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INFLUENCE OF MAGNETIZATION ON THE SHELL RATIO OF MULTIVOLTINE MULBERRY SILKWORM *(BOMBYX MORI* LINN.)

Abstract.- Per cent shell ratio of *Bombyx mori* was significantly (P<0.01) influenced due to variation in the strength of static magnetic field and exposure duration for the treatment of eggs. Maximum shell ratio (14.5%) was recorded in the case of eggs treated in 3000 Gauss magnetic field for 96 hours. Thus, the magnetization of silkworm eggs may open a new field of biotechnology for further investigation to improve certain economic characters in *Bombyx mori* resulting in the heavy production of cocoon and good quality of silk.

Key words: Magnetization, shell ratio, *Bombyx mori*, silk worm.

India has the unique distinction of being the only country producing all the four commercial variety of silk namely, mulberry, tasar, eri and munga but Indian sericulture industry depends mainly on multivoltine race. *Bombyx mori nistari* is a resistant variety of multivoltine mulberry silkworm which contributes up to great extent in the commercial production of cocoon. In order to increase the production of silk, efforts have been made to study the effect of temperature (Upadhyay and Mishra, *J. Adv. Zool*., **12**: 56-59, 1991), relative humidity (Mishra and Upadhyay, *J. Adv. Zool*. **13**:16-18, 1992), photoperiod (Mishra and Upadhyay, Nutrition efficiency of bivoltine *Bombyx mori* Linn. larvae at different photoperiod. *Proc. 80th Session Indian Sci. Congr. Goa*, pp. 54-55, 1993), artificial diet (Iwanrat and Ono, *J. Seric*., **38**: 307-315, 1969), X-rays (Kanarev and Cham, *Zhivotnov Nauky*, **22**: 47-53, 1985) etc on the performance of silkworm. The magnetization of silkworm larvae influenced the performance of

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silkworm (Chaugale and More, *Indian J. Seric.*, **28**: 115-122, 1992).

The magnetic field influenced the hormone and insuline level in rat (Udinsteve and Moroz, *Gig. Tr. Prof. Zabol.*, **12**: 54-56, 1982) and the rate of β galactosidase synthesis in bacteria (Arholt *et al., Phy. Biol. Med.*, **27**: 660–610, 1982). Keeping this in view, an attempt has been made to investigate the influence of static magnetic field on the per cent shell ratio in multivoltine mulberry silkworm (*Bombyx mori* Linn.).

Materials and methods

Seed cocoon

The seed cocoon (pupa enclosed in silken case) of multivoltine mulberry silkworm, *Bombyx mori nistari*, a native of West Bengal in India, were obtained from the silkworm grainage Bahraich, Directorate of Sericulture Uttar Pradesh and were maintained in the plywood trays (23x20x5 cm) under the ideal rearing condition (Krishnaswami *et al., F.A.O. Agric. Serv. Bull. Rome*, **15**: 1-131, 1973) in the silkworm laboratory, Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur. The temperature and relative humidity were maintained at 26±1°C and 75±5%, respectively, till the emergence of moths from seed cocoons. The newly emerged moths from seed cocoon were quickly picked up and kept sex wise in separate trays to avoid copulation. The male moths were smaller in size but more active than the female moths, which were comparatively larger and less active. The whole grainage operation was performed as per description given by Krishnaswami *et al.* (*F.A.O. Agric. Serv. Bull. Rome*, **15**: 1-131, 1973).

Copulation

Moths have tendency to pair immediately after the emergence and, therefore, the male moths, required to copulate with the female moths, were allowed their mates for copulation. A total of 360 pairs, each containing one male and one female from newly emerged moths, were allowed to mate at 26±1°C and 75±5% RH in 12 hours / day dim light condition. After four hours of mating, the paired

moths were decoupled manually. The male moths were discarded, while female moths were allowed to lay eggs.

Table I.- Effect of magnetic field on the shell ratio (per cent) of *Bombyx mori.*

Exposure duration (hrs)	Magnetic power (Gauss)				F_1 – ratio n_1=3
	1000	2000	3000	4000	
00 (Control)	11.24±0.13	11.24±0..13	11.24±0.13	11.24±0.13	
24	11.68±0.26	12.67±0.04	13.05±0.05	12.18±0.06	135.3*
48	12.24±0.03	12.79±0.06	13.57±0.05	12.49±0.07	
96	12.59±0.05	12.93±0.03	14.50±0.04	11.58±0.05	

F_2 – ratio = **158.6*** $n_2 = 3$
*$P_1 < 0.01$ *$P_2 < 0.01$
Each value represents mean ± S. E. of three replicates.

Oviposition

Just after separation, the gravid female laid eggs on the sheet of paper in dark condition at 26±1°C and 75±5% Rh maintained in BOD incubator. After 24 hours of egg laying, the female moths were individually examined for their disease freeness. The disease free layings (DFLs), thus prepared, were treated with 2% formalin for 15 min, thoroughly washed with running water to remove formalin and eggs were dried in shade. The dried eggs, thus obtained, were taken for magnetization under various experimental conditions.

Design of experiment

To observe the influence of magnetic field on the percent shell ratio of *Bombyx mori*, the DFLs, thus obtained were kept in the static magnetic field. The magnet of 1000, 2000, 3000 and 4000 Gauss were used separately for the magnetization of eggs. The DFLs were magnetized for 24, 48 and 96 hours separately with the magnet of each strength. The DFLs were kept for magnetization, just after the laying and primary processing of newly laid eggs. Zero hour magnetization means no treatment *i.e.* "control set" of experiment. For the magnetization 90 DFLs were kept with 1000 Gauss magnet of which 30 DFLs were released after 24 hours of magnetic exposure. Further 30 DFLs were released each after 48 and 96 hours of the exposure of eggs to the magnetic field. The treated eggs were transferred chronically, in separate groups, to the BOD incubator maintained at 26±1°C and 75±5% RH and 12±1 hours photoperiod in a day. The incubation of exposed eggs and further rearing of different stages was performed in the same BOD incubator. The same experiment were conducted with 2000, 3000 and 4000 Gauss magnetic strength separately. The parameters taken for observation in the present study were determined from the respective stages, obtained from the magnetized eggs.

Shell ratio

To determine the shell ratio, the weight of 20 good cocoons and 20 shells from each batch were recorded separately on the fifth day of spinning. Three replicates of each experiment were made. The shell ratio percentage was calculated based on at least 20 cocoons and 20 cocoon shells, taken at random from the good lot .

$$\text{Shell ratio (\%)} = \frac{\text{Weight of cocoon shell}}{\text{Weight of cocoon}} \times 100$$

(Ghosh, Silk realing technology, *Appropriate sericulture technique* (M.S. Jolly ed.), CSR and TI., Mysore, India, 145-172, 1987).

The data obtained were analysed statistically by two way ANOVA, regression and correlation coefficient.

Results and discussion

It is clear from the data (Table I) that change in the strength of static magnetic field and exposure duration of *Bombyx mori* eggs caused considerable influence on the shell ratio. With the increasing

duration of the exposure of eggs up to 96 hours, the shell ratio increased in 1000, 2000 and 3000 Gauss magnetized eggs, while in 4000 Gauss magnetized eggs, the shell ratio increased after 24 hours but, the shell ratio was declined after 96 hours. In 1000 Gauss magnetized eggs, the shell ratio increased with the increasing exposure period of eggs and was 12.59±0.05 after 96 hours of exposure. In 2000 Gauss magnetized eggs, a steep rise in the shell ratio was noticed in 24 hours magnetized eggs which was followed by slow increase in the shell ratio after 96 hours. In 3000 Gauss eggs, the shell ratio increased to 14.54±0.04 in 96 hours magnetized eggs. The two way ANOVA indicates that variation in the magnetic strength and exposure of eggs significantly ($P<0.01$) influenced the shell ratio of *Bombyx mori*. The regression in between independent variable [X] *i.e.* exposure duration of eggs and dependent variable [Y] *i.e.* shell ratio per cent yielded significant and positive correlation *i.e.* $Y_1=11.346 + 0.041X$ and $r_1=0.9665$, $Y_2=11.744 + 0.0150X$ and $r_2=0.8030$, $Y_3=11.95 + 0.0333X$ and $r_3= 0.8812$ while negative correlation obtained *i.e.* $Y_4= 12.05 - 0.0047X$ and $r_4= -0.0263$, where Y_1, Y_2, Y_3 and Y_4 and r_1, r_2, r_3 and r_4 are regression and correlation coefficient of eggs treated in 1000, 2000, 3000 and 4000 Gauss magnetic field, respectively (Table II).

Table II.- Effect of magnetic field on the shell ratio of *Bombyx mori.*

Magnetic power	Regression equation	r
1000 Gauss	$Y_1 = 11.34 + 0.014X$	$r_1 = 0.9663$
2000 Gauss	$Y_2 = 11.74 + 0.015X$	$r_2 = 0.803$
3000 Gauss	$Y_3 = 11.95 + 0.03X$	$r_3 = 0.8812$
4000 Gauss	$Y_4 = 12.05 + 0.0047X$	$r_3 = 0.026$

The seasonal variation influenced the production of cocoon and shell ratio (Thiagrajan *et al., J. Lepidopterist, Soc.,* **47**: 331-337, 1993). The reduced amount of food intake by parasitised silkworm larvae declined the silk production of *Bombyx mori* (Nath *et al., Indian J. Seric*., **29**: 8-12, 1990). The plant growth regulators significantly increased the shell ratio of *Bombyx mori* (Das and Vijayaraghvan, *Indian J. Seric.,* **29**: 44-53, 1993), whereas, temperature variation and variation in the photoperiod regime has been reported to cause considerable changes in the silk producing potential of *Bombyx mori* (Mishra and Upadhyay, *Sericologia,* **35**: 217-222, 1995). Magnetization caused change in cotton fiber's characters (Todoron *et al., God. Sofiiskia. Unic. Biol. Fank. Kn. 2 Bot. Microbial. Fizol. Biokhim. Rast.,* **61**: 239-250, 1966). The morphological, physiological and biochemical changes have been reported in *Drosophila* after an exposure of biological system in the magnetic field (Patnew and Mankova, *Kosm. Biol. Aviakosm. Med.*, **20**: 73 – 76, 1986). The exposure of silkworm larvae in magnetic field of 3500 Gauss caused an increase in silk yield due to the increasing protein level in silk gland (Chaugale and More, *Indian J. Seric.*, 115-122, 1992). In the present investigation, the shell ratio increased with increasing magnetization which may be due to increase in cytochrome activity resulting in an increase in the metabolic rate causing heavy production of silk protein. Higher strength of magnetic field (4000 Gauss) may however inhibit the cytochrome activity leading to reduced silk protein production, and decreased shell ratio. The beneficial effect of the magnetization of eggs on the silk producing potential may open new biotechnological approach for heavy production of good quality cocoon.

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DIVERSITY AND SEASONAL VARIATION OF COPEPOD ZOOPLANKTON IN MANCHHAR LAKE (DISTT: DADU) SINDH, PAKISTAN

Abstract.- The community analysis of copepod zooplankton was conducted at five sampling stations in sub-tropical, Manchhar lake during 2001. A total of 7 species of copepods were recorded including 4 species of

Cyclopoida, two species of Calanoida and one species of Harpacticoida. *Thermocyclops hylinus, Mesocyclops leuckerti, Heliodiaptomus cinctus* and *Onychocamptus mohammad* are the new records from Pakistan. Total copepod population exhibited a distinct peak during early summer (April) due to suitability of temperature and availability of food. The population was lowest during winter months (October – March). The genus *Cyclops* showed a peak in April, while their population was high during summer. Genus *Diaptomus* was low during the whole year with a small peak in October. Genus *Onychocamptus* exhibited a peak in June. The seasonal fluctuations are related to water temperature, food availability and reproductive activity of these zooplanktons.

Key words: Manchhar lake, Copepods, Zooplankton diversity.

Zooplankton are considered to be the ecological indicators of water bodies (Gajbhiye and Desai, *Bull. Nat. Inst. Oceangr.*, **4**: 173-182, 1981). The investigation of zooplankton forms an important aspect of limnology, since they constitute the intermediate level between the primary producers and nektons. Studies on the long-term fluctuations in the abundance of plankton are, therefore, important in relation to the conservation of aquatic resources. The availability of zooplankton as food for larval fish is thought to be one of the key factors that determine year class strength of commercial fish (Cushin, *Reu. Cons. Explor. Mer*., **173**: 107-116, 1978; Kane, *Fish. Bull*., **3**: 464-474, 1993).

Very few studies have been carried out on freshwater copepods in Pakistan. Baqai and Rehana (*Pakistan J. Zool.*, **5**: 165-168, 1973) published data on the seasonal fluctuation of freshwater copepods of Keenjhar lake, Sindh. Baqai and Rehana (*Pakistan J. Zool.*, **6**: 69-72, 1974) described quantitative and qualitative studies of freshwater calanoid zooplankton of Keenjhar lake. A new species of calanoid copepod, *Neodiaptomus kinjherensis* was described from Keenjhar lake by Baqai *et al*. (*Biologia*, **21**: 87-91, 1975). Mahoon and Zia (*Biologia*, **31**: 251-292, 1985) carried out taxonomic studies of copepoda (Calanoida and Cyclopoida) from Lahore, Punjab. Chaudhari *et al*. (*Biologia Special Suppl*., pp. 121-135, 1986) recorded aquatic flora and fauna from Nullah Dag with new records of *Daphnia rosea*, *Macrocyclops albidis*, *Macrocyclops ater*, *Eucyclops macruaus* and *Paracyclops affinis*. Present study is carried out to have basic information regarding the copepods from Manchhar lake.

Materials and methods

For taxonomic studies zooplankton samples were collected every month by plankton net (mesh size 55µ) during 2001. Samples were preserved in 4% formalin solution and brought to the laboratory. The identification was carried out with the help of taxonomic keys (Ward and Whipple, *Fresh Water Biology*, 2nd Ed., John Wiley and Sons, London, 1976; Mizuno and Takahashi, *An illustrated guide to freshwater zooplankton in Japan*. Tokai University Press, p.532, 1991; Battish, *Zooplankton of India*. Oxford & IBHP Publishing Co., 66 Janpath, New Delhi, pp. 233, 1992; Yunfang, *Atlas of freshwater biota in China*. China Ocean Press, Beijing, pp. 110-128, 1995; Pennak, *Freshwater invertebrates of the United State*. 2nd Ed., John Wiley and Sons, New York, U.S.A., pp. 803, 2001). Drawings of the specimens were made with camera lucida MNR1-01041, 10× (Poland) at magnification of 100× and 400× under the trinocular microscope model Swift M.3300D (Japan).

Water sample (10 liter) was taken from lake and strained through plankton net (55µ) to get the quantitative estimation of zooplankton. Enumeration was carried out with the help of counting tray at 100× under binocular microscope on generic level.

Results and discussion

Manchhar is a shallow eutrophic lake, situated at latitude 26°-23’ to 26°-28’and longitude 67°-34’to 67°-43’ in the province of Sindh, Pakistan. A total of seven species of copepods were recorded. These included four species of cyclopoida, *viz.* and *Thermocyclops hyalinus** and *Mesocyclops leuckerti**, *Microcyclops bicolo* and *Paracyclops affinis*, two species of calanoida *viz. Heliodiaptomus viduus* and *Heliodiaptomus cinctus**, and one species of suborder harpacticoida, *Onychocamptus mohammad**.

In one of the previous studies Manchhar Lake during the period of drought when the only source of water was MNV Drain bringing polluted water into the lake copepods were the second dominant group in the lake with 30.9% of total plankton (Mahar *et al*., In: *Proc. Nat. Sem. Env., Soc. and Cult. Impact of water Scarcity in Sindh* (eds. M.R. Arain and M.Y. Khuhawar), Dr. M.A. Kazi Institute of Chemistry, University of Sindh, Jamshoro, pp. 128-139, 2004). Cyclopoids dominated the copepod fauna with 4 species. This is a general observation that in tropical eutrophic lakes the cyclopoids dominate among copepods (Burgis, *Biology*, **4**: 535-541, 1974). Among these *Mesocyclops leuckerti* is of cosmopolitan distribution. Earlier Baig and Khan (*Pakistan J. Sci*., **28:** 33-40, 1976) described *Microcyclops bicolor* from this lake. Chaudhari *et al.* (*Biologia Special Supl*., pp. 121-135, 1986) reported *Macrocyclops albidis, Macrocyclops ater, Eucyclops macruaus, and Paracyclops affines* aquatic flora and fauna from Nullah Dag. Two species of diaptomids in Mancher lake had different sizes. *Heliodiaptomus viduus* was 1.7-2.00 mm long, whereas *H. cinctus* was 0.8-0.9 mm long. Such occurrence of sympatric species provides separate food niches (Hutchinson, *A treatise on limnology*. Vol. II. John Wiley and Sons, Inc, New York, pp. 245, 1967). The only representative of harpacticoida, *Onychocamptus mohammad,* although a benthic species was recorded among net plankton of shallow (Zm=3m), hypo-saline (2.5g/l) Manchhar lake.

Seasonal variation

Population of total copepods and cyclopoids showed a clear peak in April. During rest of the year population was higher during the period of May to August. Low population was observed in winter months (September-March) (Fig. 1A). The population of genus *Diaptomus* showed a gradual increase from August to October, and then declined and remained more or less constant till May (Fig.1B). Genus *Onychocamptus* peaked in June while in rest of the months the population remained low (Fig. 1C).

The seasonal cycle of copepods is affected by diapauses in winter (*Cyclops bicolor*) or in summer as seen in *Cyclops sternus* (Elgmork and Langeland, *Arch. Hydrobiol.*, **88**: 178-201, 1980). Dad (*Limnological studies on Chambal river with reference to pollution*. Ph.D. thesis, Vikram University, pp. 227, 1981) reported the highest population of cyclopoids in the month of June from clear water area of Chambal river as against April in Manchar lake. Zankai (*J. Plankton Res.*, **9**: 1057-1068, 1987) described three generations of *Cyclops vicinus* developed in spring, autumn and winter every year but only one generation of *Mesocyclops*

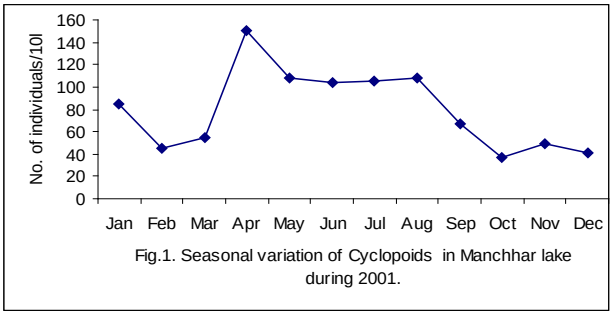

Fig.1. Seasonal variation of Cyclopoids in Manchhar lake during 2001.

A

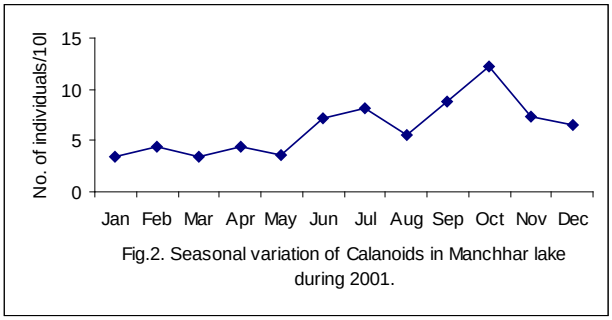

Fig.2. Seasonal variation of Calanoids in Manchhar lake during 2001.

B

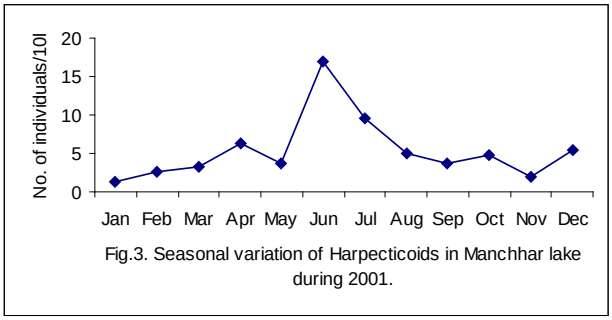

Fig.3. Seasonal variation of Harpecticoids in Manchhar lake during 2001.

C

Fig. 1. Seasonal variation of cyclopoids (A), calanoids (B), and harpecticoids (C) in Manchar lake during 2001.

*newly recorded.

leukarti in autumn. *Mesocyclops leukarti* prefers deeper water rather than shallow water (Baloch, *Species composition, abundance and seasonal variation of zooplankton in lake Ikeda, Japan*. M.Sc. thesis, University of Kagoshima, Kagoshima, pp. 87-91, 1995). In Manchhar Lake the period of diapause is not discernable.

A gradual increase was observed in the population of calanoids from January to July and then it declined till December (Fig. 1B). Baqai and Rehana (*Pakistan J. Zool.*, **6**: 69-72, 1974) reported three maxima in population of calanoids, one in April, one in July and one in October. Calanoid copepods are good indicators as oligotrophic species.

High population of *Harpicticoids* was observed during summer months (June-August), while low population was found during rest of the year (Fig. 1C). It is a well established fact that harpacticoids mostly thrive in saline water bodies (Edmondson, *Fresh water biology*. 2nd edition. John Willey and Sons, New York, pp. 127-169, 1959). According to Hynes (The ecology of running waters. In: *The ecology and fisheries of reservoirs* (ed. B.N. Saigal, B.N), pp. 46-65, ICAR. CIFRI, Barrackpore. Liverpool University Press, pp. 340, 1976) the harpacticoids can tolerate drought in summer season. The presence and seasonal fluctuation of harpacticoid species is a clear indicator of saline water condition found in Manchhar lake. Galat *et al*. (*Hydrobiology*, **82**: 281-317, 1981) have recorded seven genera of mesozooplankton and three rotifer genera in a hyposaline lake (3-5 ppt) Pyramid Lake, U.S.A.

Zooplankton community fluctuations are also altered with environmental degradation. Presence of higher density of copepod and harpacticoid in Manchhar lake indicates their tolerance of higher salinity of water.

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