Journal of Entomology

ISSN 1812-5670

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Journal of Entomology

ISSN 1812-5670
DOI: 10.3923/je.2021.29.36

Research Article
Effect of Magnetic Exposure of Eggs on DNA Contents in the Larvae and Pupae of *Bombyx mori*

Santosh Kumar Tripathi

Department of Zoology, Mahatma Gandhi Post Graduate College, Gorakhpur, 273 001, India

Abstract

Background and Objective: DNA contents in the tissues of *Bombyx mori* influences the quality and quantity of silk protein, metabolic and physiological process. The present study evaluated to the effect of magnetic field on DNA content in the silk gland, fat body and haemolymph of 5th instar larvae and fat body and haemolymph of pupae of *Bombyx mori*. **Materials and Methods:** Eggs of *Bombyx mori* magnetized in 1000, 2000, 3000 and 4000 Gauss magnetic field for 24, 48, 72 and 96 hrs magnetic exposure and the data obtained from study analyzed statistically by two way ANOVA and *post-hoc* test. **Results:** DNA contents increased in the silk gland of larvae and fat body and haemolymph of larvae and pupae with an increase in magnetic exposure of eggs up to 96 hrs and magnetic strength from 1000-3000 Gauss. DNA content was maximum (0.130±0.005 and 0.139±0.002 μ mg^{-1} haemolymph of larvae and pupae, respectively) for 96 hrs exposed eggs in 3000 Gauss magnetic field. In 4000 Gauss magnetic strength, DNA contents decreased in the tissues of larvae and pupae with an increase in magnetic exposure of eggs upto 96 hrs. **Conclusion:** Treatment of *Bombyx mori* eggs in 3000 Gauss magnetic field for 96 hrs exposure was effective to enhanced DNA contents in the tissues.

Key words: *Bombyx mori*, magnetization, silk gland, fat body, haemolymph, DNA

Citation: Santosh Kumar Tripathi, 2021. Effect of magnetic exposure of eggs on DNA contents in the larvae and pupae of *Bombyx mori*. J. Entomol., 18: 29-36.

Corresponding Author: Santosh Kumar Tripathi, Department of Zoology, Mahatma Gandhi Post Graduate College, Gorakhpur, 273 001, India

Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The innovation of India as a developing country is definitely based upon agriculture and agro-based industry. Like agriculture, sericulture plays an important role in the transformation of rural economy as it assures wide scopes in the term of regular employment and provides return round the year. The race *Nistari* is a resistant variety of multivoltine mulberry silkworm (*Bombyx mori*), which contributes up to 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 life cycle of *Bombyx mori*[1].

In order to increase the production of quality raw silk efforts have been made to investigate the effect of temperature, relative humidity[2], photoperiod[3] and X-rays[4] etc on the performance of different stages of *Bombyx mori*. The effect of magnetism on biological system has been subject of worldwide interest. Magnetic field influences larval behaviours of silkworm[5], hormone level[6], alkaline phosphatase activity in mouse[7] and seed germination[8] in plants. Its positive effect includes cell viability[9], nerve regeneration[10] and bone healing in guinea pig[11]. The magnetization of the eggs influences to the protein content[12] and acid phosphatase[13] and alkaline phosphatase[14] activities in the larvae and pupae of *Bombyx mori.* The weight of the silk gland, cocoon and cocoon shell[15] and protein content in different tissues of larvae[16] also change due to the magnetization of larvae of *Bombyx mori*.

Keeping this in view, it is hypothesized that the exposure of *Bombyx mori* eggs in the magnetic field may influence to the DNA contents in different tissues of *Bombyx mori*. This communication reports to the effects of magnetic field on the quantitative estimation of DNA content in the silk gland, fat body and haemolymph of the fifth instar larvae and fat body and haemolymph of the pupae of *Bombyx mori*. Since DNA is master molecule of the cells and has direct relation with cellular metabolic process like protein synthesis therefore, this study was performed. The study aimed to enhance the performance of larvae and pupae by increase in DNA contents in different tissues and develop interest of researchers in the field of bio-magnetism. The study is important from an academic as well as economic point of view and will help to improve the quality of silk.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Zoology, Silkworm Laboratory of Mahatma Gandhi Post Graduate College, Gorakhpur from March, 2019-April, 2020.

Seed cocoon: The seed cocoons (Pupa enclosed in silken case) of multivoltine mulberry silkworm (*Bombyx mori nistari*), a native of West Bengal in India, were obtained from silkworm grainage Behraich, Directorate of sericulture, Uttar Pradesh, India and were maintained in plywood trays (23×20×5 cm) under the ideal rearing conditions[17] in the Silkworm laboratory, Department of Zoology, Mahatma Gandhi P.G. College, Gorakhpur. The temperature and relative humidity were maintained 26±1°C and 75±5% till the emergence of moths from seed cocoons.

Copulation: Moths tend to pair immediately after emergence thus, they were allowed with their mates for copulation. A total of 510 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 4 hrs 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 conditions at 26±1°C temperature and 75±5% relative humidity. After 24 hrs of egg laying, the female moths were individually examined for their disease freeness. The females were crushed individually in a mortar with pestles and blood smears were examined by microscope under magnification of 15×45 for the detection of bacterial and protozoan pathogens.

The Disease Free Laying (DFLs) thus prepared were treated with 2% formalin for 15 min to increase 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 the shade. The dried eggs obtained, thus were taken for magnetization under various experimental conditions.

To observe, the influence of magnetic field on the DNA contents in the different tissues of the larvae and pupae of *Bombyx 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 magnetization. The

DFLs were magnetized for 24, 48, 72 and 96 hrs separately with the each strength of the magnetic field. The control set (eggs not treated in magnetic field) is also studied with experimental study. For the magnetization, 120 DFLs were kept in 1000 Gauss magnetic field and 30 DFLs were released after 24 hrs of magnetic exposure. Further 30 DFLs were released each after 48, 72 and 96 hrs of magnetization. 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 hrs photoperiod in a day. The incubation of exposed eggs and further rearing of the different stages were performed in the same BOD incubator. Similar experiments were performed with 2000, 3000 and 4000 Gauss magnetic strength. DNA content was determined from the tissues of the larvae and pupae developed from magnetized eggs.

The estimation of DNA contents was performed according to Schneider[18] by using diphenylamine and orcinol reagents. For estimation of the DNA content, 1.0 g silk gland, 0.62 mg (0.5 mL) haemolymph and 0.10 g fat body were taken from the 5th instar larvae and the same amount of fat body and haemolymph were taken from the pupae. The tissues thus obtained, were homogenized separately in 5% T.C.A. (Trichloroacetic acid) at 90°C and centrifuged at 5000 rpm for 20 min. From supernatant, 1.0mL was taken and 1.0 mL distilled water and 4.0 mL of freshly prepared diphenylamine reagent (1.0 g diphenylamine, 100 mL of glacial acetic acid and 2.5 mL concentrated H_2SO_4) were added. Now reaction mixture was kept in the boiling water-bath for 10 min. The blue colour developed and its Optical Density (OD) was measured at 600 nm with the help of a spectrophotometer. Standard curves were drawn using different concentrations of calf thymus DNA as standard.

Statistical analysis: Six replicates of each experiment were made and the data obtained were analyzed statistically by the two-way ANOVA and post-hoc test. The *post-hoc* test is used to identify significant differences between groups and calculated by mathematical Eq.[19]:

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

Where:

MS within = Mean square value within group
q = Studentized range statics
N = Number of replicates

RESULTS

DNA content in silk gland of the fifth instar larvae: The data given in Table 1 indicates that variation in the duration of magnetic exposure and strength of the magnetic field influenced to the DNA contents in the silk gland of the fifth instar larvae of *Bombyx mori.* In 1000, 2000, 3000 Gauss magnetic field, the DNA content increased with increase in the exposure duration from 24-96 hrs of the eggs while in larvae developed from eggs magnetized in 4000 Gauss magnetic field, the DNA content increased up to 24 hrs exposure of eggs and further increase in duration of exposure of eggs caused gradually decline in the DNA content. In 1000 and 2000 Gauss magnetic field, the DNA content increased slowly with an increase in the exposure duration from 24-96 hrs but variation in the 3000 Gauss magnetic field was maximum and the level of DNA content was recorded to be 0.119±0.012 µg mg^{-1} of tissues in 3000 Gauss magnetic field with 96 hrs exposure of eggs. The trend of increase in the DNA content in 4000 Gauss magnetic field was different. In the 4000 Gauss magnetic field, the DNA content increased up to 24 hrs exposure of eggs and a further increase in the exposure duration of eggs up to 96 hrs caused a gradually decline in DNA content in the silk gland. The two-way ANOVA test shows (Table 1) that variation in the strength of magnetic field (p_1<0.01) and exposure duration (p_2<0.05) of eggs significantly influenced to DNA content in the silk gland of fifth instar larvae of *Bombyx mori*. The *post-hoc* test shows (Table 2) significant group differences for the DNA content between the control and 3000 Gauss (0.032 µ mg^{-1}), 1000 and 3000 Gauss (0.026 µ mg^{-1}), 1000 and 4000 Gauss (0.071 µ mg^{-1}) and 3000 and 4000 Gauss (0.027 µ mg^{-1}) magnetic field for 24 hrs exposure of eggs. In 48 hrs exposed eggs, the significant group differences of the DNA contents were present between the control and 3000 Gauss (0.063 µ mg^{-1}), 1000 and 3000 Gauss (0.028 µ mg^{-1}) and 3000 and 4000 Gauss (0.036 µ mg^{-1}) magnetic strength whereas, the differences were significant in between the control and 3000 Gauss (0.041 µ mg^{-1}), 1000 and 3000 Gauss (0.030 µ mg^{-1}) and 3000 and 4000 Gauss (0.041 µ mg^{-1}) magnetic strength for 72 hrs exposure of the eggs. In 96 hrs exposure duration, the significant difference of DNA contents were present between the control and 2000 Gauss (0.025 µ mg^{-1}), control and 3000 Gauss (0.045 µ mg^{-1}), 1000 and 3000 Gauss (0.033 µ mg^{-1}), 2000 and 4000 Gauss (0.027 µ mg^{-1}) and 3000 and 4000 Gauss (0.047 µ mg^{-1}) magnetic strength.

Table 1: Effect of magnetic field on DNA contents (μ mg^{-1} tissues) in the silk gland of V^{th} instar larvae of *Bombyx mori*

Exposure duration (hrs)	Magnetic powers (Gauss)					F_1-ratio
	Control (X_1)	1000 (X_2)	2000 (X_3)	3000 (X_4)	4000 (X_5)	$n_1 = 4$
24	0.074±0.004	0.080±0.006	0.091±0.006	0.106±0.014	0.079±0.005	
48	0.074±0.004	0.083±0.006	0.094±0.007	0.111±0.015	0.075±0.004	8.09*
72	0.074±0.004	0.085±0.005	0.096±0.008	0.115±0.010	0.074±0.003	
96	0.074±0.004	0.086±0.006	0.099±0.010	0.119±0.012	0.072±0.004	

F_2-ratio: 3.28, ** n_2: 3, *p_1<0.01, **p_2<0.05. Each value represents Mean±SE of six replicates. X_1, X_2, X_3, X_4 and X_5 are mean value of the DNA contents in the control, 1000, 2000, 3000 and 4000 Gauss magnetic fields, respectively

Table 2: Post hoc test showed group differences for DNA contents in the silk gland of 5^{th} instar larvae of *Bombyx mori*

Mean differences between groups	Exposure durations (hrs)			
	24	48	72	96
X_1~X_2	0.006	0.009	0.011	0.012
X_1~X_3	0.017	0.020	0.022	0.025*
X_1~X_4	0.032*	0.063*	0.041*	0.045*
X_1~X_5	0.005	0.001	0	0.002
X_2~X_3	0.001	0.011	0.011	0.013
X_2~X_4	0.026*	0.028*	0.030*	0.033*
X_2~X_5	0.071*	0.008	0.011	0.014
X_3~X_4	0.015	0.017	0.019	0.020
X_3~X_5	0.012	0.019	0.021	0.027*
X_4~X_5	0.027*	0.036*	0.041*	0.047*

*Significant group difference.

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

$$= 6.10\sqrt{\frac{0.0001}{6}}$$

$$= 0.024$$

Where:

MS within : Mean square value within group

q : Studentized range statics

N : Number of replicates

Table 3: Effect of magnetic field on DNA contents (μ mg^{-1} tissues) in the fat body of 5^{th} instar larvae and pupae of *Bombyx mori*

Exposure duration (hrs)		Magnetic powers (Gauss)					F_1-ratio
		Control (X_1)	1000 (X_2)	2000 (X_3)	3000 (X_4)	4000 (X_5)	$n_1 = 4$
24							
	Larvae	0.086±0.002	0.087±0.002	0.094±0.003	0.104±0.004	0.089±0.001	
	Pupae	0.082±0.002	0.086±0.001	0.095±0.001	0.104±0.001	0.083±0.00	
48							
	Larvae	0.086±0.002	0.090±0.003	0.096±0.003	0.107±0.005	0.084±0.002	14.63*
	Pupae	0.082±0.002	0.089±0.002	0.100±0.003	0.108±0.001	0.082±0.002	29.35**
72							
	Larvae	0.086±0.002	0.092±0.004	0.098±0.006	0.110±0.007	0.080±0.003	
	Pupae	0.082±0.002	0.091±0.002	0.105±0.004	0.112±0.008	0.075±0.001	
96							
	Larvae	0.086±0.002	0.095±0.002	0.105±0.010	0.116±0.012	0.073±0.004	
	Pupae	0.082±0.002	0.095±0.002	0.111±0.004	0.118±0.005	0.071±0.002	

*Larvae, ** Pupae F_2-ratio: 6.21* and 16.21**, $n_2 = 3$. *p_1<0.01, *p_2<0.01, **p_1<0.01, **p_2<0.01. Each value represents Mean±SE of six replicates. X_1, X_2, X_3, X_4 and X_5 are mean values of the DNA contents in the control, 1000, 2000, 3000 and 4000 gauss magnetic fields, respectively

DNA content in the fat body of the fifth instar larvae and pupae: The data given in Table 3 clearly indicates that variation in the strength of static magnetic field and the exposure duration of *Bombyx mori* eggs caused a notable impact on the change in DNA content in fat body of the 5^{th} instar larvae and pupae. The DNA content increased with increase in strength of magnetic field and it was maximum in the larvae (0.116±0.012 μg mg^{-1} of tissues) and pupae (0.118±0.005 μg mg^{-1} of tissues) developed from the eggs exposed for 96 hrs in the 3000 Gauss magnetic field. In 4000 Gauss magnetic field, the DNA content increased slightly up to 24 hrs exposed eggs and a further increase in the

Table 4: Post hoc test showed group differences for DNA contents in the fat body of 5th instar larvae and pupae of *Bombyx mori*

Mean differences between groups	Exposure durations (hrs)							
	24		48		72		96	
	Larvae	Pupae	Larvae	Pupae	Larvae	Pupae	Larvae	Pupae
X_1~X_2	0.001	0.004	0.004	0.014	0.006	0.009	0.009	0.013
X_1~X_3	0.008	0.013	0.010	0.018	0.012	0.023	0.019	0.029
X_1~X_4	0.018	0.022	0.021	0.026	0.024	0.020	0.030	0.036*
X_1~X_5	0.003	0.001	0.002	0.000	0.006	0.075*	0.013	0.011
X_2~X_3	0.007	0.009	0.006	0.011	0.006	0.014	0.010	0.016
X_2~X_4	0.017	0.018	0.017	0.019	0.018	0.021	0.021	0.023
X_2~X_5	0.002	0.003	0.006	0.007	0.012	0.016	0.022	0.024
X_3~X_4	0.010	0.009	0.011	0.008	0.012	0.007	0.011	0.007
X_3~X_5	0.005	0.012	0.012	0.018	0.018	0.030	0.032	0.040*
X_4~X_5	0.051	0.021	0.023	0.026	0.030	0.037*	0.047*	0.047*

*Significant group difference,

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

Where:

MS within : Mean square value within group

q : Studentized range statics

N : Number of replicates

$$\text{For Larvae} = 6.10\sqrt{\frac{0.0002}{6}}$$

$$= 0.035$$

$$\text{For Pupae} = 6.10\sqrt{\frac{0.0002}{6}}$$

$$= 0.035$$

Table 5: Effect of magnetic field on DNA content (μ mg^{-1} tissues) in the haemolymph of 5th instar larvae and pupae of *Bombyx mori*

Exposure duration (hrs)		Magnetic power (Gauss)					F_1-ratio $n_1 = 4$
		Control (X_1)	1000 (X_2)	2000 (X_3)	3000 (X_4)	4000 (X_5)	
24							
	Larvae	0.107±0.002	0.108±0.002	0.110±0.003	0.119±0.002	0.109±0.003	
	Pupae	0.095±0.002	0.096±0.001	0.106±0.002	0.114±0.002	0.096±0.001	
48							
	Larvae	0.107±0.002	0.110±0.003	0.116±0.006	0.123±0.004	0.103±0.004	5.65*
	Pupae	0.095±0.002	0.098±0.001	0.108±0.002	0.118±0.002	0.093±0.002	2.70**NS
72							
	Larvae	0.107±0.002	0.113±0.004	0.118±0.008	0.126±0.008	0.095±0.001	
	Pupae	0.095±0.002	0.105±0.002	0.110±0.004	0.124±0.006	0.090±0.001	
96							
	Larvae	0.107±0.002	0.115±0.004	0.117±0.004	0.130±0.005	0.091±0.002	
	Pupae	0.095±0.002	0.101±0.003	0.113±0.001	0.139±0.002	0.083±0.001	

*Larvae, **Pupae F_2-ratio: 3.35*, 2.59. **$^{NS}n_2 = 3$. *p_1<0.01, *p_2<0.05, NS: Non-significant. Each value represents Mean±SE of six replicates. X_1, X_2, X_3, X_4 and X_5 are mean values of DNA contents in the control, 1000, 2000, 3000 and 4000 Gauss magnetic fields, respectively

exposure duration up to 96 hrs caused gradually decline in the DNA content in the fat body of the 5th instar larvae and pupae. The two-way ANOVA test shows (Table 3) that variation in the strength of the magnetic field and the exposure duration of eggs significantly (p_1<0.01 and p_2<0.01) influenced to the DNA content in the fat body of fifth instar larvae and pupae of *Bombyx mori*. The *post-hoc* test shows (Table 4) that no significant group difference was present for the DNA content in the fat body for 24, 48 and 72 hrs exposed eggs in larvae and 24 and 48 hrs exposed eggs in pupae with all strength of magnetic field. The significant group differences were present between the control and 4000 Gauss (0.075 μ mg^{-1}) and 3000 and 4000 Gauss (0.037 μ mg^{-1}) magnetic field for 72 hrs exposure in pupae whereas, the differences were significant between 3000 and 4000 Gauss (0.047 μ mg^{-1}) magnetic field in larvae and between the control and 3000 Gauss (0.036 μ mg^{-1}), 2000 and 4000 Gauss (0.040 μ mg^{-1}) and 3000 and 4000 Gauss (0.047 μ mg^{-1}) magnetic field in pupae for 96 hrs exposure of eggs.

DNA content in the haemolymph of the fifth instar larvae and pupae: The data given in Table 5 indicates that variation

Table 6: Post hoc test showed group differences for the DNA contents in the haemolymph of 5th instar larvae and pupae of *Bombyx mori*

Mean differences between groups	Exposure durations (hrs)							
	24		48		72		96	
	Larvae	Pupae	Larvae	Pupae	Larvae	Pupae	Larvae	Pupae
X_1~X_2	0.001	0.001	0.003	0.003	0.006	0.010	0.008	0.006
X_1~X_3	0.003	0.011	0.009	0.013	0.001	0.015	0.010	0.018
X_1~X_4	0.012	0.019	0.016	0.023	0.019	0.029	0.023	0.044*
X_1~X_5	0.002	0.001	0.004	0.002	0.012	0.005	0.016	0.012
X_2~X_3	0.002	0.010	0.006	0.010	0.005	0.005	0.002	0.012
X_2~X_4	0.001	0.018	0.013	0.020	0.013	0.019	0.015	0.038*
X_2~X_5	0.001	0.000	0.007	0.005	0.018	0.015	0.024	0.018
X_3~X_4	0.009	0.020	0.007	0.010	0.008	0.014	0.013	0.026
X_3~X_5	0.001	0.010	0.013	0.015	0.023	0.020	0.026	0.030
X_4~X_5	0.010	0.018	0.020	0.025	0.031	0.034	0.039*	0.056*

*Significant group difference,

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

$$\text{For Larvae} = 6.10\sqrt{\frac{0.0002}{6}}$$

$$= 0.035$$

$$\text{For Pupae} = 6.10\sqrt{\frac{0.0002}{6}}$$

$$= 0.035$$

Where:

MS within : Mean square value within group

q : Studentized range statics

N : Number of replicates

in the strength of static magnetic field and exposure duration of *Bombyx mori* eggs influenced to DNA contents in the haemolymph of the 5th instar larvae and pupae. The DNA content increased with an increase in exposure duration from 24 to 96 hrs and strength of the magnetic field from 1000-3000 Gauss. DNA content was maximum in the haemolymph of larvae (0.130±0.005 µg $mg^{-1}$of tissues) and pupae (0.139±0.002 µg mg^{-1} of tissues) developed from the eggs exposed for 96 hrs in 3000 Gauss magnetic field. In 4000 Gauss magnetic field, the DNA content increased slightly up to 24 hrs exposed eggs and a further increase in the exposure duration up to 96 hrs caused a gradually decline in DNA contents in the haemolymph of the 5th instar larvae and pupae. The two-way ANOVA shows (Table 5) that variation in the strength of magnetic field (p_1<0.01) and exposure duration (p_2<0.05) of eggs significantly influenced DNA contents in the haemolymph of the 5th instar larvae whereas, change in contents of DNA in the haemolymph of pupae was non-significant. The Post-hoc test shows (Table 6) that no significant difference of DNA was present in haemolymph of the fifth instar larvae and pupae for 24, 48 and 72 hrs exposed eggs with all the strength of magnetic field. The significant group difference for the DNA contents were present between the 3000 and 4000 Gauss (0.039 µ mg^{-1}) magnetic field in haemolymph of the 5th instar larvae and between the control and 3000 Gauss (0.044 µ mg^{-1}), 1000 and 3000 Gauss (0.038 µ mg^{-1}) and 3000 and 4000 Gauss (0.056 µ mg^{-1}) magnetic field of the haemolymph of pupae for 96 hrs exposed eggs.

DISCUSSION

The level of DNA content in the silk gland of *Bombyx mori* increased due to an increase in the exposure duration of eggs from 24-96 hrs in the 1000, 2000 and 3000 Gauss magnetic field and it is recorded to the maximum level in the case of 3000 Gauss 96 hrs magnetized eggs. In 4000 Gauss magnetic field, the DNA content is noticed to increase up to 24 hrs while further increase in the duration of exposure caused decline it in silk gland of the fifth instar larvae. The purified enzyme DNA polymerase activity of silk gland of *Bombyx mori* noticed most resembles the vertebrate DNA polymerases α when it compared to other eukaryotic DNA polymerases[20]. The ratio of DNA replication in the silk gland during the later period of the 5th instar larvae is probably a special feature in *Philosamia ricini* while it is the period of mainly fibroin biosynthesis in *Bombyx mori*[21,22]. The DNA production in the silk gland interrupted at each inter-molt period in

Bombyx mori[23] while interestingly, the DNA production in silk gland of *Bombyx mori* increased during the fifth instar larval growth[23,24]. The DNA content of the whole silk gland along with its wet continuously decreased during the process of spinning, till the gland is lost completely prior to pupation in *Bombyx mori*[25]. A gradual decrease in RNA:DNA ratio of the silk gland was noticed after an initial high level during middle part of 5th instar larvae[26]. The DNA synthesis in the silk gland of *Bombyx mori* stopped suddenly in the middle of the 5th instar larvae when cellular growth was over and the cell was turned on to the massive silk synthesis[22] and genomic DNA contents of silk gland cells of *Bombyx mori* dramatically increased thousand times for the larval life span through the process of endomitosis[27]. Magnetization of the eggs up to 3000 Gauss magnetic field increased protein content in the larvae and pupae but in 4000 Gauss magnetic field protein content declined in *Bombyx mori*[12]. In the present study the DNA content in silk gland is also increasing with increase in the strength of magnetic field up to 3000 Gauss and the duration of magnetic exposure for magnetization of the eggs up to 96 hrs. In the high magnetic field of 4000 Gauss, the DNA contents is increasing in the low exposure of 24 hrs but further increase in the exposure duration causes decline in DNA content in the silk gland.

The DNA contents in the fat body and haemolymph of the 5th instar larvae and pupae of *Bombyx mori* is noticed to increase in the 1000, 2000 and 3000 Gauss magnetized eggs up to 96 hrs exposure but in 4000 Gauss magnetic field, it is noticed to slightly increased in case of 24 hrs exposed eggs and further gradually declined with increase in duration of exposure. Protein level increased in fat body and haemolymph of 5th instar larvae of *Bombyx mori* due magnetization of larvae[28] whereas, changes in morphological, physiological and biochemical parameters are reported in *Drosophila* after an exposure in the magnetic field[29]. The magnetic field of 60 Hz, caused an increase in oxidation rate of cytochrome C in the cells and activation of enzyme corboxydimutase has been reported in magnetic field[30]. Magnetic exposure of eggs for 96 hrs and increase in strength of magnetic field up to 3000 Gauss caused increase in acid and alkaline phosphatase activities in the tissues of *Bombyx mori* but in 4000 Gauss magnetic field enzyme activities decreased with increase in exposure duration of eggs[14,13]. Present study also showed the increase in DNA contents in fat body and haemolymph of the 5th instar larvae and pupae with an increase in strength of magnetic field up to 3000 Gauss 96 hrs exposed eggs whereas, in the higher magnetic strength of 4000 Gauss, the DNA content is in declining trend with increasing in exposure duration of eggs of *Bombyx mori*.

CONCLUSION

It can be concluded that magnetization of eggs in 1000-3000 Gauss magnetic fields for 96 hrs increase DNA contents in the silk gland of 5th instar larvae and fat body and hemolymph of 5th instar larvae and pupae of *Bombyx mori*. The magnetization of eggs in 3000 Gauss with 96 hrs magnetic exposure is the most suitable for the elevation of DNA contents in the tissues of larvae and pupae. Since DNA is the master molecule of cell which directs protein synthesis in the tissues hence 96 hrs exposed eggs in 3000 Gauss magnetic field will increase DNA contents in the tissues which may be helpful for the greater synthesis of protein in the tissues and silk gland and this may be the cause of better growth and performance of larvae and pupae and production of quality raw silk at commercial scale.

SIGNIFICANCE STATEMENT

This study discovered that magnetization of eggs in low strength of magnetic field increases DNA contents in the tissues of larvae and pupae of *Bombyx mori* which can be beneficial for the sericulture industry. This study will help the researchers to uncover the critical areas of this field. Researchers were not able to explore that how exactly magnetic field influences to the biological system. Thus a new theory on bio-magnetism may be arrived.

REFERENCES

1. Rahmathulla, V.K., 2012. Management of climatic factors for successful silkworm (*Bombyx mori* L.) crop and higher silk production: A review. Psyche, Vol. 2012. 10.1155/2012/121234.
2. Mubashar, H., S.A. Khan, M. Naeem and M.F. Nasir, 2011. Effect of rearing temperature and humidity on fecundity and fertility of silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). Pak. J. Zool., 43: 979-985.
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*. Ind. J. Insect Physiol., 17: 743-760.
4. Adbel-Salam, K.A. and S.M. Mahmoud, 1995. Stimulating effects of low levels of 60Co gamma ray on the silkworm, *Bombyx mori* (L.). Anz. Schädlingsk. Pflanzen. Umweltschutz., 68: 147-150.

5. Chaugale, A.K. and N.K. More, 1993. Effect of magnetic energy on silkworm development and silk production. Ph.D. Thesis, Shivaji University, Kolhapur, India.
6. Anselmo, C.W.S.F., P.B. Pereira, M.T.J.A. Catanho and M.C. Medeiros, 2009. Effects of the electromagnetic field, 60 Hz, 3 μT, on the hormonal and metabolic regulation of undernourished pregnant rats. Braz. J. Biol., 69: 397-404.
7. Conely, C.C., W.J. Mills and A.G. Patricia, 1966. Enzyme activities in macrophages from animals exposed to a very low magnetic field. Proceedings of the 3rd International Biomagnetic Symposium, March 22-23, 1966, University of Illinois, Chicago, USA., pp: 13-15.
8. 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.
9. Raylman, R.R., A.C. Clavo and R.L. Wahl, 1996. Exposure to strong static magnetic field slows the growth of human cancer cells *in vitro*. Bioelectromagnetics, 17: 358-363.
10. Byers, J.M., K.F. Clark and G.C. Thompson, 1998. Effect of pulsed electromagnetic stimulation on the facial nerve regeneration. Arch. Otolaryngol. Head Neck Surg., 124: 383-389.
11. Darendetilar, M.A., A. Darendetilar and P.M. Sinclair, 1997. Effect of static magnetic and pulsed electromagnetic field on bone healing. Int. J. Adult Orthodontics Orthognathic Surg., 12: 43-53.
12. Shivpuje, M.A., V. Hanumant, S.N. Belpatre and V.B. Khyade, 2016. Influence of magnetic energy on protein contents in the fifth instar larvae of silkworm, *Bombyx mori* (L.)(Race: PM x CSR2). World Scient. News, 42: 73-86.
13. Tripathi, S.K., S.K. Shukla and V.B. Upadhyay, 2013. Acid phosphatase activity changes under magnetic exposure of eggs in *Bombyx mori*: A multivoltine mulberry silkworm. World J. Zool., 8 : 407-414.
14. Tripathi S.K. and S.K. Shukla, 2017. Alkaline phosphatase activity changes under magnetic exposure of eggs in *Bombyx mori*: A multivoltine mulberry silkworm. World J. Zool., 12: 34-41.
15. Elyamani, E.M.Y., 2020. Influence of magnetic field on some biological and biochemical aspects of silkworm, *Bombyx mori*. J. Plant Prot. Path., 11: 135-140.
16. Tripathi, S.K., 2012. Protein level changes under magnetic exposure of larvae in *Bombyx mori*: A multivoltine mulberry silkworm. Acad. J. Entomol., 5: 81-85.
17. Krishnaswami, S., M.N. Narasimhana, S.K. Suryanarayan and S. Kumaraj, 1973. Sericulture Manual-2, Silkworm Rearing. FAO., Rome, pp: 1-13.
18. Schneider, W.C., 1957. Determination of Nucleic Acids in Tissues by Pentose Analysis. In: Methods in Enzymology, Colowick, S.P. and N.O. Kaplan (Eds.). Vol. 3, Academic Press, New York, USA., pp: 680-684.
19. Allen, M., 2017. The SAGE Encyclopedia of Communication Research Methods. SAGE Publications Inc., Thousand Oaks .
20. de Turenne, M., 1978. Characterization of an α-like DNA polymerase from *Bombyx mori* silkglands. Biochimie, 60: 705-713.
21. Tashiro, Y., T. Morimoto, S. Matsumura and S. Nagata, 1968. Studies on the posterior silk gland of the silkworm, *Bombyx mori*. I. Growth of posterior silk gland cells and biosynthesis of fibroin during the fifth larval instar. J. Cell. Biol., 38: 574-588.
22. Brookes, V.J., 1966. Thymidine kinase and thymidylate kinase in the silkworm, *Antheraea pernyi*. Biochim. Biophys. Acta, Nucleic Acids Protein Synth., 119: 268-275.
23. Li, Y.F., X.Y. Chen, C.D. Zhang, X.F. Tang and L. Wang *et al*., 2016. Effects of starvation and hormones on DNA synthesis in silk gland cells of the silkworm, *Bombyx mori*. Insect Sci., 23: 569-578.
24. Morimoto, T., S. Matsuura, S. Nagata and Y. Tashiro, 1978. Studies on the posterior silk gland of the silkworm, *Bombyx mori*: III. Ultra structural changes of posterior silk gland cells in fourth larval instar. J. Cell Biol., 38: 604-614.
25. Matsuura, S., T. Morimoto, S. Nagata and Y. Tashiro, 1968. Studies on the posterior silk gland of the silkworm, *Bombyx mori*: II. Cytolytic process in posterior silk gland cells during metamorphosis from larva to pupa. J. Cell. Biol., 38: 589-603.
26. Wyatt, G.R. and B. Linzen, 1965. The metabolism of ribonucleic acid in cecropia silkmoth pupae in diapause, during development and after injury. Biochim. Biophys. Acta, Nucleic Acids Protein Synth., 103: 588-600.
27. Zhang, C.D., F.F. Li, X.Y. Chen, M.H. Huang and J. Zhang *et al*., 2012. DNA replication events during larval silk gland development in the silkworm, *Bombyx mori*. J. Insect Physiol., 58: 974-978.
28. Mulay, I.L. and L.N. Mulay, 1982. Magnetic Field Induced Mutations in *Drosophila melanogaster*. In: Biological Effect of Static Magnetic Fields: A Selective Review with Emphasis on Risk Assessment, Easterly, C.E. (Ed.)., Oak ridge National Laboratory, United States, pp: 38-40.
29. Blank, M. and L. Soo, 1998. Enhancement of cytochrome oxidase activity in 60 Hz magnetic fields. Bioelectrochem. Bioenerg., 45: 253-259.
30. Akoyunoglou, G., 1964. Effect of magnetic field on corboxydismutase. Nature, 202: 452-454.