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Acid Phosphatase Activity Changes Under Magnetic Exposure of Eggs in *Bombyx mori*: A Multivoltine Mulberry Silkworm

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Abstract: Magnetic field influences to the biological system has been reported. The aim of this study was to evaluate the effect of magnetization of eggs on the acid phosphatase activities in the silk gland, fat body and haemolymph of fifth instar larvae and fat body and haemolymph of pupae of *Bombyx mori*.The newly laid eggs after primary processing were magnetized in 1000, 2000, 3000 and 4000 Gauss magnetic field for 24, 48, 72 and 96 he xposure with the each strength magnetic field. Acid phosphatase activity was increased in the silk gland, fat body and haemolymph of the fifth instar larvae due to increase in exposure duration of eggs upto 96 h in 1000, 2000 and 3000 Gauss magnetic field, while in 4000 Gauss magnetic field, acid phosphatase activity was slightly increased upto 24 he xposure of the eggs and further increase in duration of exposure caused gradual decline to the activity. The acidphosphatase activity in the silk gland, fat body and haemolymph of the fifth instar larvae was maximum in 3000 Gauss magnetic field with 96 h exposure of eggs. Variation in the strength of magnetic field from 1000 to 3000 Gauss and exposure duration from 24 to 96 h also influenced to the acid phosphatase activity in the fat body and haemolymph of pupae. The maximum activity was recorded in 3000 Gauss magnetic field with 96 h exposure of eggs while, in 4000 Gauss magnetic strength activity was slightly increased upto 24 h and further increase in exposure duration upto 96 h caused gradual decrease to the activity of acid phosphatase. Thus, the treatment of *B.mori* eggs in 3000 Gauss magnetic field for 96 h exposure duration is most suitable to enhanced the activity of acid phosphatase in the tissues and this may be biotechnological tool to boost the sericulture industries.

Key words: Magnetization · Silk Gland · Fat Body · Haemolymph · Larvae · Pupae

INTRODUCTION

Like agriculture, sericulture plays an important role in the transformation of rural economy as it assures wide scopes in term of regular employment and provides return round the year. The race *nisrari* is a resistant variety of multivoltine mulberry silkworm (*Bombyx mori*), which contributes upto a great extent in the commercial production of silk. The ultimate aim of sericulture is to enhance the production of quality raw silk as per demand of the market. The potential of sericulture as an important source of income and generating more employment opportunities is definitely based upon the performance of the different stages of *B. mori*.

In order to increase in the production of quality raw silk efforts have been made to investigate the effect of temperature [1], relative humidity [2], photoperiod [3], X-rays [4] etc on the performance of *B.mori* larvae. The effect of magnetism on biological system has been the subject if world wide interest. Magnetic field influences morphological, physiological and biochemical characteristic of biological system [5]. Magnetic field

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influences larval behaviors of silkworm [6], hormonal level [7] and acid phosphatase activities [8] in mouse and germination of seeds [9]. Its positive effects include cell viability [10], nerve regeneration [11], skin and bone healing in guinea pig [12]. Magnetization of eggs influences incubation period [13] and protein content in larvae and pupae [14] of *B.mori*. The silk producing potential [15] and total protein content of larvae [16] of *B.mori* change due to magnetization of larvae.

This communication reports to the effects of magnetization of *B. mori* eggs on the acid phosphatase activity in the silk gland, fatbody and haemolymph of fifth instar larvae and fat body and haemolymph of pupae of *B.mori*.

MATERIALS AND METHODS

Seed cocoon: The seed cocoon (pupa inclosed in silken case) of multivoltine mulberry silkworm (*Bombyx mori nistari*), a native of West Bengal in India, were obtained from the silkworm grainage Behraich, Directerate of sericulture, Uttar Pradesh, India and, were maintained in plywood trays (23X20X5cm) under the ideal rearing conditions [17] in the silkworm laboratory Department of Zoology, DDU Gorakhpur University, Gorakhpur. The temperature and relative humidity were maintained at 26±1°C and 75±5%, respectively till the emergence of moths from the seed cocoons.

Copulation: Moths have a tendency to pair immediately after the emergence thus, they were allowed with their mates for copulation. 480 pairs each containing one male and one female from newly emerged moths, were allowed to mate at 26±1°C temperature and 75±5% relative humidity in a dim light condition. After four hour of mating, the paired coupled moths were decoupled manually. The male moths were discarded while the female moths were allowed for egg laying.

Oviposition: The gravid females laid eggs on the sheet of paper in dark condition at 26±1°C temperature and 75±5% relative humidity. After 24 h of egg laying, the female moths were individually examined for their disease freeness. To examine for the disease freeness, the females were crushed individually in mortar with pestles and blood smears were examined by microscope under magnification of15X45 for the detection of bacterial and protozoan pathogens.

The disease free laying (DFLs) thus prepared, were treated with 2% formalin for 15 minutes to increased the adhesiveness of eggs on the paper sheet and surface disinfection. Thereafter, the eggs sheets with eggs laid, were 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.

Experimental Designing: To observe, the influence of magnetic field on the acid phosphatase activity in difference tissues of larvae and pupae of *B.mori*, the newly laid DFLs just after primary processing were kept in the static magnetic field of 1000, 2000, 3000 and 4000 Gauss separately for the magnetization. The DFLs were magnetized for 24, 48,72 and 96 h separately with the magnet of different strength. Control set (no treatment) is also study with experimental study. For the magnetization, 120 DFLs were kept in 1000 Gauss magnetic field and 30 DFLs were released after 24 h of magnetic exposure. Further 30 DFLs were released each after 48, 72 and 96 h of magnetic exposure of eggs. The treated DFLs were transferred chronologically in separate groups to the BOD incubator maintained at 26±1°C temperature, 75±5% relative humidity and 12±1 h photoperiod in a day. The incubation of exposed eggs and further rearing of different stages was performed in the same BOD incubator. Similar experiments were performed with 2000, 3000 and 4000 Gauss magnetic strength. Acid phosphatase activity was determined from the tissues of larvae and pupae developed from magnetized eggs.

For estimation of acid phosphatase activity, silk gland, fat body and haemolymph tissues were taken from the fifth instar larvae and fat body and haemolymph tissues were taken from pupae. The activity of acid phosphatase was measured by method of Bergmeyer [18] and modified by Singh and Agarwal [19]. Acid phosphatase catalyses to the hydrolysis of p-nitrophenyl phosphatase in phosphoric acid and p-nitrophenol. Activity of acid phosphatase is directly proportional to the quantity of p-nitrophenol produced which gives yellow colour with NaOH due to formation of yellow anions. The optical densities were measured at 420nm. Optical densities were compared with standard which was prepared by different concentration of p-nitrophenol solution. The enzyme activity was expressed in µg p-nitrophenol liberated/ hour /mg protein.

Statistical Analysis: Six replicates of each experiment were made and the data obtained were analyzed statistically by two-way ANOVA and post-hoc test.

RESULTS

Acid Phosphatase Activity in the Silk Gland of Fifth Instar Larvae: The data given in Table-1a clearly indicates that change in duration of magnetic exposure and strength of magnetic field influenced to the acid phosphatase activity in the silk gland of fifth instar larvae of *B. mori***.** Acid phosphatase activity was increased in case of 1000, 2000, 3000 Gauss with increase in exposure duration from 24 to 96 h of eggs while in 4000 Gauss magnetized eggs, the acid phosphatase activity was increased upto 24 h exposure of eggs and further increase in duration of exposure of eggs caused gradually decline in acid phosphatase activity in the silk gland of *B.mori*. The trend of increase in the acid phosphatase activity with increase in duration of exposure was almost similar and steady in 1000 and 2000 Gauss magnetized eggs while in 3000 Gauss magnetic strength with 96 h exposure duration, the acid phosphatase activity was maximum and recorded to be 44.95±2.05µg/mg.Two way ANOVA indicates that variation in strengthof magnetic field as well as exposure duration of eggs significantly (P_1<0.01) influence to the acid phosphatase activity in the silk gland of fifth instar larvae. Post-hoc test (Table-1b) shows significance group differences for acid phosphatase activity in between control and 2000 Gauss, control and 3000 Gauss, 1000 and 3000 Gauss in 24 h exposure of eggs. The significance difference for acid phosphatase activity in between control and 2000 Gauss, control and 3000 Gauss, 1000 and 3000 Gauss, 2000 and 4000 Gauss and 3000 and 4000 Gauss magnetic field for 48 h exposure of eggs were recorded. In case of 72 h exposed eggs, the significance differences were observed in between control and 1000 Gauss, control and 2000 Gauss, control and 3000 Gauss, 1000 and 3000 Gauss, 1000 and 4000 Gauss, 2000 and 4000 Gauss and 3000 and 4000Gauss magnetic field while in 96 h exposed eggs, the significance differences were observed in between control and 1000 Gauss, control and 2000 Gauss, control and 3000 Gauss, 1000 and 3000 Gauss, 1000 and 4000 Gauss, 2000 and 3000 Gauss, 2000 and 4000 Gauss and 3000 and 4000 Gauss static magnetic field.

Table 1a: Effect of magnetic field on the acid phosphatase activity (µg p-nitrophenol liberated/ hour/ mg protein) in the silk gland of larvae

	Magnetic Power (Gauss)					
Exposure Duration (h)	Control (X_1)	1000 (X_2)	2000 (X_3)	3000 (X_4)	4000 (X_5)	F_1-ratio $n_{1=4}$
24	23.60 ±1.47	24.63 ±1.65	28.80 ±1.68	32.05 ±1.04	26.90 ±0.85	12.64*
48	23.60 ±1.47	26.72 ±1.96	30.85 ±2.02	34.25 ±1.54	24.65 ±1.06	
72	23.60 ±1.47	29.85 ±1.55	33.89 ±1.23	38.45 ±1.65	23.40 ±1.44	
96	23.60 ±1.47	33.89 ±2.01	36.02 ±2.15	44.95 ±2.05	23.20 ±1.25	

F_2-ratio=8.94* n_2=3

*P<0.01

Each value represents mean ±SE of six replicates.

X_1, X_2, X_3, X_4 and X_5 are the mean value of control, 1000, 2000, 3000 and 4000 Gauss magnetic field respectively.

Table 1b: Post-hoc test showing group differences for acid phosphatase activity in the silk gland of fifth instar larvae of *Bombyx mori*

	Exposure duration (h)			
Mean difference in between groups	24	48	72	96
$x_1 \sim x_2$	1.03	3.12	6.25*	10.29*
$x_1 \sim x_3$	5.20*	7.25*	10.29*	21.35*
$x_1 \sim x_4$	8.45*	10.65*	14.85*	21.25*
$x_1 \sim x_5$	3.30	1.05	0.20	0.40
$x_2 \sim x_3$	4.17	4.13	4.04	2.13
$x_2 \sim x_4$	7.04*	7.53*	8.60*	11.06*
$x_2 \sim x_5$	2.27	2.07	6.45*	10.69*
$x_3 \sim x_4$	3.25	3.40	4.56	8.93*
$x_3 \sim x_5$	1.90	6.20*	10.49*	12.82*
$x_4 \sim x_5$	5.15	9.70*	15.05*	21.75*

*Showing significant group difference

Honesty significant group difference (HSD) $= qv\sqrt{\frac{MS\ within}{n}}$

$= 6.10\sqrt{\frac{4.358}{6}}$

$= 5.197$

Where,

q = studentized rang static

n = number of replicates

Table 2a: Effect of magnetic field on the acid phosphatase activity (µg p-nitrophenollibereted/ hour/ mg protein) in the fat body of larvae and pupae.

	Magnetic Power (Gauss)					
Exposure Duration (h)	Control (X_1)	1000 (X_2)	2000 (X_3)	3000 (X_4)	4000 (X_5)	F_1-ratio $n_{1=4}$
24 Larvae	17.52±1.47	18.00±2.34	18.17±1.39	19.28±1.89	18.96±0.06	
Pupae	20.19±0.72	20.26±1.38	22.54±1.96	22.86±2.30	22.54±1.02	
48 Larvae	17.52±1.47	19.30±1.87	19.76±1.37	20.50±1.48	18.45±1.67	8.64*
Pupae	20.19±0.72	20.54±0.98	22.80±1.89	23.40±1.46	22.22±2.16	25.75**
72 Larvae	17.52±1.47	19.75±1.46	20.05±2.07	21.64±2.40	17.46±1.65	
Pupae	20.19±0.72	21.46±0.60	23.02±0.99	23.84±2.67	21.87±0.98	
96 Larvae	17.52±1.47	20.02±1.38	20.56±1.36	21.95±1.98	16.58±1.98	
Pupae	20.19±0.72	22.21±1.34	23.44±2.90	24.27±2.78	20.05±2.07	

F_2-ratio=1.125^{NS} and 3.52** n_2=3 *Larvae **Pupae

P_1<0.01 **P_2<0.025 NS=Non-significant

Each value represents mean ±SE of six replicates.

X_1, X_2, X_3, X_4 and X_5 are the mean value of control, 1000, 2000, 3000 and 4000 Gauss magnetic field respectively.

Table 2b: Post-hoc test showing group differencesfor acid phosphatase activity in the fat body of fifth instar larvae of *Bombyx mori*

	Exposure duration (h)			
Mean difference in between groups	24	48	72	96
$x_1 \sim x_2$	0.48	1.78	2.23	2.50*
$x_1 \sim x_3$	0.92	0.65	2.24	2.53*
$x_1 \sim x_4$	1.76	2.98*	4.12*	4.43*
$x_1 \sim x_5$	1.44	0.93	0.06	0.94
$x_2 \sim x_3$	0.17	0.46	0.30	0.54
$x_2 \sim x_4$	1.28	1.20	1.89	1.93
$x_2 \sim x_5$	0.96	0.85	2.29	3.44*
$x_3 \sim x_4$	1.11	0.74	1.58	1.39
$x_3 \sim x_5$	0.79	1.31	2.59*	3.98*
$x_4 \sim x_5$	0.32	2.05	4.18*	5.37*

*Showing significant group difference

Honesty significant group difference (HSD) = $q\sqrt{\frac{MS\ within}{n}}$

$= 6.10\sqrt{\frac{0.859}{6}}$

$= 2.30$

Where,

q = studentized rang static

n = number of replicates

Acid Phosphatase Activity in the Fat Body of Fifth Instar Larvae and Pupae: The data given in Table-2a clearly indicates that variation in the strength of static magnetic field and exposure duration of *B.mori* eggs caused notable impact on the acid phosphatase activity in the fat body of fifth instar larvae and pupae. The acid phosphatase activity in the fat body was noticed to be increased with increase in exposure duration of eggs in 1000, 2000 and 3000 Gauss magnetic field, while in 4000 Gauss, the activity was increased up to 24 h of eggs and further increase in exposure duration caused gradual decline to the acid phosphatase activity. The trend of increase in acid phosphatase activity was almost similar in 1000 and 2000 Gauss magnetic field while in 3000 Gauss magnetic field, the increase in acid phosphatase activity was steep and reached to maximum level of 21.95±1.98µg/mg (larvae) and 24.27±2.78µg/mg (pupae) in case of 96 h exposed eggs. The two way ANOVA indicates that variation in the strength of magnetic field significantly (P<0.01) influenced the acid phosphatase activity in the fat body of larvae and pupae while, variation in the exposure duration significantly (P<0.025) influenced to the acid phosphatase activity in the fat body of pupae but effect was non-significant in the larvae of *B. mori*. The post-hoc test (Table-2b) shows significance difference in acid phosphate activity due to variation in the strength of magnetic field in between control and 3000 Gauss in 48 h exposure of eggs. In 72 h exposed eggs, the significance difference for acid phosphatase activity was observed in between control and 3000 Gauss, 2000 and 4000 Gauss and 3000 and 4000 Gauss magnetic field whereas, in 96 h exposed eggs,

Table 2c: Post-hoc test showing group differencesfor acid phosphatase activity in the fat body of pupae of *Bombyx mori*

	Exposure duration (h)			
Mean difference in between groups	24	48	72	96
$x_1 \sim x_2$	0.07	0.35	1.27	2.02*
$x_1 \sim x_3$	2.35	2.61*	2.83*	2.25*
$x_1 \sim x_4$	2.67*	3.21*	3.65*	4.04*
$x_1 \sim x_5$	2.35*	2.03*	1.68*	0.13
$x_2 \sim x_3$	2.28*	2.26*	1.56*	1.23
$x_2 \sim x_4$	2.60*	2.86*	2.38*	2.06*
$x_2 \sim x_5$	2.28*	1.68	0.41	2.15*
$x_3 \sim x_4$	0.32	0.60	0.82	0.83
$x_3 \sim x_5$	0.00	0.58	1.15	3.38*
$x_4 \sim x_5$	0.32	1.18	1.97*	4.21*

*Showing significant group difference

Honesty significant group difference (HSD) = $q\sqrt{\frac{MS\ within}{n}}$

$= 6.10\sqrt{\frac{0.59}{6}}$

$= 1.91$

Where,
q = studentized rang static
n = number of replicates

Table 3a: Effect of magnetic field on the acid phosphatase activity (µg p-nitrophenol libereted/ hour/ mg protein) in the haemolymph of larvae and pupae of *Bombyx mori*.

	Magnetic Power (Gauss)					
Exposure Duration (h)	Control (X_1)	1000 (X_2)	2000 (X_3)	3000 (X_4)	4000 (X_5)	F_1-ratio $n_{1=4}$
24 Larvae	2.36±0.03	2.56±0.05	2.62±0.09	2.82±0.04	2.72±0.02	
Pupae	2.98±0.08	3.012±0.39	3.07±0.28	3.29±0.71	3.06±0.11	
48 Larvae	2.36±0.03	2.63±0.06	2.82±0.31	2.99±0.12	2.68±0.23	20.66*
Pupae	2.98±0.08	3.02±0.38	3.16±0.69	3.660.35	3.08±0.71	10.13**
72 Larvae	2.36±0.03	2.75±0.73	2.95±0.38	3.18±0.59	2.46±0.58	
Pupae	2.98±0.08	3.040±0.31	3.27±0.38	3.58±0.31	3.04±0.87	
96 Larvae	2.36±0.03	2.82±0.09	3.05±0.15	3.41±1.37	2.12±0.75	
Pupae	2.98±0.08	3.05±0.38	3.39±0.35	3.96±0.56	2.86±0.74	

F_2-ratio=2.56*^{NS} and 1.16**^{NS} n_2=3 *Larvae **Pupae
P_1<0.01 NS=Non-significant
Each value represents mean ±SE of six replicates.
X_1, X_2, X_3, X_4 and X_5 are the mean value of control, 1000, 2000, 3000 and 4000 magnetic field Gauss respectively.

the significance differences were observed in between control and 1000 Gauss, control and 2000 Gauss, control and 3000 Gauss, 1000 and 4000 Gauss, 2000 and 4000 Gauss and 3000 and 4000 Gauss magnetic strength. The Post-hoc test (Table-2c) shows significant difference for acid phosphatase in fat body of pupae due to variation in the strength of magnetic field, in between control and 3000 Gauss, control and 4000 Gauss, 1000 and 2000 Gauss, 1000 and 3000 Gauss and 1000 and 4000 Gauss in 24 h exposure of eggs while, in 48 h exposed eggs, the significance differences were observed in between control and 2000 Gauss, control and 3000 Gauss, control and 4000 Gauss, 1000 and 2000 Gauss and 1000 and 3000 Gauss magnetic field. In 72 h exposure of eggs, the significant differences for acid phosphatase activity were observed in between control and 2000 Gauss, control and 3000 Gauss, control and 4000 Gauss, 1000 and 2000 Gauss, 1000 and 3000 Gauss and 3000 and 4000 Gauss magnetic strength. In 96 h exposure of eggs, the significance difference in acid phosphatase activity were observed in between control and 1000 Gauss, control and 2000 Gauss, control and 3000 Gauss, 1000 and 3000 Gauss, 1000 and 4000 Gauss, 2000 and 4000 Gauss and 3000 and 4000 Gauss magnetic strength.

Acid Phosphatase Activity in the Haemolymph of Fifth Instar Larvae and Pupae: The data given in Table-3a shows that variation in the strength of static magnetic field and exposure duration of *B. mori* eggs influences to

Table 3b: Post-hoc test showing group differencesfor acid phosphatase activity in the haemolymph of fifth instar larvae of *Bombyx mori*

	Exposure duration (h)			
Mean difference in between groups	24	48	72	96
$x_1 \sim x_2$	0.20	0.27	0.360	0.460
$x_1 \sim x_3$	0.26	0.46	0.59	0.69
$x_1 \sim x_4$	0.46	0.63*	0.82*	1.05*
$x_1 \sim x_5$	0.36	0.32	0.10	0.24
$x_2 \sim x_3$	0.06	0.19	0.23	0.23
$x_2 \sim x_4$	0.26	0.36	0.46	0.59*
$x_2 \sim x_5$	0.16	0.05	0.26	0.70*
$x_3 \sim x_4$	0.20	0.17	0.23	0.36
$x_3 \sim x_5$	0.10	0.14	0.49	0.93*
$x_4 \sim x_5$	0.10	0.31	0.72*	1.29*

*Showing significant group difference

Honesty significant group difference (HSD) = $q\sqrt{\frac{MS\ within}{n}}$

$= 6.10\sqrt{\frac{0.42}{6}}$

$= 0.51$

Where,
q = studentized rang static
n = number of replicates

Table 3c: Post-hoc test showing group differences for acid phosphatase activity in the haemolymph of pupae of *Bombyx mori*

	Exposure duration (h)			
Mean difference in between groups	24	48	72	96
$x_1 \sim x_2$	0.03	0.04	0.06	0.07
$x_1 \sim x_3$	0.09	0.18	0.29	0.41*
$x_1 \sim x_4$	0.31	0.38*	0.60*	0.98*
$x_1 \sim x_5$	0.08	0.10	0.06	0.12
$x_2 \sim x_3$	0.05	0.13	0.22	0.33
$x_2 \sim x_4$	0.27	0.33	0.53*	0.90*
$x_2 \sim x_5$	0.04	0.06	0.00	0.19
$x_3 \sim x_4$	0.22	0.20	0.31	0.57*
$x_3 \sim x_5$	0.01	0.08	0.23	0.53*
$x_4 \sim x_5$	0.23	0.28	0.54*	1.10*

*Showing significant group difference

Honesty significant group difference (HSD) = $q\sqrt{\frac{MS\ within}{n}}$

$= 6.10\sqrt{\frac{0.022}{6}}$

$= 0.36$

Where,
q = studentized rang static
n = number of replicates

the acid phosphatase activity in the haemolymph of fifth instar larvae and pupae. The acid phosphatase activity was steadily increased with increase in the duration of exposure of eggs in 1000, 2000 and 3000 Gauss magnetic field while, the effect was adverse in 4000 Gauss magnetic field. In 3000 Gauss magnetic field, the acid phosphatase activity was steeply increased with increase in duration of exposure and reached to the maximum level of 3.41±1.37µg/mg (larvae) and 3.96±0.56µg/mg (pupae) in 96 h exposed eggs. In 4000 Gauss magnetic field the acid phosphatase activity was increased up to 24 h and further increase in exposure duration caused gradual declined tendency in acid phosphatase activity. Two way ANOVA indicates that variation in the strength of magnetic field ($P<0.01$) significantly influenced to the acid phosphatase activity in the haemolymph of larvae and pupae but effect of variation in exposure duration of eggs was non-significant. The Post-hoc test (Table-3b) shows

significance difference in acid phosphate activity in the fat body of larvae due to variation in the strength of magnetic field, in between control and 3000 Gauss in 48 h exposure of eggs. In 72 h of exposure of eggs the significant difference was observed in between control and 3000 Gauss and 3000 and 4000 Gauss magnetic strength. Significant difference in acid phosphatase activity was observed in between control and 3000 Gauss, 1000 and 3000 Gauss, 1000 and 4000 Gauss, 2000 and 4000 Gauss and 3000 and 4000 Gauss magnetic strength in case of 96 h exposure of eggs. The Post-hoc test (Table-3c) shows significance difference for the acid phosphatase activity in between control and 3000 Gauss in 48 h exposure of eggs. The significant difference was observed in between control and 3000 Gauss, 1000 and 3000 Gauss and 3000 and 4000 Gauss magnetic field in 72 h exposure of eggs while in 96 h exposed eggs, the difference was significant in between control and 2000 Gauss, control and 3000 Gauss, 1000 and 3000 Gauss, 2000 and 3000 Gauss, 2000 and 4000 Gauss and 3000 and 4000 Gauss magnetic field.

DISCUSSION

Variation in the strength of static magnetic field and duration of exposure of eggs influenced to the acid phosphatase activity in the silk gland, fat body and haemolymph of larvae and fat body and haemolymph of pupae of *B. mori*. The acid phosphatase is a lysosomal origin enzyme which helps in autolysis of cell after death [20]. The acid phosphatase was noticed to occur in higher concentration than the alkaline phosphatase in the silk gland [21]. Acid phosphatase plays significance role in the synthesis of silk protein inside silk gland and its activity is increased due to increase in temperature upto certain level [22, 23]. Since, magnetic field causes increase in metabolic rate which rises internal body temperature hence activity of acid phosphatase is found to increase in silk gland. Enzymatic reaction in the living system is influenced by the magnetic field and also noticed an enhancement in the metabolic efficiency after application of magnetic field [24]. Therefore, the activity of acid phosphatase is observed to change in the silk gland fat body and haemolymph of larvae and fat body and haemolymph of pupae of *B. mori.* Acid phosphatase activity is gradually increased and become optimum during last instar stage resulting an increased in the silk synthesis due to magnetic exposure of larvae [25]. Low magnetic field is responsible for no effect or stimulatory one whereas, high magnetic field result in an inhibitory effect [26]. An activation of some enzymes like corboxymutase [27], glutamate dehydrogenase [28] and catalase [27] has been obtained by application of magnetic field in *in vitro* studies. Low magnetic field causes an increased in mid gut protease activity in the silkworm while application of higher magnetic field has resulted an inhibition of acetylcholinesterase [27]. In present investigation, the low magnetic field upto 3000 Gauss and exposure duration upto 96 h caused increase in acid phosphatase activity in the silk gland of larvae and fat body and haemolymph of larvae and pupae but higher magnetic field of 4000 Gauss caused inhibitory effect on the activity of acid phosphatase in tissues of larvae and pupae of *B. mori.* How exactly magnetic field influences to biological system yet not clear and efforts are being made in the direction but it is hypothesized that it may be due activation of paramagnetic molecule, free radicals and cytochrome system inside the tissues. The magnetic field of 4000 Gauss for 24 h exposure of eggs caused slight increase in activity and further increase in exposure duration caused to decrease in activity which may be due to stress responses of higher magnetic field on biomolecules.

REFERENCES

1. Verma, A.N. and A.S. Atwal, 1967. Effect of temperature and food on the development and silk production of *Bombyx mori* L. Beitre Entomol., 18(1&2): 249-258.
2. Upadhyay, V.B. and A.B. Mishra, 2002. Influence of relative humidity on the nutritive potential of mulberry silkworm *Bombyx mori* L. larvae. J.adv. Zool., 23(1): 54-58.
3. Jolly, M.S., S.S. Sinha and J.L. Razdan, 1971. Influence of temperature and photoperiod on the termination of pupal diapouse in taser silkworm, *Anthearea mylita*. Indian J. Insect Physiol., 17: 743-760.
4. Kanarev, G and G.T. Chan, 1985. Effect of laser irradiation of silkworm eggs on silkworm, *Bombyx mori* development and productivity. Zhiotnov Nauki, 22: 47-53.
5. Patnev, T.P. and M.I. Mankova, 1986. Direct and indirect effect of constant magnetic field on biological objects. Kosm. Biol. Aviakosm. Med., 20: 73-76.
6. Chaugale, A.K., 1993. Effect of magnetic energy on silkworm development and silk production. Ph.D. Thesis Shivaji University, Kolhapur, India.

7. Udinstev, N.A. and V.A. Moroz, 1982. Function of hypophysial adrenal system under the effect of power frequency variable magnetic field of different regimes. Gig. Tr. Prof. Zobol., 12: 54-66.
8. Conely, C.C., W.J. Mills and A.G. Patricia, 1966. Enzyme activities in macrophages from animals exposed to a very low magnetic field. III International Biomagnetic Symposium, University of Illinois, Chicago, pp: 13-15.
9. Pittman, U.J., 1965. Magnetism and plant growth III. Effect on germination and early growth of corns and beans. Can. J. Plant Sci., 45: 549-555.
10. Ferment, J., 1994. Effect of high magnetic field on biological reactions. Bioeng., 77: 453-456.
11. Byers, J.M., K.F. Clark and G.C. Thompson, 1998. Effect of pulsed electromagnetic stimulation on the facial nerve regeneration. Arch.Otolary. Head and Neck surg., 124(4): 383-389.
12. Darendetilar, M.A., A. Darendetilar and P.M. Sinclair, 1997. Effect of static and pulsed electromagnetic field on bone healing. Int. J. Orthodon, Orthognath Surgery, 12(1): 43-53.
13. Tripathi, S.K. and V.B. Upadhyay, 2005. Magnetization of eggs influences the incubation period of multivoltine mulberry silkworm (*Bombyx mori* Linn.) eggs. J. Adv.Zool., 26(1): 24-28.
14. Upadhyay, V.B. and S.K. Tripathi, 2005. Magnetic field influences protein content in larvae and pupae of *Bombyx mori* (Linn.). J. Appl. Biosci., 31(2): 160-165.
15. Tripathi, S.K., 2010. Influence of magnetic field on the silk producing potential of *Bombyx mori*: A multivoltine mulberry silkworm. J. Adv. Zool., 31(2): 105-110.
16. Tripathi, S.K., 2012. Protein level changes under magnetic exposure of larvae in*Bombyx mori*: A multivoltine mulberry silkworm. Academic J. Entomology, 5(2): 81-85.
17. Krishnaswami, S., M.M. Narasimhanna S.K. Suryanarayan and K. Raja, 1973. Sericulturemanual 2. Silkworm rearing. F.A..O. Agric. Seroes Bull. Rome, 15(2): 1-131.
18. Bergmeyer, H.U., 1963. Methods of enzymatic analysis, Academic Press New York.
19. Singh, D.K. and R.A. Agarwal, 1989. Toxicity of piperanylbutaxidecorbaryl synergism on snail *Lymneaaccuminata*. Int. Review Derges. Hydrob., 74: 689-699.
20. Reddy, K.V.R., K.S. Nair, S.B. Magadum and R.K. Dutta, 1991. Antijuvenoid inducedmodulation on the tissues growth rate pattern and biochemical components of silkworm*Bombyx mori* L. Bangladesh J. Zool., 23(2): 221-228.
21. Shridhara, S. and J.V. Bhat, 1963. Alkaline and acid phosphatase of silkworm (*Bombyx mori* L.). J. Insect Physiol., 9: 693-701.
22. Lim, Y.Y. and O.X. Zhu, 1985. Mechanism of silk increase in silkworm by 15 N-urea administration. Sci. of Seric. (CanyeKexue,), 9: 203-213.
23. Eid, M.A., A.N. Et-Nakkady and M.A. Sailesh, 1989. Effect of supplementary amino acids on silk secretion by larvae of *Philosamiaricini* (Biosd). Indian J. Seric., 28: 248-252.
24. Alenxander, M.P. and S. Ganeshan, 1990. Electromagnetic field induces in vitro pollen germination and tube growth. Curr. Sci., 59: 276-277.
25. Chaugle, A.K. and N.K. More, 1993. Effect of magnetization on acid and alkaline phosphatase in the developing silk gland of *Bombyx mori* (L.). Entomon, 18(1&2): 1-5.
26. Mulay, I.L. and L.N. Mulay, 1964. Effect on *Drosophila melanogaster* and S-37 tumer cells. Postulate for magnetic field interpretation; In biological effect of magnetic field. M.F. Bombay (ed.) Plenum press, New York.
27. Young, W., 1969. Magnetic field and in-situ acetycholine esterase in the vagal heart system; in Biological effect of magnetic field. Barnothyet (ed.), pp: 79-102. Plenum Press, New York.
28. Akoyundoglou, G., 1965. Effect of magnetic field on corboxydimutase. Nature, 202: 452-454.