

Impact of magnetization on the performance of imported hybrid silkworm seeds *Bombyx mori* L in Egypt

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ABSTRACT

Electromagnetic field is a new technology and friendly tool that can influence insect physiology, and behaviour. During the imported silkworm seeds transfer process, some problems, such as poor storage, can have a negative effect on the physiological state of the embryo. Also, climatic changes play an important role in larvae susceptibility to being easily infected. Therefore, it was necessary to study the impact of exposing silkworm seeds before rearing season to Electromagnetic field on the immunity and the productivity of the silkworm. The results of the investigation showed that, exposed silkworm seeds to different frequencies and impact time of application have a positive effect on all biological and biochemical characters under study but at a certain level. Data revealed that, silkworm seeds exposed to (3000 G) at 10 minutes give the high results in all biological and some biochemical characteristics related to cocoon production. However, the exposure to (2000 G) at two times (5, and 10 minutes) recorded higher activity of the immunity enzymes. Exposure to a high intensity may increase the free radicals and decrease the activity of the antioxidant enzymes that lead to the increase in the mortality therefore increasing loss production. Overall, the biological systems, especially in living organisms, react differently to various magnetic fields. Different magnetic fields can stimulate or inhibit immunity enzymes in specific ways. So, we need to make a lot of studies on this technology to the point makes a balance between the immunity and the production.

Keywords: Silkworm, magnetic field, biological parameters, biochemical characters.

INTRODUCTION

Silk has an importance worldwide. It is called the (Queen) because it is an elegant, lightweight, and strong fibre with a natural shine and high dye absorbent (Tambe *et al.*, 2021). Sericulture's potential as a significant income source and job creator depends on the performance of the different stages of the *Bombyx mori* (L) life cycle. Sericulture's potential as a significant income source and job creator depends on the performance of the different stages of the *B. mori* life cycle (Rahmathulla, 2012). Recently, Egypt has given attention to this agro-industry, therefore it became interested in importing silkworm seeds hybrids for to have silk production. The imported silkworm seeds may be subjected to infection during transferring, as a result of poor storage, delay in receiving the shipment also, during the feeding period as a result of environmental conditions changes that are not favourable for it. For the larvae of mulberry silkworm feed only on mulberry leaves, mulberry variety is also a very important factor affecting silk quality (Moustafa, 2024). Therefore, researchers always looking for any tool to reduce infection expectation through different treatments as adding probiotics, proteins, amino acids, botanical extracts, photoperiods, and x-rays (Snehal and Alka, 2022).

One of the newest techniques for enhancing silkworm immunity and productivity is subjecting mulberry silkworm seeds to magnetic energy. Magnetization of silkworm eggs could lead to an advancement in biotechnological research to enhance *B. mori* economic traits resulting in increased cocoon production and improved silk quality (Upadhyay *et al.*, 2007). Exposures to the (MF) can increase the red blood cell count in the human body. It also plays a significant role in managing diseases and disabilities in humans as suggested by (Rathi, 2003). Also, The MF regulates biological functions, primarily by controlling the inflammatory process, and governing cell differentiation and gene expression (Saito *et al.*, 1999; Kulbacka *et al.*, 2009). It has the potential to influence the formation of new tissue in living organisms, indicating that it could be utilized as a tool to regulate mitotic activity (Elżbieta *et al.*, 2024). Creatures exposed to magnetic field have changes in physiological, biochemical, and morphological states (Tambe *et al.*, 2021). Silkworms have biological, and physiological changes when exposed to magnetic field (Chougale and More, 1993; Chougale *et al.*, 1995; Sonar *et al.*, 2021), increasing silk protein (Chougale, 1992), have an impact on silkworm behaviour and acid phosphatase (Kumar, 2012). The incubation period is affected by magnetization (Tripathy and Upadhyay, 2005). Enzymes activated by exposing animals to low frequencies of magnetic field (Zmy'slony *et al.*, 1998). Magnetic

energy can increase the amount of silk in the cocoon. Which might happen because it could raise the DNA content in the silk gland tissues. This might help make more silk protein (Jagtab and Khyade, 2021).

The present investigation aims to examine the impact of exposing mulberry silkworm seeds to a magnetic field, with the goal of increasing silk output and enhancing immunity. All researchers make an effort to increase silk production or even keep healthy larvae by adding different additives to mulberry leaves as botanical extract, probiotics, spraying proteins amino acids,....etc. However, in the present study has a different point of view by increasing diseased resistance and silk production through using new technology.

MATERIALS AND METHODS

The experiment was conducted during the spring 2024 at the Sericulture Research Department, Plant Protection Research Institute and Biotechnology Laboratory, Regional Centre of Food and Feed, Agricultural Research Centre, Ministry of Agriculture. The experiment was conducted by the Lake shore Vibrating Sample Magnetometer software apparatus.



Fig. 1. Vibrating Sample Magnetometer apparatus

A-Experimental design:

A bivoltine Silkworm seeds FC₁X FC₂ were imported from India. Seeds were divided into two groups. (A) represents the control group of about 1000 eggs, (B) represents the treatment group that is divided into 8 sub-groups each of 3 replicates containing 100 eggs as follow:

- T₁: Seeds exposed to 1000 Gauss at 5 minutes.
- T₂: Seeds exposed to 1000 Gauss at 10 minutes.
- T₃: Seeds exposed to 2000 Gauss at 5 minutes.
- T₄: Seeds exposed to 2000 Gauss at 10 minutes.
- T₅: Seeds exposed to 3000 Gauss at 5 minutes.
- T₆: Seeds exposed to 3000 Gauss at 10 minutes.
- T₇: Seeds exposed to 4000 Gauss at 5 minutes.
- T₈: Seeds exposed to 4000 Gauss at 10 minutes.

All groups were incubated in the incubator at 27°C till hatching. Rearing was carried out under hygienic conditions according to (Moustafa, 2020). Newly hatched silkworms reared in the spring season at 29- 32 °C and relative humidity 80-90% RH. All larvae were allowed to develop as normal up to spinning. Mulberry leaves cleaned every day then let to dry 10 minutes before feeding. Silkworm larvae fed 4 times every day till spinning. The following criteria were estimated.

A- Biological Characters:

The study involved larval weight at the end of (3rd, 4th, and 5th) larval instar, hatchability %, larval duration, survival rate at the end of (3rd, 4th, and 5th) according to (Rahmathulla *et al.* (2007) and overall growth rate for 4th, and 5th larval instar, Incubation period according to (Moustafa, 2024).

$$\text{Overall Growth Rate} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

$$\text{Survival Rate} = \frac{\text{Number of living larvae}}{\text{Total number of larvae}} \times 100$$

B- Biochemical Analysis:

Hemolymph was collected from treated larvae at the 6th day of the 5th larval instar by removal of the thoracic leg in 1.5 ml Eppendorf tubes with a small amount of phenyl thiourea crystal (PTU) as an anti-

coagulant substance, (Mahmoud, 1988). The tubes were kept at -20°C , and the hemolymph samples were centrifuged at 10000 rpm for 10 minutes at 5°C . The supernatant was collected and assayed for determining different parameters as follows:

Total protein: Total protein was determined using the method of (Bradford, 1976)

Protease Enzyme: proteolytic enzyme was determined according to (Tatchell *et al.*, 1972) with some modification of measuring the increasing of amino acid according to (Lee and Takabashi, 1966).

Peroxidase Activity: was determined according to the procedure given by (Hammerschmidt *et al.*, 1982).

Phenol oxidase: was determined according to a modification of (Ishaaya, 1971).

Esterase Enzyme: Alpha Esterase (α -esterases) and beta Esterase (β -esterases) were determined according to (Van Asperen, 1962) using α -naphthyl acetate or β -naphthyl acetate as substrates, respectively.

C- Data analysis:

The values of the measured parameters were presented as Mean \pm SE. One-way analysis of variance (ANOVA) was conducted using SAS software (SAS/STAT[®] 9.1, 2004). The Least Significant Difference (LSD) test was performed at a significant level of $p \leq 0.05$ within the same program.

RESULTS

A- Biological characters:

It was obvious that, from the data pooled in (Table, 1), silkworm seeds exposed to magnetic fields affect significantly all biological parameters. Larval weight increased about 28.8% over control at the end of 3rd instar, the maximum weight was (0.161 g.) in response to 2000 G at 10 minutes at ($p < 0.0001$). Similarly results at the end of the 4th instar the exposure to 2000 (5 and 10 m.) and 3000 G (5 m.) resulted in increasing larval weight on par significantly (0.644 g., 0.636 g., and 0.668 g.). While larval weight enhanced at the end of the 5th instar significantly (2.44 g.) at ($p < 0.0001$) by the exposure time (10 minutes) to 3000 G. Regarding the overall growth rate, exposure to 3000 G at 10 minutes increased significantly at the 4th and the 5th by about 20% over control at ($p < 0.0001$) recorded (383% and 268 %), respectively. Interestingly, the growth rate at the end of the 4th decreased in (T₁, T₂, and T₄) compared to control. However, it increased at all treatments at the end of the 5th larval instar and recorded (201, 191, 255, 198, and 237%) for (T₁, T₂, T₃, T₄, and T₅), respectively.

Table 1. The effect of magnetization on some biological parameters of mulberry silkworm *B. mori*

Treatment Parameters	3 rd larval weight (g.) \pm SE	4 th larval weight (g.) \pm SE	5 th larval weight (g.) \pm SE	4 th overall growth rate (%) \pm SE	5 th overall growth rate (%) \pm SE
T1	0.137 \pm 0.004 b	0.546 \pm 0.011 c	1.63 \pm 0.022e	301 \pm 0.12 c	201.086 \pm c
T2	0.131 \pm 0.003 bc	0.614 \pm 0.017b	1.72 \pm 0.023d	360 \pm 0.10 ab	191 \pm 0.11 c
T3	0.132 \pm 0.004 bc	0.500 \pm 0.009 d	1.80 \pm 0.014 cd	281 \pm 0.14 c	255 \pm 0.09 ab
T4	0.161 \pm 0.003 a	0.644 \pm 0.012 ab	1.88 \pm 0.045 c	302 \pm 0.13 c	198 \pm 0.11 c
T5	0.141 \pm 0.004 b	0.636 \pm 0.010 ab	2.13 \pm 0.021b	359 \pm 0.17 ab	237 \pm 0.09 b
T6	0.139 \pm 0.004 b	0.668 \pm 0.010 a	2.44 \pm 0.030 a	383 \pm 0.16 a	268 \pm 0.09 a
T7	-	-	-	-	-
T8	-	-	-	-	-
C	0.125 \pm 0.004 c	0.514 \pm 0.009 d	1.37 \pm 0.054 f	318 \pm 0.23 bc	161 \pm 0.15 d
LSD	0.011	0.032	0.093	0.446	0.249

*SR: Survival Rate %.

The precise time of hatching was calculated from the time that silkworm seeds were exposed to magnetic field till the time of larval hatched out. Table (2) revealed that no effect of magnetization on the time of hatching (incubation period) in all treatments except (T7 and T8) larvae hatched out after 168 hrs. from the exposure date. However, in all treatment's the hatchability was recorded 100% on par without significance. Undoubtedly all magnetic fields had strength effects on larval duration, T6 decreased significantly with mean duration (624hrs.) followed by T4 (at 645.6hrs.). Whereas the other treatments had equal times (648hrs.), control was the longest value ever (696hrs.).

Survival Rate % is a very important evaluated criterion. Using magnetic field indicated that the untreated group (control) recorded significantly the lowest value of survival rate at ($p < 0.0001$) during the 3rd, 4th, and 5th larval instars significantly, that recorded (67.73, 50.86, and 55.15%), respectively. Otherwise, all treatments were positively affected in response to the magnetic intensities that increased in (T2, and T3) significantly at the end of 3rd larval instar (96.60 %).

Whereas, at the end of 4th and 5th larval instars (T₂, T₃, T₄, T₅, and T₆) recorded the highest par values that were (94.23, 91.02, 89.94, and 89.50%) , (75.09, 75.91, 73.50, 88.64, and 89.01%), respectively.

B- Biochemical Analysis:

The change in the static magnetic field and exposure time of mulberry silkworm seeds caused positive variations in all enzymes under study. Total protein increased significantly, in response to 3000 G at 10 minutes (T₆) that was (50.80 mg/ml) at (p<0.0001), whereas, decreased significantly, by decreasing the strength of the magnetic field, also, and recorded (45.50, 44.00, 43.13, 39.60 36.30 mg/ml) for (T₅, T₄, T₃, T₂, and T₁), respectively. The lowest value significantly, was determined for the control 36.00 mg/ml.

Exposure to the magnetic field at different times affects positively the protease enzyme, which increased gradually by increasing the strength and the time of exposure as shown in Table (3). It significantly recorded 192.33, 192.00, 241.66, 244.33, 245.00, and 259.00 ug alanine/min/ ml for (T₁, T₂, T₃, T₄, T₅, and T₆) respectively at p<0.0001. The control was the lowest significant value 203.66 ug alanine/min/ ml.

On the other hand, immunity enzymes (peroxidase, phenol oxidase, and Esterase) took another direction. T₃ and T₄ were significantly the highest results in the previous enzymes, recorded (4.23, and 4.00 Δ O.D./min/ ml), (8.36, and 8.26 O.D. units/min/ ml), and (366.66, and 364.33 ug α- naphthol/min/ ml), respectively in response to 2000 G at 5, and 10 minutes exposure time at (p<0.0001). The lowest values were determined for T₅ and T₆ (2.94, and 2.82 O.D./min/ ml), (6.71, and 5.94 O.D. units/min/ ml), and (309.00, and 243.33 ug α- naphthol/min/ ml), respectively Table (3).

Table 2. The Effect of magnetization on some biological parameters of mulberry silkworm larvae *B. mori*.

Treatments parameters	3 rd S.R%± SE	4 th S.R%± SE	5 th S.R%± SE	Larval Duration (hrs)	Incubation period (hrs)	Hatchability %± SE
T ₁	81.06±2.23 c	84.96±2.5 c	72.7±3.69 b	654 b	144 b	100±0 a
T ₂	96.60±0a	94.23±1.28 a	75.09±3.58 ab	654b	144 b	100±0a
T ₃	96.60±0a	91.02±1.28 ab	75.91±2.77 ab	654 b	144 b	100±0a
T ₄	88.86±1.13 b	89.94±1.28 ab	73.50±2.02 ab	633 c	144 b	100±0a
T ₅	88.60±0 b	89.50±1.99abc	88.64±4.19 a	654b	144 b	100±0a
T ₆	88.60±0 b	87.17±1.13 bc	89.01±2.14 a	624d	144 b	100±0a
T ₇	-	-	-	-	168 a	100±0a
T ₈	-	-	-	-	168 a	100±0a
C	67.73±2.94 d	50.86±0.86 d	55.15±7.50 c	696a	144 b	100±0a
LSD	4.429	4.757	13.20	ns	ns	ns

Table 3. The effect of magnetization on some enzymatic activity of silkworm *B. mori* L.

Treatment Parameters	Total Protein (mg/ml)	Protease (ug alanine/min/ml)	Peroxidase (Δ O.D./min/ml)	Phenol oxidase (O.D. units / min / ml)	Esterase (ugα-naphthol/ min / ml)
T ₁	36.30±0.317 cd	192.33±2.43 d	3.28±0.05 bc	6.69±0.115 c	338.33±4.74 cd
T ₂	39.60±0.592 c	192±1.66 d	3.54±0.07 b	7.58±0.386 b	351±2.43 bc
T ₃	43.13±0.763 b	241.66±1.15 b	4.23±0.10 a	8.36±0.233 a	366.66±2.82 a
T ₄	44±0.568 b	244.33±1.28 b	4±0.04 a	8.26±0.215 a	364.33±2.95 ba
T ₅	45.5±0.723b	245±2.69 b	2.94±0.03 d	6.71±0.055 c	309±2.56 e
T ₆	50.8±0.80 a	259±1.79 a	2.823±0.02 d	5.94±0.086 d	243.33±3.84 f
T ₇	-	-	-	-	-
T ₈	-	-	-	-	-
C	36±2.51 d	203.66±2.95 c	3.21±0.05 c	6.25±0.132 cd	328.66±3.33 d
LSD	3.41	8.82	0.263	0.619	14.02

DISCUSSION

Electromagnetism is undeniably one of the key pillars of physics, driving extensive research in the fields of physics, material science, and engineering. The impact of magnetism on everyday life has spurred significant interest and ongoing exploration. Magnetic field Utilization is an intriguing avenue for enhancing the production and the immunity of silkworm, *B. mori* by exposing silkworm seeds to certain strengths of magnetic fields.

A- Biological Characters:

It was clear from the previous results that, the change in the magnetic field at a certain level strength and exposure time affects positively all biological parameters but at a certain level. That increased significantly at 10 minutes of 3000 Gauss and was inhibited by exposure up to 3000 Gauss. These results go in line with (Surendra, 2007) who referred that treated eggs with stronger magnetic field (4000 Gauss) may reduce silk and shell quality by affecting cytochrome activity. Furthermore, (Prasad and Upadhyay, 2011) confirmed that raising the cocoon exposure time from 24 to 96 hours in a 1000, 2000, and 3000 Gauss magnetic field reduces larval duration, and increases larval weight and survival rate while decreasing at (4000 Gauss). Moreover, exposure to a magnetic field led to increased larval weight due to enhanced metabolic activities and higher food consumption (Shivpuje *et al.*, 2016). Also, Elyamani (2020) illustrated exposure time to the magnetism plays an impact role on larval weight. The rapid growth of insects and plants that were exposed to stimulators such as ultraviolet radiation, magnetic and electromagnetic fields, microwaves, ultrasound, and low-dose irradiation (Kim *et al.*, 2015; Zhikrevetskaya *et al.*, 2015; Araujo *et al.*, 2016). However, (Taha, 2018) reported that silkworms gained heavy weight at (5000 Gauss).

The current research indicated varying duration of incubation period to hatching in the bivoltine mulberry silkworm resulted from a biotic stress (high dose of magnetic field). Duration of up to six days was noted in the structures T₇ and T₈. Our results agree with the findings of (Lertsatitthanakorn *et al.*, 2006; Sharma and Kalita, 2017). The development of embryos in silkworm eggs achieves uniformity and avoids mixed age characteristics in balanced environmental conditions. Also, (Tripathy and Upadhyay, 2005) showed that silkworm seeds exposed to 4000 G negatively on incubation period.

Seeds coded T₂, T₃, T₄, T₅, and T₆ exposed to the magnetic field showed high survival rates, and structures which showed a positive correlation but while, it was poor in T₁ and control. The reduction in the larval period of silkworms observed in T₆, and T₄ conformed to the findings of earlier workers (Chougale and More, 1992). Also, (Qadri *et al.*, 2006) reported that both the feeding and moulting periods were concomitantly reduced without any adverse effect on the larval growth.

B- Biochemical Analysis.

Undoubtedly, the magnetic field and the time of application have a pronounced effect on all physiological processes in the larvae as the effect decreased by increasing the strength. The haemolymph total protein increased significantly at (3000G) by exposure to 10 minutes. Santosh (2012) supported our results, all enzymatic activities especially total protein increased at (3000 G) but high strength caused a stress response. Whereas process of magnetized eggs significantly impacts the amino acid composition within the haemolymph of *B. mori* larvae as suggested by (Tripathy *et al.*, 2012).

Also, (Prudhomme *et al.*, 1985) referred that at 10 days of embryonic life, the silk gland initiates the synthesis of silk protein, which continues until the start of the spinning process.

As pooled previously protease enzymes have the same direction of the total protein that increased significantly by exposure to (3000 G) strength of magnetic field at 10 min. Our results are in line with (Shivpuje *et al.*, 2016) who confirmed that the exposure to the magnetization has the potential to increase levels of amino acids, subsequently resulting in an accelerated rate of protein synthesis in the 5th instar larvae of silkworm. Efficient utilization of magnetic energy supports the growth of silkworm larval instars, (Tambe *et al.*, 2021) which aligns with the study's findings.

On the other hand, our study showed a fluctuation in the strategy of immunity and antioxidant enzyme defence. The chart showed greater variability in the activity of Peroxidase, Phenoloxidase, and Esterase enzymes responding to different strengths of the static magnetic field and apply time. Silkworm seeds exposed to low static magnetic field (2000 G) increased the immunity and antioxidant enzyme under studied. These results coincide with (Arafat *et al.*, 2020) who observed an increase in antioxidant enzyme activity in response to the static magnetic field (SMF). However, (Todorovic *et al.*, 2012) reported that invertebrates are more sensitive to electromagnetic fields during embryonic development, especially at low magnetic field frequencies. Rearing silkworms in a 12-hour magnetic field increased antioxidant enzymes levels more than in 24, 6, 3, or 0-hour exposures (Tambe and Aherkar, 2021)

CONCLUSION

Studying magnetic field's environmental effects is increasingly important. The productivity and profitability of sericulture depend on healthy larvae. Many potential interactions between magnetic field and biological systems have been studied. It is important to expose silkworm seeds to magnetic field to stimulate the immunity system, increasing the activity of antioxidant enzymes and increasing silk productivity especially that imported from different countries because of being exposed to certain conditions during the transportation

process or any environmental conditions that not suitable for rearing. Magnetic field offering a new biotechnological approach for higher-quality cocoon production.

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