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MAGNETIZATION FOR THE IMPROVEMENT OF SILK PRODUCING POTENTIAL IN MULTIVOLTINE MULBERRY SILKWORM (*BOMBYX MORI* LINN).

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ABSTRACT
The magnetic field activates the physiological and developmental processes in the living beings there fore; the magnetization of silkworm cocoon has been studied to observe any possibility for the heavy production of cocoon. The experiments were conducted with the static magnetic field of 1000, 2000, 3000 and 4000 gauss. Variation in the strength of static magnetic field ($p_1 < 0.01$) and exposure duration ($p_2 < 0.05$) of cocoon significantly influenced the weight of silk gland in *Bombyx mori*. The weight of silk gland increased from 0.201g (control) to the maximum of 0.246g in case of 3000 gauss -72 hrs exposed cocoons. The weight of cocoon increases with the increasing exposure duration and magnetic strength up to 3000 gauss. The maximum weight of cocoon was recorded to be 1.072g in case of 3000 gauss-72 hrs exposed cocoons while it was minimum (0.812g) in 4000 gauss- 96 hrs exposed cocoons. The weight of cocoon shell increased with the increasing exposure duration of cocoons from 24 to 96 hrs in 1000, 2000 and 3000 gauss magnetic field and it was maximum (0.199g) in 3000 gauss-72 hrs exposed cocoons.

INTRODUCTION

The silk industry has developed as a popular cottage industry providing self- employment to more than ten million rural persons in the unorganized sector. An analysis of the trends in international silk production suggests that sericulture has better prospects for growth in the developing countries than in advanced countries. 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 (Upadhyay and Mishra, 1991) relative humidity (Mishra and Upadhyay, 1992) photoperiod, (Mishra and Upadhyay, 1993) artificial diet (Iwanvat and Ono, 1969), X-rays (Kanarev and Cham, 1985) etc on the performance of silkworm. The magnetization of eggs influences silk producing potential (Upadhyay and Tripathi, 2006), incubation period of eggs (Tripathi and Upadhyay, 2005) and reproductive potential (Upadhyay and Tripathi, 2005). The magnetic field influences the level of hormone (Udinstev and Moroz, 1982) and the rate of beta-glactosidase synthesis (Arholt *et al.,* 1982). It is hypothesized that if the cocoon of *Bombyx mori* are exposed in different magnetic strength, there may by 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 weight of silk gland, weight of cocoon and weight of cocoon shell in multivoltine mulberry silkworm (*Bombyx mori*).

MATERIALS AND METHODS

The seed cocoons of multivoltine mulberry silkworm (*Bombyx mori* Nistari) were obtained from the silkworm grainage Behraich, Directorate of Sericulture Uttar Pradesh and were maintained in the plywood trays (23x20x5cm) under the ideal rearing conditions (Krishnaswami *et al*., 1973) in the silkworm laboratory. The temperature and relative humidity were maintained in the BOD incubator at 26 ± 1ºC and 80 ± 5% RH respectively until 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 Krishnaswami *et al.,* (1973).

Moth have a tendency to pair immediately after the emergence, therefore sufficient pairs, each containing one male and one female from newly emerged moth were allowed to mate at 26 ± 1ºC and 80 ± 5%RH in 12 ± 1 hr/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 hrs of eggs laying the female moths were individually examined for their disease free ness 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 designing

To observe the influence of magnetic field on the reproductive potential 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 hrs 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 with in the magnetic field range of 1000 gauss of which 90 cocoons were released after 24 hrs of magnetic exposure. Further, remaining 3 groups of 90 magnetized cocoons were released each after 48, 72 and 96 hrs 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 ± 1ºC, 80 ± 5% RH and 12 ± 1 hrs photoperiod a day. Further the incubation of exposed cocoons and the rearing of 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 stages obtained from the magnetized cocoons.

Weight of silk gland

For determining the weight of silk gland, the larvae were dissected in distilled water on the 8th day of 5th instar and complete silk gland was taken out. The removed silk gland was cleaned and gently soaked with filter paper to remove the adhered water on it. For the weight of silk gland, nine silk glands (Three batches of three silk glands in each batch) were weighed for each replicate. Three replicates of each experiment were made. The data obtained were analyzed statistically by two way ANOVA and Post- hoc test.

Weight of cocoon

To estimate the weight of single cocoon, 30 cocoons (Three batches of 10 cocoons in each batch) were weighed for each replicate. Three replicates of each experiment were made. The weight of cocoon was taken on 5th day from the beginning of spinning. The data obtained were analysed statistically by two way ANOVA and Post-hoc test.

Weight of shell

For the weight of single shell, 30 shells (Three batches of 10 shells in each batch) were weighed for each replicate. Three replicates were made. The weight of shell was also taken on the 5th day from the beginning of spinning. The data obtained were analyzed statistically by two way ANOVA and Post- hoc test.

RESULTS

Weight of silk gland

The data given in table 1a clearly indicate that variation in the intensity of static magnetic field and exposure duration of *B. mori* cocoons caused considerable influence on the weight of silk gland. With the increasing exposure duration of cocoons from 24 to 96 hrs, the weight of silk gland increased in 1000, 2000 and 3000 gauss magnetized cocoons while in 4000 gauss magnetized cocoons, the weight of silk gland increased up to 24 hrs of exposure and further declined up to 96 hrs exposed cocoons. The trend of the rate of increase in the weight of silk gland 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 weight of silk gland was very high. The maximum weight of silk gland was noticed to be 0.246 ± 0.007g in 3000 gauss magnetized cocoons exposed for 72 hrs. Two-way ANOVA indicates that the magnetic strength and exposure duration of cocoons significantly ($p_1 < 0.01$, $p_2 < 0.05$) influenced the weight of silk gland in *B. mori*. Post-hoc test (Table 1b) shows significant group difference in the weight of silk gland in between control and 4000 gauss in case of 24 hrs exposed cocoons; control and 2000 gauss, control and 3000 gauss and 3000 and 4000 gauss in case of 48 hours exposed cocoons, and control and 2000 gauss, control and 3000 gauss, 2000 and 4000 gauss, and 3000 and 4000 gauss magnetic field in case of 72 hours exposed cocoons. In case of 96 hours exposed cocoons, the significant group difference was observed in between control and 2000 gauss, control and 3000 gauss, 1000 and 4000 gauss, 2000 and 4000 gauss and 3000 and 4000 gauss magnetic strength.

Weight of cocoon

The data presented in Table 2a clearly indicate that variation in the strength of magnetic field and exposure duration did not cause considerable influence on the weight of cocoon. With the increasing exposure duration up to 96 hrs, the weight of cocoon increased from 0.903 ± 0.004 g (control) to the level of 0.934 ± 0.006, 0.964± 0.006 and 0.985 ± 0.002 g in 1000, 2000 and 3000 gauss magnetized cocoons respectively while in 4000 gauss magnetized cocoons, the weight of cocoon increased to the level of 0.948± 0.021 g in 24 hrs magnetized cocoons which further declined to the level of 0.812 ± 0.22 g in 96 hrs exposed cocoons. The increase in the weight of cocoon, obtained from 24 hrs exposed cocoons, were steady in 1000 and 2000 gauss magnetized cocoons while the increase in cocoon weight was considerable in 3000 and 4000 gauss magnetized cocoons. The maximum weight of cocoon was noticed to be 1.072 ± 0.001 g in case of 72 hrs exposure of 3000 gauss magnetized cocoon. Two-way ANOVA indicates that variation in the exposure duration and static magnetic strength did not cause significant change in the weight of *B. mori* cocoons. The Post-hoc test (Table 2b) shows significant group difference in between control and 3000 gauss, 1000 and 3000 gauss and 3000 and 4000 gauss magnetic strength in case of 72 hrs exposed cocoons. In case of 96 hours exposed cocoons, the significant group difference was observed in between 1000 and 4000 gauss, 2000 and 4000 gauss and 3000 and 4000 gauss magnetic strength while 24 and 48 hrs exposed cocoons of each magnetic strength did not cause significant group difference.

Weight of cocoon shell

The data given in table 3a clearly indicate that the variation in the magnetic strength and exposure duration of *Bombyx mori* cocoons, did not influence the weight of cocoon shell. With the increasing exposure duration of cocoons up to 96 hrs under the influence of 1000 and 2000 gauss magnetic strength the trend of increase in the weight of cocoon shell was steady while in 3000 gauss magnet exposed cocoons, the rate of increase in the weight of cocoon shell, with the increasing

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

Exposure duration (hrs)	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	0.20 ±0.011 (100)	0.201±0.014 (104.47)	0.216 ±0.003 (107.46)	0.219 ±0.006 (108.95)	0.226 ±0.009 (112.43)	
48	0.20 ±0.011 (100)	0.214±0.011 (106.46)	0.228 ±0.003 (113.43)	0.236 ±0.006 (117.41)	0.026 ±0.003 (102.48)	12.50*
72	0.20 ±0.011 (100)	0.218±0.006 (108.45)	0.232 ±0.004 (115.92)	0.246 ±0.007 (119.40)	0.190 ±0.003 (94.52)	
96	0.201±0.011 (100)	0.225±0.002 (111.94)	0.234 ±0.005 (116.41)	0.238 ±0.005 (118.40)	0.180 ±0.006 (89.55)	

F2 –ratio = **3.75****; **n2 = 3;** ***p1** < 0.01; ****p2** < 0.05

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 weight of silk gland in control, 1000, 2000, 3000 and 4000 gauss magnetic strength Figures in parentheses indicate per cent value when control was taken as 100%

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

Mean difference in between groups	Exposure duration (hrs) 24	48	72	96
$X_1 \sim X_2$	0.009	0.013	0.017	0.024
$X_1 \sim X_3$	0.015	*0.027	*0.031	*0.033
$X_1 \sim X_4$	0.018	*0.035	*0.045	*0.037
$X_1 \sim X_5$	*0.025	0.005	0.011	0.021
$X_2 \sim X_3$	0.006	0.014	0.014	0.009
$X_2 \sim X_4$	0.009	0.022	0.028	0.013
$X_2 \sim X_5$	0.016	0.008	0.028	*0.045
$X_3 \sim X_4$	0.003	0.008	0.014	0.004
$X_3 \sim X_5$	0.010	0.022	*0.042	*0.054
$X_4 \sim X_5$	0.007	*0.030	*0.056	*0.137

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

$= 5.05\sqrt{\frac{0.00008}{3}}$

$= 0.025$

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 weight of silk gland in control, 1000, 2000, 3000 and 4000 gauss magnetic strength respectively.

Table 2b: ost-hoc Test showing effect of cocoon magnetization on the weight of cocoon (gm) of *Bombyx mori*

Mean difference in between groups	Exposure duration (hrs) 24	48	72	96
$X_1 \sim X_2$	0.007	0.015	0.023	0.031
$X_1 \sim X_3$	0.017	0.028	0.490	0.061
$X_1 \sim X_4$	0.036	0.065	*0.169	0.082
$X_1 \sim X_5$	0.045	0.028	0.008	0.091
$X_2 \sim X_3$	0.008	0.013	0.026	0.030
$X_2 \sim X_4$	0.027	0.050	*0.146	0.051
$X_2 \sim X_5$	0.038	0.013	0.031	*0.122
$X_3 \sim X_4$	0.028	0.037	0.120	0.021
$X_3 \sim X_5$	0.030	0.000	0.057	*0.152
$X_4 \sim X_5$	0.011	0.037	*0.177	*0.173

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

$= 5.05\sqrt{\frac{00.0017}{3}}$

$= 0.121$

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 weight of cocoon in control, 1000, 2000, 3000 and 4000 gauss magnetic strength respectively.

exposure duration was steep. The higher level of the weight of cocoon shell, obtained in case of 96 hrs exposed cocoons were 0.176 ± 0.005, 0.190 ± 0.007 and 0.191 ± 0.002g in 1000, 2000 and 3000 gauss magnetized cocoons respectively. In 4000 gauss magnetized cocoons, the weight of shell was maximum to be 0.176 ± 0.004g in 24 hrs exposed cocoons. The maximum weight of cocoon shell (0.199 ± 0.010g) was noticed in 72 hrs exposed 3000 gauss magnetized cocoons. Two-way ANOVA indicates that variation in the static magnetic field and exposure duration of cocoons did not cause significant effect on the weight of cocoon shell in *B. mori*. The Post-hoc test (Table 3b) shows significant group difference only in between 2000 and 4000 gauss and 3000 and 4000 gauss magnetic strength in case of 96 hrs exposed cocoons,

Table 2a: Effect of cocoon magnetization on the weight of cocoon (g) of *Bombyx mori*

Exposure duration (hrs)	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	0.903±0.004 (100)	0.910±0.006 (100.97)	0.918 ±0.003 (101.66)	0.937 ±0.009 (103.76)	0.948 ±0.021 (104.98)	
48	0.903±0.004 (100)	0.918±0.005 (101.66)	0.931 ±0.005 (103.10)	0.968 ±0.006 (107.19)	0.931 ±0.032 (103.10)	3.24*
72	0.903±0.004 (100)	0.926±0.004 (102.54)	0.952 ±0.005 (105.42)	1.072 ±0.001 (118.71)	0.895 ±0.013 (99.11)	
96	0.903±0.004 (100)	0.934±0.006 (103.43)	0.964 ±0.006 (106.75)	0.985 ±0.002 (109.08)	0.812 ±0.022 (89.92)	

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 weight of cocoon in control, 1000, 2000, 3000 and 4000 gauss magnetic strength Figures in parentheses indicate per cent value when control was taken as 100%

Table 3a: Effect of cocoon magnetization on the weight of cocoon shell (g) of *Bombyx mori*

Exposure duration (hrs)	Magnetic power(gauss) Control(X1)	1000(X2)	2000(X3)	3000(X4)	4000(X5)	F1-ratio n1 = 4
24	0.153 ± 0.001 (100)	0.158 ± 0.011 (103.26)	0.163 ± 0.008 (106.53)	0.172 ± 0.010 (112.41)	0.176 ± 0.004 (115.03)	
48	0.153 ± 0.001 (100)	0.166 ± 0.014 (108.49)	0.172 ± 0.012 (112.41)	0.181 ± 0.008 (118.30)	0.172 ± 0.015 (112.41)	1.25*
72	0.153 ± 0.001 (100)	0.173 ± 0.003 (113.07)	0.183 ± 0.013 (119.60)	0.199 ± 0.010 (130.06)	0.147 ± 0.005 (96.07)	
96	0.153 ± 0.001 (100)	0.176 ± 0.005 (115.03)	0.190 ± 0.007 (124.18)	0.191 ± 0.002 (124.83)	0.102 ± 0.003 (66.66)	

F_2 –ratio = **0.375***; 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 weight of cocoon shell in control, 1000, 2000, 3000 and 4000 gauss magnetic strength

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

Table 3b: Post-hoc Test showing effect of cocoon magnetization on the weight of cocoon shell (g) of *Bombyx mori*

Mean difference in between groups	Exposure duration (hrs) 24	48	72	96
$X_1 \sim X_2$	0.005	0.013	0.020	0.023
$X_1 \sim X_3$	0.010	0.019	0.030	0.037
$X_1 \sim X_4$	0.019	0.028	0.046	0.038
$X_1 \sim X_5$	0.023	0.019	0.006	0.051
$X_2 \sim X_3$	0.005	0.006	0.010	0.014
$X_2 \sim X_4$	0.014	0.015	0.026	0.015
$X_2 \sim X_5$	0.018	0.006	0.026	0.074
$X_3 \sim X_4$	0.009	0.009	0.016	0.001
$X_3 \sim X_5$	0.013	0.000	0.036	*0.088
$X_4 \sim X_5$	0.004	0.009	0.052	*0.089

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

$= 5.05\sqrt{\frac{0.0008}{3}}$

$= 0.081$

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 weight of cocoon shell in control, 1000, 2000, 3000 and 4000 gauss magnetic strength respectively.

while 24, 48 and 72 hrs exposed cocoons of all the magnetic strength did not cause significant group difference in the weight of cocoon shell of *B. mori*.

DISCUSSION

The weight of the silk gland of *Bombyx mori* was considerably influenced due to change in the strength of static magnetic field and exposure duration of cocoons. With the increasing exposure duration of *B. mori* cocoons from 24 to 96 hrs, the weight of silk gland increased in case of 1000, 2000 and 3000 gauss while in 4000 gauss magnetized cocoons, the weight of silk gland increased up to 24 hrs of exposure and further declined in case of 96 hrs exposed cocoons. The trend of increase in the weight of silk gland was almost similar in 1000 and 2000 gauss while in 3000 gauss magnetized cocoons, the rate of increase in the weight of silk gland was very high (Table 1a). The morphological, physiological and biochemical changes take place after the exposure of magnetic field on the biological system (Patnev and Mankova, 1986). Magnetic field caused significant increase in the protein content of silk gland of 5th instar *Bombyx mori* larvae (Upadhyay and Tripathi, 2005). Magnetization of eggs significantly increased the weight of silk gland of *B. mori* (Upadhyay and Tripathi, 2006). The application of 3500 gauss magnetic field on the 5th instar *B. mori* larvae caused an increase in the production of silk (Chaugale, 1993). Thus, it may be concluded that the magnetization of cocoons may cause the activation of cellular activities in silk gland by the consumption of more and more food by silkworm larvae in low magnetic field while higher strength of magnetic field exposure may cause stress response leading to decrease in the growth of silk gland.

Variation in the strength of static magnetic field and its exposure duration notably influenced the weight of cocoon. The minimum weight of cocoon was noticed to be 0.812g in case of the cocoons exposed at 4000 gauss magnetic field for 96 hrs, whereas, it reaches to the maximum level of 1.072g in 3000 gauss for 72 hrs exposed cocoons (Table 2a). The treatment of *Bombyx mori* larvae in the magnetic field of 3500 gauss increases the production of silk of about 10% (luca *et al.,* 1967), whereas, the exposure of silkworm larvae to low magnetic field caused an increase in the protein metabolism as result the larvae utilized more mulberry leaves (Ishaaya *et al.*, 1971). Magnetization of eggs increases the weight of cocoon while at higher magnetic power of 4000 gauss the weight of cocoon of *B. mori* decreased (Upadhyay and Tripathi, 2006). The exposure of *B. mori* larvae in the magnetic field of 3500 gauss at various times causes an increase in the weight of cocoon (Chaugale and More, 1992). In the present investigation the weight of cocoon is increased with the increasing strength of magnetic field up to 3000 gauss which may be due to the increase in the enzyme activities resulting the increase of the general metabolic rate while the higher range of magnetic field may decline the enzymatic activities due to stress resulting in the reduction of the cocoon weight.

The weight of cocoon shell was influenced due to variation in the strength of static magnetic field and exposure duration of *B. mori* cocoons. With the increase in exposure duration up to 96 hrs in 1000, 2000 and 3000 gauss magnetic field, the weight of cocoon shell increased and reached to the maximum level of 0.199 g in case of 3000 gauss exposed cocoons (Table 3a). The treatment of silkworm larvae in gamma radiation, caused an increase in the production of cocoons (Adbel Salam and Mahmoud, 1995), whereas, magnetization caused change in the characters of cotton fibre (Todoran *et al*., 1966). The increase in the protein metabolism and utilization of more mulberry leaves by the larvae was increased by the exposure

of silkworm larvae in low magnetic field (Ishaaya *et al.,* 1971). The functional significance of sequence homology can be illustrated by cytochrome C, an iron containing mitochondrial protein that transfers electron during biological oxidation in eukaryotic cells (Dickerson, 1972) and magnetic field below 0.3T caused an increase in oxidation rate due to increased reducing power of cytochrome C in cells (Blank, 1998). Magnetization of eggs has also been reported to influence the weight of cocoon shell of *B. mori* (Upadhyay and Tripathi, 2006). Thus, it may be concluded that the low magnetic field exposure of cocoon may cause an increase in the metabolic rate as result the food intake of larvae becomes more and more and the cellular activities of silk gland increased, causing increased cocoon shell weight, whereas, higher magnetic field may cause stress response, resulting in the decline of cocoon shell weight.

Thus, the exposure of *B. mori* cocoons under 3000 gauss magnetic strength for 72 hrs enhances the vital activity in different stages of silkworm hence the weight of silk gland, weight of cocoon and weight of shell were at the maximum of the performance level. 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.

The biotechnological knowledge as explicited from the revealations may give an insight in the field of bio-magnetic researches in future.

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