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Biotechnological Importance of Cocoon Magnetization with Particular Reference to the Larval Performance of Multivoltine Mulberry Silkworm (*Bombyx mori* Linn.)

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Abstract: The application of magnetic field on *Bombyx mori* cocoon has been proved to be of biotechnological significance in the sericulture industry. Variation in the static magnetic field significantly (P_1<0.05) influenced the larval performance of *Bombyx mori* in terms of larval duration, larval weight and survival of larvae. The larval weight and survival of larvae increased with the increasing exposure duration of cocoon from 24 to 96 hours in 1000, 2000 and 3000 gauss magnetic field. The larval duration of *Bombyx mori* decreased with the increase in exposure duration of cocoons from 24 to 96 hours in case of 1000, 2000 and 3000 gauss magnetic strength. The maximum larval weight (1.961 ±0.08 gm) and survival of larvae (93.58 ±0.76%) was noticed in case of 3000 gauss-72 hours exposed cocoons. The minimum larval duration was recorded (23.75 ±0.65days) in case of 3000 gauss-72 hours exposed cocoons showing good development of *Bombyx mori* larvae. The experiments were conducted in the year 2007. The cocoons were magnetized for 24, 48, 72 and 96 hours separately with the magnet of each strength just after 3rd day of spinning. The bio-magnetic interactions, if applied tactfully, may be useful for boosting up the sericulture industry as well as the economy of silkworm rearing.

Key words: Cocoon magnetization · Larval performance · Exposure duration · *Bombyx mori*.

INTRODUCTION

Nistari is a resistant variety of multivoltine mulberry silkworm (*Bombyx mori*) which contributes up to a great extent in the commercial production of cocoon. The larval duration, larval weight and survival of larvae are the most important factors which influence the production of cocoon on commercial scale. The efforts are being made to evolve new technologies that are effective, labour saving and eco-friendly. In order to increase, the production of silk, efforts have been made to study the effect of temperature [1], relative humidity [2], photoperiod [3], artificial diet [4], X-rays [5] etc on the performance of silkworm. The magnetization of silkworm larvae also influences the performance of silkworm [6]. The Magnetization of eggs influences silk producing potential [7, 8] and incubation period of eggs [9 and 10]. Now a days biotechnology has become leading field of scientific researches which are directly concerned with the life quality of human beings. It is hypothesized that if the cocoon of *Bombyx mori* are exposed to different magnetic strength, there may be some beneficial effects on the life pattern of silkworm and the productivity of cocoon. Keeping this in view, an attempt has been made to investigate the bio-magnetic effect of cocoon magnetization on the larval duration, larval weight and survival of larvae in multivoltine mulberry silkworm (*Bombyx mori*). This investigative study may be helpful in devicing the biotechnological application of magnetic field for the heavy production of silkworm cocoon as well as new magnification in the field of biophysics.

MATERIALS AND METHODS

The seed cocoons of multivoltine mulberry silkworm (*Bombyx mori* Nistari) were obtained from the silkworm grainage Beharich, Directorate of sericulture Uttar Pradesh and were maintained in the plywood trays (23 x 20 x 5 cm) under the ideal rearing conditions [11] in the silkworm laboratory. The temperature and relative humidity were maintained in the BOD incubator at 26 ± 1°C and 80 ± 5%

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RH respectively till the emergence of moths from the seed cocoons. The newly emerged moths were quickly picked up and kept sex-wise in separate trays to avoid copulation. The whole grainage operation was performed as per description given by [11].

Moth have a tendency to pair immediately after the emergence and therefore, sufficient pairs, each containing one male and one female from newly emerged moth, were allowed to mate at 26 ± 1°C and 80 ± 5% RH in 12 ± 1 hour/ day dim light condition. After four hours of mating, the paired moths were decoupled manually. The female moths were allowed for egg laying. After 24 hours of eggs laying, the female moths were individually examined for their disease freeness and after formaline treatment eggs were transferred to the incubator for hatching. After hatching, the larvae were reared on the mulberry leaves given as food in the trays. After completion of 5th instar, the ripe worms ceased feeding and ready for spinning. Small mountages were provided to the ripe worms and thus, sufficient number of cocoons were obtained from the silkworm larvae reared in the laboratory. Further, the cocoons were taken for magnetic exposure.

Experimental Design: To observe the influence of magnetic field on the larval performance of *Bombyx mori,* the cocoons, thus obtained were kept in the static magnetic field. The magnets of 1000, 2000, 3000 and 4000 gauss were used separately for the bio-magnetization of silkworm cocoons. The cocoons were magnetized for 24, 48, 72 and 96 hours separately with the magnet of each strength. The cocoons were kept for magnetization just after 3rd day of spinning. The control set of experiment i.e no magnetization of cocoons was also arranged. For the purpose of magnetization, initially 360 cocoons were kept within the magnetic field range of 1000 gauss of which 90 cocoons were released after 24 hours of magnetic exposure. Further, groups of 90 magnetized cocoons were released each after 48, 72 and 96 hours of exposure to the static magnetic field of 1000 gauss. These four groups of magnetized cocoons were separately transferred to the BOD incubator chronically, maintained at 26 ± 1°C, 80 ± 5% RH and 12 ± 1 hours photoperiod a day. Further, the incubation of exposed cocoons and the rearing of the different stages of silkworm were performed in the same BOD incubator. All the parameters of observations in the present study were determined from the respective stage obtained from the magnetized cocoons.

Larval duration: The time required from the hatching of larvae to the third day of spinning by the fifth instar larvae was considered. For this purpose, 90 larvae (three batches of 30 larvae in each batch) were taken for observation. Three replicates of each experiment were made.

Larval weight: For the determination of larval weight the weight of 30 larvae (three batches of 10 larvae in each batch) were recorded. Three replicates of each experiment were made. The larval weight was taken on the day when fifth instar larvae stop feeding.

Survival of Larvae: For determining the survival of larvae, 90 larvae (three batches of 30, 1st instar, larvae in each batch) were taken under the observation. The number of larvae which attained the pupal stage was counted for the calculation of the survival of larvae as following:

$$\text{Per cent survival of larvae} = \frac{\text{No. of larvae pupated}}{\text{No. of 1}^{st}\text{ instar larvae taken for observation}} \times 100$$

RESULTS

Larval Weight: The data given in table 1 clearly indicates that variation in the intensity of static magnetic field and exposure duration of *B. mori* cocoons influenced the weight of 5th instar larvae. With the increasing exposure duration of cocoons from 24 to 96 hours, the weight of 5th instar larvae increased in 1000, 2000 and 3000 gauss magnetized cocoons while in 4000 gauss magnetized cocoons, the larval weight increased up to 24 hours of exposure and further increase in exposure duration caused decline in the larval weight. The trend of the rate of increase in the weight of 5th instar larvae, with the increasing exposure duration, was almost similar and steady in 1000 and 2000 gauss magnetized cocoons while in 3000 gauss magnetized cocoons the rate of increase in the larval weight was high. The maximum weight of 5th instar larvae was noticed to be 1.961 ±0.08 gm (20.08% increase as compared to control) in 3000 gauss magnetized cocoons exposed for 72 hours.

Two-way ANOVA indicates that variation in the magnetic field and exposure duration of cocoons did not cause significant effect on the larval weight. The Post-hoc test (Table 1b) indicates significant group difference in the larval weight, in between 1000 and 4000 gauss,

Table 1a: Effect of cocoon magnetization on the larval weight (g) of 5th instar *Bombyx mori* larvae

Exposure duration (hours)	Magnetic power (gauss) Control (X_1)	1000 (X_2)	2000 (X_3)	3000 (X_4)	4000 (X_5)	F_1-ratio n_1= 4
24	1.633 ± 0.01 (100)	1.654±0.009 (101.28)	1.761 ± 0.02 (107.83)	1.818 ± 0.01 (111.32)	1.882 ±0.04 (115.24)	
48	1.633 ± 0.01 (100)	1.743 ± 0.02 (106.73)	1.882 ± 0.01 (115.24)	1.892 ± 0.01 (115.86)	1.862 ±0.03 (114.02)	3.11*
72	1.633 ± 0.01 (100)	1.803 ± 0.02 (110.41)	1.925 ± 0.01 (117.88)	1.961 ± 0.08 (120.08)	1.592 ±0.02 (97.48)	
96	1.633 ± 0.01 (100)	1.917 ± 0.06 (117.39)	1.930 ± 0.03 (118.18)	1.911 ± 0.06 (117.02)	1.421 ±0.02 (87.01)	

F_2-ratio = 0.352* n_2 = 3

*Non Significant

Each value represents mean ± S.E. of three replicates

X_1, X_2, X_3, X_4 and X_5 are the mean values of larval weight in control, 1000, 2000, 3000 and 4000 gauss magnetic strength

Figures in parantheses indicate per cent value when control was taken as 100%

Table 1b: Post-hoc Test showing effect of cocoon magnetization on the larval weight (g) of 5th instar *Bombyx mori* larvae

Mean difference in between groups	Exposure duration (hours) 24	48	72	96
$X_1 \sim X_2$	0.021	0.110	0.170	0.284
$X_1 \sim X_3$	0.128	0.249	0.292	0.297
$X_1 \sim X_4$	0.185	0.259	0.328	0.278
$X_1 \sim X_5$	0.249	0.229	0.041	0.212
$X_2 \sim X_3$	0.107	0.139	0.122	0.013
$X_2 \sim X_4$	0.164	0.149	0.158	0.006
$X_2 \sim X_5$	0.228	0.119	0.211	*0.496
$X_3 \sim X_4$	0.057	0.010	0.036	0.019
$X_3 \sim X_5$	0.121	0.020	0.333	*0.509
$X_4 \sim X_5$	0.064	0.030	0.369	*0.490

Honesty Significant difference (HSD) = $q\sqrt{\frac{MSwithin}{n}}$

$= 5.05\sqrt{\frac{0.017}{3}}$

$= 0.378$

MS = Mean square value of ANOVA Table

q = Studentized range static

n = No. of replicates

* = Shows significant group difference

X_1, X_2, X_3, X_4 and X_5 are mean values of larval weight in control, 1000, 2000, 3000 and 4000 gauss magnetic strength respectively.

Table 2a: Effect of cocoon magnetization on the larval duration (days) *Bombyx mori* larvae

Exposure duration (hours)	Magnetic power(gauss) Control (X_1)	1000 (X_2)	2000 (X_3)	3000 (X_4)	4000 (X_5)	F_1-ratio n_1= 4
24	26.52 ± 1.15 (100)	25.98 ± 0.05 (97.96)	25.75 ± 1.21 (97.09)	25.58 ± 0.64 (111.32)	25.32 ± 0.62 (95.47)	
48	26.52 ± 1.15 (100)	25.87 ± 0.02 (97.54)	25.29 ± 0.35 (95.36)	24.92 ± 0.65 (93.75)	24.75 ± 0.68 (93.32)	
72	26.52 ± 1.15 (100)	25.72 ± 0.58 (96.98)	24.94 ±0.056 (94.03)	23.75 ± 0.65 (89.55)	26.56 ± 1.20 (100.15)	3.75*
96	26.52 ± 1.15 (100)	25.62 ± 1.25 (96.60)	24.83 ± 0.70 (93.62)	24.30 ± 1.01 (91.62)	27.65 ± 0.60 (104.26)	

F_2-ratio = 0.304** n_2 = 3

*$P_1 < 0.05$ **Non Significant

Each value represents mean ± S.E. of three replicates

X_1, X_2, X_3, X_4 and X_5 are the mean values of larval duration in control, 1000, 2000, 3000 and 4000 gauss magnetic strength

Figures in parantheses indicate per cent value when control was taken as 100%

Table 2b: Post-hoc Test showing effect of cocoon magnetization on the larval duration (days) of *Bombyx mori* larvae

Mean difference in between groups	Exposure duration (hours) 24	48	72	96
$X_1 \sim X_2$	0.54	0.65	0.80	0.90
$X_1 \sim X_3$	0.77	1.23	1.58	1.69
$X_1 \sim X_4$	0.94	1.60	*2.77	*2.22
$X_1 \sim X_5$	1.20	1.77	0.04	1.13
$X_2 \sim X_3$	0.23	0.58	0.78	0.79
$X_2 \sim X_4$	0.40	0.95	1.97	1.32
$X_2 \sim X_5$	0.66	1.12	0.84	2.03
$X_3 \sim X_4$	0.17	0.37	1.19	0.53
$X_3 \sim X_5$	0.43	0.54	1.62	*2.82
$X_4 \sim X_5$	0.26	0.17	*2.81	*3.35

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

$= 5.05\sqrt{\frac{0.582}{3}}$

$= 2.222$

MS = Mean square value of ANOVA Table

q = Studentized range static

n = No. of replicates

* = Shows significant group difference

X_1, X_2, X_3, X_4 and X_5 are mean values of larval duration in control, 1000, 2000, 3000 and 4000 gauss magnetic strength respectively.

2000 and 4000 gauss and 3000 and 4000 gauss in case of the cocoons exposed for 96 hours while exposure of 24, 48 and 72 hours of cocoons of each static magnetic field did not cause significant group difference in the weight of 5th instar larvae.

Larval Duration: The data given in table 2 clearly indicates that both the duration of exposure and strength of magnetic field influenced the larval duration of silkworm. In 1000, 2000 and 3000 gauss magnetized cocoons for 72 hours the larval duration decreased slowly and slowly which reached to the level from 26.52 ±1.15 days (control) to 25.72 ±0.58, 24.94 ±0.056 and 23.75 ±0.65 days respectively. In case of 4000 gauss magnetized cocoons for 24 hours of exposure, the larval durations slightly decreased to 25.32 ±0.62 days but further increase in the exposure duration up to 96 hours caused an increase in the larval duration. Notable decrease in the larval duration in case of very short duration of exposure (24 hours) was recorded in 4000 gauss magnetized cocoons. The trend of variation in the larval duration in 1000, 2000 and 3000 gauss magnetized cocoons is almost of similar pattern with the varying exposure duration while in 4000 gauss magnetized cocoons, the trend of variation in the larval duration with exposure duration is different. The larval duration was noticed to be minimum of 23.75 ±0.65 days (10.45% decrease as compared to control) in 3000 gauss magnetized cocoons exposed for 72 hours.

Two-way ANOVA indicates that variation in the strength of static magnetic field significantly ($P<0.05$) influenced the larval duration of *B. mori* while variation in the exposure duration did not cause significant effect. The Post-hoc Test (table 2b) shows significant group difference in the larval duration in between the control and 3000 gauss and 3000 and 4000 gauss strength of magnetic exposure in case of 72 hours exposed cocoons. In case of 96 hours exposure of cocoon, the significant group difference between control and 3000 gauss, 2000 and 4000 gauss and 3000 and 4000 gauss magnetic strength was observed while 24 and 48 hours exposure of each magnetic strength did not cause significant group difference.

Survival of Larvae: The data presented in table 3 clearly indicates that both, the exposure duration and magnetic strength, influenced the survival of larvae. With the increasing exposure duration of cocoons, per cent survival of larvae was noticed to be increased in 1000, 2000 and 3000 gauss magnetized cocoons while in 4000 gauss magnetized cocoons, the trend of per cent survival

Table 3a: Effect of cocoon magnetization on the survival per cent of larvae *Bombyx mori*

Exposure duration (hours)	Magnetic power(gauss) Control (X_1)	1000 (X_2)	2000 (X_3)	3000 (X_4)	4000 (X_5)	F_1-ratio n_1= 4
24	79.66 ± 0.64 (100)	81.85 ± 0.72 (102.74)	82.22 ± 1.11 (103.21)	84.52 ± 1.24 (106.10)	85.02 ± 0.98 (106.72)	
48	79.66 ± 0.64 (100)	83.25 ± 1.80 (104.50)	83.84 ± 0.92 (105.24)	87.46 ± 0.83 (109.79)	82.04 ± 1.07 (102.98)	
72	79.66 ± 0.64 (100)	84.96 ± 1.26 (106.65)	86.78 ± 0.64 (108.93)	93.58 ± 0.76 (117.47)	79.60 ± 1.21 (99.92)	6.17*
96	79.66 ± 0.64 (100)	85.25 ± 1.15 (107.01)	87.96 ± 1.20 (110.41)	90.75 ± 0.99 (113.92)	75.20 ± 1.29 (94.40)	

F_2-ratio = 0.490** n_2 = 3

*P_1 < 0.01 **Non Significant

Each value represents mean ± S.E. of three replicates

X_1, X_2, X_3, X_4 and X_5 are the mean values of survival per cent of larvae in control, 1000, 2000, 3000 and 4000 gauss magnetic strength

Figures in parantheses indicate per cent value when control was taken as 100%

Table 3b: Post-hoc Test showing effect of cocoon magnetization on the survival percentage of *Bombyx mori* larvae

Mean difference in between groups	Exposure duration (hours) 24	48	72	96
$X_1 \sim X_2$	2.19	3.59	5.30	5.59
$X_1 \sim X_3$	2.56	4.18	7.12	8.30
$X_1 \sim X_4$	4.86	7.80	*13.92	*11.09
$X_1 \sim X_5$	5.36	2.38	0.06	4.46
$X_2 \sim X_3$	0.37	0.59	1.82	2.71
$X_2 \sim X_4$	2.67	4.21	8.62	5.50
$X_2 \sim X_5$	3.17	1.21	5.36	*10.05
$X_3 \sim X_4$	2.30	3.62	6.80	2.78
$X_3 \sim X_5$	2.80	1.80	7.18	*12.76
$X_4 \sim X_5$	0.50	5.42	*13.98	*15.55

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

$= 5.05\sqrt{\frac{9.39}{3}}$

$= 8.93$

MS = Mean square value of ANOVA Table

q = Studentized range static

n = No. of replicates

* = Shows significant group difference

X_1, X_2, X_3, X_4 and X_5 are mean values of survival percentage of larvae in control, 1000, 2000, 3000 and 4000 gauss magnetic strength respectively.

of larvae was different. The survival per cent in 72 hours exposed cocoons were noticed to be 84.96 ±1.26, 86.78 ±0.64, 93.58 ±0.76 and 79.60 ±1.21 in 1000, 2000, 3000 and 4000 gauss magnetized cocoons respectively which indicates that the exposure of cocoons under very high intensity (4000 gauss) of magnetic field was not suitable for the survival of larvae. The maximum level of the survival of larvae was noticed to be 93.58 ±0.76% (17.47% increase as compared to control) in case of the cocoons magnetized for 72 hours in the static magnetic field of 3000 gauss. Two-way ANOVA indicates that variation in the strength of the static magnetic field significantly (P_1< 0.01) influenced the survival of larvae while exposure duration did not cause significant effect. The Post-hoc test (Table 3b) indicates significant group difference in between control and 3000 gauss and 3000 and 4000 gauss in case of 72 hours exposed cocoons. In case of 96 hours exposed cocoons, the significant group difference was observed in between control and 3000 gauss, 1000 and 4000 gauss, 2000 and 4000 gauss and 3000 and 4000 gauss magnetic strength while 24 and 48 hours exposed cocoons of each magnetic field did not cause significant group difference.

DISCUSSION

Larval Weight: Variation in the strength of magnetic power and exposure duration of silkworm cocoons considerably influenced the weight of 5th instar *Bombyx mori* larvae. The larval weight increased gradually with the increasing exposure duration of cocoons from control to 96 hours in static magnetic field of 1000, 2000 and 3000 gauss. The highest larval weight was recorded in case of 3000 gauss magnetic exposure of 72 hours. The weight of 5th instar larvae, obtained from 4000 gauss-96 hours exposed cocoons was of lowest level. The larval weight of *Bombyx mori* also vary with the variation in the varieties of some host plant [12]. The larval weight of *Bombyx mori* was affected due to the starvation of larvae [13]. The refrigeration of *Bombyx mori* eggs at blue pigmentation stage caused reduction in the larval weight [14] while refrigeration of silkworm eggs at blue eye spot stage caused considerable increase in the weight of larvae [15]. The increase in the refrigeration duration of *Bombyx mori* eggs caused considerable decrease in the weight of silkworm larvae [16]. The weight of *Bombyx mori* larvae significantly influenced due to variation in the range of relative humidity while photoperiod regime has no significant influence on the larval weight [17]. The nutritional quality of mulberry leaf has also been noticed to be the major factor in deciding the weight of silkworm larvae [18-20]. The highest larval weight was noticed during winter while it was lowest during the summer season [21]. The application of magnetic field in the biological system caused enhancement of metabolic activities [22] and the low magnetic field caused stimulatory effect while higher magnetic field caused inhibitory effect [23]. The exposure of silkworm larvae to low magnetic field caused increase in the protein metabolism and larvae utilized more mulberry leaves [24]. The exposure of *Bombyx mori* larvae in the magnetic field of 3500 gauss at various durations of exposure caused an increase in the weight of cocoon [6] and also influenced the protein content in the larvae and pupae of *Bombyx mori* [25]. The magnetization of cocoon has been proved to be useful in improving the silk producing potential [8] and reproductive potential [10]. Thus, magnetization of cocoon in low magnetic field caused, an increase in the larval weight due to increased rate of metabolism resulting in the consumption of more food by the silkworm larvae while exposure in higher strength of magnetic field may cause stress response leading to the decrease in the larval weight of *Bombyx mori.*

Larval Duration: The change in the strength of static magnetic field and the exposure duration of cocoons influenced the larval duration of *Bombyx mori.* In 1000, 2000 and 3000 gauss magnetized cocoons, the larval duration decreased gradually with the increase in the exposure duration of cocoons, whereas, in case of 4000 gauss, the larval duration decreased slightly up to 24 hours of exposure but further increase in the exposure duration of cocoons caused an increase in the larval duration. The minimum larval duration was noticed in case of the cocoon exposed for 72 hours in 3000 gauss magnetic strength while maximum larval duration was recorded in 4000 gauss magnetized cocoons for 96 hours exposure of cocoons. The duration of larval period is an important parameter to measure the relative growth rate. It is well known that an ideal race is one, which has a shorter larval duration thus causing low consumption of leaf [26]. Temperature variation influenced the physiological ability of insect, therefore, larval duration varied significantly due to seasonal changes [27]. The rich nutrients of mulberry leaves enhanced its nutritional status causing reduction in the larval duration [28]. When the silkworm larvae were exposed to 24 hours light a day, the larval span was prolonged, resulting in good production of cocoon [29]. The magnetic field acts on enzyme molecules and produces conformational changes in enzyme, which is concerned for the activation of enzyme system, whereas, at higher magnetic field strength the change brought about is negative hence the enzyme get inhibited [30]. [6] recorded reduction in the larval duration under 20 min. exposure at 3500 gauss. The low magnetic field caused the activation of enzymatic activity due to conformational change in the enzyme molecule, whereas, high level of magnetic field caused inhibitory changes in the enzyme molecule and thus, inhibited enzyme activity [30]. Magnetic field stimulates the level of transcription where the field interact with electrons, moving along with stacked base pairs in DNA [31]. Thus, it may be concluded that reduction in the larval duration may occur due to stimulation of enzyme activities and acceleration in the metabolic rate under the low strength magnetic exposure.

Survival of Larvae: The change in the strength of static magnetic field and exposure duration of cocoons influenced the survival of larvae. With the increasing exposure duration of cocoons, the survival of larvae increased in 1000, 2000 and 3000 gauss magnetized cocoons while in 4000 gauss a decrease in the larval survival was noticed. The maximum survival of the larvae

was in case of 3000 gauss-72 hours exposed cocoons which was minimum in case of 4000 gauss magnetic exposure for 96 hours. The average temperature between 23.9 to 25.8°C along with 90.9% relative humidity has been proved to be favourable for the best survival of *Bombyx mori* larvae [32]. The ecological factors [17] and genome of silkworm [33] caused considerable influence on the survival of *Bombyx mori* larvae.

Thus it is concluded that the higher survivability of larvae may be due to the resistance developed in the larvae at low magnetic strength while the higher strength of magnetic field may cause stress response resulting is the high mortality of larvae.

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