oryzophila* and *L. lophiara*.

Different species of the genus Lernaea have been reported from other countries of the world. Shariff (1981) reported L. piscinae from bighead carp, Aristichthys noblis in Selangor, Malaysia. Lewis et al. (1984) reported L. cruciata from the pumpkinseed, Lepomis gibbosus in Southern Quebec, Canada. Noga (1986) collected L. cruciata (LeSuerer) from the mouthbass, Micropterus salmoides in the Chowan River, North Carolina and USA. Berry et al. (1991) found L. cyprinacea on stocked rainbow trout (Oncorhynchus mykiss) in Utah State, USA. Marcogliese (1991) reported L. cyprinacea on 3 species of fishes in Belews Lake, North Carolina. Kularatne et al. (1994) reported the L. minuta (Kuang) on the Javanese carp, Puntius gonionotus (Bleeker) in Malaysia.

Robinson and Avenant-Oldewage (1996) collected L. cyprinacea from Labeo rosae and Oreochromis mossambicus in the Olifants River in the Kruger National Park, South Africa. Ho and Kim (1997) reported five species of the genus Lernaea i.e., L. arcuata Soejanto from Puntius gonionotus, L. cyprinacea L., from Puntius partipentazona, L. oryzophila Monod from Puntius gonionotus, L. polymorpha Yu from Cyprinus carpio and L. taipila from Oreochromis mossambicus in freshwater fishes of Thailand.

Lernaea spp., have also been reported from Punjab, Pakistan. Tasawar and Hussain (1999) reported five species of Lernaea namely, L. polymorpha, L. cyprinacea, L. oryzophila, L. arcuata and L. lophiara from Labeo rohita. Tasawar and Khurshid (1999) recovered five species of the genus Lernaea, L. cyprinacea, L. polymorpha, Lernaea sp., L. oryzophila and L. lophiara from mori, Cirrhinus mrigala. Tasawar and Naseem (1999) collected four species of the genus Lernaea namely, L. cyprinacea, L. polymorpha, L. lophiara and L. ctenopharyngodonis from Ctenopharyngodon idella. Lernaea cyprinacea, L. polymorpha, L. oryzophila and L. lophiara were recovered from

Labeo rohita (Arshad, 1999). From hybrid (Catla catla x Labeo rohita), L. cyprinacea, L. polymorpha, L. lophiara and L. oryzophila were recorded (Nazli, 1999). Six species of the genus Lernaea, L. cyprinacea, L. polymorpha, L. oryzophila, L. lophiara, L. ctenopharyngodonis and L. arcuata were reported from silver carp (Shahzad, 1999).

The results of the present study are only comparable with studies conducted by Arshad (1999), Nazli (1999), while the results are not in agreement with studies conducted by Tasawar and Hussain (1999), Tasawar and Khurshid (1999), Tasawar and Naseem (1999), Shahzad (1999) from Pakistan as well as in other parts of the world. This difference could be accounted on the basis of different hosts examined and due to different climatic conditions (when compared world-wide).

The overall prevalence of Lernaeid parasites of C. catla

A total of 168 ectoparasites were recovered from 120 *C. catla*. Out of 120 specimens 44 were infested. The percentage of infestation of parasites of *C. catla* was calculated (Table I) and it was observed that all four species of ectoparasites showed considerable variations with maximum values for *L. cyprinacea* (26.67%), *L. polymorpha* (25.83%), *L. oryzophila* (4.17%) and *L. lophiara* (2.5%).

Table I: The overall prevalence of Lernaeid parasites of Catla catla from Fish Hatchery Pirowal (Punjab).

Name of parasite	No. of fish examined	No. of fish infested	Prevalence (%)
Lernaea cyprinacea	120	32	26.67
L. polymorpha	120	31	25.83
L. oryzophila	120	5	4.17
L. lophiara	120	3	2.50

Various workers in Pakistan have studied the overall prevalence of *Lernaea* spp. For example Tasawar *et al.* (1999) examined the overall prevalence of parasites of *Cirrhinus mrigala*. The prevalences were *L. cyprinacea* (43.33%), *L. polymorpha* (34.16%), *Lernaea* sp. (14.16%), *L. oryzophila* (7.5%) and *L. lophiara* (4,16%). From *Labeo rohita* the prevalences for various species were *L. polymorpha* (5.83%), *L. cyprinacea* (5%), *L. oryzophila* (1.66%) and *L. lophiara* (1.66%) (Arshad, 1999). The prevalences of *L. polymorpha* (6.66%), *L. cyprinacea* (9.16%), *L. lophiara* (3.33%), *L. oryzophila* (2.5%), *L. ctenopharyngodonis* (2.5%) and *L. arcuata* (1.6%) were reported from silver carp examined by Shahzad (1999).

It is obvious from the results of the present study that *Lernaea* spp., have higher prevalences when compared with Arshad (1999) and Shahzad (1999). This could be explained on the basis that *C. catla* is more susceptible to lernaeid parasites as compared to other fishes present within the same habitat. While the prevalence of *Lernaea* spp., recorded during the present study is lower when compared with Tasawar *et al.* (1999) this could be due to the chemical control measures, taken at Pirowal Fish Hatchery in order to kill the parasites. This reasoning is supported due to initial similarity observed

between the prevalence of *Lernaea* spp., during the present study with Tasawar *et al.* (1999).

Mixed infestation of Lernaeid parasites of C. catla

The mixed infestation of parasites of *C. catla* was studied (Table II) and it was found that eleven fishes were infested with *L. cyprinacea*. *Lernaea polymorpha* was present on twelve hosts, while *L. cyprinacea* and *L. polymorpha* infested 14 fishes. Only one fish out of 120 was infested with *L. cyprinacea* and *L. oryzophila*. Same number of fish were infested with *L. cyprinacea* and *L. lophiara*. Three hosts were infested with *L. cyprinacea*, *L. polymorpha* and *L. lophiara* were present on one host. One host was infested with *L. cyprinacea*, *L. polymorpha*, *L. oryzophila* and *L. lophiara*. The overall results of multiple infestation showed that *L. cyprinacea* was the most abundant species than other parasites.

Table II: Mixed infestation of lernaeid parasites of Catla catla from Fish Hatchery Pirowal (Punjab)

Species combination	No. of fish examined	No. of fish infested
Lernaea cyprinacea	120	11
L. polymorpha	120	12
L. cyprinacea + L. polymorpha	120	14
L. cyprinacea + L. oryzophila	120	1
L. cyprinacea + L. lophiara	120	1
L. cyprinacea + $L.$ polymorpha + $L.$ oryzophila	120	3
L. cyprinacea + L. polymorpha + L. lophiara L. cyprinacea + L. polymorpha + L. oryzophila	120	1
L. lophiara	120	1

According to Tasawar and Hussain (1999) *L. oryzophila* was the most abundant species than the other ectoparasites. *Lernaea polymorpha* was reported as the dominant species by Tasawar and Naseem (1999), while *L. cyprinacea* and *L. polymorpha* were reported as the dominant species by Tasawar and Khurshid (1999), Arshad (1999), Nazli (1999) and Shahzad (1999). The results of the present study are not comparable with the studies conducted by Tasawar and Hussain (1999), Tasawar and Khurshid (1999), Tasawar and Naseem (1999), Arshad (1999), Nazli (1999) and Shahzad (1999).

Stdies have shown that if two species of parasites are present within the same environment, competition will force one of them to be eliminated, or reduction in the number of one parasite is observed, due to competition for and food space (Noble and Noble, 1982). More combinations made by *L. polymorpha*, *L. cyprinacea* and *L. oryzophila* could be explained on the same basis that these species are more competent as compared to *L. lophiara* and *L. arcuata*.

Relationship between body weight and lernaeid parasites of C. catla

The maximum parasite infestation was recorded in the weight group (214-413 g) followed by 614-813, 14-213, 414-613, 814-1013, 1214-1413, 1014-1213 and 1414-1613 g, which had ectoparasites (74, 38, 23, 25, 3, 3, 1 and 1) respectively. There was no parasite in the weight groups 1614-1813, 1813-2013 and 2014-2220 g (Table III).

Table III: Relationship between body weight and lernaeid parasites of Catla catla from Fish Hatchery Pirowal (Punjab)

Species	Body weight of fish (g)										
Name	14-	214-	414-	614-	814-	1014-	1214-	1414-	1614-	1814-	2014-
	213	413	613	813	1013	1213	1413	1613	1813	2013	2220
L.cyprinacea	12	36	17	14	1	1	1	1			
L.polymorpha	10	34	5	24	2		2				
L.oryzophila	2	3	7722	100-				<u> </u>			
L.lophiara	1	1	1								

It was concluded that minimum number of parasites in the highest weight group could be due to the acquired immunity by fish against these parasites. A decrease in infestation has also been reported by Tasawar and Hussain (1999), Tasawar and Khurshid (1999), Tasawar and Naseem (1999), Arshad (1999), Nazli (1999) and Shahzad (1999).

It could be possible that parasites are less able to establish themselves and mature on older fish. This may be due to the result of an immunological response in older fish.

Relationship between body length and lernaeid parasites of C. catla

Relationship between body length and parasites of *Catla catla* (Table IV) was recorded maximum in length group 26.5-32.4 cm followed by 20.5-26.4, 32.5-38.4, 14.5-20.4, 8.5-14.4 and 38.5-44.0 cm) which had ectoparasites (51, 49, 42, 20, 5 and 1) respectively.

Table IV: Relationship between body length and lernaeid parasites of Catla catla from Fish Hatchery Pirowal (Punjab)

Species name		Body length of fish (cm)						
	8.5-	14.5-	20.5-	26.5-	32.5-	38.5-		
	14.4	20.4	26.4	32.4	38.4	44.0		
L. cyprinacea	2	10	22	33	15	1		
L. polymorpha	3	7	26	14	27			
L. oryzophila		2	1	2				
L. lophiara	-	1		2	177			

The results of the present investigation have revealed that length of the host seems to have influence on the parasite numbers. Similar findings have been reported by Tasawar and Hussain (1999), Tasawar and Khurshid (1999), Tasawar and Naseem (1999), Arshad (1999), Nazli (1999) and Shahzad (1999). Structure of the scales (in case of small fish) and the acquired immunity (in case of large fish) could be responsible for lower prevalences of these parasites.

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ELECTROPHORETIC ANALYSIS OF SERUM PROTEIN FRACTIONS IN HYPOTHYROID WOMEN

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Abstract: Clinical facility, for the present study, was available at the Institute of Nuclear Medicines and Oncology (INMOL) and Sheikh Zayed Hospital (SZH), Lahore. Serum samples of healthy women and hypothyroid patients were obtained along with the radioimmunoassay values of triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH). Samples were diluted in phosphate buffer (pH 7.2) and proteins were denatured by heating with loading dye. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), based on the method of Laemmli (1970), was employed for studying the protein profile of healthy and hypothyroid subjects. Gels were photographed and their images were stored for quantification of various protein fractions by UVP Gel Base Software Programme that provides the data of molecular weights and percent areas covered by each of the fractions. Data was employed in finding the enhancement or reduction and the appearance or disappearance of particular protein fractions for comparison between healthy and hypothyroid groups. Thirty-eight fractions were detected in normal whereas 49 were detected in hypothyroid subjects. Most of the fractions in hypothyroid group were found to be significantly enhanced when compared to healthy group. Fractions of molecular weights 156 and 26 kDa, detected in healthy group, could not be expressed in hypothyroid group, however, thirteen new fractions of molecular weights 325, 123, 91, 68, 65, 62, 60, 55, 50, 40, 31, 17 and 12 kDa were found to appear in hypothyroid group which could not be detected in healthy group.

Key words: Gel electrophoresis, hypothyroidism, protein fractions.

INTRODUCTION

he thyroid gland secretes two significant hormones thyroxine and triiodothyronine, commonly called T₃ and T₄, that have profound effect of increasing the metabolic rate of the body (Guyton, 1991). Different consequences follow a primary alteration in the rate of hormone supply. For example, in hyperthyroid states, hypersecretion of hormone leads to an increase in total hormone concentration. As a result, the concentration of unoccupied binding sites on total binding globulin decreases and the concentration of both free and bound hormone

rise. Converse consequences occur when the supply of hormone is decreased, as in hypothyroidism (Ingbar, 1985).

Inadequate synthesis of thyroid hormone leads to hypersecretion of TSH, which in turn produces both goiter and stimulation of all steps in hormone biosynthesis capable of response. This compensatory response may be inadequate and goiter with hypothyroidism or cretinism results. Hence, hypothyroidism follows upon an inadequate supply of active thyroid hormone to the peripheral tissues. The term implies failure of adequate production of hormone within the thyroid gland.

Thyroid hormone increases the metabolic rate of almost all tissues, one important exception being the adult brain. Through their stimulating effect on protein, carbohydrate and fat metabolism, thyroid hormones profoundly affect the functions of almost every organ system of the body. A protein anabolic effect of thyroxine has been demonstrated in the young hypophysectomized rat. The growth of nearly all tissues may be stimulated by thyroxine in the absence of pituitary hormones. The protein content of pelt, including hair is greatly increased.

Physiologic doses of thyroxine must be employed in order to demonstrate protein anabolic effects; toxic amounts of the hormones, however, do not stimulate growth (Scow, 1955). In hypothyroid children, small doses of thyroid hormones cause a positive nitrogen balance because they stimulate growth, but large doses cause protein catabolism similar to that produced in the adult. In essence, it is believed that thyroxine has little specific direct effect on protein metabolism but does have an important general effect in increasing the rates of both normal anabolic and normal catabolic protein reactions (Guyton, 1991).

Polyacrylamide gel electrphoresis (PAGE) of serum proteins has an important role as a diagnostic investigation. Attempts to manipulate various metabolites by endocrine intervention have been made for last several years and protein metabolism in relation to thyroid hormones has been extensively studied. However, the studies regarding electrophoretic protein profile in response to thyroid pathophysiology are meagre and almost non-existent. By keeping in view the importance of proteins in so many physiological phenomenon and the role thyroid hormones play in protein metabolism, the present investigation is carried out to emphasize the effect of thyroid hormone excess on serum protein fractions of female subjects resolved by sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

MATERIALS AND METHODS

Serum samples of healthy women and hypothyroid patients were obtained from the Institute of Nuclear Medicines and Oncology and Sheikh Zayed Hospital, Lahore along with the radioimmunoassay values of T_3 , T_4 and TSH (Fig.1). Samples were ultrafiltered in phosphate buffer (pH 7.2) and proteins were denatured by heating with loading dye.

Polyacrylamide gels, 8% for high and 15% for low molecular weight protein fractions, were prepared using the method of Laemmli (1970). Protein size markers and each of the samples were loaded in separate wells and gels were electrophoresed at a current supply of 12 mA and voltage of 150 V, in a cooling chamber maintained at 4°C

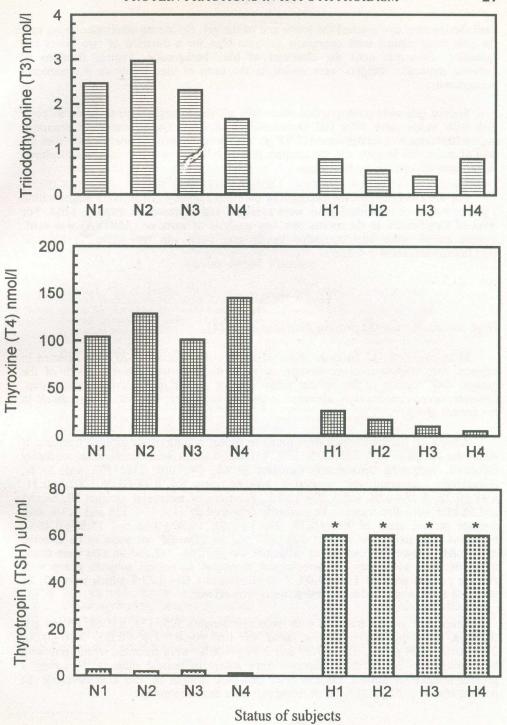


Fig. 1: Serum hormonal levels of healthy and hypothyroid women collected from hospitals. Abbreviations used: N = Normal; H = Hypothyroid, * = TSH level $> 60~\mu\text{U/ml}$.

until the tracking dye reached the lower end of the gel. Following electrophoresing run, the gels were stained with coomassie brilliant blue for a duration of two hours and destained afterwards until the clearance of blue background. Protein fractions of different molecular weights were visible in the form of blue bands on a transparent background.

Stained gels were photographed afterwards and their images were saved on a floppy disk with image store 5000 Gel Documentation System. Quantification of separated protein fractions was carried out by UVP gel base software programme that provides the data of molecular weights and the total areas covered by each of the fractions displayed in the form of peaks in the histogram.

For the assessment of the variation in the results among the different stages within a phase, values of individual goats were averaged and expressed as mean \pm SEM. For level of significance in the means one way analysis of variance (ANOVA) was used. Sample means were also compared by Student t-test and the minimum level of significance was set at P < 0.05.

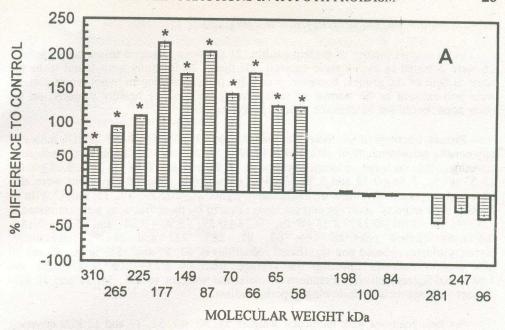
RESULTS

High molecular weight protein fractions (Fig. 2A)

In an overview, 17 fractions appeared in normal subjects and 23 were detected in subjects with hypothyroidism. Sixteen of these fractions were same in both of the groups. One fraction of the normal group did not appear in the hypothyroid group, however, seven new fractions appeared in the hypothyroid group, which were absent in the normal group.

Among 16 fractions which were found to appear in both of the groups, fractions of molecular weights, 310, 265, 225, 177, 141 and 88 kDa were found to be markedly enhanced, indicating pronounced increases of 63, 94, 110, 216, 173 and 204%, respectively, covering the respective average areas of 3.18 ± 0.06 , 3.20 ± 0.11 , 6.48 ± 0.22 , 5.28 ± 0.06 and $3.28\pm0.07\%$. Fractions of molecular weights 70, 66, 65 and 58 kDa were also found to be markedly enhanced by 143, 174, 126 and 125% with average percent areas of 9.85 ± 0.15 , 29.43 ± 0.60 , 15.30 ± 0.36 and $17.88\pm0.48\%$. Fractions with molecular weights 198, 100 and 84 kDa did not seem to be affected appreciably. However, fractions of molecular weights 281, 247 and 96 kDa were found to be declined adequately, in pathological compared to normal subjects group with average percent areas of 1.20 ± 0.00 , 1.50 ± 0.00 and $1.60\pm0.23\%$ which were 40, 24 and 34% lower compared to normal subjects, respectively.

Seven new protein fractions with molecular weights 325, 123, 91, 68, 65, 62 and 60 kDa, covering the respective areas of 1.65 ± 0.08 , 1.10 ± 0.00 , 3.10 ± 0.00 , 2.93 ± 0.11 , 3.38 ± 0.06 , 5.45 ± 0.29 and $2.98\pm0.06\%$, were recorded in subjects with hypothyroidism. All of these fractions were absent in normal subjects. However, a protein fraction of normal subjects with molecular weight of 156 kDa covering the percent area of $2.20\pm0.21\%$ failed to express after endocrinopathy.



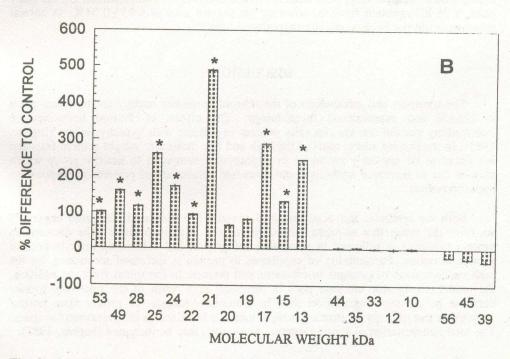


Fig. 2: Percent difference to control of high (A) and low (B) molecular weight protein fractions resolved by SDS-PAGE, in healthy and hypothyroid groups. Values are mean \pm SEM. *Sigificance at P<0.05.

Low molecular weight protein fractions (Fig. 2B)

In an overall picture of protein profile, 21 fractions appeared in normal, whereas, 26 were detected in hypothyroid subjects. Of these, 20 fractions were found to be the same in both of the groups, however, 6 new fractions appearing in hypothyroid patients were non-existent in the normal subjects. One fraction of the healthy subjects, on the other hand, could not be detected after hypothyroidism.

Protein fractions of the molecular weights 53, 49, 28, 25, 24 and 22 kDa indicated appreciable enhancements of 100, 159, 117, 261, 172 and 93% in hypothyroid subjects, covering the average percent areas of 8.25 ± 0.18 , 8.88 ± 0.11 , 8.08 ± 0.18 , 15.53 ± 0.36 , 5.58 ± 0.22 and $3.38\pm0.04\%$, respectively. Marked elevations were also observed in protein fractions of molecular weights 21, 20, 19, 17, 15 and 13 kDa in hypothyroid subjects. Average percent areas covered by these fractions were estimated at 7.80 ± 0.08 , 3.90 ± 0.11 , 3.13 ± 0.19 , 9.23 ± 0.17 , 3.90 ± 0.24 and $5.50\pm0.18\%$ indicating marked rises of 486, 63, 81, 285, 132 and 244\%, respectively. Hypothyroidism induced non-significant reductions of 23, 27 and 35% in 56, 45 and 39 kDa protein fractions covering the average areas of 2.23 ± 0.08 , 2.00 ± 0.35 and $1.68\pm0.05\%$, respectively. Fractions of molecular weights 44, 35, 34, 11 and 10 kDa did not vary appreciably following hypothyroidism.

Six new fractions with molecular weights 55, 50, 40, 31, 17 and 12 kDa covering the average areas of 2.10 ± 0.00 , 3.53 ± 0.16 , 2.50 ± 0.14 , 1.68 ± 0.06 , 3.20 ± 0.00 and $2.50\pm0.00\%$, respectively, were detected in subjects with hypothyroidism. On the other hand, a 26 kDa protein fraction, covering the percent area of $3.95\pm0.04\%$, in normal subjects, could not be detected after hypothyroidism.

DISCUSSION

The transport and metabolism of the thyroid hormones occupy an important place in clinical and experimental thyroidology. The effects of thyroid hormones on intermediary metabolism are clinically evident in patients with hypothyroidism (Ingbar, 1985). In the present study, most of the high and low molecular weight protein fractions are found to be markedly enhanced in hypothyroid compared to healthy group which may be due to increased anabolism and decreased catabolism of proteins in response to hypothyroidism.

Both the synthesis and degradation of proteins are decreased, the latter especially so, with the result that nitrogen balance is usually slightly positive. The decrease in protein synthesis is reflected in retardation of growth and a lessened effectiveness of growth hormone. Permeability of capillaries to protein is increased accounting for the high concentration of proteins in effusions and perhaps in the spinal fluid. In addition, the total exchangeable albumin pool is increased as a result of the relatively greater decrease in albumin degradation than in albumin synthesis. A greater than normal proportion and quantity of exchangeable albumin is localized in the extravascular space. The total concentration of serum proteins, as a result, may be increased (Ingbar, 1985).

Changes in plasma and other extracellular fluid proteins are prominent in human hypothyroidism. Plasma low density lipoprotein levels increase nearly three fold, reflecting a decrease in catabolism and turnover out of proportion to effects on synthesis

(Walton et al., 1965). Net production of albumin also increases and despite reduction in both synthesis and degradation there is a rise in total body miscible albumin pool. The latter increase in total exchangeable albumin is attributable entirely to an estimated 20-70% increase in extravascular pool and is accompanied by increased capillary permeability to albumin (Schwartz, 1955; Lewallen et al., 1959). Most pronounced impact of thyroid hormone deficiency, in the present investigation also, has been observed on albumin (66 kDa) and proalbumin (58 kDa) fractions, indicating appreciable enhan ements of 174% and 125%, respectively, in hypothyroid compared to healthy subjects. Enhanced capillary permeability may also account for a proportion of the approximate doubling in average protein content of the cerebrospinal fluid in hypothyroidism (Bronsky et al., 1958) as well as for some of the protein both in interstitial fluid and in various serious effusions associated with this condition (Loeb, 1978). In hyperthyroidism, on the other hand, there is increased anabolism of proteins resulting in positive nitrogen balance and high serum albumin concentration (Varley et al., 1980).

Conclusively, in hypothyroidism, synthesis of secreted, functional and structural proteins in many tissues is impaired, most obviously in children. Protein catabolism is also impaired. For several proteins, such as lipoproteins and albumin, degradation is impaired more than synthesis and thus increased quantities are found (Parving *et al.*, 1979). All these functions, in hypothyroid patients, are restored to normal by replacement doses of thyroid hormone (Ingbar, 1985).

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NATURAL VARIATION AMONG HELICOVERPA ARMIGERA (HÜBNER) POPULATIONS TO BACILLUS THURINGIENSIS Cry1Ac TOXIN

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Abstract: Natural variation in susceptibility of field collected cotton American bollworm (Helicoverpa armigera) to purified Bacillus thuringiensis (Bt) Cry1Ac toxin was investigated to establish a geographic baseline for comparison of future population responses to the increased use of Bt based insect control products like Bt transgenic crops and commercial formulations. Populations of H. armigera were evaluated for their susceptibility to purified Bt Cry1Ac toxin. The range of LC50's among selected populations from Lahore, Multan, Rahim Yar Khan and Hyderabad in response to Cry1Ac toxin was more than 16 fold. The data provide a strong basis for monitoring changes in susceptibility of H. armigera populations to future use of Bt toxins in Bt transgenic crops program in Pakistan.

Key words: Insect control, BT toxins, American bollworm, susceptibility, future responses.

INTRODUCTION

he development of insecticides based on Bacillus thuringiensis (Bt) δendotoxin proteins has increased in response to the need of efficacious, environmentally safe and selective pesticides with unique modes of action. Advances in Bt formulation technology and genetic engineering, and the discovery of Bt strains with a broader spectrum of activity, have resulted in new microbial products with increased potency and greater stability (Khan et al., 1995, 1995a). In addition, genetically modified crops such as cotton, corn and potato have already been commercialized (James and Krattiger, 1996). The advantages of genetically engineered plants include more efficient delivery of active ingredient to the target pest, less input in terms of the costs of pesticide application, and reduced environmental impact from pesticide use. Although, genetically altered plants producing their own protective insecticides provide an exciting new approach to insect control, a large scale introduction of these crops could rapidly lead to the development of resistance to Bt toxins within pest populations (Tabashnik, 1993). The combined impact of transgenic rice and increased use of Bt formulation for rice lepidopteran pests management increase the likelihood for resistance development which would negate the economic and environmental benefits of this important management option. Once commercially

available, these new microbial and plant products will play an important role in insect management (Karim and Riazuddin, 1997).

Selection for *Helicoverpa armigera* to Bt toxins is expected to be intense and is likely to result in the evolution of resistance to Bt endotoxins (for details, see Karim *et al.*, 1999a). An effective resistance management program will also be needed to foresee the long-term utility of Bt technology (Karim and Riazuddin, 1999b). The US Environmental Protection Agency (EPA) has approved conditional registrations for several Bt crops and is requiring the development of scientifically sound resistance management strategy by the year 2001 (Mellon and Rissler, 1998). The currently favored resistance management strategy for Bt crops is the "high dose/refuge strategy" (Gould, 1994, 1998).

Helicoverpa armigera is an important economic target for many Bt based products, but variation in susceptibility among populations has not been investigated. One of the most important research needs currently in Bt resistance management programs is the development of baseline susceptibility studies of key insect pests in all ecosystems where Bt will be used (Whalon, 1992). The present study reports the results from experiments to determine if differences exist in susceptibility to Cry toxins among H. armigera field populations collected from Lahore, Multan, Rahim Yar Khan and Hyderabad.

MATERIALS AND METHODS

Insects

Helicoverpa armigera larvae were collected from cotton growing areas like Multan, Rahim Yar Khan and Hyderabad. Larvae were reared on an artificial diet (Makhdoom et al., 1998) at $28\pm2^{\circ}$ C and a photoperiod of 16:8 (light: dark). Field collected larvae were the generous gift from IRAC, Pakistan. The bioassays with H. armigera were carried out with neonatal first instar larvae on artificial diet. A total of $100~\mu l$ of toxin dilutions were layered on diet in glass vials and air-dried. One larvae each was placed per vial because of their cannibalistic behavior. A total of 30 larvae per toxin dilution were used in each set of experiment. The mortality rates were recorded after 5 days and lethal concentration of toxin (LC₅₀) 95% fiducial limits was calculated using Probit analysis (Leora Software, 1987). At least five concentrations per toxin were used to estimate LC₅₀ value. Each set of experiment was repeated at least 4 times.

Purification and activation of Bt Cry1Ac δ-endotoxin

The Cry1Ac protein was obtained as recombinant proteins expressed in *Escherichia coli* (Karim *et al.*, 1999b). Bt δ -endotoxins were purified from *E. coli* as documented (Karim *et al.*, 1999b). Inclusion bodies from Cry1Aa, Cry1Ab, Cry1Ac, Cry1C and Cry2A were solubilized in Alkalic buffer (50mM Sodium carbonate, 10mM Dithiothreitol, pH 9.5) for 2 hrs at 37°C. The solubilized protoxin of Cry2A was dialyzed against 50mM sodium carbonate buffer (pH 10.5). The purity of protoxins and toxins was examined by Sodium dodecyl sulfate 10% Polyacrylamide gel electrophoresis (Laemmli, 1970). Protein concentrations of the protoxins and toxins were determined by the Bradford method (Bradford, 1976).

Statistical analysis

Data generated through biotoxicity assays of *H. armigera* were statistically analysed through Probit analysis using computer program Quantal software (LeOra software, 1987). IRRISTAT software (1997) was used to estimate ANOVA and correlation for individual toxin and insect strain effect.

RESULTS AND DISCUSSION

Significant differences in susceptibility to *Bacillus thuringiensis* Cry1Ac toxin exist among the *H. armigera* populations tested in the present study. Briese (1981), Georghiou (1988) and Rossiter *et al.* (1990) suggested that if the natural variation in susceptibility within a population is heritable, resistance can develop. Because of the extensive resistance of insects to chemical insecticides monitoring for the potential development of resistance to Bt is crucial. The results of present investigation indicate that similar natural variation exists among *H. armigera* and may be responsible for differences in susceptibility unrelated to prior exposure of Bt toxin.

Insect population sensitivity was investigated to *Bacillus thuringiensis* Cry1Ac toxin. Figure 2 shows a 10% SDS-PAGE containing the trypsin activated Cry toxins used in these studies. The significant difference was found in sensitivity of population of *H. armigera* to Cry1Ac toxin. However, no obvious geographic trends in susceptibility for *H. armigera* were observed (Table I). The sensitivity of *H. armigera* populations to Bt Cry1Ac toxin protein is mentioned in Table I. LC₅₀ ranged from 411 ng/cm² (Lahore) to > 6400 ng/cm² (Multan); this difference represented an 16-fold in susceptibility to Cry1Ac toxin (Table I). Hyderabad population was found most susceptible to Cry1Ac with LC₅₀ value 256 ng/cm². Multan population of *H. armigera* was least susceptible to Cry1Ac. Hyderabad population was also susceptible to Bt toxins with LC₅₀ value 518*ng/cm².

Table I: Susceptibility of field collected H. armigera to Bt Cry1Ac toxin.

Location	LC ₅₀ ng/cm ²	Fiducial limits	Slope ± SE
Lahore	411	369-713	4.84 ± 2.46
Multan	>6400	6400-51952	38.13 ± 5.23
Rahim Yar Khan	518	399-599	6.11 ± 2.11
Hyderabad	256	352-497	7.85 ± 3.11

The difference in susceptibility among *H. armigera* populations was almost 1-fold minimum and 16-fold maximum (Table I). Correlation coefficient provided a qualitative evaluation for the behavior of insect population towards Bt toxins (Table II) in relation to each other. These populations were collected from an area not known to have received prior exposure to Bt, and probably do not represent a resistant population. Surveys of susceptibility to Bt in other pest species have shown that considerable inter-population variation exists (reviewed by Tabashnik, 1993). Although, we have not done an extensive sampling of target pests, variation has been reported in other Lepidoptera including the diamond back moth, *Plutella xylostella* (Tabashnik *et al.*, 1990), Indian meal moth, *Plodia interpunctella* (Hubner) (Pyralidae) (Kingsinger and McGaughey,

1979), cotton bollworm, *Heliothis virescens* (F.) (Noctuidae), corn earworm, *Helicoverpa zea* (Boddie) (Noctuidae) (Stone and Sims, 1993) and gypsy moth, *Lymantria dispar* L. (Lymantridae) (Rossiter *et al.*, 1990), European Corn borer, *Ostrinia nubilalis* (Pyralidae) (Siegfried *et al.*, 1995), Spruce budworm, *Christoneura (Immiferana* (Clemens) (Tortricidae) (Van Frankenhuyzen *et al.*, 1995) and rice pests differences in tolerance are probably the result of natural variation among the populations (Robertson *et al.*, 1995) and unrelated to prior exposure to the Bt based insecticide.

Table II: ANOVA for the variation in the susceptibility among *H. armigera* populations to Bt CrylAc toxin.

Source	Sum of squares	D.F.	Mean square	F-ratio	Prob.
Toxin Population ERROR TOTAL	7183945 13539862.375 13539862.375 34263669.875	1 3 3 7	7183945.125 4513287 4513287.458	1.592 1.000	0.2963 0.5000

Significant difference was found in *Helicoverpa* populations for their susceptibility towards Cry1Ac toxin (Fcal. 1.0 < Ftab. 0.5).

The results obtained from a limited number of *H. armigera* populations sampled suggest that variability in susceptibility to Bt may exist among geographically distinct populations (Table I). However, large unexplained variations in susceptibility of field populations have been observed (Sawicki, 1987; Ffranch-Constant and Roush, 1990). Further investigation is required to establish that differences in susceptibility are genetically controlled and not the result of other unidentified factors. We recommended that stably transformed Bt cotton be used as standards for comparison with Bt purified proteins.

The data provide an important baseline information on the susceptibility of *H. armigera* to variety of different purified Cry toxins. However, the relationship between population susceptibility to Bt and the potential for field resistance remains to be established. Further monitoring for potential resistance genes in *H. armigera* populations would facilitate the establishment of a discriminating dose based on the frequency of resistance alleles in the populations. This information is critical to the development of an effective resistance monitoring program and implementation of resistance management strategies. However, it is important to document the extent of this variability throughout the known range of *H. armigera* to establish a diagnostic Bt concentration or dose that could be used in more extensive resistance monitoring program. Further, a baseline susceptibility against a variety of Bt toxins, as reported here, provides a basis for determining if resistance is developing as a result of increased exposure to Bt toxins as might be expected with increased use of Bt based products. Such information is essential to develop resistance management strategies designed to maintain the efficacy of these environmentally sound and economically important management options.

In lab, greenhouse and field assays (Halcom et al., 1996; Sachs et al., 1998) totton genetically modified to contain a Bt δ -endotoxin protein has consistently demonstrated

efficacy against Heliothis species. These field trials indicate that genetically modified Bt cotton has been effective throughout cotton growing countries. The development of strategies for implementing genetically modified Bt crops within integrated pest management offers an exciting challenge to pest management specialists and a considerable potential benefit to cotton growers. We analyzed natural variation to variety of different Bt Cry1Ac toxin in field collected insects of H. armigera. These results suggest that natural tolerance to Bt toxins is present in four different populations of both insects towards Bt Cry1Ac toxin. Therefore, it is suggested that high dose/refuge strategy should be evaluated on the basis of natural variations among distinct populations of insect pests. Now, a question arises, if natural variation among pest populations are present, then what would be the range of high dose? Does it vary from locality to locality for same pest? The currently proposed resistance management strategy for Bt cotton, the high dose/refuge strategy (Gould, 1998) requires a) plant tissue express enough Bt toxin to kill all heterozygotes, b) the resistance alleles be very rare, and c) susceptible insects are within an effective mating distance of resistant insects. The high dose/refuge strategy would not be useful for resistance management if high natural tolerance exit among different populations of insect pests. However, at higher dosages all individuals are expected to be susceptible. The practical importance of this natural variation will depend on whether these insects can survive on Bt cotton. In presence of natural variations, the high dose/refuge strategy is subject to a number of stringent prerequisites that may be difficult to meet in practice. More robust resistance management options are imperative.

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ON BIOLOGY OF HOUBARA BUSTARD (CHLAMYDOTIS UNDULATA MACQUEENII) IN BALOCHISTAN, PAKISTAN: SOME FIELD OBSERVATIONS ON BEHAVIOUR

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Abstract: Field observations on behaviour of Houbara wintering in Balochistan (Pakistan) suggest that though it becomes alert to the approaching man or jeep from a distance of some 500 m, yet it allows their approach up to some 50 m. The reaction to such stimuli varies with type, mode of application, recent experience, physiological and psychological state of individual bird, size of the flock and general foraging conditions. Different body postures indicate different psychological states of individuals. The bird comes in open place and takes few brisk steps before taking to its wings. Distance maintained from the human settlement directly increases with the size of settlement. The bird retires from active foraging during the middle part of the day but does not go for a complete rest. Houbara selects an open place between shrubs for night roosting, where it maintains an alert sitting posture and keeps on changing the roosting places during the night. The species prefers to stay on ground during dust storm. It can more efficiently avert falcon attack while on ground. It has a perfect camouflaging, especially when it is still or squatting.

Key words: Interference, falcon, storm, human settlements, rest, camouflage.

INTRODUCTION

nowing behavioural responses and needs help in understanding the basic biological requirements of an animal species. This knowledge can be exploited in development of future experimentation, conservation strategy and planned hunting programmes (Ralls, 1995). Researches and observations on behaviour in bustards (Ardeotis australis: Fitzherbert, 1979; Tetrax tetrax: Schulz, 1985; Eupodotis melanogaster: Schulz and Schulz, 1986; Otis tarda: Hellmich, 1987; Choriotis nigriceps: Ali and Rahmani, 1982; Manakadan, 1985) and/or Houbara Bustard (Collins, 1985) have been generally overshadowed by records on habitat, migration, food and population as per practical problems associated with behavioural study on desert species in the wild (Launay and Paillat, 1990).

Present study was instituted with the hope that field observations, gathered from different parts of wintering range of Houbara in Balochistan, will allow us to reach at

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some broad preliminary generalisations on general behaviour, which can be used in management programmes designed for the species.

MATERIALS AND METHODS

Field observations were recorded on selected bird/birds, in different localities of Balochistan (Pakistan), regarding their general behaviour and reaction of adult Houbara to different stimuli, as and when allowed by suitability of conditions. After spotting bird/birds in a general broad locality, some suitable observation point was carefully selected without disturbing the bird. A man, group of men or a jeep was asked to approach the bird cautiously, trying not to disturb it. Observation on reaction of the bird was recorded through unaided eyes and/or field binoculars (8 X 40, field 6.5; Enbeeco, Concord) till the bird remained within the visual approach. Activity of the bird was also later traced through foot prints, wherever possible. Sudden movements and/or high pitched sounds (horns, gun fires, etc.) were avoided so as not to cause severe disturbance in the general area.

Reaction to dust storm was recorded through direct observations on selected birds, spotted when dust storm was on in the central highlands, with mid day temperature touching freezing points. Bird/birds were observed in their normal behaviour and after disturbing it by approaching man and jeep.

Reaction of Houbara to attacking falcon was recorded while we had a chance sharing of the general area with a falconry party in central lowlands.

Records were maintained on distance of each of the sighted bird/flock from the nearest human settlement, using speedometer of the jeep and a general guess, along with information on size and type of the settlement. Observations, thus collected, were organised to develop generalisations on the influence of size of settlement on Houbara.

For studies on night roosting/day time rest, the birds were spotted in an area in the evening and/or in the morning, when their active foraging activities started subsiding. Day time activities of birds were followed through general physical observations, with unaided eyes and/or available binoculars (8 X 40). Foot tracks were also traced to collect supporting information. Places of night roosting and day time rest were located through physically observing the roosting/resting bird or through foot tracks or marks of the sitting bird/birds. Observations were recorded on posture and activity level of bird and the physico-biotic conditions in/around roosting/day time rest sites.

RESULTS

Interference

Human

Two men walking through a dried water course, with a good vegetative cover, remained unnoticed to a male Houbara coming from nearby hilly undulates (in Rakshan valley, Punjgur) for the evening foraging. It noticed the presence of the men only when

they were at a distance of some 100 m. At this stage, the bird started moving away from the approaching men, keeping its neck outstretched and low, trying to take the advantage of the edges of the water course and associated vegetation. The bird moved out of the water course when the approaching men remained at a distance of some 30-40 m. At this stage approaching men spotted the bird, when they got excited and showed some abrupt movements, making the bird to fly away.

At another occasion, a flock of four birds in Grashia valley (central Khuzdar), having relatively flat plain and evenly distributed shrubs (0.3-0.6 m height; general cover 3.13%), was tried to be approached by a group of three men. Houbara noticed the approaching men from some 300 m. All the birds (scattered with a distance of some 20-30 m from one another) immediately started moving away from the stimulus, maintaining a casual gait and relaxed posture but keeping at unconcerned eye on the men. Speed and concern of the birds gradually increased as the men got closer. Birds took to their wings when the men were at a distance of 50-70 m, and settled after taking a low flight of about 500 m. In an attempt to approach this flock a second time the birds showed more concern. This time these took to their wings when the men were still some 200-250 m away, and took a longer flight to get out of the range of the available binoculars.

In Sibi, where a flock of 9 birds persisted in cultivated fields (being exploited regularly by this flock for night foraging) despite the presence of the farmers (engaged in their routine activities). Two men tried to approach the birds through dried natural vegetation. Birds noticed the approaching men and became vigilant; but did not show an appreciable change in their activities. The birds tried to maintain a distance of 100-400 m from the men by walking and/or taking short flights. The birds persisted in the cultivations despite repeated disturbance/change for more than an hour.

Jeep

General vegetation: A single bird when approached with a jeep (CJ 7; blue with black canvas top) in Gat (western Chagai; having an almost pure stand of shrubs of *Anabasis* sp.) got vigilant to the jeep from a distance 500 m, yet remained calm and unconcerned. It, however, kept casually walking ahead of the jeep, exploiting relatively better vegetative cover in the water course. When the bird was approached to a distance of about 50 m, if walked out of the water course into a comparatively open plain, took a few brisk steps, became air borne and went out of the available vision.

In Dasht-e-Kani (Turbat), a bird initially allowed the approach of jeep (green, hard top) up to some 200 m, remaining almost unconcerned. On a closer approach the bird started moving away. It walked for some 30 m with its out stretched but low neck and took to its wings. The bird settled after some 500 m. In an attempt to chase the bird a second time, it immediately became air borne, settled at some 1.5 km and lost in the dunes.

A pair of birds was spotted in Hab (Lasbella) while grazing at a distance of some 4-10 m from each other, mainly in the bare area having a background of grey stones and some sprouting herbs. The birds allowed the approach of the jeep to a distance of about 500 m, when they started moving in front of the gradually approaching jeep. The birds walked majestically with upright neck appearing almost unconcented to the stimulus, though with watchful eyes and moving the head in low jerks. The pair continued

moving, side by side for a distance of some 300 m, without seeking refuge. On persistence of the chase, one of the birds took to its wings, while the other continued walking rather briskly with some degree of outstretched neck and took shelter in a small shrub (0.4 m). On a closer approach, the bird opted flight and settled at the distance of some 400 m and started walking briskly, showing concern. The bird was again chased when it allowed the approach to a distance of about 700 m, resorted to flight and was lost. The bird remained watchful of the jeep throughout this period. The other bird, still hiding itself in the shrub, was tried to be approached. On seeing the jeep at a distance of some 500 m, the bird took to its wings. It settled after taking a long flight and was lost in the undulates.

A flock of 6 birds in Grashia valley (central Khuzdar, a flat plain) allowed the approach of a jeep (Suzuki, Green hard top) to a distance of 50-100 m, though the birds were vigilant from a distance of about 500 m.

A pair of birds (in Gat, western Chagai) took to their wings when the jeep was still at a distance of about 300-400 m and disappeared. It could not be adequately confirmed whether this pair had already faced a chase, though a vehicle with hunters was later spotted in the area.

At two other occasions (in Sibi), Houbara was spotted in the general vegetation. On seeing the approaching jeep from a distance of 1 km the birds took to their wings and settled after a flight of about 1 km. The birds were lost in the general vegetation, after an identical reaction on the second chase.

Cultivations: In Sibi, a flock of 9 birds tried to maintain a distance of 500-700 m from the jeep, while on their evening foraging in the cultivations, being regularly exploited for night foraging. At one occasion, jeep happened to come close to the flock (50 m) and caught the birds in a surprise. At this stage the birds became chaotic, immediately took to their wings and even moved towards the jeep. On noticing the jeep under them, the birds suddenly changed the direction of their flight in an attempt to find a safer distance. On a second chase, birds became alert from a distance of 300 m, but took to their wings when these were some 50 m away and settled after some 50 m. These were again chased when these became air borne from a distance of about 100 m; and settled after a flight of some 100 m. The birds when chased a fourth time, took to their wings from some 300 m and settled after a flight of about 100 m, when these disappeared taking advantage of a scanty shrub cover.

At another occasion, the birds spotted in the fallow land cultivation (not used as regular foraging grounds), allowed the approach of jeep up to 200 m, when these started walking in front of the jeep. These birds tried to maintain a distance of 200-300 m. The birds took to their wings after walking for more than 1 km and settled after about 500 m.

A single bird present in a cultivated field (Kodalip, Khuzdar) maintained a safe distance from the approaching jeep. The bird avoided to take to its wings and tried to adjust its position in the field maintaining a distance of 200-300 m from the jeep, giving an impression that it cannot fly. However, when the chase persisted for some 15 minutes, the bird took a longer flight (1 km) and was lost in low undulates.

A lone female was present in a cultivated field (Kodalip, Khuzdar) where the wheat had just started sprouting. The bird remained almost unconcerned to the approaching jeep till it was some 400 m away. The bird then started moving briskly away from jeep to take shelter in a *Rhazya stricta* bush (0.2 m tall) and remained undetected to the workers in the jeep. The bird moved in the centre of the field when the jeep reached close to the bush (50 m). It continued walking briskly, showing cautious attitude, but did not opt to fly. On persistence of the chase the bird took a short flight and settled at about 100 m, in the distal part of the field. On a further chase, the bird took to its wings when the jeep was still some 200 m away, and settled after some 500 m on the other side of the hilly undulate. This bird remained in the cover of general vegetation, feeling itself safe from the approaching jeep till it reached a distance of some 50 m. At this stage, it moved around the bush (*Astragalus hyrcanus*), remaining undetected by the occupants of the jeep. When the bird was spotted, the jeep stopped suddenly in excitement of the occupants. It caused the bird to resort to flight and settle after taking a much longer flight with Houbara standards (about 2 km).

Dust storm

A pair of birds was spotted at about 11:30 a.m. in western Chagai, having a sparse vegetation in comparatively open area and a better cover in a dried water course with a gentle edges. A very low temperature (almost freezing) was prevailing and strong dust storm was on. The birds persisted around *Anabasis* sp. bushes. When the birds were disturbed by a directly approaching jeep, they moved out of the bushes into the open area. The male attempted flight, but almost immediately grounded itself. The bird went back into the water course, crossed it diagonally and settled in the side of one of the bushes. On persistence of the chase, the bird once again tried to become air borne; but decided against and found refuge in an other bush of *Anabasis* sp. (0.3 m high: 0.35 m diameter). The female was lost during this process and could not be studied for a similar behaviour.

A similar reaction was observed at another occasion (Kharan). The bird resorted to seek refuge in a shrub (0.1-0.2 m high) after finding itself incapable of taking to its wings.

Attacking falcon

A comparatively heavy male Houbara placed near a low bush squatted on the ground on seeing the attacking falcon. It fell flat with the head and the neck placed on the ground and slightly raised back. The tail feathers were prominent giving the appearance of a bush. The neck frill was erect. The bird remained undetected to the falcon.

At another occasion, a very heavy male was attacked by the falcon while it was on ground. The bird immediately erected its neck frill and tail feathers and started giving a tough fight, finally forcing the attacking falcon to give up. At this stage, a second falcon was allowed by the falconer, which too was finally exhausted by Houbara.

Human settlement

Houbara was generally observed at an average distance of some 2-3 km from human settlement of about 100 houses; at 3-4 km from a rather busy RCD highway in

Yakmuch; at about 1 km from a settlement of some 20-25 houses, at 750 m from a settlement of 5-10 houses. In Bisemah, the birds, however, came to a distance of 500-1,000 m from the town (about 500 houses), to forage during the night in the cultivated tracts (providing the only foraging grounds in the area). The birds retired to deeper parts of natural vegetation during the day. Occasional birds have been sometimes spotted in the vicinity of human setlements and/or busy road, especially when located in the mid of natural Houbara habitat.

Rest

Day time rest: In Patak (Punjgur), the birds moved to relative desolated hilly undulates after morning foraging, where these spent the middle parts of the day. Foot prints of some rather slow walking birds could be traced in depressions and general area; but a sitting and/or mark of a sitting bird could not be spotted, despite out hectic efforts. There was also no indication to suggest exploitation of the bush/bushes for day time rest.

In Sibi, the birds persistently retired to the dry general vegetation in the morning after night foraging in the cultivations. The birds could neither be spotted nor their day time activity be traced through foot prints in the presence of a comparatively stabilised soil.

At two occasions, the birds have been seen at about 10.00 a.m. in Kharan, walking along dried water courses moving towards desolated hilly undulates away from relatively disturbed general valley, having cultivated fields. The temperature appeared tolerable. The birds exhibited little interest in grazing and looked like moving casually. The birds persistently exploited depressions.

At about mid day, in Dasht-e-Kani (Turbat, temperature 30°C), a bird was observed walking rather casually and trying to exploit the shades of small shrubs of *Haloxylon salicornicum*. The bird though stood for some time exploiting the shade of prevalent shrubs, yet it kept on showing some movements of feet/body. Throughout the period the bird maintained an upright alert posture.

In different areas the birds persisted in the general vegetation and exhibited some degree of activity throughout the day. The level of the activity was low during the middle parts of the day. During low activity periods, the birds appeared to rest for a while along some protective bush. A low level of casual foraging continued during such periods.

Night roosting: A pair of roosting birds was spotted in the western Chagai. These birds were sitting at a distance of 2.5 m from each other in the dried water course having gentle edges. The roosting places were at a distance of 2-3 m from an Anabasis sp. bush (about 1 m in diameter). A bird was spotted sitting in the dried water course while roosting in Dalloh valley (Urmagai) during night. The bird selected its roosting place right in the middle of the water course having smooth edges and at a distance of 2.5 m from the nearest shrub. At two different points, Houbara was seen sitting in open tracts between 0.3-0.4 m tall shrubs in Harrah (Lasbella) valley.

The impressions of the sitting posture appearing on the loose sand suggested that the bird roost with its tarsi totally placed on the ground and bearing the total weight of the body. Very little of the other parts of the body appeared touching the sand. Bird appeared to have remained in the upright* position. At only one occasion, the faecal pellets were seen at the roosting place.

Camouflaging

A Houbara was spotted in Rakshan valley (Punjgur) in the centre of a cultivated field (some 1.5 hectare), having sprouting wheat but giving a general aspection of a loose sandy patch. On seeing the approaching men the bird became still, maintaining an upright posture. The bird matched with the background so perfectly that it remained undetected by approaching men (experienced hunters) for a considerable time, although they were pointed towards the general location of the bird and they were sure of its presence in the field. The bird was spotted through the rising sand trails, only when it started moving.

DISCUSSION

Detailed studies on behaviour require massive inputs in the form of manpower, time and finances so that patient observations could be recorded. Such studies are more difficult on Houbara in its wintering grounds, showing extensive movements. The radio tracking could be a useful alternate but in the absence of efficient trapping techniques (Taylor, 1985; Launay *et al.*, 1996) it has a little practical value in this bustard species. Physical observations on selected birds supported by track tracing has been previously exploited in Great Indian Bustard (Ali and Rahmani, 1982; Manakadan, 1985).

Some careful behavioural studies have been instituted in recent years on captive flock (Launay and Paillat, 1990; Combreau and Launay, 1996; Jacquet and Launay, 1996), but, no study is available on behavioural responses of the species under field conditions, except for some observations recorded on Canary Island race (Collins, 1985). The present study is grossly below a desired level and hence demands detailed studies; yet is the first to provide some direct field observations on this race.

On the basis of the available results, the following pattern of behavioural responses can be evolved.

Reaction to stimuli

- 1. Houbara moves away from the approaching stimulus or predator to find a suitable shelter *i.e.*, physical or vegetative cover. It spots the on-coming predator (man) or disturbance (jeep) from a distance of 300-500 m, when it starts taking protective measures, mainly relying upon its camouflaging armoury. However, its concern increases as the stimulus continues approaching, when it tries to find a suitable shelter. The bird takes to its wings only when it is sure that camouflaging is grossly insufficient and being air borne is safe. This is in general conformity with observations recorded in different reviews (Ali and Ripley, 1983; Cramp and Simmons, 1980).
- 2. Behaviour of the bird varies with recent experience and physiological state of the individual. It allows the approach of the stimulus to a closer distance (50 m) if it has not met a recent chase but flies away when the stimulus is still at a longer distance (300-400 m) if it was chased in recent past. However, on acclimatisation of the bird/birds to the

human activities, especially when these are not chase oriented, a little concern is exhibited to approaching man.

The response of the bird is more pronounced when it is alone. The intensity of response decreases as the number of the birds within visible range increases. This appears to be a group behaviour.

The severity of response also decreases while the bird is in its foraging grounds, especially where these are limited and the bird has to wait long before foraging. This appears to be a forced behaviour, where need for food dominates the protective and wary nature of the species.

3. The response of Houbara varies with the type and mode of application of the stimulus. The bird shows more concern to the chasing man than a slow moving gradually approaching jeep. Such a behaviour has been frequently reported from this area (Mian and Surahio, 1983; Mian, 1984, 1988) and has sometimes been exploited by the hunters.

The bird is less concerned about a slow moving jeep than when it stops. The stopping of the jeep probably results in a sudden change in the mode of application of the stimulus, when the reflexes of the bird go chaotic. Such a reflex action is universal in animals.

4. Different body postures indicate different psychological states of the bird. The upright and stretched neck indicate a cautious psychological state, while the outstretched but lowered neck reflects a severe concern. The relaxed posture suggests the lack of concern. These conclusions on the species behaviour have been partially reflected in previous accounts (Ali and Ripley, 1983; Cramp and Simmons, 1980) and studies (Collins, 1984; Hinz and Heiss, 1989; Launay and Paillat, 1990).

Change in the general posture, in response to strength of the stimulus, appears to have its own adaptive value for the species. A cautious bird requires to keep an eye on the activities of the on-coming stimulus. Upright neck places the eyes on a higher position, equipping the bird to better perceive the signals. Movements of the head facilitate eyes for a better perception of signals. Bird searching for a refuge can theoretically expect an attack from the stimulus (predator) and hence it has to move rapidly to a safer place. Outstretched low neck, under such conditions, can save the bird from a possible stoop on the head/neck, which can be fatal for the bird.

Flight

The bird takes a few brisk steps before being air borne. For the purpose the bird comes out of the denser shrub vegetation. This appears to be a basic requirement of this bird species, having heavy body and large wings. Open areas can save the larger wings from being entangled in the shrubs. Our studies with phytohabitat requirements of Houbara suggest that Houbara requires stretches of bare plains as part of its habitat and cannot live in thick growth of tall grasses, shrub or trees (Mian, unreported).

Human settlements

The distance maintained by Houbara from a human settlement directly increases with the increasing size of the settlement. This goes in line with many previous reports (Roberts and Savage, 1971; Surahio, 1981, 1982; Ali and Ripley, 1983; Mian and Surahio, 1983; Mian, 1984, 1988) suggesting that Houbara lives away from large human settlements though scattered nomadic camps have no influence on distribution of the species.

Rest

1. Available data suggest that the bird species does not exploit well-defined night roosting or day time resting sites. This is a general behaviour of bustards (Cramp and Simmons, 1980). The bird prefers open places for night roosting, but no roosting has been recorded in large stretches of bare plains and cultivations. This helps the bird to detect the on-coming predator (especially carnivores, like, fox, cat, jackal and/or wolf) and to take necessary protective measures. General absence of feathers and/or faecal material from the roosting sites suggests that Houbara continues changing its roosting places throughout the night. This suits the alert nature of the bird and may allow some casual foraging during night.

Present observations suggest that the bird cares a little to seek protection of the physical or vegetative obstacle against cold breeze while roosting. The race appears to prefer chill over sacrificing occlusion of its vision.

- 2. The sitting posture of the roosting bird suggests an alert state, similar to the one suggested previously for Houbara (Hinz and Heiss, 1989) and for Great Bustard (Hummel, 1985). This suggests that the bird remains active throughout the night. These findings go in conformity with the experiences of many hunters, suggesting that the bird is hard to be taken while asleep (Mian, 1988). Relaxed sitting posture has been suggested for captive flock (Launay and Paillat, 1990) but such a posture is still to be recorded for wild birds.
- 3. The activities of the bird though subsides considerably at middle part of the day, depending upon the physiological needs, available food and general temperature, yet it does not go for a complete mid day rest. It may however, retire from foraging tract to relatively desolated parts. This may be an adaptive behaviour for optimal utilisation of habitat through feeding and protective optima. There are some indications to suggest exploitation of bush/bushes for day time rest, especially where day time temperature rises to 30°C or above. This is in conformity with our previous report (Mian, 1988).

Dust storm

Houbara appears to minimise its activities and prefers to stay on ground during dust storm, especially when there is chill associated with it. This reaction appears to be equally shared by other desert birds. Hardly any bird activity can be observed under such conditions (Mian, unreported). This appears to be a protective behaviour of the birds in the highland cold deserts.

Falcon attack

A tough fight given by Houbara, especially by males, to the falcon has been previously recorded (Ali and Ripley, 1983). It appears that the bird can protect itself more efficiently while on ground as compared with that when it is in the air.

Camouflaging

A perfect camouflaging of Houbara with the desert background has been frequently quoted in literature (Cramp and Simmons, 1980). The camouflaging plumage has a special value when the bird is still. This behaviour is the first line of defence of the species.

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EFFECT OF ORAL ADMINISTRATION OF HEXAVALENT CHROMIUM ON TOTAL BODY WEIGHT, CHROMIUM UPTAKE AND HISTOLOGICAL STRUCTURE OF MOUSE LIVER

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Abstract: Administration of different concentrations (500, 750, 1000, 1500 and 2000 ppm) of potassium dichromate (K₂Cr₂O₇) mixed in drinking water to adult mice, *Mus musculus ad libitum* for 8 weeks resulted in dose-related decreased body growth and reduced feed intake, during the first 4 weeks of administration. Chromium was accumulated in the liver in a dose dependent manner, accompanied by some hepatolobular derangements in the liver. Excessive chromium deposition, retarded body growth and hepatotoxicity as indicated by histological abnormalities indicate dichromate toxicity.

Key words: Potassium dichromate toxicity, tannery effluent, Mus musculus, total body growth rate, heavy metal toxicity, metal induced pathological changes in liver.

INTRODUCTION

hromium is one of the heavy metals which in the ambient air, originates from industrial sources particularly ferrochrome production, ore refining, chemical and refractory processing and combustion of fossil fuels (Jworski, 1980). A controllable source of chromium is waste water from chrome plating and metal finishing industries, textile plants and tanneries (Cheremisinoff and Habib, 1972).

High concentration of chromium are toxic, carcinogenic, mutagenic and teratogenic (Petrilli and Deflora, 1977; Luli *et al.*, 1983; Nair and Krishnamurthi, 1991). Chromium (Cr) exists in two valance states *i.e.*, trivalent state (Cr⁺³) and hexavalent state (Cr⁺⁶) and these two forms differ markedly in a number of their biological properties (Levis and Bianchi, 1982). Hexavalent forms Cr(VI) are more toxic and mutagenic than the more common trivalent forms, Cr(III). Chromate and dichromate ions are strong oxidizing agents. Derivatives of Cr(III) are water soluble at neutral pH and can be removed from medium in the form of Cr hydroxide, while Cr(VI) are highly insoluble (Cary, 1982; Levis and Bianchi, 1982; Ohtake *et al.*, 1990; Yamamoto *et al.*, 1993; Vishnyakov *et al.*, 1992).

Chromium (III) is considered to be an essential trace nutrient serving as a component of the glucose tolerance factor (Mertz, 1969). It is a cofactor for insulin

action and has a role in the peripheral activities of this hormone by forming a tertiary complex with insulin receptors, facilitating the attachment of insulin to these sites.

Trivalent chromium salts have little or no mutagenic activity in bacterial system. Since there is preferred uptake of hexavalent form by cells and it is the trivalent form that is metabolically active and binds with nucleic acids within the cell, it has been suggested that the causative agent in chromium mutagenesis is trivalent chromium bound to genetic material after reduction of the hexavalent form.

The major lesions caused by acute and generally accidental poisoning with chromium (VI) involve the kidneys and liver (Franchini and Mutti, 1985). In workers which were exposed to chromium (VI), a mild severe rhinopathy was the most common ailment. It has also been shown that most affected organs are the digestive system, respiratory tract and the skin. In case of skin lesions, chronic ulcers and irritative dermatitis is attributable to the oxidizing properties of chromium (VI) and eczematous dermatitis caused by cutaneous sensitization.

The present study aims at determining the toxic effects of hexavalent chromium on the total body growth rate and relative growth rate of liver, heart and kidney of male albino mice *Mus musculus*, administered with K₂Cr₂O₇ in drinking water. Histopathological effects of hexavalent chromium have also been assessed in liver of mouse.

MATERIALS AND METHODS

Mice and their maintenance

One hundred mature male albino mice, *Mus musculus*, Swiss strain maintained in the Animal House of Department of Zoology (temperature $25\pm2^{\circ}$ C) were used for this study. The animals kept in iron cages in groups of three per cage, were fed on chick feed No.3 (Punjab Feeds, Lahore) and provided with drinking water *ad libitum*. Saw dust was used for bedding which was replaced on alternate days.

Six groups, one control and five experimentals, each of 9 mice, were maintained in separate cages. The control group was provided with simple drinking water, whereas experimental groups were administered with different concentrations (500, 750, 1000, 1500 and 2000 ppm) of aquous solution of potassium dichromate (K₂Cr₂O₇) as drinking water for 8 weeks. Total body weight was recorded every week, whereas daily water and feed intake was also recorded for each mice.

After eight weeks of experiment, the mice from each group were weighed and sacrificed. The blood was drained off. Different body organs including liver, heart and kidney, were dissected out, and wet weighed. For determination of their dry weights, these organs were placed in incubator at 120°C for 12 hours until the organs completely dried. Small pieces of liver from each group were fixed in Bouin's fixative for histological sections. Routine procedure for section cutting was adopted. Sections (6-8 μ m) were cut and stained with haematoxylin and eosin. The slides were studied under stereomicroscope and the selected sections were microphotographed.

Chromium accumulation in liver

Pieces of liver from control as well as experimental groups of mice were used for determination of Cr. Colorimetric method (Kunicka *et al.*, 1992) was followed for this purpose. One hundred milligrams of each tissue were taken and homogenized in 3 ml distilled water. After this 2 ml H₂SO₂ and 5 ml HNO₃ were added in each sample. The solutions were made 50 ml by adding distilled water and boiled for 15 minutes, cooled, filtered and again made 50 ml each. The pH was adjusted at 0.8 by using 0.2N H₂SO₄. The final volume of each sample was made 100 ml and 2 ml color reagent, diphenyl carbazide solution (0.5% in acetone) was added. After 10 minutes the absorbance was noted at 540 nm and Cr concentration was calculated according to the method.

All the data were analysed by using Student's 't' test.

RESULTS

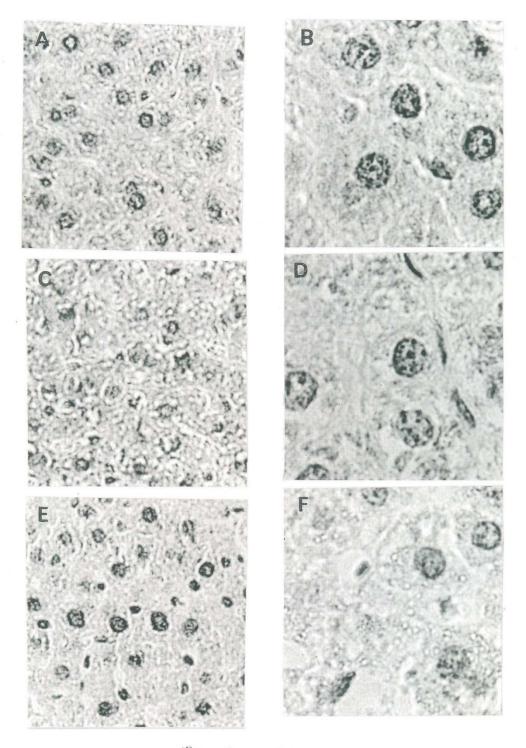
Body weight

Table I shows effect of $K_2Cr_2O_7$ administration on the total body weight of male mice. The mice on the average, weighed 30.66 ± 3.72 g on the first day of the experiment in controls. After two weeks the body weight increased 5% and after 4 weeks the increase was 12.5%, showing thereby an average increase of 0.42% per day (0.128 g/day). Administration of CrVI reduced the total body weight. Within one day $K_2Cr_2O_7$ administration, the body weight decreased 27% and 18% after 1500 and 2000 ppm of CrVI, respectively. After 2 weeks of CrVI administration, the decrease was 5%, 7.25%, 28.5% and 14.5% after 500, 750, 1500 and 2000 ppm. After 4 weeks of CrVI administration at a dose of 500, 750, 1000, 1500 and 2000 ppm, the decrease in body weight was respectively, 8.7%, 10.64%, 15.48%, 28.52% and 20.29%.

The average growth rate in mice administered with 500 ppm, 750 ppm, 1000 ppm, 1500 ppm and 2000 ppm was 0.036% per day (0.011 g/day), 0.170% per day (0.05 g/day), 0.31% per day (-0.022 g/day), 0.348% per day (0.077 g/day), 0.31% per day (0.078 g/day) as against 0.42% per day (0.128 g/day) of control mice.

Liver, kidney and heart weights

Table II shows the effect of $K_2Cr_2O_7$ on the relative weights of liver, kidney and heart. After 8 weeks of CrVI administration the liver of control mice had 0.45 ± 0.05 (wet weight) and 0.15 ± 0.08 gm (dry weight). After hexavalent Cr treatment, both the wet and dry weights of liver increase significantly. After administration of CrVI at a dose of 500, 750, 1000, 1500 and 2000 ppm, the increase in liver weight is respectively, 5.11 fold, 4.4 fold, 3.33 fold, 5.62 fold and 2.6 fold. The increase in dry weight is respectively 7.73 fold, 2.13 fold, 2.06 fold, 2.6 fold and 2.06 fold, respectively. The control liver has 66.66% of water content, whereas in 500, 750, 1000, 1500 and 2000 ppm groups the water content of liver constitute 49.56%, 83.84%, 79.33%, 84.58% and 73.50%, respectively.



(For captions, see facing page)

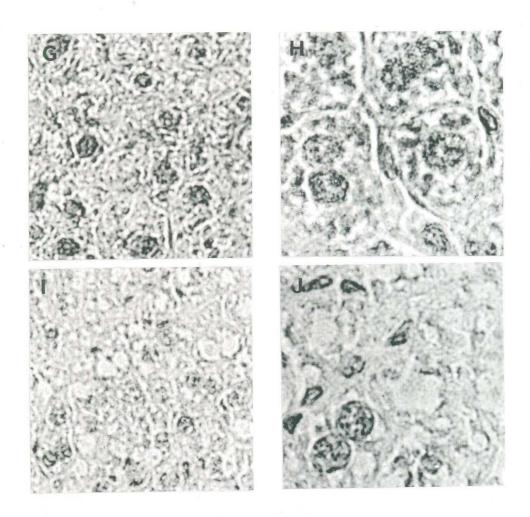


Fig. 1: Histological structure of liver of mouse given different concentrations of hexavalent chromium. A and B: Control group with quite normal hepatic structure. C and D: 750 ppm dose group showing increased sinusoidal space. E and F: 1000 ppm dose group with hepatic fibrosis and cirrhosis. G and H: 1500 ppm dose group with increased sinusoidal space and fibrosis. I and J: 2000 ppm dose group showing increased sinusoidal space, cirrhosis and nuclear pyknosis.

Table I: Effect of chromium on body weight of mice exposed to different concentrations of $K_2Cr_2O_7$.

Treatment	DOSE (ppm) K ₂ Cr ₂ O ₇					
(day)	Control	500	750	1000	1500	2000
lst	30.66a	31.16	29.33	29.83	22.33**	25.16
	± 3.72	± 1.67	± 5.73	± 4.13	± 4.26	$\pm 4.37^{*}$
7th	31.33	30.00*	28.33	28.16	21.83***	24.16*
*	± 1.88	± 1.29	± 4.85	± 4.21	± 3.43	± 3.89
15th	32.16	30.66*	29.83	29.83	23.00***	27.50*
	± 1.77	± 1.37	± 4.91	± 4.01	± 2.76	+3.40
22nd	33.83	29.83**	29.33**	28.00***	23.83***	28.16*
	± 2.91	± 0.68	± 3.90	± 3.91	± 1.95	+3.23
30th	34.50	31.50**	30.83*	29.16**	24.66***	27.50*
	± 2.36	± 1.38	± 4.17	+3.48	± 1.49	+2.21

 $a = Mean \pm S.D. (gm); * = P < 0.05; ** = P < 0.01; *** = P < 0.001.$

Table II: Effect of K₂Cr₂O₇ on different body organs of mice.

K2Cr2O7		LIV	LIVER		KIDNEY		ART
(ppm)	Body	Wet	Dry	Wet	Dry	Wet	Dry
Control (9)	30.66ª	0.45	0.15	0.25	0.07	0.07	0.05
	± 3.72	± 0.05	± 0.08	± 0.02	± 0.01	± 0.02	± 0.01
500 (9)	31.50	2.30	1.16	1.16	0.13	1.17	0.02
	± 1.38	± 0.08	± 0.08	± 0.08	± 0.05	± 0.02	± 0.01
750(9)	30.83	1.98	0.32	1.18	0.03	1.17	1.02
	± 4.18	± 0.45	± 0.03	± 0.02	± 0.01	± 0.01	± 0.01
1000 (9)	29.16	1.50	0.31	1.16	0.02	1.16	0.02
	± 3.48	± 0.59	± 0.02	± 0.05	± 0.01	± 0.01	± 0.01
1500 (9)	24.66	2.53	0.39	1.22	0.04	1.17	0.62
	± 1.49	± 0.27	± 0.08	± 0.03	± 0.01	± 0.01	± 0.07
2000 (9)	27.50	1.17	0.31	1.16	0.03	1.16	0.02
	± 2.21	± 1.06	± 0.06	± 0.02	± 0.01	± 0.04	± 0.00

 $a = Mean \pm S.D.$ (gm); () = No. of animals.

Table III: Effect of different concentrations of K₂Cr₂O₇ on feed intake by mice.

		K ₂ Cr ₂ O ₇ (ppm)						
Weeks	Control (n=9)	500 (n=9)	750 (n=9)	1000 (n=9)	1500 (n=9)	2000 (n=9)		
1	5.04a	4.37*	5.42	4.25*	3.81**	4.34		
1	+1.11	+0.77	+0.54	±1.06	±0.98	± 0.95		
2	6.20	5.85	5.45**	5.30**	4.34***	5.58**		
2	+1.02	+0.74	+0.54	±0.52	± 0.73	± 0.40		
	6.61	6.17*	5.38***	5.92**	5.18***	6.08**		
9	+0.29	+0.38	+0.57	± 0.65	±0.30	± 0.64		
4	7.22	6.30**	5.75***	5.55***	5.35***	5.10***		
4	+0.78	±0.69	±0.38	±0.51	± 0.22	±0.20		

a = gm/animal (Mean \pm S.D.); * = Significantly decreased against controls (P<0.05); ** = Significantly decreased against controls (P<0.01); *** = Significantly decreased against controls (P<0.001).

Table IV: Accumulation of chromium ($\mu g/gm$) in liver of mice administered orally different aquous concentrations of $K_2Cr_2O_7$.

Dose	K ₂ Cr ₂ O ₇ treat	K ₂ Cr ₂ O ₇ treatment (weeks)			
(ppm)	4	8			
Control (n = 4) 500 (n = 4) 750 (n = 4) 1000 (n = 4) 1500 (n = 4) 2000 (n = 4)	0.0106 ± 0.0005 $0.0180 \pm 0.0003^{***}$ $0.0163 \pm 0.0002^{***}$ $0.0225 \pm 0.0008^{***}$ $0.0210 \pm 0.0008^{***}$ $0.0615 \pm 0.0011^{***}$	0.0146 ± 0.0001 $0.0393\pm0.0011^{***}$ $0.1147\pm0.0027^{***}$ $0.0555\pm0.0023^{***}$ $0.1155\pm0.0055^{***}$ $0.1428\pm0.0015^{***}$			

After 8th week, the kidney of controls weighed 0.25±0.02 (wet) and 0.07±0.01 (dry) gm. The weight of kidney increased 2.32, 4.72, 2.32, 4.88 and 2.32 fold after administration of CrVI at 500 ppm, 750 ppm, 1000 ppm, 1500 ppm and 2000 ppm, respectively. This increase for the dry weight was respectively 1.85 fold, 0.428 fold, 0.285 fold, 0.571 fold and 0.428 fold. The control kidney had 72% water content, whereas in mice administered with CrVI at 500 ppm, 750 ppm, 1000 ppm, 1500 ppm and 2000 ppm, kidney had water content 88.8%, 94.91%, 98.27%, 96.72% and 97.41%, respectively. The dry and wet weights of heart also follow almost the same pattern. After 8 weeks of experimental period the heart in control mice weighed 0.11±0.01 g (wet weight) and 0.03±0.01 g (dry weight).

The heart wet weight in treated mice is about 17 fold that of control. Likewise the dry weight of heart is increased 0.4 fold, 20.4 fold, 0.4 fold, 12.4 fold and 0.4 fold, respectively in mice group administered with 500, 750, 1000, 1500, and 2000 ppm K₂Cr₂O₇.

DISCUSSION

The greater toxicity of hexavalent chromium (VI) may be due to its higher rate of absorption through gastrointestinal tracts (Danielsson *et al.*, 1982; Outridge and Scheuhammer, 1993).

All these observations indicate that hexavalent chromium (VI) has caused toxic effects even at low concentrations, when these were given for a long time. These findings are quite in conformity with some previous studies done to evaluate intoxication of hexavalent chromium (VI) in biological systems.

The trivalent chromium Cr(III) is a dietary requirement in trace amounts (Starich and Blincoe, 1983), but hexavalent chromium has been shown by epidemiological studies to cause respiratory cancers (EPA, 1984; Langard, 1990). Witmer *et al.* (1989, 1991) reported tissue distribution of chromium after 14 days of oral administration of sodium and calcium chromates to the rats. The chromium level in blood was highest (1800 μ mole Cr/gm) while other body organs including liver, kidney, lung, muscle, heart, skin, bone and gonads also contained high concentrations of chromium.

During some other studies, fatal poisoning was caused by the ingestion of 1-3 gm of chromate compounds (Kaufmann *et al.*, 1970; Pedersen and Morch, 1978). This concentration is much more higher than the normal chromium concentration, which is known to be 20-30 mg/ml in the blood and 15-40 mg/gm in the liver and kidney (Hyodo *et al.*, 1980; Goyer, 1986).

Kim and Na (1990) investigated acute toxic effects of sodium dichromate on metabolism of rats. Intraperitoneal injection of sodium dichromate (20 or 40 mg/kg) caused significant increases in serum lactate, pyruvate and creatinine concentrations within 15 min after intoxication. Severe hypoglycemia occurred thereafter, as a result of increased hepatic glycogenolysis. Serum total amino acids were decreased, with a subsequent increase in blood urea nitrogen concentration. In another study, Cr(VI) has been found cytotoxic in osteogenic cells (Puleo and Huh, 1995; Thompson and Puleo, 1995).

The oxidation state of chromium is a critical factor in determining the activities of various chromium compounds in experimental tests (Mathur *et al.*, 1977). Regardless of chromium species, only the hexavalent chromium compounds had an effect on the cellular metabolism. Chromium (VI) compounds, unlike most other metal compounds, are actively transported through cell membranes and thus can reach genetic targets. Consequently, the genotoxic effects of chromium have been studied with a variety of short-term tests covering the entire range of genetic damages (Sunderman, 1978; Bianchi *et al.*, 1983).

Intercellular reduction of chromium occurs either in the cytoplasm or in the nucleus. The intercellular reduction generates the carcinogenic chromium species (DeFlora and Wetterhahn, 1989). Chromium (V) is considered to be a candidate for one of these ultimate carcinogenic species since it has been observed both *in vivo* during the biological reduction of Cr(VI) to the final intercellular stable oxidation state (Cr(III).

Recently, Sugden and Wetterhahn (1996) have suggested formation of Cr(IV) upon disproportionation of Cr(V). Chromium (IV) oxidizes the nucleotide deoxyribose sugar

moiety via a phosphate-bound intermediate. A consequence of this intercellular reduction is the production of a wide array of adverse biological effects including DNA lesions induced by oxidative stress (DNA strand breaks) and chromium directly metalated to the DNA strands (Cr-DNA adducts). At the genetic level, chromium has been shown to affect the expression of some inducible genes and to interact with oxidative stress inducible transcription factors in the signal transduction pathway.

In a recent study on toxicity of chromium (VI) on osteogenesis, chromium appeared to produce a substantial decrease in calcium incorporation, especially at doses between 5 and 10% TC₅₀ (Thompson and Puleo, 1995).

It is concluded that the chronic exposure of mice to comparatively high concentrations of potassium dichromate (500, 750, 1000, 1500, 2000 ppm) during the present study found toxic for mice. In spite of chromium in traces being essential for living systems, exposure to high concentrations of hexavalent chromium is dangerous.

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NEW TERMITES FROM IRAN*

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Abstract: Termite fauna of Iran was surveyed during 1990 to 1995. As a result, 19 species were collected from Iran. In the present report five new species, Postelectrotermes bidentatus n.sp., P. zabulniensis n.sp., Anacanthotermes gurganiensis n.sp., Psammotermes prohybostoma n.sp. and Amitermes paravilis n.sp. are described. Different morphological features, their ranges, standard deviation, mean values and co-efficient of variability are discussed.

Key words: Termite, new species, Iran.

INTRODUCTION

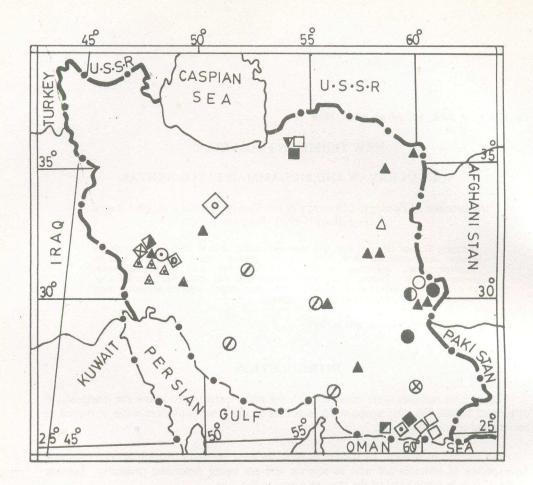
Studies on termites were initiated with the main intention to know the composition of termite fauna. For this purpose 75 localities in different provinces were surveyed to understand termite diversity.

As a result of survey, 19 species of termites were collected from Iran. Out of these, five species of termites are new to science and are being described presently. Termite fauna of Iran is comprised of the species given in the map.

MATERIALS AND METHODS

he present studies are based on the collection of termites from different provinces of Iran, except Kurdistan and Azarbijan that could not be sampled.

Termite colonies collected from the field and houses were given field number and preserved in 80% ethanol, for subsequent studies in the laboratory. The specimens were examined under Leitz Stereoscopic microscope with built-in magnification changer.



- O Postelectrotermes bidentatus, n.sp.
- Postelectrotermes pasniensis Akhtar
- O Postelectrotermes zabuliensis, n.sp.
- ▼ Anacanthotermes gurganiensis, n.sp.
- △ Anacanthotermes iranicus Ravan & Akhtar
- Anacanthotermes turkestanicus (Jacobsen)
- ▲ Anacanthotermes vagans (Hagen)
- Psammotermes prohybostoma, n.sp.
- Psammotermes rajasthanicus Chhotani
 & Bose

- ♦ Heterotermes indicola (Wasmann)
- Microtermes mycophagus (Desneux)
- Microcerotermes buettikeri Chhotani
 & Bose
- Microcerotermes diversus (Silvestri)
- o Microcerotermes gabrielis Weidner
- ♦ Amitermes baluchistanicus Akhtar
- Amitermes paravilis, n.sp.
- ☐ Amitermes stephensoni Harris
- ♠ Amitermes vilis (Hagen)

Map of Iran, showing distribution of termite species.

Measurements were taken with the aid of an ocular micrometer. Drawings were made with the help of a Camera Lucida. The type material and duplicate specimens are distributed as follows:

Holotypes: Deposited in the Department of Zoology, Entomology Laboratory, presently under the custody of Prof. Dr. Muhammad Saeed Akhtar, Paratypes and duplicates: in the Department of Zoology, University of the Punjab, Lahore; and in Iran.

Terminology

Taxonomic terms and measurements presently used are as explained by Light and Wilson (1936), Emerson (1945, 1952), Ahmad (1950, 1955a, 1965), Krishna (1961) and Akhtar (1975). Standard deviation (S.D.) and co-efficient of variability (C.V.) was worked out according to Sokel and Rohlf (1973).

Postelectrotermes bidentatus, n.sp.

Major soldier (Fig. 1)

Head light reddish brown, area near frons and antennal carinae darker than head, dark brown; antennae and labrum light brown; mandibles reddish brown at bases, black

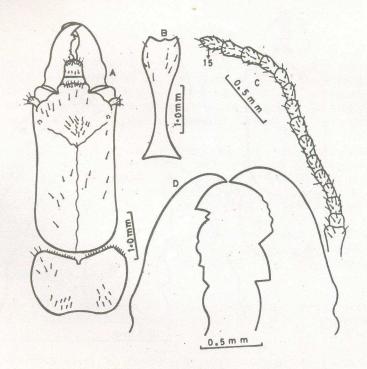


Fig. 1: Major soldier of *Postelectrotermes bidentatus*, new species: A, head and pronotum from above; B, postmentum from below; C, antenna; D, mandibles.

above; postclypeus darker than head; pronotum slightly lighter than head, meso-andmetanota lighter than pronotum; abdomen and legs brownish yellow. Head with a few scattered hairs; labrum with few long bristles on anterior margin and a few short bristles in middle of disc; postclypeus with distinctly long bristles near anterior margin. Postmentum with a few bristles on anterior margin; pronotum with few short hairs. Head elongate, rectangular, lateral sides, slightly depressed in middle; antennal carinae distinct, lighter than head; Y-suture present; ocelli area below antennal carinae weakly indicated. Labrum dome-shaped, sides weakly convex; anteclypeus white, trapezoid. Mandibles long, stoutly built, basal humps distinct; left mandible with apical, 5 marginal teeth and molar plate, inner cutting edge of the apical tooth concave; first marginal teeth well developed second located very close to first tooth, third, fourth and fifth weakly developed; right mandible with two well developed marginal teeth. Postmentum long, club-shaped, widest in anterior half, narrowest below middle (Table I). Antennae with 13-15 segments, first longest; second shortest. Pronotum wider than head, anterior margin deeply concave, lateral sides weakly convex, posterior side indistinctly emarginate in middle. Tibial spurs 3:3:3; mid tibia with an extra spine close to outer apical spur. Tarsi four-jointed.

Minor soldier (Fig. 2)

Head light reddish brown, area near frons and antennal carinae darker than head, dark brown; antennae and labrum light brown; mandibles reddish brown at bases, black

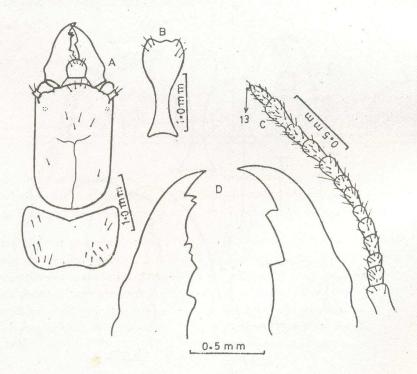


Fig. 2: Minor soldier of *Postelectrotermes bidentatus*, new species; A, head and pronotum from above; B, postmentum from below; C, antenna; D, mandibles.

above; postclypeus darker than head; pronotum, meso-and-metanota lighter than head; abdomen and legs brownish yellow. Head with a few scattered hairs; labrum with a few long bristles; postclypeus with a few long bristles near anterior margin; pronotum with a few bristles on anterior margin. Postmentum with a few short bristles. Head elongate, rectangular, lateral sides, parallel; antennal carinae distinct, lighter than head; Y-suture present; ocelli area below antennal carinae weakly indicated. Labrum dome-shaped, sides weakly convex; anteclypeus white. Mandibles, short, well developed, basal humps distinct; left mandible with apical, 5 marginal teeth and molar plate, inner cutting edge of the apical tooth concave, first marginal tooth well developed, second located very close to the first, third, fourth and fifth weakly developed; right mandible with two well developed marginal teeth. Postmentum, club-shaped, widest in anterior half narrowest below the middle (Table II). Antennae with 13-15 segments, first longest; third shortest. Pronotum wider than head, anterior margin deeply concave, lateral sides weakly convex; posterior sides indistinctly emarginate in middle. Tibial spur 3:3:3; mid tibia with an extra spine close to outer apical spur. Tarsi four-joined.

Table I: Measurements (in mm) of major soldiers of *Postelectrotermes bidentatus*, n.sp. (number of specimens measured is given in parenthesis).

	Locality: Zabul (Jalahi)				
	Holotype	Range (n=3)	Mean \pm S.D.	C.V.	
Length of head to side base of mandibles	3.50	3.00-3.50	3.20±0.26	8.12	
Length of left mandible	1.60	1.50-1.60	1.43 ± 0.06	3.72	
Length of right mandible	1.60	1.50-1.60	1.53 ± 0.06	3.72	
Width of head at side base of mandibles	1.45	1.30-1.45	1.38 ± 0.08	5.50	
Width of head at postero-lateral ends of antennal carinae	1.60	1.40-1.60	1.53±0.11	7.18	
Maximum width of head	1.85	1.65-1.85	1.76 ± 0.10	5.68	
Length of postmentum	2.70	2.65-2.70	2.66 ± 0.03	1.05	
Maximum width of postmentum	0.85	0.80-0.85	0.83 ± 0.03	3.37	
Minimum width of postmentum	0.30	0.25-0.30	0.28 ± 0.03	10.00	
Length of pronotum	1.25	1.10-1.25	1.15 ± 0.09	7.47	
Width of pronotum	2.25	1.90-2.25	2.08 ± 0.17	8.17	

Comparisons

The soldier of *P. bidentatus* comes close to *P. pasniensis* described from Pakistan, but differs in having first and second marginal teeth located very closely.

Type locality

Zabul (Jalahi) (Type locality), major soldiers (Holotype, paratype), minor soldiers (Morphotype, Paramorphotype), nymph, collected by S. Ravan on 16.6.1991 from *Populus caspica*.

Table II: Measurements (in mm) of minor soldiers of *Postelectrotermes bidentatus*, n.sp. (number of specimens measured is given in parenthesis).

	Locality: Zabul (Jalahi)				
	Holotype	Range (n=3)	Mean \pm S.D.	C.V.	
Length of head to side base of mandibles	2.85	2.15-2.85	2.50±0.49	19.60	
Length of left mandible	1.45	1.25 ± 1.45	1.35 ± 0.14	10.37	
Length of right mandible	1.50	1.20-1.50	1.35 ± 0.21	15.55	
Width of head at side base of mandibles	1.30	1.15-1.30	1.22 ± 0.10	8.19	
Width of head at postero-lateral ends of antennal carinae	1.50	1.30-1.50	1.40 ± 0.14	10.00	
Maximum width of head	1.70	1.55 ± 1.70	1.62 ± 0.10	6.17	
Length of postmentum	2.35	1.35-2.35	1.85 ± 0.70	37.83	
Maximum width of postmentum	1.00	0.75-1.00	0.87 ± 0.17	19.54	
Minimum width of postmentum	0.30	0.25-0.30	0.27 ± 0.04	12.96	
Length of pronotum	1.05	0.90-1.05	0.97 ± 0.10	10.30	
Width of pronotum	2.00	1.75-2.00	1.87 ± 0.17	. 9.09	

Postelectrotermes zabuliensis, n.sp.

Major soldier (Fig. 3)

Head light reddish brown, area near postclypeus and antennal carinae darker than rest of head; antennae and labrum light brown; mandibles dark brown at bases, black

above; pronotum, meso-and-metanota lighter than head; abdomen and legs yellowish brown. Head with scattered hairs and a few bristles; labrum with a few long bristle on anterior margin and a few in middle; postclypeus with a few long bristle near anterior margin. Postmentum with a few bristle on anterior half; pronotum with few scattered hairs. Head elongate, rectangular, sides slightly depressed in middle; antennal carinae distinct; Y-suture present; ocelli area below antennal carinae weakly indicated. Labrum dome-shaped, sides weakly convex. Anteclypeus white, trapezoid. Mandibles long, stoutly built, basal humps distinct; left mandible with apical and 4 marginal teeth and molar plate, inner cutting edge of the apical tooth concave, first marginal tooth well developed, second, third and fourth weakly developed; right mandible with two well developed marginal teeth. Postmentum long, club shaped, widest in anterior half, narrowest below middle (Table III). Antennae with 13-15 segments, first longest, second and third sub-equal. Pronotum wider than head, anterior margin deeply concave, lateral sides weakly convex. Tibial spurs 3:3:3; mid tibia with an extra close to outer apical spur. Tarsi four-jointed.

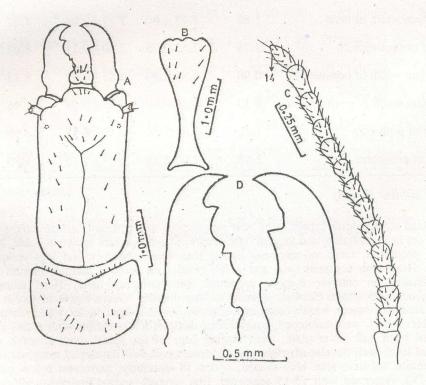


Fig. 3: Major soldier of *Postelectrotermes zabuliensis*, new species: A, head and pronotum from above; B, postmentum from below; C, antenna; D, mandibles

Table III: Measurements (in mm) of major soldiers of Postelectrotermes zabuliensis, n.sp. (number of specimens measured is given in parenthesis).

		Locality: 2	Zabul (Jalahi)	
	Holotype	Range (n=3)	Mean±S.D.	C.V.
Length of head to side base of mandibles	3.20	2.95-3.20	3.07±0.17	5.53
Length of left mandible	1.44	1.45-1.55	1.50 ± 0.07	4.66
Length of right mandible	1.55	1.45-1.55	1.50±0.07	4.66
Width of head at side base of mandibles	1.40	1.35-1.40	1.37±0.04	2.55
Width of head at postero-lateral ends of antennal carinae	1.60	1.50-1.60	1.55±0.07	4.51
Maximum width of head	1.80	1.75-1.80	1.77 ± 0.04	1.97
Length of postmentum	2.75	2.45-2.75	2.60 ± 0.21	8.07
Maximum width of postmentum	0.90	0.80-0.90	0.85 ± 0.07	8.23
Minimum width of postmentum	0.30	0.25-0.30	0.27 ± 0.04	2.96
Length of pronotum	1.15	1.05-1.15	1.10±0.07	6.36
Width of pronotum	2.15	2.00-2.15	2.07 ± 0.10	4.83

Minor soldier (Fig. 4)

Head light reddish brown, area near postclypeus and antennal carinae darker than rest of the head; antennae and labrum light brown; mandibles dark brown at bases, black above; pronotum, meso-and-metanota lighter than head; abdomen and legs yellowish brown. Head with scattered hairs and a few bristles on anterior margin; labrum with long bristles on anterior margin; pronotum with scattered hairs. Head elongate, rectangular, sides almost parallel; antennal carinae distinct; Y-suture present, ocelli area below antennal carinae weakly indicated. Labrum dome-shaped, sides weakly convex. Mandibles, short, well developed, basal humps distinct; left mandible with apical and 4 marginal teeth and molar plate, inner cutting edge of the apical tooth concave, first marginal tooth well developed; right mandible with two well developed marginal teeth. Postmentum, medium size, club-shaped, widest in anteriorly, narrowest below middle (Table IV). Antennae with 13-15 segments, first longest, second and third sub-equal. Pronotum wider than head, anterior margin deeply concave, lateral sides almost parallel and weakly convex. Tibial spurs 3:3:3; mid tibia with an extra spine close to outer apical spur. Tarsi foun-jointed.

Table IV: Measurements (in mm) of minor soldiers of Postelectrotermes zabuliensis, n.sp. (number of specimens measured is given in parenthesis).

		Locality: Z	abul (Jalahi)	
• • • • • • • • • • • • • • • • • • •	Holotype	Range $(n=3)$	Mean±S.D.	C.V.
Length of head to side base of mandibles	2.40	2.25-2.40	2.32±0.10	4.31
Length of left mandible	1.40	1.30-1.40	1.35 ± 0.07	5.18
Length of right mandible	1.40	1.30-1.40	1.35 ± 0.07	5.18
Width of head at side base of mandibles	1.25	1.20-1.25	1.22±0.04	2.86
Width of head at postero-lateral ends of antennal carinae	1.40	1.35-1.40	1.37±0.04	2.55
Maximum width of head	1.70	1.60-1.70	1.65 ± 0.07	4.24
Length of postmentum	1.95	1.50-1.95	1.72 ± 0.31	18.02
Maximum width of postmentum	0.80	0.75-0.80	0.77 ± 0.04	4.54
Minimum width of postmentum	0.30	0.30-0.30	0.30 ± 0.00	0.00
Length of pronotum	0.90	0.85-0.90	0.87 ± 0.04	4.02
Width of pronotum	1.85	1.70-1.85	1.77 ± 0.10	5.64

Comparisons

Postelectrotermes zabuliensis, n.sp., can be distinguished by the known species in having first and second of left mandible marginal teeth widely separated.

Type locality

Zabul (Jalahi) (type locality), major soldier (holotype, paratype), minor soldiers (morphotype, paramorphotypes), nymphs, collected by S. Ravan and Noor-u-din-Farooqi on 20.6.91 from *Populus caspica*.

Anacanthotermes gurganiensis, n.sp.

Major soldier (Fig.5)

Head dark reddish brown; antennae, lighter than head; postclypeus slightly darker than head; antennal carinae nearly as dark as postclypeus; mandibles dark reddish brown at bases, blackish above; pronotum, mesonotum and metanotum lighter than head, meso-

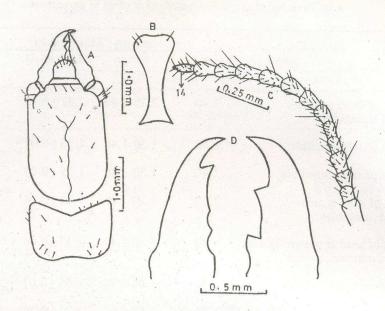


Fig. 4: Minor soldier of *Postelectrotermes zabuliensis*, new species: A, head and pronotum from above; B, postmentum from below; C, antenna; D, mandibles.

and-metanota with two round spots near anterolateral corners along posterior margin; abdomen and legs yellowish brown. Head with scattered hairs and bristles; postclypeus with a few hairs and long bristles near anterior margin; labrum with scattered hairs and bristles in the middle of disc; postmentum with a few long bristles in anterior half; pronotum with a few scattered hairs on the anterior margin and scattered bristles on posterior half. Head squarish, sides almost parallel, without depression in middle; antennal carinae well developed; Y-suture present; eyes small with lateral sides slightly convex; ocelli weakly developed, minute spots, slightly incurved. Left mandible with apical and 4 marginal teeth; molar plate protuberance like, inner cutting edge of the apical tooth, anterior edge of first marginal tooth of left mandible convex from tip to junction of the inner edge of apical tooth, posterior cutting edge of first marginal tooth concave below tip to slightly convex at base, second marginal tooth with anterior edge more convex than that the of first marginal tooth, 3rd and 4th marginal tooth minute elevation molar plate without serrations; right mandible with inner cutting edge of apical convex at base; concave above tip to anterior margin of first marginal tooth convex; posterior margin concave in upper half; second marginal tooth with anterior margin convex, posterior margin concave in anterior half, molar plate with distinct serration. Postmentum medium size, flate, variable shape, widest in middle (Table V). Antennae with 25-26 segments, the first longest, second twice as long as third, which is shortest. Pronotum well developed raised anteriorly in middle, posterior margin slightly depressed in middle; mesonotum with weak depression below anterior lateral corners,

lateral sides convex below, posterior margin wavy, depressed in middle. Tibal Spurs 3:4:4. Tarsi fourjointed.

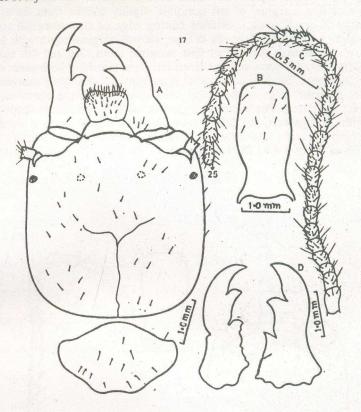


Fig. 5: Major soldier of Anacanthotermes gurganiensis, new species: A, head and pronotum from above; B, postmentum from below; C, antenna; D, mandibles.

Minor soldier (Fig. 6)

Head reddish brown, antennae lighter than head; postclypeus slightly darker than head; labrum, antennal carinae slightly lighter than postclypeus; mandibles dark reddish brown at bases, dark black above; pronotum, mesonotum lighter than head; metanotum lighter than mesonotum; in mesonotum two round sports near anterolateral corners and two thick lines along posterior margin present. Legs yellowish brown. Head with scattered hairsl and bristles; postclypeus with a few hairs and long bristles along anterior margin; labrum with scattered hairs and bristles in middle of disc; postmentum with a few long bristles in anterior half; pronotum with a few scattered hairs on the anterior margin and scattered bristles in posterior half. Head squarish, sides almost parallel. Antennal carniae well developed; Y-suture present; eyes small, ocelli weakly developed, minute spots, slightly raised than surface of head. Anteclypeus, white, trapezoid.

Mandibles well developed, slightly incurved; left mandible with apical and 4 marginal teeth, molar plate protuberance like; inner cutting edge of the apical tooth concave; anterior edge of first marginal of left mandible slightly convex from tip to junction of the inner edge of apical tooth, posterior cutting edge of first marginal tooth slightly concave in upper half; slightly convex at base; second marginal tooth with anterior edge slightly more convex than that the of first marginal tooth, 3rd and 4th marginal teeth minute elevations; molar plate without serration; right mandible with inner cutting edge of first marginal tooth. Slightly concave; first marginal tooth slightly convex from tip to junction with the inner edge of the apical tooth posterior margin concave; molar plate with indistinct serrations. Postmentum, widest in middle; variable in shape (Table VI). Antennae with 25 segments, the first longest, second twice as long as third, pronotum well developed raised anteriorly in middle, antero-lateral corner with short projection. Tibial Spurs 3:4:4. Tarsi four-jointed.

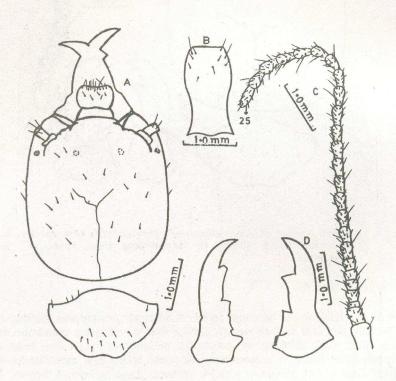


Fig. 6: Minor soldier of Anacanthotermes gurganiensis, new species: A, head and pronotum from above; B, postmentum from below; C, antenna; D, mandibles.

Table V: Measurements (in mm) of major soldiers of Anacanthotermes gurganiensis, n.sp. (number of specimens measured is given in parenthesis).

		Locality	: Gurgan	
	Holotype	Range $(n=3)$	Mean±S.D.	C.V.
Length of head to side base of mandibles	3.80	3.10-3.80	3.50±0.36	10.28
Length of left mandible	2.40	2.25-2.50	2.38 ± 0.12	5.04
Length of right mandible	2.15	1.95-2.30	2.13 ± 0.17	7.98
Width of head at side pase of mandibles	2.60	1.85-2.60	2.23±0.37	16.59
Width of head at postero-lateral ands of antennal carinae	2.70	1.95-2.70	2.38±0.38	15.96
Maximum width of head	3.60	3.10-3.60	3.41±0.27	7.91
ength of postmentum	2.25	1.65-2.25	1.93 ± 0.30	15.54
Maximum width of postmentum	1.20	1.00-1.25	1.15±0.13	11.30
Minimum width of postmentum	0.80	0.70-0.80	0.76±0.06	7.50
ength of pronotum	1.50	1.40-1.50	1.46±0.06	3.90
Width of pronotum	2.70	2.25-2.70	2.51±0.23	9.16

Comparisons

The soldier of A. gurganiensis, comes close to A. peshawarensis, described from Pakistan. Howevfer, it can be distinguished by its antennae having 25 segments (29 in A. peshawarensis Akhtar).

Type locality

Gurgan (Tappay Mirza biayl), (Type locality); Soldiers (Holotype, Paratype), nymph, collected by S. Ravan on 8.8.90 from A. cinerascens and S. babylonica.

Table VI: Measurements (in mm) of minor soldiers of Anacanthotermes gurganiensis, n.sp. (number of specimens measured is given in parenthesis).

- Company - Residence - Company - Co				
		Localit	y: Gurgan	
	Holotype	Range $(n=3)$	Mean±S.D.	C.V.
Length of head to side base of mandibles	2.95	2.36-2.95	2.65 ± 0.42	15.84
Length of left mandible	2.10	2.00-2.10	2.05 ± 0.07	3.41
Length of right mandible	1.90	1.90-1.90	1.90 ± 0.00	0.00
Width of head at side base of mandibles	1.80	1.80-1.95	1.87 ± 0.10	5.34
Width of head at postero-lateral ends of antennal carinae	1.90	1.90-1.95	1.92 ± 0.04	1.82
Maximum width of head	2.75	2.60-2.75	2.67 ± 0.10	3.74
Length of postmentum	1.65	1.50-1.65	1.57 ± 0.10	6.36
Maximum width of postmentum	0.90	0.80-0.90	0.85 ± 0.07	8.23
Minimum width of postmentum	0.65	0.60-0.65	0.62 ± 0.04	5.64
Length of pronotum	1.25	1.00-1.25	1.12±0.17	15.17
Width of pronotum	2.05	2.05-2.20	2.12±0.10	3 4.71

Psammotermes prohybostoma, n.sp.

Major soldier (Fig. 7)

Head pale golden yellow; antennae and labrum dark brown, postclypeus darker than head; mandibles dark brown at bases, black above; pronotum, meso-and-metanota lighter than head; abdomen and legs brownish yellow. Head with many hairs and bristles. Pronotum with long bristles and small hairs on antero-posterior margin; labrum with many long bristles and hairs on anterior and posterior margin; postclypeus with many hairs on near anterior margin; postmentum with one or two small bristles on the anterior half. Head, rectangular, sides almost paralle, thick, with whole at the centre of the head; labrum, subpentagonal and "crown shaped", sides curved inward; distal lobe triangular with a roundly pointed tip. Mandibles, long, sabre-shaped or sickle-shaped incurved at apex, thick and strong basally, slender and sharply pointed apically; right mandible with 4-6 irregular teeth; left mandible with 6-8 irregular teeth. Postmentum, long, club-shaped, widest in anterior half, narrowed in posterior half, both anterior and posterior margin straight (Table VII). Antennae with 14-15 segments; first and third is longest, second shortest. Pronotum, saddle-shaped, distinctly notched in anterior

margin, slightly emarginate to almost straight in posterior margin. Tibial spurs 3:2:2. Tarsi four-jointed.

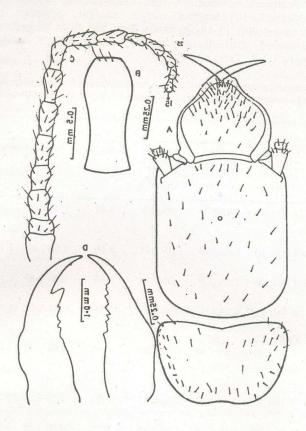


Fig. 7: Major soldier of *Psammotermes prohybostoma*, new species: A, head and pronotum from above; B, postmentum from below; C, antenna; D, mandibles.

Minor soldier (Fig. 8)

Head brownish yellow to yellowish brown; antennae and labrum darker than head; mandibles, light rown at bases, reddish brown above. Postclypeus darker than head; pronotum, meso-and-metanota lighter than head; abdomen and legs yellowish brown. Head with few scattered bristle and hairs, labrum with few long bristles on the anterior margin; pronotum with many bristles and hairs on the anterior and posterior margin; postmentum without any bristles. Head subrectangular, sides almost parallel, with whole in the centre of the head. Labrum dome-shaped sides slightly convex. Mandibles, thin,

sabre-shaped or sickle-shaped, incurved at apex, thick and strong basally, slender and sharply pointed apically; right mandible with 4-5 irregular teeth; left mandible with 6-7 irregular teeth. Postmentum, medium size, club shaped, widest in anterior half, narrowed in posterior half, anterior margin straight, posterior margin concave (Table VIII). Antennae with 13-14 segments; first and thirs longest, second shortest. Pronotum saddle-shaped, distinctly notched in anterior margin, straight in posterior margin. Tibial spurs 3:2:2. Tarsi four-joined.

Table VII: Measurements (in mm) of major soldiers of *Psammotermes prohybostoma* n.sp. (number of specimens measured is given in parenthesis).

			LOC	CALITIES			
	Chabahar (Tees) C = 1	Chabahar (Teezkopan) C = 2	Chabahar (Vashname) C = 3	Hologype	Range	Mean±S.D.	C.V.
	(n=12)	(n=6)	(n=6)		(n=24)		
Length of head to side base of mandible	1.40-1.95	1.45-1.55	1.40-1.50	1.95	1.40-1.95	1.54±0.20	12.98
Length of left mandible	1.00-1.60	1.10-1.25	1.05-1.15	1.60	1.00-1.60	1.19±0.21	17.64
Length of right mandible	1.00-1.60	1.15-1.25	1.00-1.15	1:60	1.00-1.60	1.19±0.22	18.48
Width of head at base sides of mandibles	0.90-1.25	0.95-1.05	0.90-0.95	1.25	0.90-1.25	1.00±0.13	13.00
Width of head at postero-lateral ends of antennal carinae	0.90-1.40	1.00-1.10	0.95-1.00	1.40	0.90-1.40	1.05±0.18	17.14
Maximum width of head	1.10-1.55	1.15-1.20	1.10-1.15	1.55	1.10-1.55	1.20±0.17	14.16
Length of postmentum	1.05-1.25	0.95-1.10	1.05-1.15	1.25	0.90-1.25	1.09±0.10	9.17
Maximum width of postmentum	0.50-0.60	0.45-0.50	0.50-0.55	0.60	0.45-0.60	0.51 ± 0.05	10.00
Minimum width of postmentum	0.30-0.45	0.30-0.35	0.30-0.35	0.45	0.30-0.45	0.34±0.06	10.29
Length of pronotum	0.50-0.90	0.55-0.65	0.55-0.60	0.90	0.50-0.90	0.62±0.14	22.58
Width of pronotum			0.95-1.00	1.35	0.90-1.35	1.03 ± 0.16	15.53

C = Colony

Comparison

Psammotermes prohybostoma, comes very close to P. hybostoma (Desneux). The soldier of P. prohybostoma is, however, comparatively smaller in size, labrum is broader and is provided with 14-15 antennal segments (16-17 in P. hybostoma).

Type of locality

Chabahar (Tees), (type locality), soldiers (holotype, paratype) workers, collected by S. Ravan and Hamid Sanjarani on 5.7.90 from *Acacia seyal*. Chabahar (Teezkopan), soldiers, workers, collected by S. Ravan on 28.7.90 from *A. seyal*. Chabahar (Vashname), soldiers, workers, collected by S. Ravan on 15.6.91 from *A. seyal*.

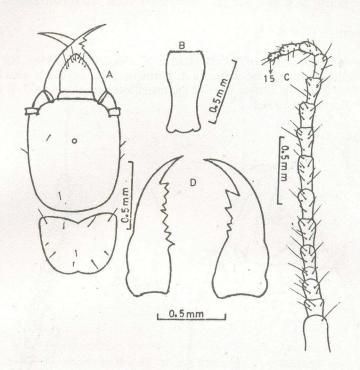


Fig. 8: Minor soldier of *Psammotermes prohybostoma*, new species: A, head and pronotum from above; B, postmentum from below; C, antenna; D, mandibles.

Amitermes paravilis, n.sp.

Soldier (Fig. 9)

Head brownish yellow to yellowish brown; antennae light brown; labrum light yellow; mandibles brownish yellow at bases, reddish brown to dark brown above; pronotum lighter than head, meso-and-metanota, abdomen pale; legs yellowish brown. Head with a few bristles and short hairs, frontal projection slightly more hairy, than the rest of the body; labrum with few long bristles and short hairs; postclypeus with few

bristles near anterior margin. Head weakly rectangular, thick, slightly narrowed anteriorly, widest almost in middle, posterior margin weakly convex. Labrum domeshaped, wider than long, lateral sides slightly convex; anteyclypeus bilobed. Mandibles, sickle-shaped, thick, outer margin below tooth depressed; tooth sharply pointed, posterolaterally directed, placed almost in middle. Postmentum widest in anterior half and narrowest in posterior half (Table IX). Antennae with 14 segments, first longest, second shortest. Pronotum saddle-shaped, indistinctly notched anteriorly; posterior margin emarginate to almost straight. Tibial spurs 3:2:2. Tarsi four-jointed.

Comparison

Amitermes paravilis, comes very close to A. vilis (Hagen, 1858), but differs in the shape of head capsule and mandibles. In A. paravilis, head capsule is weakly rectangular (oval in vilis), while the cutting edge of the mandibles just above tooth is much broader than apices that are narrower.

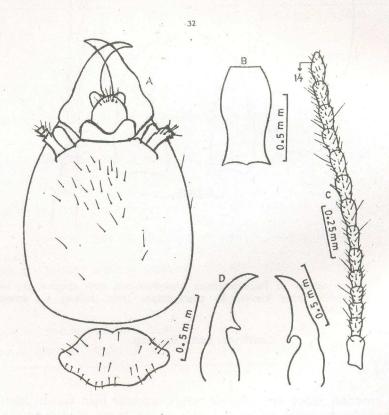


Fig. 9: Soldier of *Amitermes paravilis*, new species: A, head and pronotum from above; B, postmentum from below; C, antenna; D, mandibles.

Table VIII: Measurements (in mm) of minor soldiers of *Psammotermes prohybostoma* n.sp. (number of specimens measured is given in parenthesis).

			LO	CALITIES	S		
	Chabahar (Tees) C = 1	Chabahar (Teezkopan) C = 2	Chabahar (Vashname) C = 3	Hologype	Range	Mean ± S.D.	C.V.
	(n=12)	(<i>n</i> =6)	(n=6)		(n=24)		
Length of head to side base of mandible	1.20-1.35	1.25-1.35	1.25-1.35	1.35	1.20-1.35	1.29±0.07	5.11
Length of left mandible	0.95-1.15	0.85-0.95	0.80-1.00	1.05	0.80-1.15	0.95 ± 0.12	12.63
Length of right mandible	0.95-1.15	0.80-0.95	0.80-1.05	1.10	0.80-1.15	0.95 ± 0.13	13.68
Width of head at base sides of mandibles	0.80-1.00	0.75-0.85	0.75-0.90	0.95	0.75-1.00	0.84 ± 1.00	11.54
Width of head at postero-lateral ends of antennal carinae	0.85-1.00	0.85-0.90	0.80-0.95	1.00	0.80-1.00	0.89 ± 0.07	8.20
Maximum width of head	1.00-1.15	1.00-1.05	1.05-1.10	1.10	1.00-1.10	1.05 ± 0.06	5.52
Length of postmentum	0.90-1.15	0.95-1.00	0.90-1.05	1.10	0.90-1.15	0.99 ± 1.00	9.79
Maximum width of postmentum	0.45-0.50	0.45-0.45	0.45-0.50	0.45	0.45-0.50	0.46 ± 0.003	5.43
Minimum width of postmentum	0.30-0.35	0.30-0.35	0.35-0.35	0.30	0.30-0.35	0.33 ± 0.03	7.57
Length of pronotum	0.50-0.60	0.45-0.50	0.45-0.50	0.60	0.45-0.60	0.50 ± 0.05	10.8
Width of pronotum	0.80-0.95	0.80-0.95	0.60-0.85	0.90	0.60-0.95	0.82 ± 0.12	14.63

C = Colony

Type locality

Dezful (type locality) soldiers (holotype, paratype), workers, collected by S. Ravan on 12.8.90 from *P. caspica*. Shoushtar, soldier (paratype), workers, collected by S. Ravan on 20.7.91 from *P. caspica*. Ahwaz, soldiers (paratype), workers, collected by S. Ravan and Bijan Bajalan on 9.5.90 from *Morus alba*. Andimashk, soldiers (paratype), workers, collected by S. Ravan on 20.4.91 from *Salix babylonica*.

Table IX: Measurements (in mm) of soldiers of Amitermes paravilis, n.sp. (number of specimens measured is given in parenthesis).

,				LOCA	ALITIES			
	Dezful C=1	Shou- shtar C=2	Ahwaz C=3	Andi- mashk C=4	Holotype	Range	Mean±S.D	C.V.
	(n=15)	(n=10)	(n=5)	(n=10)		(n=40)		
Length of head to side base of mandibles	1.25- 1.35	1.20- 1.30	1.25- 1.35	1.05- 1.40	1.40	1.05- 1.40	1.26 ±0.11	8.73
Length of left mandible	0.75- 0.90	0.75- 0.85	0.75- 0.80	0.75- 0.90	0.80	0.75- 0.90	0.80 ±0.07	8.37
Length of right mandible	0.80- 0.90	0.75- 0.85	0.75- 0.75	0.75- 0.85	0.85	0.75- 0.85	0.80 ±0.06	7.37
Tooth of left mandibles from tip	0.35- 0.40	0.35- 0.40	0.40- 0.40	0.35- 0.40	0.40	0.35-0.40	0.38 ± 0.03	6.57
Width of head at base sides of mandibles	0.75- 0.80	0.75- 0.80	0.70- 0.80	0.75- 0.80	0.80	0.70- 0.80	0.76 ±0.04	4.86
Width of head at posterolateral ends of antennal carinae	0.85- 0.90	0.85- 0.90	0.75- 0.80	0.85- 0.90	0.90	0.75- 0.90	0.85 ±0.05	6.23
Maximum width of head	1.05- 1.15	1.05- 1.10	1.10- 1.15	1.05- 1.15	1.15	1.05- 1.15	1.10 ±0.05	4.18
Length of postmentum	0.70- 0.80	0.65- 0.80	0.65- 0.75	0.75- 0.80	0.75	0.65- 0.80	0.73 ± 0.06	8.76
Maximum width of postmentum	0.30- 0.35	0.35- 0.40	0.35-	0.30- 0.35	0.35	0.30- 0.40	0.35 ±0.40	10.57
Minimum width of postmentum	0.20- 0.25	0.25- 0.30	0.25- 0.25	0.25- 0.30	0.30	0.20- 0.30	0.25 ± 0.03	12.80
Length of pronotum	0.35- 0.40	0.30- 0.40	0.30- 0.40	0.35-	0.35	0.30- 0.40	0.36 ±0.04	12.22
Width of pronotum	0.70- 0.85	0.70- 0.75	0.70- 0.70	0.65- 0.75	0.75	0.65- 0.85	0.72 ±0.06	8.19

C = Colony

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MITOSIS: A METHOD OF REPLICATION IN THE HAEMOCYTES OF COMMON GARDEN SNAIL FROM LAHORE, PAKISTAN

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Abstract: Mitosis as a method of replication in molluscan haemocytes is being reported for the first time in common garden snail *Bensonies jacquemonti* from Lahore, Pakistan.

Key words: Land snail, haemocytes, mitosis.

INTRODUCTION

ood deal of disagreement on the origin, type and mode of replication of molluscan haemocytes has been reviewed by Cheng (1981), Cowden and Curtis (1981), Sminia (1981) and Bayne (1983). The molluscan haemocytes/blood cells are known to divide while they are in the haemopoetic tissue both in vitro (Bohuslav, 1933a,b; Bourne, 1935; Gatenby and Hill, 1934; Gatenby et al., 1934) and in vivo (Lie and Heyneman, 1976; Lie et al., 1975). However, Bohuslav (1933 and 1933a), Bourne (1935) and Gatenby (1932) observed no mitotic division in the cells of Helix maintained in vitro. However, mitotic cell division has been reported in the haemocytes of Modiolus modiolus (Gressen, 1937), Philine aperta (Fretter, 1937), Crassostrea virginica (Feng, 1967; Cheng and Rifkin, 1970; Feng et al., 1971) and Lymnaea rubiginosa (Dondero et al., 1977). Cytoplasmic fragmentation has been reported by Bourne (1935 in Helix) and Foley and Cheng (1972 in Crassostrea virginica). Proliferation of epithelial layers of various tissues and various parts of connective tissues has also been noted in Helix pomatia (Wagge, 1951, 1955), L. stagnalis (Müller, 1956), Biomphalaria glabrata (Pan, 1958), Doto sp. (Kress, 1968), Bulinus sp. (Kinoti, 1971) and L. stagnalis (Sminia, 1974). Endogenous budding as a method of replication in the haemocytes of Angiospira alternata alternata Say have also been reported by Khan (1986).

The present investigation is aimed to further investigate this method in a common land snail *Bensonies jacquemonti*.

MATERIALS AND METHODS

Specimens of *B. jacquemonti*, collected from the Jinnah Garden, Lahore were maintained in the laboratory in large earthen pots half filled with humus soil at room temperature. The snails used in this study measured $1.80\pm0.25~\text{mm}$ - $3.0\pm0.55~\text{mm}$ in shell width. Their blood samples were obtained by inserting a micropippette directly into the heart after removing a piece of shell above this region (Guida and Cheng, 1980). The haemolymph was allowed to spread on glass slides for 30 minutes at room temperature and fixed in 1% glutaraldehyde in Sörensen's buffer (pH 7.4) at 5°C. These cells were stained with lead haematoxylin and basic fuchsin as described by Guida and Cheng (1980) and microphotographs were made at an enlargement of x1000.

RESULTS AND DISCUSSION

It was observed that 2-3% of the total blood cells of *B. jacquemonti* were binucleated and showed an unequal or equal amitotic division of the nucleus (Plate 1; Figs.1-4). These cells measured from 19-49 μ m in diameter (29.21 \pm 22.14 μ m). The mother nuclei measured 5-10 μ m in diameter (9.34 \pm 1.89 μ m) and daughter nuclei measured 6-11 μ m in diameter (6.9 \pm 3.71 μ m). The cell to nucleus ratio comes to 6:1 (Table I). Almost similar morphometric data has been reported by Khan *et al.* (1998) in this snail species.

In one of these daughter nuclei accumulates some portion of the cytoplasm cumulated from the parent cell and developed as an endogenous bud which then separated from the parent body. These buds immediately after separation were extremly small with a very poorly formed ectoplasm, a very high nucleus to cytoplasm ratio and showed limited pseudopodial activity (Plate 1, Fig. 5a) but they gradually grew into normal haemocytes, a sequence of this growth is shown in Plate 1, Fig. 5,a,b,c,d. It appears that although the question of a definite haemopoietic tissue still remains somewhat unsettled, there is ample proof to show that mature granulocytes and hyalinocytes undergo amitotic division and that new cells are formed through endogenous bud formation.

Although Feng et al. (1971) have reported the presence of fine structure like protocentrioles and associated microtubules in the mitotically dividing granulocytes of Crassostrea virginica. However, in the present investigation it was not possible to find out such details. Mitosis as a mode of cell division in molluscan haemocytes has been generally accepted by Malek and Cheng, (1974) and Sminia (1981). However, there are other workers who consider it as very rare phenomenon (Dondero et al., 1977)

The presence of an amoebocyte producing organ between the pericardium and the posterior epithelium of mantle cavity has been suggested by Lie and Heyneman (1976) and Lie et al. (1976) with the emphasis that this organ became hyperactive during pathological conditions. Although their observations indicated that mature amoebocytes can generate daughter cells, special pool of stem cells or embryonic cells, as suggested by several other authors (Muller, 1956; Kinoti, 1971; Lie et al., 1975).

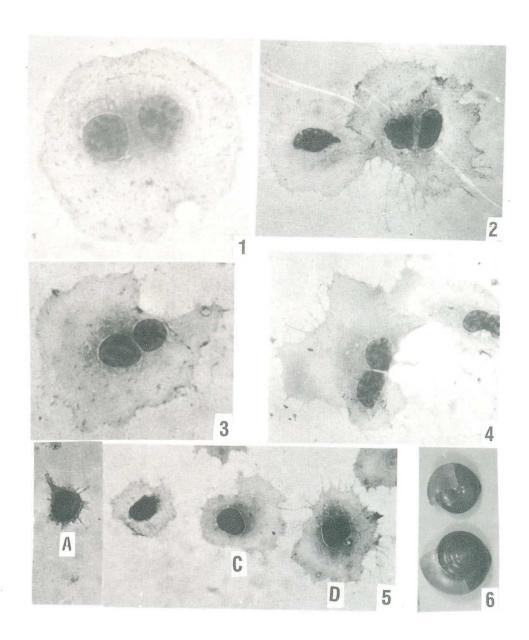


Plate 1: Stained blood cells of *Bensonies jacquemonti* x 1000. Fig.1. Mitotically dividing hyalinocyte. Fig.2. Mitotically dividing granulocyte merging with hyalinocyte. Fig.3. Mitotically dividing granulocyte. Fig.4. Mitotically dividing granulocyte merging with another granulocyte. Fig.5. Young granulocytes showing growth sequence. Fig.6. Specimens of *Bensonies jacquimonti*.

Morphometric data (mean \pm S.D.) of stained binucleated granulocytes of Bensonies jacquimonte (n=60). Table I:

Cell		Mother	nucleus	Daughter	. nucleus	Cell to	Cell to	I	seudopodi	a
Diameter (μm) (Range)	Area $(\mu m)^2$ (Range)	Diameter Area $(\mu m)^2$ (Range) (Range)	Area $(\mu m)^2$ (Range)	Diameter (μm) (Range) (Area $(\mu m)^2$ (Range)	mother nucleus ratio	dauthter nucleus ratio	Total number (Range)	Minimum Maximum rr length length c) (Range) (Range)	Maximum length (Range)
29.21	1732.12	9.34	87.13	6.9	74.12	68:1	6:1	14.21	5.14	46.21
22.14	531.18	1.89	21.44	3.71	18.13			6.33	4.21	10.44
(19-49)	(390-1918)	(5-10)	(47-121)	(6-11)	(24-98)			(6-21)	(6-13)	(14-58)

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ON CERVUS PUNJABIENSIS BROWN FROM THE SIWALIK HILLS OF PAKISTAN AND AZAD KASHMIR*

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Abstract: A well preserved right molar is described from Dhok Pathan, Chakwal district, the Punjab province, Pakistan and a basal part of left antler from Jari Kas, Mirpur district, Azad Kashmir, Pakistan. A comparison of the specimens under study with the known material of the genus *Cervus* has shown that they are referable to the species *Cervus punjabiensis* Brown. The material studied gives additional information on the known species.

Key words: Ceryus, Dhokpathan, Antler and Jarikas.

INTRODUCTION

ervids are a group of mammals which belong to the class Mammalia, family Cervidae and order Artiodactyla. They are characterized by antlers and prominent lacrymal depressions anterior to the eyes. According to Colbert (1935) the Siwalik cervids may be placed in four species i.e., *Cervus triplidens* Lydekker, *C. simplicidens* Lydekker, *C. sivalensis* Lydekker, *C. punjabiensis* Brown. Arif and Shah (1991) erected the new species of cervids from the Upper Siwaliks of Pakistan by describing a right mandibular ramus having P₃-M₃. The distinct features of *C. rewati* are the small size of the teeth, the presence of accessory columns and pronounced anterior folds on the molars.

Recently, several cervid remains comprising dental, partial skulls and antler fragments have been found by Arif and Shah (1991) from the Upper Siwaliks of Mirpur, Azad Kashmir. The comparative studies of these specimens have shown common occurrence of *Cervus punjabiensis* and *C. triplidens*. Sarwar and Farooq (1991) have also indicated the presence of *C. punjabiensis* from the Middle Siwaliks of Lehri, Jhelum district. The comparative anatomical studies of the specimens under study have shown that they belong to the species *C. punjabiensis*. These specimens have been given the number with a prefix P.U.P.C. (Punjab University Palaeontological Collection). The classification is based on Simpson (1945).

Systematics

Order Suborder Infraorder Superfamily Family Subfamily Genus Species Artiodactyla Owen Ruminantia Scopoli Pecora Linnaeus Cervoidea Simpson Cervidae Gray Cervinae Baird Cervus Linnaeus

Cervus punjabiensis Brown (Figs. 1-2).

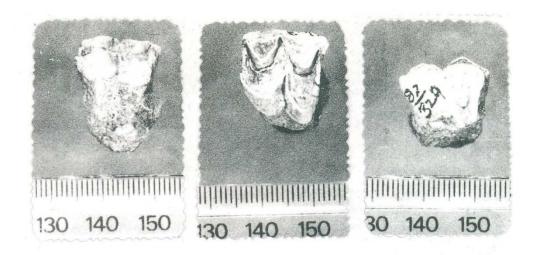


Fig. 1: Cervus punjabiensis Brown (P.U.P.C. No. 87/329), an isolated right first upper molar, collected from Dhok Pathan, Chakwal district, the Punjab province, Pakistan. i) Inner view, ii) Crown view, iii) Outer view.

Holotype

Amer. Mus. No. 19911, an incomplete skull with antlers.

Locality

Chandigarh, Indian Punjab, India.

Horizon

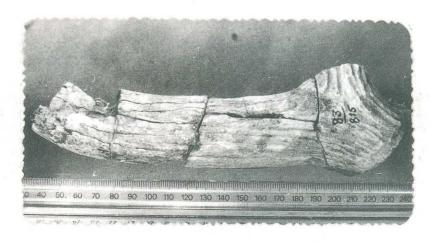
Upper Siwaliks.

Diagnosis

The teeth hypsodont, with open crescents and enfolded enamel, lacking accessory inner columns, surface smooth, Brown (1926).

Material Studied

P.U.P.C. No. 87/329, a right M^1 and P.U.P.C. No. 83/815, a basal part of left antler.



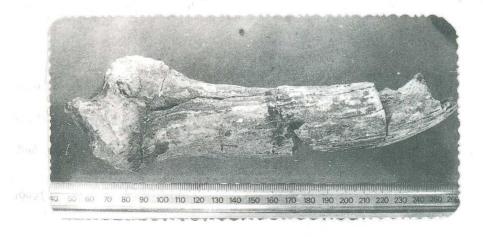


Fig. 2: Cervus punjabiensis Brown (P.U.P.C. No. 83/815), a basal part of left antler, collected from Jari Kas, Mirpur district, Azad Jammu & Kashmir, Pakistan. i) Outer view, ii) Inner view.

Locality

Dhok Pathan for P.U.P.C. No. 83/329, Chakwal district, the Punjab province, Pakistan. Jari Kas for P.U.P.C. No. 83/815, Mirpur district, Azad Kashmir, Pakistan.

Discussion

As stated by Brown (1926), the antler, though incomplete, appears to be long and is circular in outline. Its surface is smooth with faint longitudinal grooves.

The terminal part of the antler under study is completely missing but in the basal part the brow tine is represented by its basis only. In size and structure the antler compares favourably with the type described by Brown (1926). It appears to be quite long.

In size and structure the tooth compares favourably with the right M^1 of the type specimen. There is no accessory inner column and the enamel surface is perfectly smooth. The anterior half of the tooth under study is wider than the posterior one as in the type specimen, though this feature is not mentioned by Brown (1926). However, this character is quite evident from his diagram. Externally, the posterior part of the tooth is slightly damaged but it shows the basic features perfectly well. The maximum length x width of the tooth is 17 mm x 19 mm respectively. The same figures in the type specimen, as taken from the diagram, are 19 mm x 19 mm.

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STUDIES ON THE INVESTIGATIONS OF FEEDING POTENTIALS FOR GRASS CARP FARMING IN MULTAN

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Abstract: Thirteen different types of aquatic and terrestrial plants proposed to be used as fodder for grass carp were collected for analysis of different nutritional elements like dry matter, proteins, lipids, carbohydrates and ash contents. Dry matter was significantly highest in Zea mays and lowest in Marsilia quadrifolia. Sorghum bicolor showed significantly highest protein while Marsilia quadrifolia had lowest. Lipid was significantly highest in Sesbania sesban and lowest in Hydrilla sp. and Trifolium alexandrinum. Carbohydrate was significantly highest in Zea mays and lowest in Potamogeton sp. Ash content was significantly highest in Chara sp. and lowest in Typha angustifolia. Economically, the cheap source of protein was calculated as Sorghum bicolor with a rate of Rs.6.29 per 1000 g of protein in the local market. But it has no advantage over other fodder due to non-availability throughout seasons.

Key words: Grass carp, Ctenopharyngodon idella, fodder, composition, economics, aquatic weeds.

INTRODUCTION

he population of the world is increasing by geometrical way while the available food resources are increasing arithmetically. This population explosion is going to threaten the mankind by causing food problem. A protein deficient diet has its own ill effects. Fish is the best source of animal protein (Sinha and Ramchandran, 1985). Therefore, to overcome food shortage, fish culture in the country requires to be promoted. Aquaculture is receiving world wide attention as many types of protein which are not directly consumed by man can be upgraded through this practice into highly acceptable and well relished products (Pillay, 1990).

Fish flesh is converted into body tissues more efficiently than all other meats of farm animals such as sheep, goat, cow etc. (Sinha and Ramchandran, 1985). Also the percentage of edible tissue in a fish is overall greater than cattle, pigs or chicken (Barnabe, 1994).

The main objective of fish culture is the production of high value products for human consumption, which are not obtained in sufficient quantity by fishing. Another

objective of fish culture is to produce the maximum fish in a given volume of water in shortest time and at lowest possible cost. Achieving this objective required not only the management and optimization of growth of farm stock, but also obtaining them in first place (Barnabe, 1994). Fish culture can yield much more per acre per year than other animals without involving much of capital inputs, scientific and technical know-how (Pillay, 1990). Success in fish farming essentially lies on thorough knowledge about the food and feeding habits of the cultureable varieties of fishes taken for culture (Rath, 1993). The nutrients that fish require are the same as those required by other animals *i.e.*, proteins, carbohydrates, lipids, vitamins and minerals. The demand for these elements forms the basis for the preparation and selection of artificial fish feeds (NACA, 1989).

The grass carp, Ctenopharyngodon idella (Val.) along with three other species of fish namely, the snail carp, Mylopharyngodon piceus (Richardson), the silver carp, Hypophthalmichthys molitrix (Cuv. and Val.) and bighead, Aristichthys nobilis (Richardson) are most popular for pond culture in China now-a-days. In Pakistan grass carp was introduced in 1964, mainly for weed control purpose, at Halaji Lake, Sind (Naik, 1972). However, during the year 1986-87 grass carp and silver carp have been introduced for culture in Punjab under concept of polyculture system to increase the fish production per unit area (Javaid, 1990). Carps appear to be especially suited for pond culture as they feed on low trophic levels and it is possible to produce maximum food required through fertilization and maturing of ponds which reduce the production costs (Pillay, 1990).

Grass carp is a typical herbivorous fish, which is cultured in many countries of the world due to its tasty flesh and rapid growth rate. Its additional characteristics is that it can eat grasses and alagae more than its weight per day therefore, it is useful for prevention of water algae and weeds (NACA, 1989). The grass carp start feeding on macro vegetation when about 3 cm in length. It stops feeding at temperature 10-12°C. At temperature above 20°C, it eats large amount of grasses (Pillay, 1992, 1994). Due to its growing popularity in Pakistan, more attention is needed of the fisheries scientists because very little attempts were made in the past to collect biological data about grass carp in Pakistan.

The present study is one of a series (Salam et al., 1993) deals with the composition of proposed natural and supplementary diet for grass carp in order to test their effect on growth rate under laboratory conditions. Furthermore, economics of the fodder has been calculated to determine its feasibility as a source of diet for this important herbivorous fish.

MATERIALS AND METHODS

Composition

For composition some aquatic weeds and terrestrial grasses proposed to be used as fodder/ supplementary food for grass carp were collected. The list of samples collected is as under, local names are in parenthesis:

1.	Chara sp. (Funjali patta)	8.	Eragrostis sp. (Dub)
2.	Hydrilla sp. (Jalla)	9.	Avena sativum (Javvi)
3.	Marsilia quadrifolia	10.	Trifolium alexendrinum
	(Tripattra godhi)		(Barsheem)
4.	Potamogeton sp. (Phus)	11.	Sesbania sesban (Jantar)
5.	Typha angustifolia (Pan bori)	12.	Sorghum bicolor (Juar)
6.	Trianthema petendra (It-sit)	13.	Zea mays (Makki)
7.	Stellaria media (Morlia).		

To estimate the water content in each sample the pre-weighed sample was placed in pre-weighed aluminum foil tray for drying in as electric oven (Memmert-laborerate 8540) at 70-80°C till the weight became constant. The dried sample was crushed in a pestle and morter and powdered in a Moulinex electric blender. To calculate ash content in each individual sample, 1 g of the sub sample was taken in a pre-weighed, heat resistant, China clay crucible and ashed in a muffle furnace (RJM 1.8-10, China) for 6 hours at 550°C and weighed after cooling.

The fat content was estimated from dry tissues using dry extraction method in which 0.5-1.0 g of powdered dry tissue was mixed well into 10 ml of chloroform and methanol mixture in a ratio 1:2 (Bligh and Dyer, 1959; Cui and Wootton, 1988; Salam and Davies, 1994). The said mixture was left overnight and then centrifuged at 1000 rpm. (Centrifuge-YJ03-043-4000) for 2 minutes. After centrifugation, the clear supernatant was removed carefully into washed, dried and pre-weighed small bottle. The bottle was then placed in an electric oven at 40-45°C for evaporating the mixture till dryness. Lipid fraction left after evaporation was weighed on an electric balance (MP-3000) to nearest 0.01 g. A weighed sample (0.3-0.4 g) of the material is homogenized, with a volume of alcohol chloroform mixture of 2:1 ratio, centrifuged and supernatant discarded. The residue was dried at room temperature. Then the protein was extracted by 1N NaOH solution. The estimation of protein in the plant material was done by Biurete method is given below as described by Gornall et al. (1949).

The carbohydrate content was determined by the difference from the mass of other main sample constituents like ash, fat, water and protein following Salam and Davies (1994).

Economics

Economics of the fodder was calculated on the basis of data collected from the local market. The economic feasibility was calculated for 1 kg of protein by the following formula:

Rate kg = Protein (Rs.)

Price kg of sample

$$x 100$$

Protein/kg of sample

Excel 97 and Minitab 11 were used for all data and statistical analysis.

RESULTS

Composition

The significantly highest % protein (wet weight) (P<0.001) was found in Typha angustifolia (aquatic plant) and Sorghum bicolor (Fodder) basis and in dry weight was found in Hydrilla sp. (aquatic plant) and Sorghum bicolor (fodder). The significantly highest % fat (wet weight) (P<0.001) was found in Typha angustifolia (aquatic plant) and Sesbania sesban (fodder) and in dry weight were found in Typha angustifolia (aquatic plant) and Eragrostis sp. and Settleria media (fodder). The significantly highest % carbohydrate (P<0.001) (wet weight) was present in Typha angustifolia and Zea mays and in dry weight was present Marsilia quadrifolia and Avena sativum (fodder).

The significantly highest % ash (wet weight) (P < 0.001) was found in *Chara* sp. and *Zea mays* and in dry weight was found in *Chara* sp. and *Trianthema petendra*. The significantly highest % water (P < 0.001) was present in *Marsilia quadrifolia* and *Stellaria media* and lowest in *Typha angustifolia* and *Sorghum bicolor* (Table I, II; Fig.1,2).

Table I: Composition of samples on wet weight basis.

Fodder	water (%)	Protein (%)	Lipids (%)	Carbohy-drates (%)	Ash (%)
Chara sp.	84.51	1.37	0.92	6.08	7.12
Hydrilla sp.	90.00	2.48	0.2	4.72	2.60
Marsilia quadrifolia	94.30	0.10	0.56	3.57	1.47
Potamogeton sp.	92.57	1.29	0.07	3.1	2.97
Typha angustifolia	83.55	3.44	1.97	10.08	0.96
Trianthema petendra	88.74	2.06	0.89	5.84	2:47
Stellaria media	89.61	3.52	1.55	3.46	1.86
Eragrostis sp.	86.89	2.54	1.96	7.04	1.57
Avena sativum	82.69	3.69	1.03	11.04	1.55
Trifolium alexandrinum	88.80	3.25	0.22	6.05	1.68
Sesbania sesban	79.42	6.97	2.88	9.09	1.64
Sorghum bicolor	74.60	10.33	1.01	11.52	2.54
Zea mays	76.30	3.97	2.37	14.52	2.84

The significantly lowest % protein (wet weight) was present in Marsilia quadrifolia and Trianthema petendra and in dry weight were present in Marsilia quadrifolia and Zea mays. The significantly lowest % fat (wet weight) (P<0.001) as well as in dry weight was present in Potamogeton sp. and Trifolium alexendrinum. The significantly lowest % carbohydrate (wet weight) (P<0.001) was present in Potamogeton sp. and Trianthema petendra and in dry weight was present in Chara sp. and Stellaria media. The significantly lowest % ash (wet weight) (P<0.001) was found in Typha angustifolia and avena sativum and in dry weight was found in Typha angustifolia and Sesbania sesban (Table I, II; Fig.1,2).

Economics

Rates per kg proteins obtained from fodder are given in Table III on wet and dry weight basis respectively.

Results clearly show that *Sorghum bicolor* is the cheapest source of protein among fodders both on wet and dry weight basis.

Table II: Composition of samples on dry weight basis.

Fodder	Proteins (%)	Lipids (%)	Carbohy-drates (%)	Ash (%)
Chara sp.	9.00	6	39.00	46
Hydrilla sp.	24.24	2	47.76	26
Marsilia quadrifolia	2.45	10	61.55	26
Potamogeton sp.	17.25	1	41.78	40
Typha angustifolia	20.93	12	61.07	6
Trianthema petendra	18.32	8	51.68	22
Stellaria media	34.03	15	32.97	18
Eragrostis sp.	19.92	15	53.08	12
Avena sativum	21.49	6	63.56	9
Trifolium alexandrinum	29.08	2	53.92	15
Sesbania sesban	33.87	14	44.13	8
Sorghum bicolor	40.69	4	45.31	10
Zea mays	16.77	10	61.23	12

Table III: Economic feasibility of fodders proposed to be used as food for grass carp on wet and dry weight basis.

Fodder	Rate/kg (Rs.) Dry wt.	Protein content/kg (g)	Rate/1000g/protein (Rs.)
Avena sativum (wet)	0.60	36.9	16.26
Trifolium alexandrinum (wet)	0.60	32.5	18.46
Sorghum bicolor (wet)	0.65	103.3	6.29
Sesbania sesban (wet)	1.00	69.7	14.34
Zea mays (wet)	0.70	39.7	17.63
Avena sativum (dry)	0.60	214.9	2.79
Trifolium alexandrinum (dry)	0.60	290.5	2.06
Sorghum bicolor (dry)	0.65	406.9	1.59
Sesbania sesban (dry)	1.00	338.7	2.95
Zea mays (dry)	0.70	167.7	4.17

Economics feasibility of aquatic plants to be used as fodder for grass carp has not been calculated due to non-availability for sale in the market, however they could be utilized as a cheap source.

DISCUSSION

Green fodders include aquatic plants and terrestrial plants mainly used as feed for grass carp and some times for common carp, crucian carp and tilapia. Green fodders contain mostly water and cellulose. However, they also contain the principle nutrients, *i.e.*, fat, protein and carbohydrate and are rich in vitamins. Green fodders are the principle feed for grass carp and wuchang fish and serves as supplementary feed for other cultivable fish (NACA, 1989).

Little attempts have been made to investigate the composition of fodders in Pakistan. However, percentage composition of some fodders used as food for grass carp has been reported in China (NACA, 1989). In the present study water content of fodders range between 74.6-94.3% similar in range as reported earlier *i.e.*, 74.61-96.77%. These results however, show higher protein contents 0.1-10.33% than reported earlier *i.e.*, 0.61-4.17%. While fats in the present study range between 0.2-2.88%, similar to previous studies *i.e.*, 0.09-1.27%. Carbohydrates have the values between 3.1-14.52% which is in agreement to the studies reported earlier *i.e.*, 1.65-14.24% (Table I; NACA, 1989).

Results of the present study are in line with earlier researches, however slight difference may be due to differences in sampling or experimental methods adopted. However, efforts have been made to minimize the error. The fodders analyzed in this study belong to the different species, varieties and localities.

A certain proportion of plant material in the diet apparently plays an important role in facilitating ingestion and digestion of food and in supplying vitamins and carbohydrates necessary for respiration and proper growth (Shireman and Smith, 1983).

Sutton (1977) reported that grass carp fed on duck weed (Lemna sp.) show significantly higher growth than fed on Naajas quadalupensis or Chara sp. Proximate analysis revealed that duck weed had 22.8% crude protein compared to 9.2-13.1% in other plants. Therefore, protein rich diet promotes growth in fish than any other feed.

Economically among fodders Sorghum bicolor is the cheapest source of protein with a rate of Rupees 6.29 per 1000 g of protein. Sesbania sesban, Avena sativum, Zea mays and Trifolium alexandrinum have the rates of 1000 g protein as Rupees 14.34, 16.26, 17.63 and 18.46 respectively (Table III). Cattle are said to be very fond of this fodder and from its manifesting a power to survive for more than one year and is perhaps better suited than any other form for the purpose of fodder (Watt, 1972). It is, therefore, recommended on the basis of present analysis that Sorghum bicolor may be used as a very effective fodder for grass carp farming in Pakistan. However, due to non-availability of all the fodders in one season, each has its own merits to be used as food for grass carp.

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ECHINOCOCCUS GRANULOSUS: HISTOPATHOLOGY OF NATURALLY INFECTED SHEEP LIVER*

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Abstract: Histological sections of both uninfected and naturally infected sheep liver with hydatidosis were studied. Intensity of histopathological changes was found proportional to the size of cyst. Small cysts (1-2 mm diameter) showed pathological changes only close to the cyst wall. The hepatocytes were deformed due to reduced cytoplasmic contents and large gaps were seen around the fibrous cyst wall. Sinusoidal spaces were however expanded forming aster like channels around the cyst wall. Some of the hepatic cells were also found swollen. Medium (1 cm) and large (3-4 cm) cysts showed prominent changes near to the cyst wall, like pyknotic nuclei, heavy leucocytic infiltration and fibrosis. Hepatocytes also showed cytoplasmic and nuclear vacuolation near the wall of medium and large cysts. Some of the cysts also showed pressure atrophy, which was more common in larger cysts. Hepatic architecture was completely destroyed while prominent changes near the cyst wall included increase in sinusoidal spaces and bile canaliculi. Portal veins and lymphatics became indistinguishable. The histopathology of hepatocytes surrounding the cyst were noted at various distances from the cyst wall with the findings that the damage was inversely proportional to the distance from the cyst.

Key words: Echinococcus granulosus, sheep, liver, histopathology.

INTRODUCTION

andey (1971) has reported that the damage produced by the hydatid cyst in liver, lungs and spleen of goats was both mechanical and toxic. Svilenov et al. (1978) studied histopathology of Echinococcus multilocularis cysts in the liver of Meriones unguiculatus and reported that connective tissue membrane around the cysts was rich in lymphocytes, histiocytes and occasional eosinophils. Remaining liver tissue showed dystrophic-granulomatous changes. Logachev and Bat'kaev (1979) studied multilocular hydatids in 6 to 130 Marmota bobac in Kazakh (U.S.S.R.). In 2 cases, all the organs and the serous membrane of the abdominal cavity were affected. In the remaining 4 animals, the cyst almost entirely filled the liver and included numerous chambers of varying size, up to 3

^{*}Part of the Ph.D. thesis of first author.

cm in diameter. Khan (1982) worked on incidence in sheep (8.27%), goat (7.55%), cow (9.59%) and buffalo (12.25%) in Lahore district, Pakistan and reported that females were more susceptible than males among all the four groups of animals. Singh et al. (1988) studied the pathology of experimental E. granulosus in sheep, guineapigs, hamsters and mice. The gross pathological changes seen were congestion and haemorrhages in the internal organs. Hepatomegaly and splenomegaly were observed in the guinea pigs and mice. Microscopic examination showed cut sections of the cysts in internal organs, possessing a laminated cyst wall and an inner layer of germinal epithelium. The cyst wall was found surrounded by a zone of necrotic tissue and a connective tissue capsule infiltrated mostly with mononuclear cells and few polymorphs. Matto et al. (1990) reported that in buffaloes liver cysts were usually large in size and surrounded by infiltration of foreign body giant cells, macrophages, lymphocytes and a thick fibrous capsule. Markovics et al. (1992) studied four large and several smaller cysts of E. granulosus in the liver of a baboon. Three of the large cysts had an atypical structure and were packed with a semi-solid mass of collapsed daughter cysts, small cysts containing fluid and involuted membranes. Present study was aimed to work out the histopathological changes in sheep liver and also to investigate the effective range of hydatid cyst fluid (HCF) and cystic pressure towards the damage of hepatic parenchyma.

MATERIALS AND METHODS

Out of one thousand livers examined at local slaughter house, only five uninfected and ten which harbored unilocular hydatid cysts of various sizes were collected and fixed quickly after the animal was slaughtered. The infected tissue was cut in long strips of $0.5 \times 0.5 \times 3.0$ cm in such a way that a portion of the cyst was also included at one end of the strip, while the other end was farthest from the cyst. The sections (4-5 μ m thick) were cut and stained in hematoxylin and eosin. Both progressive and regressive methods were used. The stained sections were studied to observe pathological changes.

RESULTS

Fig.1 showed normal hepatic architecture of sheep. The cysts were divided in three categories according to their diameter, small (1-2 mm), medium (1 cm) and large (3 cm).

Small cysts

Figure 2 showed a tissue containing very small cysts. The germinal layer was covered by laminated layer and well pronounced fibrous tissue layer surrounded the cyst. This layer seemed to be formed by highly compressed cells in which the membranes had been obliterated. The hepatic nuclei were elongated, dark and solid showing indications of pyknosis. The depth of the fibrous area extended from 0.022 to 0.047 mm (Fig.3). There were some indication of abnormal cytoplasmic vacuolation in the area beyond the fibrous tissue along with slight disturbance in the arrangement of hepatic cords and with no signs of any active repair process. Some of the hepatocytes were deformed due to reduced cytoplasmic contents. Large gaps in the tissue were seen around the fibrous cyst wall. Sinusoidal spaces were however, expanded forming aster like channels around the cyst wall (Fig.2).

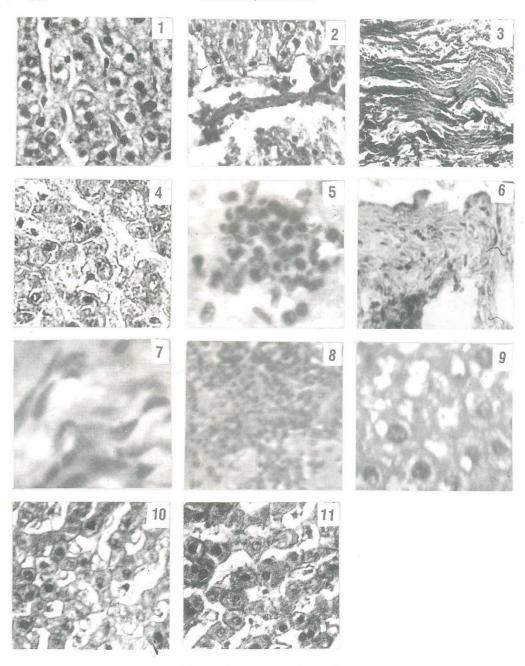
Medium cysts

The histopathology of such cyst was noted at the distance of zero to 0.5, 0.5-1, 1-1.5 and 1.5-2.0 cm from the cyst. Zone of zero to 5 cm showed that considerably thickened cyst wall in which laminated layer was covered by fibrous area measuring 0.136-0.181 mm. The nuclei (not visible in compact cyst wall but in loose tissue) in the fibrous areas were definitely pyknotic and the cell membranes not discernible. The cells near fibrous layer seemed to be compressed with disturbed arrangement of hepatic cords, occasional cytoplasmic vacuolation, heavy infiltration of leukocytes along with visible change in the nuclear size of hepatic cells. In the zone of 0.5-1.0 cm the nuclei of the hepatocytes appeared comparatively normal and hepatic cords were better arranged with reduced cytoplasmic vacuolation while the blood vessels were packed with lymphocytes. However, some of the cells were slightly swollen. In the zone 1.0-1.5 cm, arrangement of hepatic cords was unaltered with almost normal nuclei. Number of lymphocytes in the blood vessels were less as compared to previous zone. There was no sign of pressure atrophy. At distance 1.5-2.0 cm from the cyst the arrangement of hepatic cords, hepatocytes and their nuclear sizes were almost normal. Structurally, cells and the nuclei were functional while number of lymphocytes in the blood vessels was higher than normal but lesser than those found in the previous zone (Figs.4,5).

Large cysts

Largest cysts processed during present study were between 3.0-4.0 cm in diameter. Their cyst wall was well developed, thick, fibrous and stained poorly with eosin. Main zone, 0-0.5 cm was subdivided into three sub zones: Subzones SZ1, 0.0-0.1 cm, SZ2 0.1-0.4 cm, SZ3 0.4-0.5 cm from cyst. SZ1 (Figs.6-8) consisted of a germinal (cellular) and laminated (acellular) layer which was again covered by a fibrous layer that extended from 0.036 to 0.091 mm (Fig.6). The cyst wall was followed by a narrow cellular area in which cell membrane had been lost completely. Parenchyma in the close vicinity of the cyst wall was completely lost which formed very large intercellular spaces that were exwardly directed (in which the tissue had disappeared). Cells where present, were very small in size and lobular pattern was entirely missing. In some regions majority of the nuclei were deshaped probably due to pressure and/or toxic atrophy. The number of nuclei per cell were not countable in most of the cells. Some of the nuclei were much elongated. Sinusoidal spaces, bile canaliculi, protal veins and lymphatics were not visible in the close vicinity of cyst wall. A large number of lymphocytes, polymorphs and eosinophils were present in this region. In SZ2, the cells were highly compressed (Fig.9) with deshaped nuclei. Some of them were pyknotic, the cell membranes were diffused, blotted and vague. The arrangement of hepatic cords were greatly disturbed. Sinusoidal spaces, bile canaliculi, portal veins and lymphatics were not distinct. The cytoplasm showed vacuolation and colloidal disintegration. Portal veins were visible, hepatic lobules damaged but with visible boundaries. Kupffer cells were present representing the phagocytic action. SZ3 showed recognizable cells but with poor hepatic architecture and large sinusoidal spaces, representing oedemic condition. Majority of the nuclei were pyknotic but portal areas were visible. Hepatic arteries and veins in this area were thick walled with smaller diameter and tightly packed cells in some areas. Kupffer cells were present indicating on going of the repair process. Number of the nucleoli per nucleus was not countable in most of the nuclei because of pyknosis. Cytoplasm was highly granulated with diffused cell membranes. Most of the veins and arteries were broken and degenerated in this zone. Erythrocytes were also seen in the sinusoidal spaces (Fig. 10).

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(For captions, see opposite page)

In zone 0.5-1.0 cm most of the nuclei were pyknotic, others possessed 2-3 nucleoli per nucleus. The nuclear membranes were clear, solid and the nuclei were large in size. Vacuolation was only present in some of the nuclei. Large number of lymphocytes were present in the blood vessels while a few hepatocytes showed division representing a little repair activity. Condition of cell membrane was still very poor, while the cells were spherical to oblong. Arrangement of hepatic cord and general condition of the cell was very poor. The size of the cell was however, improved, while bile ducts were thick walled, dilated with free bile patches, visible in sinusoidal spaces. Kupffer cells were occasionally present and lymphatic vessels were large in this zone. Condition of bile canaliculi were very poor due to desolated and dilated hepatic cords. Blood vessels were however, compact thick walled and no pressure atrophy was seen in the hepatocytes. Sinusoidal spaces were expanded representing oedemic condition (Fig.11).

Zone 1.0-1.5 cm showed slight improvement in the arrangement of hepatic cords. The number of nucleoli were usually two per nucleus. Nuclear and cytoplasmic presence vacuolation. Nuclear membranes were clear and well demarcated. Only a few nuclei were undergoing mitosis. Nuclear size was larger than the nuclei present in the previous zone but smaller than control. Condition of cell membrane was improved, cells were spherical to oval but their typical hexagonal condition was not maintained. Arrangement of hepatic cord was in better position. Bile ducts were close to the normal. Kupffer cells were present but lymphatic vessels still expanded. No pressure atrophy was seen in this zone but sinusoidal spaces were still expanded and free bile patches were seen. Hepatic arteries and veins were thick walled but not compressed.

In zone 1.5-2.0 cm proportion of pyknotic nuclei was comparatively less. The degree of vacuolation and colloidal disintegration was unchanged. The cells were swollen but in some cells there were three nucleoli per nucleus. Some of the nuclei were hypertrophied and the cells were hexagonal, hepatic cord were improved and hepatocytes were arranged according to hepatic architectural. Bile canaliculi were visible in this zone but were highly expanded. No pressure atrophy was seen in this zone. Sinusoidal spaces were packed with erythrocytes, polymorphs, eosinophils, monocytes and lymphocytes.

Fig. 1: Showing a normal hepatic architecture of sheep with sharp and well demarkated cell membrane, cytoplasm clear and large & active nuclei (X1000); Fig. 2: Showing a small cyst (1.0 mm diameter). Fibrotic layer around the cyst is formed by highly compressed cells. Large spaces in the tissue are seen around the fibrous cyst wall. Sinusoidal spaces have been expanded forming aster like channels around the cyst wall (X400); Fig. 3: Closest to a small cyst showing fibrosis in the cyst wall. Many macrophages are penetrating in the cyst wall (X1000); Fig. 4: Showing a medium cyst (1.0 cm diameter). Some of the hepatic nuclei are pyknotic with prominent, cytoplasmic vacuolation. The arrangement of hepatic cords is remarkably disturbed. Sinusoidal spaces have been enlarged and expanded (X1000); Fig. 5: Close to a medium cyst (1.0 cm diameter) showing pyknotic nuclie, cytoplasmic disintegration, numerous lymphocytes, polymorphs and kupffer cells (X1000); Fig. 6: Showing a large cyst (4.0 cm diameter) with visible fibrosis in thick cyst wall alongwith many macrophages penetrating in the thick cyst wall (X1000); Fig. 7: 1.0 mm from the cyst (4.0 cm diameter) showing hepatocytes surrounding the cyst wall have undergone necrosis, while nuclear and cell membranes are dissolved. Both hepatocytes and nuclei have been elongated due to pressure atrophy and also by toxic invasion of HCF (X1000); Fig. 8: 0.01-0.10 cm from a large hydatid cyst (4.0 cm diameter) showing hepatic nuclei are pyknotic. Cytoplasm shows colloidal disintegration. The arrangement of hepatic cords has been disturbed. Numerous defensive cells are visible (X400); Fig. 9: 0.1-0.4 cm from the large cyst (4.0 cm diameter) showing hepatic nuclei are with dissolved membranes. Cytoplasm shows colloidal disintegration and vacuolation. The arrangement of hepatic cords has been disturbed (X1000); Fig. 10: 0.4-0.5 cm from a large hydatid cyst (4.0 cm diameter) showing hepatic nuclei are with dissolved membranes. Cytoplasm shows colloidal disintegration. The arrangem

In zone 2.0 to 2.5 cm nuclear and cytoplasm vacuolation was absent, nuclear and cell membranes were clear and well demarcated. Hepatic lobules were intact while, sinusoidal spaces were packed with erythrocytes and lymphocytes. Hepatic arteries and veins were normal and filled with blood cells.

Zone 2.5-3.0 cm was close to that of normal. The nuclei were spherical, with no nuclear or cytoplasm vacuolation. Bile ducts, arteries, veins, lymphatic vessels and hepatic lobules were all normal. A few Kupffer cells were however, present.

DISCUSSION

The appearance of vacuole, atrophy of some of the hepatic cells and extensive hypertrophy of hepatic nuclei were the typical signs of hepatotoxicity. The atrophy of the hepatic cells indicate some abnormalities in cell membrane permeability. Hypertrophy of some of the hepatic nuclei at 1.00 cm of largest cyst may be because of increased nucleic acid content. This has also been supported by increased nucleic acid content (Anwar and Tanveer, 1996) in rabbits infected with hydatidosis. Two facts were already known that the cyst wall was permeable and that when released in the body the hydatid fluid can induce a severe anaphylactic reaction in the body. The growth rate of the cyst was very slow (Chandler and Read, 1961). The present findings showed that in case of small cysts there was practically no prominent effect on the host tissue and cells and nuclei were also normal and functional. This is expected because at this stage neither the size of the cyst is large enough nor the host tissue has been exposed to high quantities of HCF. As the cyst grow in size the cells in the immediate vicinity of the cyst showed a pressure atrophy. The cell membranes of hepatocytes were destroyed and nuclei become elongated and pyknotic and the progressive fibrosis started. There was heavy leukocytosis which showed damage to the tissue. In large growing cysts in addition to this the host tissue showed fibrosis, cirrhosis, even upto a distance of 2.0 cm from cyst. The HCF and/or autolysis based proteolytic enzymes were definitely in action and damaged the plasma membranes of hepatocytes and interfered the permeability of the plasma membrane, which probably resulted in the seepage of such cytoplasmic enzymes, metabolites and plasma constituents out of cell membrane. The remarkable damage in the hepatocytes upto 2.0 cm from the large cyst can be attributed to the pressure atrophy as well as to the toxins present in the fluid that had been continuously seeping out of the cyst in quantities varying according to the cyst size. From Pakistan no significant work has so far been done on this aspect of hydatidosis which is of great medical and veterinary importance.

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CONTROL OF HYDATIDOSIS THROUGH LOCAL PLANTS: HAEMATOLOGICAL STUDIES

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Abstract: Out of thirty-eight healthy rabbits acclimatized to the optimal laboratory conditions, six were kept as normal control and rest were inoculated intraperitoneally and subcutaneously with 45,000 protoscoleces of Echinococcus granulosus of sheep origin. They were randomly divided into four groups i.e., one infected control (n=8) and three treated groups (having 8 rabbits in each group). These treated rabbits were individually given (2g/day) leaves of Aloe vera or ovary part of Punica granatum or fruit of Azadirachta indica along with ordinary green fodder. Rabbits were individually weighed and their blood samples were pooled for haematological analyses up to 90 days with an interval of 10 days. The body weight (g) in the experimental control rabbits decreased (<0.001) and in treated rabbits body weight initially decreased but later on improved gradually (P<0.001). Among shaematological parameters total erythrocyte counts (TEC) (100 mm³), haemoglobin (g/dl), packed cell volume (% ml); total leukocyte counts (TLC) (thousands/mm³), and erythrocyte sedimentation rate (mm/hr) fluctuated in the rabbits inoculated with protoscoleces of sheep origin. However, these values improved gradually in the rabbits treated with different plants/parts of plants. The improvement was significant in the rabbits treated with leaves of A. vera and fruit of A. indica as compared to those given ovary part of P. granatum. Their respective haematological indices i.e., mean corpuscular haemoglobin (picogram) and mean corpuscular haemoglobin (picogram) and mean corpuscular haemoglobin. The above results showed that all the three plants play important role in curing the haematological parameter in experimentally induced hydatidosis. These plants may have some chemical that can antagonise the effects of protoscoleces.

Key words: Hydatidosis, sheep, protoscoleces, rabbits, haematology, herbal control.

INTRODUCTION

ydatidosis is medically and economically one of the most important parasitic diseases and both unilocular (*Echinococcus granulosus*) and multilocular (*E. multilocularis*) of the disease are widely and actively expanding their range into areas previously considered to be free of this infection (Schwabe, 1986). It is also an important zoonosis and the chances of its high infection in man are more in those areas where sheep and cattle grazing is aided by the dogs (FAO Report, 1981) as in Pakistan. Its wide prevalence in the livestock of Pakistan has been given consideration by Munir (1980), Khan and Haseeb (1984), Iqbal *et al.* (1986), Pal and Jamil (1986), Hussain (1987) and Iqbal *et al.* (1989). Its seriousness in human beings is also far

greater than what the published work shows and its incidence is much higher than what is generally believed to be (Chaudhry et al., 1992; Naveed et al., 1993; Junejo et al., 1995).

The suitable climate, poor hygienic conditions and unawareness about this disease have made the conditions favourabe for the growth and completion of the life cycle of E. granulosus. That is why livestock in Pakistan is constantly at risk of hydatidosis that has direct effect on the economy of the country and results in loss of million of rupees each year. It has also worsen the protein deficiency for human consumption in terms of condemned organs and lowered products of infected animals. Keeping in view their medical and veterinary importance it was considered desirable to find out some remedy. Although mebendazole and albendazole are now routinely used in cystic echinococcosis (Smyth, 1994). Besides them many plant drugs have also been in practice since ancient times for the treatment of parasitic infections in man and animals (Nadkarni, 1954; Chopra et al., 1956; Said, 1969). Akhtar (1987) tested the anthelmintic activity of several plants (in powdered and extract form) in goats, buffaloes and calves and found number of plant drugs and their extracts that possess interesting potent and safe anthelmintic principle for veterinary usage. Anwar et al. (1997, 1997a) successfully used indigenous plants to control hydatidosis in experimentally infected rabbits. In the present investigation 3 plants (Aloe vera, Punica granatum and Azadirachta indica) were orally given to experimentally infected rabbits with common fodder and their effects were studied in terms of changes/ improvements they induce in their haematology.

MATERIALS AND METHODS

Thirty-eight healthy rabbits maintained under optimal laboratory conditions were fed twice a day on seasonal green fodder. They were individually weighed in the beginning of experiment and then before each blood sampling.

Hydatid cyst fluid (HCF)

Hydatid cyst fluid (HCF) was aspirated from the cyst present in liver, lungs and spleen of infected sheeps at local slaughter house and only viable protoscoleces were selected for inoculation. HCF was centrifuged at 500 rpm for 5 minutes and the pallet thus formed was washed with saline solution, containing the Penicillin and Streptomycin sulfate (Ohnishi, 1985). The protoscoleces were counted in the chamber of white blood cells of haemocytometer. Two inoculums each having 22500 protoscoleces were injected sub-cutaneously and intra-peritoneally in the left flank of rabbit after taking all antiseptic precautions.

Grouping of rabbits

Rabbits were divided into 5 groups. For each plant treatment 3 treated (eight rabbits each) and one experimental control (n=8) along with one normal control (n=6) were maintained. Following plants and their parts were used:

	Name of Plant	Local Name	Parts of
1.	Aloe vera	Kwargandal	Plants Leaves
2.	Punica granatum	Anar	Ovary part

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3. Azadirachta indica

Neem

Fruit

Preparation of plant/herbal extracts

The dried plants and their parts (Anar, Neem) were ground to fine powder, 2 g of each powdered plant was mixed with 25 ml of tap water and then given to rabbits. The fresh leaves of kwargandal were mashed and 2 g mixed with 2.5 ml of water was given to rabbits. Three treated groups were orally given 2 g/day of A. vera or P. granatum or A. indica upto 90 days. The experimental control (without herbal treatment) and normal control were also maintained.

Blood sampling and haematological studies

After every 10 days 4.0 ml blood was pooled from the marginal vein of each rabbit and mixed with EDTA (anticoagulant). This blood was used for the estimation of haemoglobin (Hb) contents according to Van-Kampan and Zijistra (1961), Packed cell volume (PCV; haematocrit) according to microhaematocrit method of Strumia et al., 1954), total erythrocyte counts (TEC) and total leukocyte counts (TLC) were made according to Dacie and Lewis (1991). The data thus obtained was utilized for calculating different haematological indices, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) according to Dacie and Lewis (1991). For the determination of erythrocyte sedimentation rate (ESR) Westergren's method reported by Swarup et al. (1986) was followed.

RESULTS

Body weight

The initial body weight (g) in the normal control, experimental control and treated rabbits was 1134.3 ± 36.4 , 1104.5 ± 58.2 , 1122.0 ± 34.2 , 1108.3 ± 36.2 , 1121.4 ± 48.3 . Body weight (g) gradually increased in the normal control rabbits and reached up to 41.5% after 90 days. However, in the experimental control the body weight (g) increased only 18.05%. In the rabbits treated with A. vera, P. granatum and A. indica the body weight increased 24.98, 28.2 and 28.43% respectively (P < 0.001) (Fig.1).

Treatment with A. vera

TEC $(10^6 \mathrm{m}^3)$ in the beginning of experiment was 6.3 ± 9.0 , 4.8 ± 0.9 and 6.4 ± 0.8 in the normal control and experimental rabbits. In the normal control group these values remained almost constant throughout the experiment with slight decrease (5.6%) in the end. However, the similar values decreased constantly in the rabbits inoculated with protoscoles of sheep origin except on the 40th day of experiment. In the end they faced an overall decline (27.08%). However, in the treated rabbits TEC number also decreased in 20th, 30th and 40th day but later on improved positively and in the end decrease was only 6.25% as compared to the 27.08% in the infected control rabbits (Fig.2A).

In the normal control group the haemoglobin (g/dl) contents slightly fluctuated and finally decreased (6.83%). However, these values declined (72.9%) in the infected

control rabbits while improved in the rabbits treated with A. vera. Although, decline of 6.96% was also noted in this group (P<0.01; Fig.2B).

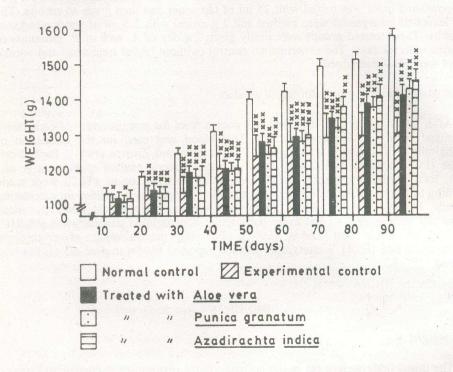


Fig. 1: Normal control, Experimental control, Treated with Aloe vera, Treated with Punica granatum, Treated with Azadirachta indica.

Packed cell volume (PCV) (%/ml) remained almost unaltered in the normal control group with a slight decrease (3.3%) in the end. These values increased (58.9% and 8.02%) in the experimental control and rabbits treated with A. vera and A. indica (Fig.2C). Their haematological indices (MCV, MCH, MCHC) computed from the results of TEC, Hb and PCV also reflected the similar changes. After an initial alterations their values reached near control values (P<0.001) except in the rabbits of experimental control group (Fig.2D, E, F).

TLC (thousands/mm³) showed slight fluctuations and in the end increased up to 3.06% in the normal control rabbits. However, in the infected controls these values increased up to 65.6%. The treated rabbits showed increase in the 20th and 30th day but later on they reached near the normal values and increased up to 4.08% only (P<0.01; Fig.2G).

Erythrocyte sedimentation rate (ESR) (mm/hr) increased 74.86, 215.01 and 58.3% in the normal control, infected control and treated rabbits (P<0.01; Fig.2H).

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Treatment with P. granatum

In this group the TEC remained almost unaltered in the normal control rabbits. However, slight decrease (5.1-6%) was noted in the end. These values drastically decreased in the infected control rabbits up to 27.08 percent. However, in the rabbits treated with P. granatum TEC decreased continuously and reached up to 33.33% in the end (P < 0.01; Fig.3A). In this case no improvement due to P. granatum was noted Hb contents showed slight variations but in the 90th day they showed 6.32% decrease. However, P. granatum treated rabbits showed initial decrease but after 40th day of treatment they improved and reached 8.4 ± 0.8 (g/dl) when compared with the normal control values 8.8 ± 0.8 (g/dl) (P < 0.05; Fig.3B).

PCV values in the control rabbits showed slight variations and in the end decreased up to 3.3% as compared to the infected control rabbits which showed 58.9% increase (P<0.001). However, PCV values in the rabbits given 2 g/day of A. granatum showed slight improvement and in the end they increased up to 10.2% (P<0.05; Fig.3C). As far as their haematological indices are concerned MCV, MCH and MCHC showed fluctuation as compared with the normal control values but ultimately showed improvements. While in the experimental controls these values changed drastically (P<0.001; Fig.3D, E, F).

TLC of normal control rabbits showed slight alterations in 50th day of experiment when they dropped to 7.9 ± 1.3 and afterwards they again improved and reached the normal values. Similar values in their corresponding experimental controls increased upto 65.6% in the end (P<0.001; Fig.3G). ESR values increased in the normal control, experimental control and treated control rabbits up to 74.8, 218.5 and 35.5% respectively (P<0.001; Fig.3H).

Treatment with A. indica

TEC also showed improvement (3.7%) in the rabbits given (2 g/day) A. indica as compared to the decrease of 27.08% and 5.6% faced by the infected control and normal control rabbits respectively (P < 0.01; Fig.4A).

Hb contents showed slight changes in the normal control rabbits except in the end with an overall reduction of 6.38%. Infected control rabbits faced a gradual decline that reached up to 72.9% in the end (P < 0.01; Fig.4B) as compared to the slight improvements (2.83%) faced by the treated rabbits.

PCV also showed little fluctuations throughout the course of experiment with an overall decrease of 3.3% in normal control rabbits and 0.30% in infected controls as compared to 58.9% increase in the infected controls (P<0.001; Fig.4C).

The haematological indices like MCV increased and MCH and MCHC decreased (P<0.001; Fig.4D, E). Overall increase noted in the TLC of normal control and infected control was 3.07 and 65.6% respectively. Increased TLC was also noted in treated groups but after 50th day of treatment they improved and in the end increase was only up to 13.18% (P<0.001; Fig.4G). ESR increased up to 74.8, 218.5 and 35.5% in the normal control, infected control and treated rabbits respectively (P<0.001; Fig.4H).

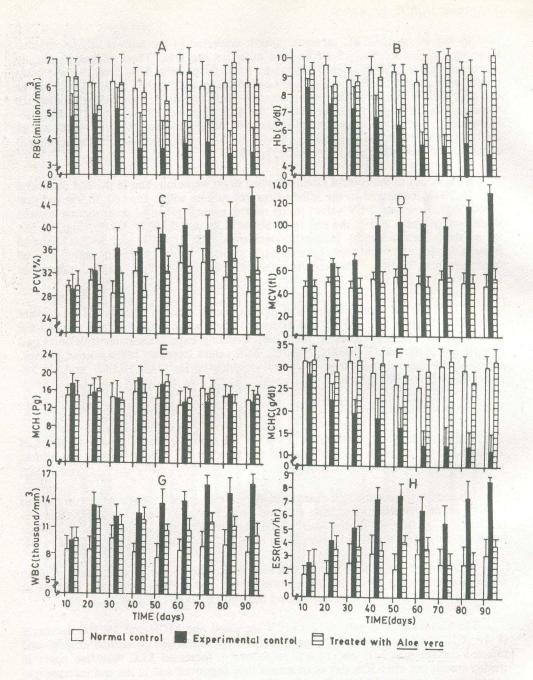


Fig. 2: Normal control, Experimental control, Treated with Aloe vera.

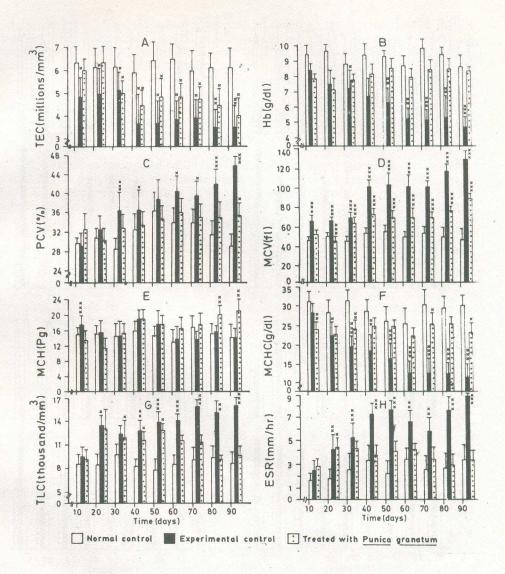


Fig. 3: Normal control, Experimental control, Treated with Punica granatum.

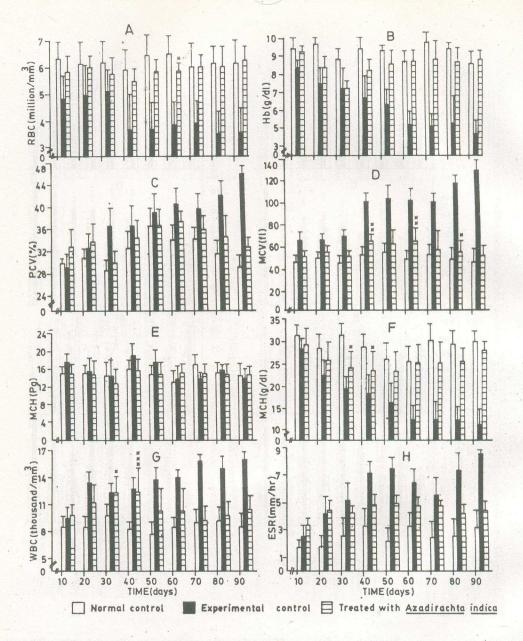


Fig. 4: Normal control, Experimental control, Treated with Azadirachta indica.

DISCUSSION

Rabbits maintained as infected control and those orally given A. vera, P. granatum and A. indica initially showed sharp decline in their body weight (g) when compared with their respective control ones. It is suggested that this decrease in body weight may be due to the combined effect of some toxicity present in the inoculated protoscoleces. Decreased body weight due to hydatidosis have also been reported by Pandey (1971) who studied its effects on liver, lungs and spleen of goats and reported gross changes in these tissues. He suggested that toxicity of hydatid cyst fluid may have changed the surrounding cells thereby resulting in organ condemnation which leads to weight loss. It also leads to economic losses through low quality and reduced yield of milk, meat and retarded growth (FAO, 1981; Schwabe, 1986). Animal suffering from hydatidosis also showed protein deficiency that ultimately lead in weight reduction (Iqbal et al., 1989). Toxic effect induced by the incoming protoscoleces also have affected some of their physiological phenomenon which have made them, lethargic, inactive and minimized their food intake that ultimately aided in their weight reduction.

Present results showed that body weight of treated rabbits showed more improvement as compared to the experimental control rabbits. Although this increase in weight was less as compared to the normal controls. Most probably this was due to the development of some resistance/ immunity or some inhibitory effect of plants against the incoming protoscoleces. Almost similar findings have been reported by Anwar et al. (1997) and Tanveer et al. (1997, 1998, 1998a).

Erythrocytes play a very important role in the transport of haemoglobin which carries oxygen to different parts of the body by converting it to metahaemoslobin. A nice balance kept between normal and destroyed erythrocytes in normal conditions was disturbed by the inoculation of protoscoleces that have decreased or destroyed them. Similar findings have been reported by Aminzhanov (1977) who noted decreased TEC in sheep naturally infected with E. granulosus. Decreased TEC due to alveolar hydatidosis in mice (Hinz and Gehring, 1987) and due to the inoculation of hydatid cyst fluid have been reported in rabbits (Anwar et al., 1997; Tanveer et al., 1997, 1998, 1998a). The decrease thus produced have caused anaemia. Anaemic condition noted in the present investigation was probably produced by the increased blood cell destruction. Damaged erythrocytes and reduced erythropoiesis are also related to decreased TEC that ultimately led to anaemia. Anaemia can also be induced by the reduced TEC (due to destruction), PCV and Hb contents, etc. However, an initial decrease in TEC was followed by a gradual improvement in the rabbits treated with A. vera, P. granatum and A. indica. Among the three plants tested, more promising results were achieved by A. vera and A. indica. However, the normal control rabbits showed minor fluctuations in their TEC. Improvement in the TEC may be due to some chemical present in the plants that have antagonized the harmful impact of incoming protoscoleces.

Hb transport oxygen to all parts of the body and bring carbon dioxide back. During present studies the Hb in normal control rabbits fluctuated but remained within normal range, while in infected control groups it decreased continuously. The rabbits, orally given A. vera, P. granatum and A. indica, also showed decreased Hb in the beginning, that later on improved with the passage of time. This improvement reached near normal values in case of P. granatum and A. indica, while in case of A. vera this value increased more than the control values. Reduced TEC in the infected control rabbits and treated rabbits (in the beginning) was mainly attributed to haemolysis that leads to binding of haemoglobin to plasma haemoglobin. During this process the extra

haemoglobin is excreted through urinc (Gottstein et al., 1993). Hb is partly converted to methaemoglobin prior to conversion to bilirubin. This haemolysis factor probably have increased haemoglobin in each RBC (Charles and Norman, 1966; Eastham, 1986; Dacie and Lewis, 1991). Gradual decline in the Hb level have also been reported in rabbits after administration of hydatid cyst fluid of sheep origin (Anwar et al., 1997). However, improved Hb contents in treated rabbits may be due to some chemical in the plants that may have antagonise/ produced resistance against the toxicity of protoscoleces. This increase was further confirmed by their increased MCV.

PCV depends up on number and size of erythrocyte. Decrease in PCV is normally associated either with decreased erythrocytic count or MCV or increased blood plasma level. In the absence of any significant change in Hb, TEC and MCV the only possibility is that decrease in PCV may be due to increased plasma level. In this case the erythrocyte increased in volume in infected control group so their volume increased more than the erythrocytes of rabbits orally given plants. Here the MCV values increased in the infected groups while the MCH and MCHC decreased. However, in the present investigation size of erythrocyte may have increased due to some toxic effect of protoscoleces and not due to haemoglobin formation. This increase may be associated with increased bone marrow activity, anemia, haemolysis and some deficiency of haemopoetic factor (Benjamin, 1985). MCHC in normal control rabbits was 30.1 g/dl which is close to the maximum value of Hb that RBC can hold (Sachdew, 1991). Increase MCV in infected rabbits showed macrocytic normochromic anemia (Benjamin, 1985; Sachdew, 1991). If MCH reached the upper normal limit it showed that the erythrocytes are of bigger size and therefore, contain/hold maximum amount of haemoglobin. Such a cell is always associated with macrocytosis (Swarup et al., 1986). General trend of increased MCV and decreased MCH and MCHC in infected control rabbits have also been reported by Anwar et al. (1997) and Tanveer et al. (1997, 1998, 1998a) in rabbits inoculated with hydatid cyst fluid of sheep origin.

The role of leukocytes is defensive in the body. They always require certain foreign stimulus for their increase. Hydatid cyst fluid have reported to stimulate the leukocytosis (Tanveer et al., 1997, 1998, 1998a). In the present work TLC sharply increased in rabbits administered with protoscoleces but later on the pace of increase slowed down in the treated rabbits. This increase in infected rabbits may be due to some resistance develop through the plants. Increase in TLC in the initial stage may be attributed to tissue damage (Eastham, 1985). Similar findings have earlier been reported by Hinz and Gehring (1987) and Alkarmi and Khan (1989) in mice infected with E. multilocularis. The phagocytic activity of blood monocytes in mice infected with E. granulosus and increase in WBC due to monocyte increase was noticed by Alkarmi and Behbehani (1989) and Wangoo et al. (1989). The effect of plant treatment (Prosopis glandulosa and Embelia ribes) with some curing effects have been reported by Anwar et al. (1997).

Inoculation of protoscoleces of sheep origin have also altered the ESR of rabbits. ESR depends on the rouleaux formation, which in turn depends on the concentration of fibrinogens and globulins (Frayha and Haddad, 1980) so their administration in the rabbits is responsible for the increased ESR values. However, these values, gradually came down in the rabbits orally given A. vera, P. granatum and A. indica.

In the present investigation, different haematological parameters of rabbits have been investigated after inoculating 45000 protoscoleces of sheep origin. The alterations shown in various parameters were found improving only in those rabbits which were

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given (2 g/day) A. vera (leaves), P. granatum (ovary) and A. indica (fruit). The results clearly showed that these plants have some potential in antagonising the harmful/toxic effects of protoscoleces. Further studies in this connection are needed.

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ISOLATION AND CHARACTERIZATION OF PENTACHLOROPHENOL DEGRADING BACTERIA FROM INDUSTRIAL WASTES*

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Abstract: Effluent samples collected from tanneries of Kasur and paper industry Lahore were spread on agar plates containing glucose deficient M9 media in which the pentachlorophenol (PCP) was added as the only carbon source. Four bacterial strains CB17 to CB20 were isolated and on the basis of physical and biochemical characterization identified as belong to genus Listeria sp., Lactobacillus sp., Pseudomonas sp. and Xanthomonas sp., respectively. The pure cultures of these isolates were prepared and their tolerance for PCP was determined upto 12 μ g/ml. The minimum inhibitory concentration (MIC) for CB17 was 10 µg/ml, for CB18 it was 11 $\mu g/ml$, for CB19 and CB20 it was 12 $\mu g/ml$., Optimum growth conditions of these isolates were determined. The optimum temperature for the growth of all these isolates was 37°C. The optimum pH for CB17 strain was 6.0, for CB18 and CB19, 5.5 and for CB20 it was 7.0. Growth curves for these isolates were prepared. They showed log and stationary phases. The span of log phase was 8, 8, 12 and 24 hours in 4 strains, respectively. The bacterial isolates were also tested for resistance against some heavy metals i.e., Cu^{+2} , Cr^{+6} and Pb^{+2} . The MIC for Cu^{+2} was 175, 350, 400 and 275 $\mu g/ml$, for Pb^{+2} it was 100, 200, 500 and 250 $\mu g/ml$ and for Cr^{+6} 200, 275, 175 and 225 µg/ml respectively for these strains. All isolates showed resistance to antibiotics such as urixin, erythromycin, cefazolin and SXT. The MICs of these isolates against PCP proved that these insecticide degrading bacteria although, can be used for the purpose of environmental cleanup but they showed relatively, moderate potential for degradation of PCP.

Key words: Decontamination, bioremediation, chlorinated phenols, microbial degradation, biodegradation, polychlorophenols, chlorinated phenols, microbial degradation, Lactobacillus sp., Listeria sp., Pseudomonas sp., Xanthomonas sp.

INTRODUCTION

uge amount of chemicals are being used in the form of insecticides, herbicides, bactericides, rodenticides, dyes' food additives, cosmetic products, pharmaceuticals and fertilizers (Winter and Street, 1992; Carrara et al., 1993; Conning, 1993; Francis, 1994; Lin and Ho, 1994; Timbrell, 1995). The most dangerous pollutants are pesticides and industrial compounds such as halogenated aliphatics, polychlorinated biphenyls, chlorinated phenols and polycyclic

aromatic hydrocarbons (Nichols et al., 1995; Orser, 1996; Chauhan et al., 1998; Livingston et al., 1998; Rawlings et al., 1998).

The three main groups of insecticides, which are being used these days, are organophosphorous (OP), organochlorine (OC) and pyrethroids which are extremely toxic to non-target animals in both acute and chronic studies (Shakoori *et al.*, 1982, 1992; Shakoori and Haq, 1987; Ali *et al.*, 1988, 1997; Ahmad *et al.*, 1995; Ali and Shakoori, 1996, 1998, 1999; Ali and Mir, 1998). Organochlorine compounds are serious pollutants in the environment. They become concentrated, instead of dispersal, in food chain. They are not easily degraded into their metabolites (Focardi *et al.*, 1986; Masud and Hasan, 1992; Winter and Street, 1992; Redetzke and Applegate, 1993). Their toxicity is being increasingly reported in terrestrial, marine and freshwater animals including fish, birds, dairy animals and other mammals. After entering into the system, variable quantity of these pesticides and thus metabolites are ultimately accumulated in various tissues of animals including man.

Pentachlorophenol (PCP) is one such compound used as wood preservative fungicide, bactericide, molluscicide, herbicide and insecticide etc. During last several decades large quantities of PCP have found their way into the environment, resulting in significant environmental contamination at many wood processing sites (McGinnis et al., 1994; Hryhoxzuk et al., 1998). It is very stable compound with high cummulative potential in animals, plants and food chains. Significant amounts of this compound have been reported in human serum, adipose tissue and urine (Feit et al., 1998; Osman et al., 1998; Petroske et al., 1998). PCP is even found in people not occupationally exposed to it or not living in PCP treated log houses (Jorens et al., 1991). The acute pentachlorophenol intoxication is dangerous for the life. Chronic PCP exposure may increase the incidence of several diseases e.g., immunodeficiency, blood disorders, reproductive abnormalities, tissue lesions, mutagenicity and malignancies (Vasarhelyi et al., 1993; Gichner et al., 1998; Hryhorczuk et al., 1998; Gerhard et al., 1999; Umemura et al., 1999). Acute and chronic poisoning may occur by dermal absorption, inhalation or ingestion. Chronic poisoning occurs mainly in saw mill workers or people living in log houses treated with PCP - containing wood protection formulations. PCP poisoning may be classified systematically into effects on the skin metabolism, the haematopoietic tissues, the respiratory systems, central and peripheral nervous systems, the kidney and gastrointestinal tract (Jorens and Schepens, 1993).

Increasing public concern over the contamination of the environment with industrial wastes has elicited considerable research into methods of removing them from soil and water (Kirk et al., 1995; Karamanev et al., 1998). Microbial degradation is of considerable interest. In such experiments investigation generally follow the disappearance of polychlorinated organic compounds by dehalogenation, oxidation, reduction, hydrolysis, ring cleavage by condensation or conjugate formation (Nakatsugawa and Morelli, 1976; Gibson and Skett, 1993; Parkinson, 1996). Microorganisms are the scavengers in nature, responsible for recycling most natural and synthetic waste materials into harmless or non-toxic compounds, when faced with an increasing array of synthetic compounds such as various compounds manufactured by chemical industries for common use. Thus such xenobiotic compounds tend to accumulate in the nature exerting their toxic effects on animals and humans upon their entry into the body, mostly as the part of food chain.

In present study an attempt is being made to isolate and characterise some indigenous bacteria which may prove beneficial for degradation of highly persistent chlorinated compound, the pentachlorophenol from contaminated soil and water.

MATERIALS AND METHODS

Collection of samples and bacterial isolation

The waste water samples to isolate useful bacteria were collected in sterilized bottles from waste effluents of paper industry, Lahore, and tanneries in Kasur.

Media used for bacterial reening

Three type of media were used for bacterial isolation i.e., M9 Agar medium, LB (Luria Bertani) Agar medium and LB broth medium (Maniatis et al., 1982).

Pollutant used and its preparation

The technical grade insecticide, fungicide i.e., pentachlorophenol (PCP) was used for these studies. The stock solution was prepared by dissolving 10 mg PCP/ml acetone. This stock solution was added @ 50 μ l (=0.5 mg) per 100 ml of M9 medium.

Screening of PCP resistant bacteria

Waste effluent samples (50 μ l) were spread on the plates containing M9 medium in which PCP was added as the only carbon source. Two types of control media were used. First with glucose only and second without glucose and insecticide. Four bacterial types successfully grew at plates containing M9 media in which the toxicant PCP was added as the only carbon source @ 5 μ g/ml. For pure culture single colony was picked and streaked on LB agar plates. These strains were stored as glycerol stocks.

Study of PCP resistance of isolates

The recovered bacterial strains were further tested for determination of minimum inhibitory concentration (MIC). The PCP concentrations used for this purpose were 6, 8, 10 and 12 μ g/ml of medium.

Identification and characterization of isolates

Various physical, morphological and biochemical tests were performed to determine the major biochemical activities of the isolates, which are later on used for their identification. They were also characterized for their optimum growth conditions. All these strains were cultured in different media with the determination of optimum conditions of temperature, pH, inoculum size etc. They were also checked for resistance against some heavy metals and antibiotics.

Study of optimum growth conditions of isolates

For determination of optimum growth conditions, for each isolate, two parameters i.e., temperature and pH were considered. Following procedure was adopted to find out the optimum temperature at which the strains showed maximum growth.

For each isolate 12 LB broth culture tubes were prepared for each temperature. These tubes were autoclaved and after cooling incubated at 37°C for checking contamination. Fresh culture (50 μ l) of each isolated strain was inoculated in each tube and incubated at 25°C, 30°C, 37°C and 42°C for 8 hours. The light absorbance which is a measure of growth was noted at 600 nm wavelength using Hitachi 2000 double beam spectrophotometer. The values were plotted in graphical form and the peak of graph indicated the optimum temperature of strain.

Determination of optimum pH of isolates

To study the optimum pH for maximum growth of bacterial strains, following procedure was adopted.

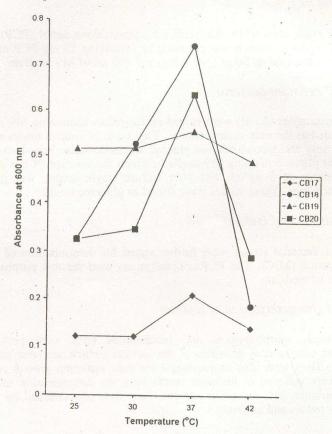


Fig. 1: Graph showing the optimum temperature for growth of four bacterial isolates.

LB broth (5 ml), taken in triplicate, each with 9 tubes was used for each isolate. The pH in each set was adjusted at 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 of 1M HCl and 1M NaOH solutions. All test tubes were autoclaved cooled and inoculated with fresh pure culture of isolates, followed by incubation in shaking water bath for 8 hours at 37°C to check the growth of each bacterial isolated. The pH showing highest optical density was considered as the optimum pH of that isolate.

Preparation of growth curves of isolates

To determine the growth curves of each isolate, 39 test tubes with 5 ml LB broth in each, were used. These were adjusted at their optimum pH, and were autoclaved and incubated at 37°C overnight. Fresh bacterial culture (50 ml) was inoculated in each tube and incubated in shaking water bath at 37°C for 10 hours consecutively and then after 12, 24 and 36 hours. Their light absorbance was measured and graph was plotted.

Antibiotic and heavy metal resistance of isolates

The resistance of all isolates was also checked against toxic heavy metals like Cu^{+2} , Cr^{+6} and Pb^{+2} . The starting minimum concentration was 300 μ g/ml, 1 mg/ml and 100 μ g/ml of LB broth. These concentrations were successively increased or decreased to determine MIC depending upon the results obtained with each isolate.

For checking antibiotic resistance, sensitivity discs of four antibiotics (urixin, erythromycin, SXT and cefazolin) were placed separately in 4 LB agar plates, which were inoculated previously with 4 different bacterial isolates. These were incubated overnight. Clear area around the antibiotic discs indicated the sensitivity of isolates for that particular antibiotic. While the growth of isolates around antibiotic disc indicated its resistance.

RESULTS

The four PCP tolerant isolates, recovered from industrial waste samples were tested for various characteristics. These include the evaluation of minimum inhibitory concentration (MIC), heavy metal resistance and antibiotic resistance. Their optimum growth and growth conditions were determined. The isolates were also characterized physically, morphologically and biochemically for their identification. Four bacterial isolates which grew successfully in PCP containing M9 agar media were designated as CB-17, CB-18, CB-19 and CB-20.

Minimum inhibitory concentration (MIC) of isolates

The four bacterial isolates slightly differ in MIC against PCP which was 10 μ g for the MIC of PCP for CB-17 was 10 μ g/ml, for CB-18 it was 11 μ g/ml and for CB-19 and CB-20, 12 μ g/ml. The MIC values indicated that these four isolates have almost similar potential as far as PCP degradation is concerned.

Characterization of isolates

Different physical and morphological characteristics shown by the four isolates have been shown in Table I, while biochemical characterization is mentioned in Table II. On the basis of these tests, the isolates CB-17 to CB-20 were identified as *Listeria* sp., *Lactobacillus* sp., *Pseudomonas* sp. and *Xanthomonas* sp., respectively.

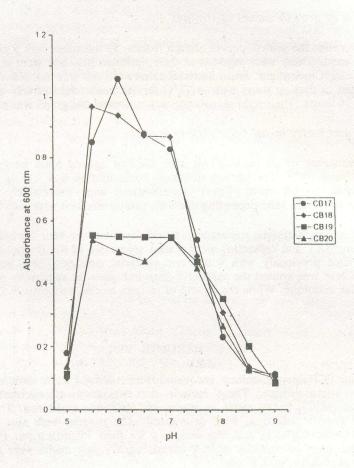


Fig. 2: Graph showing the optimum pH range for growth of four bacterial isolates.

Optimum growth conditions and growth curves

For the study of optimum growth conditions, these isolates were cultured at different temperatures and pH. The mean optimum temperature for the growth of all four isolates was 37°C (Fig.1), while the optimum pH for CB-17 bacterial isolate was 6.0, for CB-18 and CB-19, 5.5 and for CB-20 it was 7.0 (Fig.2). The growth curves, using light absorbance of the media, for CB-17 to CB-20 were prepared. They clearly

showed log phases (Fig. 3).

Cross heavy metal resistance of isolates

The resistance of above four bacterial strains were checked against different heavy metals i.e., Cu^{+2} , Cr^{+6} and Pb^{+2} . The MIC shown by CB-17 was 175 μ g/ml for copper, 200 μ g/ml for chromium and 100 μ g/ml for lead. The MIC for CB-18 was higher i.e., 350 μ g/ml for copper, 275 μ g/ml for chromium and 200 μ g/ml for lead. CB-19 showed 400 μ g/ml MIC for copper, it was 175 μ g/ml for chromium and 500 μ g/ml for lead. CB-20 showed highest MIC 275 μ g/ml for copper, 225 μ g/ml for chromium and 250 μ g/ml for lead.

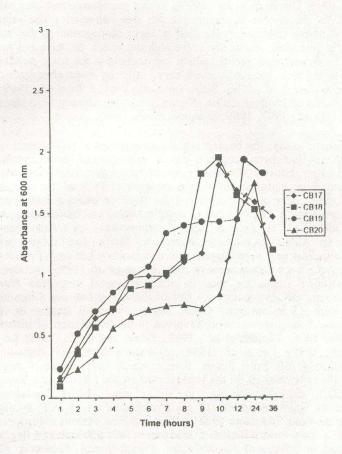


Fig. 3: Graph showing the normal growth curves for isolated four bacterial strains under optimum conditions of temperature and pH.

Resistance against antibiotics

The bacterial isolates were tested for resistance against different antibiotics i.e., rerythromycin, cefazolin, urixin and SXT. CB-17 showed sensitivity for erythromycin, urixin and SXT while it was resistant to cefazolin. CB-18 and CB-19 were resistant to all four antibiotics and CB-20 showed resistance against urixin and cefazolin while sensitive to erythromycin and SXT.

DISCUSSION

Chlorinated hydrocarbons are notorious for their ability to induce genotoxicity, mutagenicity and carcinogenicity (Saracci et al., 1991; NTP working group, 1993; Pitol and Dragan, 1996; Umemura et al., 1999). In fact persistence and long stability of their molecules in the ecosystem is responsible for this behaviour. The remedy from the destruction of these chemical pollutants is their biodegradation by microorganisms having specific potentials, especially in the soil and water. Bacteria and fungi have been proposed as bioremediation agents, which can transform the toxic pollutants into nontoxic products by their metabolic machinery, utilizing these chemicals for their own metabolic needs (Lee et al., 1998; Stahl and Aust, 1998; Fahr et al., 1999; Tayal et al., 1999). The studies on biodegradation of insecticides has already been reported from this laboratory (Ali et al., 1997, 1999; Shakoori et al., 1999).

In the present study, the biodegradation potential of some bacterial isolates against pentachlorophenol has been studied, which were isolated from industrial waste of tanneries and paper industry. The four types of bacterial colonies appeared/ survived in the media containing 5 μ g/ml initial concentration of PCP as the only carbon source. The isolates were, later on, characterized for their optimum growth conditions along with their identification. On the basis of morphological and biochemical characterization they were identified as Listeria sp., Lactobacillus sp., Pseudomonas sp. and Xanthomonas sp. All strains were rod shaped i.e., bacilli. The first two isolates (Listeria sp. and Lactobacillus sp.) were Gram-positive rods while last two (Pseudomonas sp. and Xanthomonas sp.) were Gram-negative rods. Farah et al. (1999) isolated 4 endosulfan (an OC insecticide) degrading bacteria which belonged to genera Planococcus sp., Marinococcus sp., and Acetobacter sp. Out of these, last one was Gram-negative, while other two were Gram-positive. Other reports showed strains of Pseudomonas, Flavobacterium, Sphingomonas were involved in biodegradation of insecticides (Orser, 1996; Pfender, 1996; Pfender et al., 1997; Saboo and Gealt, 1998; Lo et al., 1998; Schmidt et al., 1999). Lee et al. (1998) collected a novel PCP degrading strain from PCP contaminated soil from Pusan, Korea which can tolerate 1 mg PCP/ml and identified as Pseudomonas sp. In the present studies, the Listeria sp., showed the growth up to 10 mg/l, Lactobacillus sp., up to 11 mg/l, while Pseudomonas sp. and Xanthomonas sp. grow up to the 12 mg/l concentration of PCP as the only carbon source in the medium. Mannisto et al. (1999) reported various chlorophenol degrading bacteria from a long term polluted groundwater. Polychlorophenol degrading capacity has been found in isolates of Nocardioides, Pseudomonas, Ralstonia, Flavobacterium and Caulobacter previously not known to degrade these compounds. According to this study a Gram-positive isolate was sensitive to PCP with tolerance level of 5 mg/l. Isolates belonging to Cytophaga/ Flexibacter Bacteroides phylum shown to tolerate 25-63 mg/l concentrations of chlorophenols. The various strains of Sphingomonas sp., were also reported to degrade chlorinated phenols (Rutgers et al., 1998; Mannisto et al.,

1999). Buitron *et al.* (1996) isolated and characterized the microorganisms responsible for degradation of a mixture of phenol, 4-chlorophenol, 2,4-dichlorophenol and 2,4,6-trichlorophenol in terms of their degradation rate which, according to this study, was sequential with phenol and 4-chlorophenol degraded first followed by 2,4-dichlorophenol and then 2,4,6-trichlorophenol. It has also been reported that presence of highly chlorinated compounds in the mixture, increase the degradation of less chlorinated compounds significantly (Lu *et al.*, 1993).

The optimum temperature for growth of all four isolates was 37°C. It has been observed that at 45°C the first PCP dechlorination steps were very rapid but later dechlorination occurred with difficulty. Below 15°C the dechlorination was very slow (Juteau et al., 1995). These findings were also true for our studies in the present experiment in which less growth was observed below 15°C. The pH of the media has also profound effect on the biodegradation potential of the microorganisms. The Listeria sp., in the present study showed successful growth at pH 6, Lactobacillus sp., and Pseudomonas sp., at pH 5.5, while Xanthomonas sp., at pH 7. According to Sloff et al. (1991) the mobility of the chlorophenols in soil is dependent on the characteristics of the compound and those of the soil. It can vary from extremely mobile to almost mobile. Whether a chlorophenol occur in a water phase as neutral hydrophobic compound or as a soluble phenolate anion, depends on the pka of the chlorophenol concerned as well as pH of its aquatic environment. In environmentally relevant pH ranges between 6-8, the solubility increases sharply. The Pseudomonas sp. and Xanthomonas sp., showed almost stable growth zone in pH range 5.5-7.0. The Lactobacillus sp., also behaved almost similarly in the above range. Rutgers et al. (1998) reported that PCP exhibited stronger toxicity at low pH working with Sphingomonas sp., strain P-5. The reults suggest that increase in environmental pH, may reduce the risk of chlorophenol toxicity. It has been observed that for successful biodegradation system, a suitable combination of physical and chemical factors is required for each organism under which that can work efficiently. The growth curves of the isolates prepared using optimum growth conditions, showed log and stationary phases. No lag phase appeared because the isolates have already passed this during 24 hours pre-inoculation incubation at 37°C. It has also been reported that lag phase prior to PCP biotransformation increase with decrease in temperature. The log phase of 72 hours was observed at 31°C (Cole et al., 1996). In this study, under present set of conditions Listeria and Lactobacillus showed 12 hours log phase while Pseudomonas sp. and Xanthomonas sp., showed 24 and 30 hours log phase respectively. Similar studies by other workers indicated variable response of isolated bacteria against PCP (Lee et al., 1998; Stuart and Woods, 1998; Schmidt et al., 1999).

Keeping in view the MIC of PCP and various heavy metals against these isolates, it can be concluded that these isolates can be used for the purpose of environmental cleanup. As far as comparative biodegradation potential is concerned these isolates showed moderate capacity to degrade PCP. There is a need to extend these studies for screening of microorganisms having better biodegradation potential.

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TOXICITY OF METHAMIDOPHOS IN PREGNANT MICE AND DEVELOPING FETUSES

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Abstract: The effects of an organophosphorus insecticide, methamidophos were evaluated in pregnant mice and developing fetuses. To calculate LD50 values for pregnant mothers and developing fetuses different concentrations (5 to 20 μ g/g BW) of the insecticide were given orally to pregnant mothers on day 6 of gestation. The mothers were watched after the dose administration up to 24 hours. The dose of 15 and 20 μ g/g BW proved 100% mother lethal. The mothers that survived for 24 hours after dose administration were found recovered from insecticide's toxic effects as the usual symptoms of eye, oral and anal secretions and limb weakness subsided. The LD50 values for mothers were turned out 9 μ g/g BW. The fetuses from mothers survived after 24 hours of treatment were recovered on day 15 of gestation. Dead fetuses were noted and were fixed in Bouin's fixative. The LD50 value for fetus was calculated as 8.2 μ g/g BW of mother.

Key words: Organophosphates, methamidophos, maternal toxicity, fetotoxicity.

INTRODUCTION

versince the time, humans came to occupy the earth they found themselves competing with other animals for existence. There was competition for food as well as space. Humans by all virtue of superior intelligence outmaneuvered their competitors and created all sources of habitats in which they lived severely. With the increase in their agricultural skills, they started growing and cultivating many new crops to supplement their food. But to their dismay, they found that many animals like their crops and took away a lot of their yield. Among these animals, insects were the main culprits. To combat this situation, an organized pest control programme had to be developed. Many poisons were used to kill these insects and were named insecticides.

The use of these insecticides has increased many folds during the last three decades. It has been estimated, that about 2.7 billion kg of insecticides were used in one year (Munnecke, 1980). Unfortunately where this extensive use of insecticides had rendered great service to mankind, it also led to many problems. The most important of these problems is that these insecticides proved harmful to many non-target organisms (Newsom, 1967; Tucker and Leitzke, 1979) as it turned out that most vulnerable of these non-target organisms were the human beings themselves. Lahman *et al.* (1990) studied a case of polyneuropathy with flaccid paresis of the extremities and accompanying

vegetative system impairments in a 39-year old woman farmer after acute poisoning with phytosol. Another study was done on a group of 48 workers employed at production of organophosphorus (OP) pesticides. In a clinical picture, the subjective symptoms complex was predominant including pains, vertigo, feeling of fatigue after work, difficulties with attention, concentration, memorizing paresthesia and polyneuropathy (Halina, 1990).

The primary action of OP insecticides is inhibition of AChE at cholinergic nerve terminals which has been extensively reported (Aldrin *et al.*, 1947; Holmstedt, 1959; Usdin, 1970; Davis and Richardson, 1980; Ecobichon, 1983).

It is quite apparent that much more research will have to be carried out before we know more specifically, about the effects of these chemicals on pregnant mothers and the unborn and thus plan and design ways and means for the use of these insecticides in the most appropriate way.

MATERIALS AND METHODS

Swiss Webster variety of *Mus musculus* was used in this experiment. Breeding stock was kept in steel cages in controlled environmental conditions in the form of 12 hours light/dark cycles and temperature of 28±1°C and humidity 40-55%. Each cage had wood shavings as bedding material, which was replaced every other day. Chick feed No.3 (Punjab Feeds, Lahore) was given to these mice.

For experiment, first objective was to mate male and female under controlled conditions so that exact conception time and day "0" of gestation can be determined accurately. Three estrus females were kept with one male overnight in each cage. Next morning, these females were examined for presence of vaginal plug, which was considered as confirmation of fertile mating. These females were isolated in separate cages and marked day "0" of gestation.

During this research, maternotoxic and embryotoxic effects of methamidophos were tested. Various concentrations of methamidophos were used to determine LD $_{50}$ values. Since no such values for pregnant mice were found in literature, so it was decided to try a wide range of concentrations (5 to $20~\mu g/g$ BW) to reach LD $_{50}$ values. These doses were prepared by dissolving the insecticide in water in such a way that 0.1~ml of solution contains desired concentration. The doses were given to pregnant mice with the help of 1 ml glass syringe to which a capillary rubber tubing was attached. The doses were pumped into the gullet, which were readily gulped by the mice. By this technique, there was minimum escape of dose. These doses were given at day 6 of gestation. The treated mothers who survived after 24 hours of dose application were kept singly in different cages till day 15 of gestation. The control groups were maintained by giving only distilled water.

On day 15 of gestation, the pregnant mothers were weighed and then anaesthesized with ether. After giving a cesarian section, the two horns of uterus were taken out of the body and weighed. The live and dead/resorbed fetuses were counted and were dissected out of the uterus and then fixed in Bouin's fixative for 48 hours. In cases, where the fetuses were resorbed, such fetuses were not taken out of the uterus.

Both control and treated fetuses were prepared for morphological and anatomical studies. The fetuses, which were preserved in Bouin's fluid, were then shifted to 70% alcohol for overnight. Then they were preserved in 80% alcohol. Morphological and morphometric studies involved wet weight as well as crown-rump length measurement of each fetus. These fetuses were closely examined by placing under binocular dissecting microscope. The photographs were taken with the help of camera fitted with close-up-lenses.

Data Analysis

 LD_{50} values were calculated by probit analysis (Finny, 1964) and plotting regression line between dose concentrations and maternal as well as fetal mortality. The other data were analyzed by student "t" test.

RESULTS

Single treatment of all concentrations (5-20 μ g/g BW) was given to pregnant mothers on day 6 of gestation. In order to see the effects of insecticide on mother's morphology and behaviour, some observations were made such as lordosis, heavy sweetening, oral and anal secretions and weakness. When higher doses (15-20 μ g/g BW) were administered mothers died immediately after treatment.

After calculations and plotting regression line, the LD₅₀ value for pregnant mothers was found to be 9.00 μ g/g BW for 24 hours (Table I, Fig.1). Some recovered fetuses were found dead. In some of these cases, all fetuses were resorbed within the uterus (Fig.2). For fetuses, LD₅₀ value was turned out as 8.2 μ g/g BW of pregnant mothers (Table II, Fig.1).

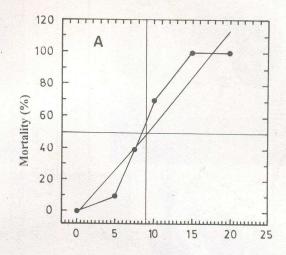
From 20 different mice, 151 control fetuses were recovered (Table III). These fetuses were uniform in appearance and in their other anatomical details. The average weight of fetuses was 431.76±88.16 mg and average CR length was 14.02±1.50 (Table III). These fetuses were well developed (Fig.2).

After the exposure of fetuses to different concentrations of methamidophos, different morphological observations were made. It was found that low dose (5 μ g/g BW) also proved to be toxic for some mothers themselves. With the administration of higher doses, rate of maternal mortality increased from 10 to 100% as a result of single dose of 5 to 20

Calculation of log-dose/probit line for pregnant mice exposed to 6 different doses of methamidophos, administered orally on day 6 of gestation.

Table I:

6	5	4	ω	2	1				
0.00	5.00	7.5	10	15	20	μg/g BW	conc.	Dose	1
20	20	20	20	.20	20		animals used	# of	2
0.00	10	40	70	100	100		Dead	0.00	3
00	10	40	70	100	100	P	ted % Dead	Correc-	4
1	0.699	0.875	1.00	1.176	1.3	×	the	Log of	5
1	3.72	4.75	5.52	ı.	1		probits	Empi-	6
1	3.9	4.7	5:5	6.5	7.2	1	probits	Expec-	7
1	3.74	4.75	5.53	7.01	5.69	y	efficient	Weig-	8
i.	0.405	0.616	0.581	0.269	0.092			Weight	9
	8.1	12.32	11.62	5.38	1.84	¥ 100 E			10
	5.66	10.78	11.62	6.326	2.392				1
	5.66 30.294	58.52		5 37.714	10.469	2,7	1000		12
1	3.81	4.78		6.44			1,1		13



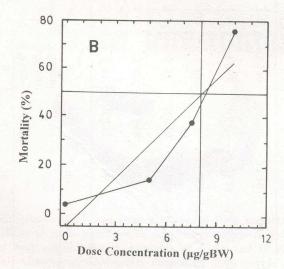


Fig.1: Regression lines of toxicity of methamidophos given on day 6 of gestation to the pregnant mothers (A) as developing fetuses (B), recovered on day 15 of gestation.

Table II: Calculation of log-dose/probit line for mouse fetuses exposed to 6 different doses of methamidophos, administered orally on day 6 of gestation.

6	S	4	w	2	-				
0.00	5.00	7.5	10	15	20	μg/g BW	conc.	Dose	1
1.51	124	74	83	. 00	00		animals used	# of	2
3.97	14.51	37.8	75.9	1	I		Dead	%	3
4	15	38	76	1	1	P	ted % Dead	Correc-	4
00	0.699	0.875	1.00	1.176	1.3	×	the dose	Log of	5
3.25	3.96	4.69	5.71	00	00		rical probits	Empi-	6
3.1	4.6	5.00	5.3	5.6	5.9	Y	ted probits	Expec-	7
3.246	4.068	4.70	5.52	3.42	2.83	Ψ.	ing co- efficient	Weig-	8
0.180	0.601	0.637	0.616	0.558	0.471			Weight	9
27.18	74.524	471.38	51.128	00	000	Ж			10
00	52.092	41.245	51.128	00	00	хм			11
88.226 2.92	52.092 303.163	.245 221.548	.128 282.22 5.1	00	00	úм			12
2.92	4.42	4.8	5.1	5.44	5.71	. 'Y			12 13

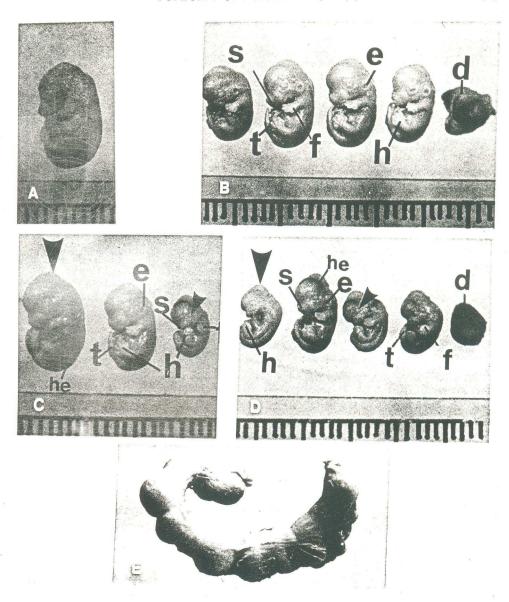


Fig.2: Macroj hotographs of 15-days fetuses recovered from mothers exposed to different concentrations of methamidophos on day 6 of gestation. A, a control fetus with well developed body organs; B,C,D, members of 5, 7.5 and 10 μ g/g BW dose groups, respectively showing developmental anomalies; E, a gravid uterus with resorbed fetuses, recovered from 10 μ g/g BW dose group. Note: dead fetus (d), small pina (e), paddle shaped forelimb (f), hind limb (h), skin haemorrhage (he), abnormal snout (s), short tail (t), microphthalmia (small arrow head) and abnormal brain parts (large arrow head).

μg/g BW, respectively (Table I).

The fetuses recovered from survived mothers were studied and it was found that they were negatively affected. There was tendency towards dwarfism. Their body weights and CR lengths were reduced. For a dose of 5 μ g/g BW to mothers, the recovered fetuses had an average body weight of 393.13±9.83 mg while CR length was 13.65±0.19 mm. Following the doses of 7.5 and 10 μ g/g BW, the corresponding figures for BW and CR length were 364.58±11.74 mg and 13.38±0.17, 66.36±3.41 and 8.12±0.16 mm, respectively (Table III). In dose of 10 μ g/g BW, the reduction in BW and CR length was highly significant (P<0.001) against controls.

The fetal abnormalities were also observed in recovered fetuses. In dose of $5.00~\mu g/g$ BW, out of 29 fetuses 8 were dead while 31% were microphathalmic 48% were with short pinnae, 20% had short tail, 35% had limb defects and 55% found having skin haemorrhages. Following an administration of 7.5 $\mu g/g$ BW, out of 33 fetuses 5 were dead, 45% were microphathalmic, 33% were with short pinnae, 9% had short tail, 36% had limb defects, 58% showed haemorrhagic spots and 27% developed with abnormal snout.

Following a dose of $10~\mu g/g$ BW, the mortality rate was 70% (Table I), 71% had small eyes, 76% found having short pinnae, 67% had short tails, 67% had brain abnormalities. All these fetuses were developed with abnormal snout and defected limbs (Table III).

Table III: Developmental abnormalities caused by an organophosphorus insecticide, methamidophos in mouse fetuses.

	The state of the s			
Dose μg/g BW				
С	5.00	7.5	10.00	
45	29	33	22	
2	8	5	5	
$431.75 \pm$	393.13±	364.56±	66.36±	
88.16	9.83*	11.74**	3.41***	
$14.02 \pm$	13.65±	13.38±	8.12±	
1.5	0.19	0.17*	0.16**	
10	31	45	71	
00	48	33	76	
00	20	9	67	
00	35	36	100	
8	55	58	67	
00	00	27	100	
5	00	00	67	
	45 2 431.75± 88.16 14.02± 1.5 10 00 00 00 00 8	C 5.00 45 29 2 8 431.75± 393.13± 88.16 9.83* 14.02± 13.65± 1.5 0.19 10 31 00 48 00 20 00 35 8 55 00 00	C 5.00 7.5 45 29 33 2 8 5 431.75± 393.13± 364.56± 88.16 9.83* 11.74** 14.02± 13.65± 13.38± 1.5 0.19 0.17* 10 31 45 00 48 33 00 20 9 00 35 36 8 55 58 00 00 27	

Asterisks show significant difference against controls (*P<0.05; **=P<0.01; ***=P<0.001).

DISCUSSION

The toxic effects of a commonly used OP insecticide, methamidophos were worked out in pregnant mice. LD_{50} values for the insecticide were calculated as 9 μ g/g BW for pregnant mothers and 8.2 μ g/g BW (mother) for developing fetuses.

It was observed that maternal mortality was greater at higher dose concentrations. After the administration of doses of 15-20 μ g/g BW, there was immediate death of mothers. There was also a high mortality rate (70%), even at a dose of 10 μ g/g BW (Table I). It is quite apparent from these observations that methamidophos is highly toxic to mice. This insecticide caused acute respiratory distress and restlessness, which may be due to neurotoxic effects of the insecticide.

In many previous studies as well, it has been shown that OP insecticide are toxic to the nervous system by inhibiting AchE activity. Inhibition of AchE results in an accumulation of free acetylcholine in nervous tissues (Kobayashi et al., 1980) and smooth muscles (Narahashi, 1979) producing undesirable side effects including death (Murphy, 1980). The cause of death by OP poisoning was thought to be a combination of excessive bronchial secretions, pulmonary edema, bronchospasm, respiratory muscle paralysis and paralysis of respiratory medulla, in short, "Respiratory Failure" (Du Toit et al., 1981: Zwiener and Ginsburg, 1988; Bardin et al., 1989). It had been discovered that the cardiorespiratory system is the first to be involved during OP poisoning with death resulting from asphyxia and/or impaired circulation (Heath, 1961; O'Brien, 1967; Taylor, 1980). Bakima et al. (1989) described that experimental Dichlorovos toxicosis induced changes in the mechanics of breathing and pulmonary haemodynamics associated with diffused bronchoconstriction and cardiac insufficiency, respectively. This type of poisoning also has been identified in humans. Jamil (1989) reported that 1900 cases of poisoning admitted to the Department of Intensive Care at Jinnah Postgraduate Medical Centre, Karachi between 15th January 1978 to 31st December 1985. Out of which 755 cases were of OP insecticide poisoning, forming 39.7% of the total poisoning cases and mortality rate of OP poisoning was twice as high (3.8%) as the mortality rate from all other poisons excluding OPs (1.8%). In another report respiratory failure developed in 43 (40.2%) of 107 patients with acute OP poisoning out of which 22 (51.2%) died (Tsao et al., 1990). The observations made on fetuses during the present study have also proved that these OPs are embryotoxic and teratogenic in mice.

As far as the effects of organophosphate, methamidophos on BW and CR length are concerned, there was significant (P<0.001) decrease in all treated fetuses as compared to controls (Table III). Morphological studies also supported the toxic effects of the insecticide. Some cases of hydrocephaly, fetal distortion, hydroneurocoel, deformed limbs and tail, microphthalmia and abnormal ear were found (Fig.2).

It is quite apparent from the results that methamidophos is embryotoxic. These results are not surprising because many of the previous studies or investigations have also shown that OP insecticides are highly toxic to mammalian embryonic systems, even at lower concentrations. Budreau and Singh (1973) tested two OP insecticides, Demeton and Fenthion for their teratogenic effects on mice. It was discovered that Demeton administered in the form of a single dose of 7 to 10 mg/kg or in the form of doses of 5 mg/kg between 7th and 12th day of gestation, proved to be only mildly teratogenic.

Karlow and Marton (1961) found that a continuous administration of malathion for a 10 week period to rats before and during pregnancy also resulted into dwarfism and increased mortality. Recently, Machin and McBride (1989) also studied the effects of a 100 mg/kg dose of malathion in rabbits, given on day 7-12 of gestation and found that although this dose produced no significant fetal abnormalities, still at least one case of fetal resorption was recorded. Lechner and Abdel Rehman (1984) also found an increased incidence of external haemorrhagic spots on rat fetuses following a treatment of 50 mg/kg of malathion. The present results are also supported by the studies of Staples and Goulding (1979) that developmental alterations are produced in mice exposed to OPs.

All these studies including the present study indicate, in spite of being non-accumulative and biodegradable, these OP insecticides are still harmful to adult animals and potentially dangerous to developing fetuses when given comparatively higher concentrations.

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CLINICAL CHEMISTRY PROFILE OF AN INSECT, DINOTHROMBIUM TINCTORIUM

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Abstract: Dinothrombium tinctorium, the mites of family Thrombididea, are administered routinely in the Unani system of medicine, with reference to human fertility regulation. These mites have been found rich in steroidal hormones and lipids, the probable cause of their therapeutic property. The present study looks at a yet unexplored aspect of these mites, clinical chemistry, through quantification of ions like calcium, phosphorus, potassium and sodium, in addition to parameters such as creatinine, uric acid, bilirubin, alkaline phosphatase, albumins, globulins, urea, high density lipoproteins, low density lipoproteins, lactate dehydrogenase, creatine phosphokinase, glutamate oxaloacetate transaminase and glutamate pyruvate transaminase.

Key words: Dinothrombium tinctorium, insect, aphrodisiac, clinical chemistry

INTRODUCTION

inothrombium tinctorium as a treatment of male infertility in the Unani system of medicine, is administered orally, both as total animal powder as well as metabolites collected on rice (Kabir, 1937; Board of Ayurvedic System of Medicine, 1971). Studies have been carried out to scientifically determine the efficacy of the drug on animal models (Subhan et al., 1988, 1989a, 1989b, 1990a, 1990b; Subhan and Khan, 1991; Subhan, 1995).

Earlier studies (Subhan et al., 1995; Subhan and Tahir, 1996) have revealed that these mites are rich in terms of the androgenic steroid as well as its precursors, the lipids, suggesting the possible causes of androgenic property of these mites.

In order to further the information on these insects, a study was carried out to determine the clinical chemistry of these mites, with the aim that findings of this preliminary study would contribute to the presently unexplored arena of entomological science.

MATERIALS AND METHODS

Insect collection

Live Bir Bahuty (*Dinathrombium tinctorium*) was commercially obtained, during the period July/ August, 1996.

Analyte extraction

Bir Bahuty was killed and ground into powder form. The powder was dissolved with an organic solvent in the ratio of 1:3. The organic solvent consisted of ethyl alcohol and n-hexane in the ratio of 3:2. The extract was obtained by centrifugation at 700 g for 15 minutes.

Sample preparation

The clinical chemistry was studied by dividing the samples into two groups. In each group, the purified extract was resuspended in ethyl alcohol in the ratio of 1:10. The second group extract was centrifuged to remove the debris and collect the clear supernatant.

Clinical chemistry analysis

Clinical chemistry of the two groups was studied by analysing high density lipoproteins (HDL), low density lipoproteins (LDL), lactate dehydrogenase (LDH), creatine phosphokinase (CPK), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), potassium, sodium, calcium, phosphorus, bilirubin, alkaline phosphatase, urea, creatinine, uric acid, total protein and albumin. The analysis was carried out using the standardized ELISA method on a Labsystem Chemistry Analyser. Each group was represented by a sample size of 150.

RESULTS AND DISCUSSION

Table I represents the results of the study. A comparison between the two groups indicates that removal of cell drbris improves the recovery of all the analytes from the extract.

Creatinine was not detected in the uncentrifuged samples, while HDL, LDL, LDH, CPK, SGOT, SGPT, potassium, sodium and urea were not detected in any of the groups, suggesting that their levels were far below detectable limits.

Higher values exhibited by the centrifuged group can either be attributed to the interference caused in the spectrometric analysis by the cell debris or improved extraction due to centrifugation.

The albumin: globulin ratio in the uncentrifuged sample was 13 times, as compared with the centrifuged group. This was due to the 13-fold difference in the globulin levels, among the two groups (the centrifuged group having the higher values).

Table I: Clinical chemistry profile of Dinothrombium tinctorium.

Analyte*	alpariti	Uncentrifuged sample	Centrifuged sample
Calcium	(mg%)	0.2±0.01	1.9±0.02
Phosphorus	(mg%)	9.7 ± 0.03	10.2 ± 0.05
Creatinine	(mg%)	N.D.	1.1 ± 0.01
Uric acid	(mg%)	0.3 ± 0.01	1.7 + 0.08
Bilirubin	(mg%)	1.1 ± 0.05	3.2 ± 0.06
Alkaline phosphatase	(IU/L)	55.0 ± 1.07	73.1 ± 2.18
Total proteins	(gm%)	4.2 ± 0.04	7.9 ± 0.12
Albumin	(gm%)	3.9 ± 0.01	4.0 ± 0.03
Globulin	(gm%)	0.3 + 0.03	3.9 ± 0.01
A:G ratio		13.00	1.03
Urea	(mg%)	N.D.	N.D.
HDL	(mg%)	N.D.	N.D.
LDL	(mg%)	N.D.	N.D.
LDH	(IU/L)	N.D.	N.D.
CPK	(IU/L)	N.D.	N.D.
SGOT	(IU/L)	N.D.	N.D.
SGPT	(IU/L)	N.D.	N.D.
Potassium	(meq/L)	N.D.	N.D.
Sodium	(meq/L)	N.D.	N.D.

^{*}For abbreviations see Material and Method section.

In short, this study presents the first report upon the clinical chemistry profile of D. tinctorium, which will add to the basic information regarding this insect, which is widely used as an aphrodisiac medicine in the Unani system of medicine.

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FISH AND FISHERIES OF LORA-PISHIN IN PAKISTAN

Abstract: The fish and fisheries of the Lora-Pishin river in Baluchistan (Pakistan) has been briefly discussed, which include the present status and previous reports of different genera and species.

NTRODUCTION

he river Lora-Pishin originates from Toor-Murgha Manda in the Toba-Kakar ranges and the Zarghoon mountains in the Urak valley of Quetta District. It drains the districts of Pishin, Quetta, Qila Abdullah, Kalat and Chaghai in the northwestern Balochistan and after taking a short turn in Afghanistan, it ends in a lake — Hamun-i-Lora — near Noshki. Its length is about 400 km

The fish and fisheries of this river has not been properly studied. There are some brief papers on the fish fauna only. The present paper is based on the fish collections done by the 1st author from Quetta, Mastung, and Pishin during 1963-65, which was supplemented by the 2nd and 3rd authors in recent years (1996-1998).

The fish fauna is composed of West Asian, High Asian and South Asian forms in addition to three exotic species. The following species have so far been recorded:

ORDER: SALMONIFORMES

	FAMILY:	SALMONIDAE	
1.	Salmo trutta fario Linnaeu	s	Exotic
	ORDER: FAMILY:	CYPRINIFORMES CYPRINIDAE	
2.	Carassius auratus (Linnae	us)	Exotic
3.	Crossocheilus diplocheilus	(Heckel)	South Asian
4.		n (Day)	
5.	Garra rossica (Nikolsky).		West Asian

	FAMILY: NEMACHELLIDAE
6. 7.	Schistura kessleri (Gunther)West AsianTriplophysa brahui (Zugmayer)High Asian
	ORDER: CYPRINODONTIFORMES FAMILY: POECILLIDAE
8.	Gambusia affinis (Baird & Girard) Exotic

Of these, Crossocheilus diplocheilus was not recorded by Mirza and Naik (Pakistan J. Sci., 17: 83-86, 1965). One specimen of Carassuis auratus (the goldfish) measuring 31 cm in total length and 495 g in weight was collected from the river Lora-Pishin near Hameed Bridge in Pishin District. The brown trout Salmo trutta fario is restricted to the Orak valley and the Spin karez. The other species remain small in size.

One species, Garra gotyla (Gray) [recorded as Garra lamta by Zugmayer (Abh. Bayer. Acad. Wiss. (math-phy; Klasse, 26: 1-35, 1913) from Pishin] has not so far been collected by us. Coad (Biologia, 42: 1-18, 1996) recorded Cyprinion milesi (Day) from Lora-Pishin also. But this species is not present in our collection.

One specimen of *Schitura lindbergi* (Banarescu & Mirza) was collected from Lake Khushdil Khan from Pishin by Mr. M. Naeem Javed but was lost, so it has not been included in the present list.

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