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### Influence of Magnetic Field on some Biological And Biochemical Aspects of Silkworm, *Bombyx mori*

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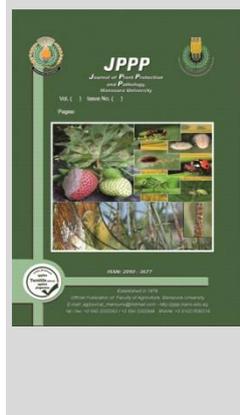


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#### ABSTRACT

The application of 180 milli-tesla (mT) on larvae of silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) was investigated to assess the effect of its different exposure periods; 1, 2, 4 and 8 minutes on some biological traits and haemolymph biochemical constituents. The magnetization each of the third and the fourth larval instar was carried out once on the first day before first feeding. Exposing silkworm larvae during each of the third or the fourth instars to the tested magnetic field for eight minutes increased mature larval weight followed by four minutes magnetization during both instars. Control group and the larvae exposed to the magnetic field for eight minutes manifest the highest cocoon shell weight of the resulting cocoons. Silk ratios were significantly higher in two minutes exposure to the magnetic field during the fourth larval instar followed by one minute exposure during the third larval instar. Variation in the exposure periods of the magnetic field remarkably influenced the biochemical component of the haemolymph of mature silkworm larvae. Stimulatingly, Alanine amino transferase activity, total protein content and Amylase activity were highest in haemolymph of the larvae exposed to the tested magnetic field during the third or the fourth larval instar for eight minutes, while Aspartate amino transferase activity and total lipid content were found to be the highest in the haemolymph samples taken from larvae exposed to the same magnetic field during the third larval instar for four and eight minutes, respectively.

**Keywords:** Silkworm, *Bombyx mori*, magnetization, 180 milli-tesla, biological and biochemical aspects



#### INTRODUCTION

Sericulture plays an important role in the alteration of the rural economy as it declares wide choice in term of regular employment and provides yield round the year. The potential of sericulture as an important source of expenditure and generating more job opportunities is absolutely based upon the protein synthesis inside haemolymph, silk gland and other tissues of *Bombyx mori* Tripathi (2012). Efforts have been made to examine the more equipment that is effective, toil saving and eco-friendly in order to increase the production of noble silk. Magnetism is a physical implement which has some biological influence on any living organism. Prasad and Upadhyay (2011) said that, the bio-magnetic fields, when applied successfully sounds useful for promoting up the sericulture industry as well as the economy of silkworm rearing.

The effect of magnetism on biological system has been the subject of worldwide interest. Magnetic field influences the morphological, physiological and biochemical characters of biological systems (Patnev and Mankova, 1986). The exposure of *B. mori* larvae in the magnetic field at various times increased in the weight of cocoon (Chaugale and More, 1992). Magnetic field also affects larval behaviors of silkworm (Chaugale, 1993), increases the protein metabolism and utilization of mulberry leaves (Alexander and Ganeshan, 1990). The magnetization of silkworm, eggs, larvae and cocoons influences incubation period, the performance of silkworm and silk producing potential (Tripathi and Upadhyay, 2005), (Upadhyay, and Tripathi, 2006) and (Upadhyay,

and Prasad, 2010). Applying the magnetic field on *Bombyx mori* cocoon proved to be of biotechnological importance in the sericulture manufacturing (Prasad and Upadhyay, 2014). Magnetic flux gave a positive response in egg hatchability, larval duration, larval, pupal and cocoon weights, as well as, gradual improvement in the effective rate of rearing, cocooning and pupation percentages (Taha 2018). Magnetization causes an increase in the amino acid level which followed by an acceleration of protein rate synthesis in the tissues of 5<sup>th</sup> larval instar of silkworm, *Bombyx mori* (Madhuri *et al.*, 2016).

#### Aim of work

This study was carried out to investigate the effect of the magnetic field with power 180 milli-tesla on some biological and biochemical aspects of silkworm, *B. mori*.

#### MATERIALS AND METHODS

##### Materials:

- 1- Silkworm eggs of the commercial Bulgarian hybrid (H1\*KK\*G2\*V2) were obtained from Sericulture Research Department of Plant Protection Research Institute, Agricultural Research Center (ARC).
- 2- Mulberry leaves (Balady variety) were used.
- 3- A magnetic device with the power equal 180 milli-tesla (mT) which designed by the technical help of faculty of engineering, Menoufia university was used.
- 4- The kits of biomed- EGY-CHEM for lab technology (Badr city, Egypt) were used to measure the total proteins, glucose levels and the activities of transaminase enzymes.

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5 - The kits of Bio-diagnostic Co. Egypt with CAT. No. TL 20 10 and AY 10 50 were used to measure the total lipids and amylase enzyme activity.

**Method:**

The present studies were carried out during the spring season of 2019 in the laboratory of Sharkia Branch of Sericulture Research Department, Plant Protection Research Institute (PPRI), Agriculture Research Center (ARC), Egypt.

Rearing procedures were achieved under laboratory conditions of  $25 \pm 2^\circ\text{C}$  and  $70 \pm 5\%$  RH according to (Krishnaswami 1978) methods. The larvae reared in plastic trays ( $40 \times 30 \times 7$  cm) and fed four times\ day as recommended on the local mulberry leaves, *Monus alba* (Balady variety).The mulberry leaves were collected twice daily i.e. at 8 am and 4 pm, then washed and left to dry in the room temperature from hatching to cocooning. Small larval instars provided with Fresh chipped clean mulberry leaves until the third larval instar, while the larvae of the fourth and the fifth instar were fed the whole clean mulberry leaves. Cleaning nets were used to remove the remaining dried mulberry leaves and faces. Each of the third and fourth instar larvae were exposed separately to the magnetic field by fixing them inside a glass test tube (Diameter = 1.5 cm) between the two magnetic poles of the magnetic device.

**Experimental design:**

The tested larvae exposed to the magnetic field power 180 ml.t. as follow:-

**T1:** 50 individual from the third larval instar for 1 minute.

**T2:** 50 individual from the third larval instar for 2 minute.

**T3:** 50 individual from the third larval instar for 4 minute.

**T4:** 50 individual from the third larval instar for 8 minute.

**T5:** 50 individual from the third larval instar without exposure as control.

**T6:** 50 individual from the fourth larval instar for 1 minute.

**T7:** 50 individual from the fourth larval instar for 1 minute.

**T8:** 50 individual from the fourth larval instar for 1 minute.

**T9:** 50 individual from the fourth larval instar for 1 minute.

**T10:** 50 individual from the fourth larval instar without exposure as control

**Biological aspects:**

Larval weight at the end of the fifth larval instar, pupal weight, fresh cocoons weight (g.), weight of cocoon shells (g.) and silk content ratios (%) were measured. Silk content ratio (%) was calculated according to Tanaka (1964) formula:

$$\text{Silk content ratio (\%)} = \frac{\text{Weight of cocoon shell} \times 100}{\text{Weight of fresh cocoon}}$$

**Biochemical parameters:**

At the end of the fifth larval instar the haemolymph were collected in micro-tubes by removing one of the abdominal legs of the larvae and bending the body to expose the sternum at the position of the leg removed. 2 ml haemolymph were taken from each treatment for biochemical studies of silkworm *B. mori* haemolymph. 0.01 mg phenylthiourea added immediately inside the collecting tubes to prevent melanization. Samples were centrifuged at 14,000 rpm for 10 min. The supernatant was removed and kept in  $-20^\circ\text{C}$  for analysis.

Total proteins, glucose level and the activities of Alanine amino transferase (ALT) and Aspartate amino

transferase (AST) were measured according the methods of Yatzidis, (1987), Vassault, *et al.*, (1986), Tietz, (1976) and Henry, (1964), respectively. Meanwhile, total lipids and the activity of amylase enzyme were measured according to the methods of Zöllner and Kirsch, (1962) and Caraway, (1959), respectively.

**Statistical analysis:**

Statistical analysis was performed using analysis of variance (ANOVA); means were compared using Duncan's test ( $\leq 0.05$ ) according to Snedecor and Cochran (1982) using Costat V.6.311 (2005) Software.

## RESULTS AND DISCUSSION

**Biological characters**

The obtained data of larval weight, pupal weight, cocoon weight, cocoon shell weight and silk ratio were recorded in Table 1 and illustrated graphically by figures 1, 2, 3, 4, and 5, respectively.

**Larval weight:**

Data obtained cleared that, there were significant differences between larval weight means when silkworm larvae exposed to the magnetic field 180 ml.t. Exposure silkworm larvae during the third larval instar for eight or four minutes increased mature larval weight to 3.676 and 3.354 g followed by 3.298 and 3.216 g for two and one minutes compared to 3.008 g for control.

The exposure during the fourth larval instar for eight minutes increased mature larval weight to 3.474 g followed by 3.366 and 3.246 g for four and two minutes magnetization as compared to 3.008 g for control (Table 1 and Fig 1).

**Pupal weight:**

Concerning pupal weight, the difference between means was non-significant in all exposure periods and larval instars (Table 1 and Fig 2).

**Fresh cocoon weight:**

No significant differences were noticed of cocoon weights among all exposure periods and larval instars (Table 1 and Fig 3).

**Cocoon shell weight:**

As shown in Table (1) and Fig (4) the differences between cocoon shell weight means were significant. Non exposed group (control) gave the highest cocoon shell weight 0.244 g followed by 0.238 g for cocoon shell which obtained from larvae exposed to the magnetic field 180 ml.t once for eight min. during the third larval instar.

**Silk ratio:**

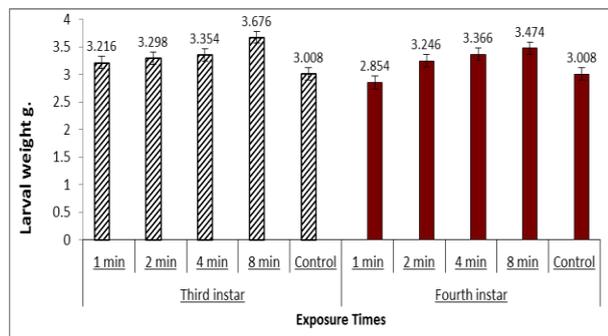
Obtained data in Table (1) and Fig (5) cleared that the exposer of silkworm, *B. mori* to magnetic field 180 ml.t for two minutes during the fourth larval instar increased the silk ratio to 19.838 % compared to 19.306 % of untreated control group.

The silk ratio for cocoons obtained from the larvae exposed to the same magnetic field for one minute during the third larval instar increased the silk ratio to 19.674 % as compared to 19.306 % for the control group. Cocoons obtained from larvae exposed to the power 180 ml.t for 4 min. during the third larval instar gave the least silk ratio recording 16.338 %.

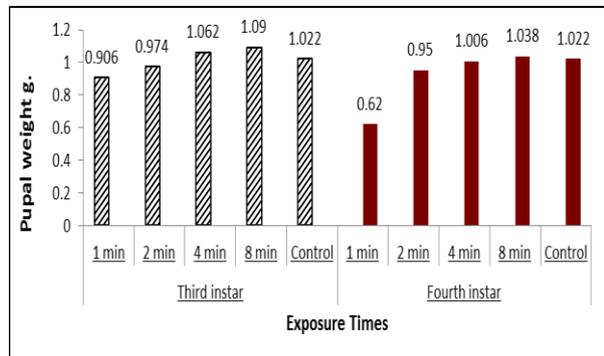
**Table 1. The effect of the exposure the third larval instar or the fourth larval instar of silkworm *B. mori* to the magnetic field on larval, pupal, cocoon, cocoon shell weights and silk ratio.**

Treatment instar	Exposing period (min.)	Larval weight (g)	Pupal Weight (g)	Cocoon weight (g)	Cocoon shell weight (g)	Silk ratio (%)
Third instar	1 min.	3.216	0.906	1.136	0.222	19.674
	2 min.	3.298	0.974	1.184	0.214	18.126
	4 min.	3.354	1.062	1.274	0.206	16.338
	8 min.	3.676	1.090	1.34	0.238	17.808
	Control	3.008	1.022	1.278	0.244	19.306
Fourth instar	1 min.	2.854	0.962	1.182	0.220	18.878
	2 min.	3.246	0.950	1.180	0.230	19.838
	4 min.	3.366	1.006	1.236	0.220	19.306
	8 min.	3.474	1.038	1.276	0.232	18.294
Control	3.008	1.022	1.278	0.244	19.306	
LSD <sub>0.05</sub>		0.3 07	ns	ns	0.019	1.60
P <sub>≥0.05</sub>		0.0132*	0.678	0.2349	0.0208*	0.0033**

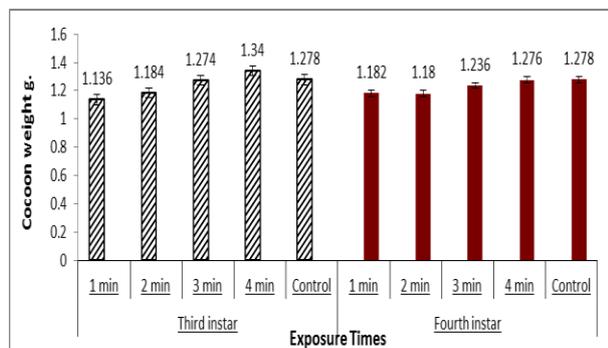
ns, \*, \*\* denote not significant, significant and highly significant differences 0.001 levels of probability



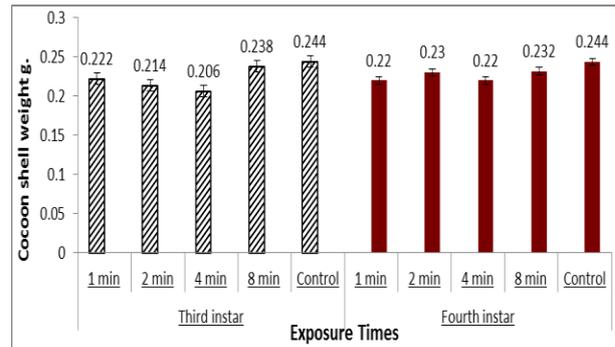
**Fig.1. Effect of exposure of 3<sup>rd</sup> and 4<sup>th</sup> larval instar to the magnetic field 180 ml.t on larval weight (g) of silkworm, *Bombyx mori*.**



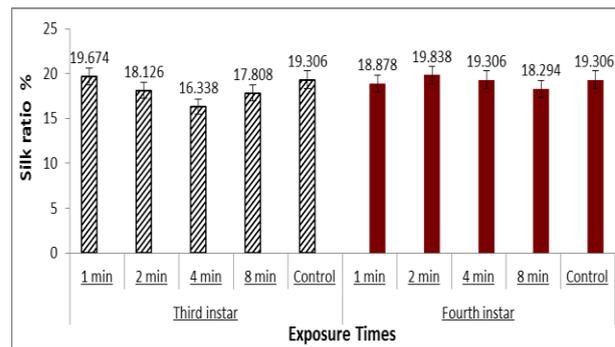
**Fig. 2. Effect of exposure of 3<sup>rd</sup> and 4<sup>th</sup> larval instar to the magnetic field 180 ml.t on pupal weight (g) of silkworm, *Bombyx mori*.**



**Fig. 3. Effect of exposure of 3<sup>rd</sup> and 4<sup>th</sup> larval instar to the magnetic field 180 ml.t on fresh cocoon weight (g) of silkworm, *Bombyx mori*.**



**Fig. 4. Effect of exposure of 3<sup>rd</sup> and 4<sup>th</sup> larval instar to the magnetic field 180 ml.t on shell weight (g) of silkworm, *Bombyx mori* cocoon.**



**Fig. 5. Effect of exposure of 3<sup>rd</sup> and 4<sup>th</sup> larval instar to the magnetic field 180 ml.t on silk ratio (%) of silkworm, *Bombyx mori* cocoon.**

These results are in partial accordance with the findings of Prasad and Upadhyay (2011) who said that, raising exposure duration of cocoon from 24 to 96 hr. in case of 1000, 2000 and 3000 gauss magnetic field strength decreased larval duration of *B. mori*, increased larval weight and survival of larvae.

Also, Taha, (2018) indicated that, 5 kilo Gauss recorded the heaviest *B. mori* larval weight, the heaviest pupal weight, the highest values for cocoon weight, cocoon shell weight and cocoon shell ratio among all tested groups.

The cocoon magnetization has been proved to be useful in improving the silk producing potential and gave the heavy production of silkworm cocoons by (Upadhyay and Prasad, 2010) and (Prasad and Upadhyay, 2011). They found that the cocoon weight and cocoon shell weight increased with the increasing exposure duration of cocoons from 24 to 96 hrs and magnetic strength up to 3000 gauss, while at higher magnetic power of 4000 gauss the cocoon weight of *B. mori* decreased (Upadhyay and Tripathi, 2006). Moreover, Chaugale and More, (1992) studied the exposure of *B. mori* larvae in the magnetic field and found that, various durations of exposure at 3500 gauss caused an increase in the cocoon weight and an increase in the production of silk.

Tripathi, (2010) stated that, magnetic exposure of silkworm, *B. mori* eggs up to 3000 Gauss gradually increased cocoon weight and influenced the weight of cocoon shell (Upadhyay and Tripathi, 2006). Alenxander and Ganeshan, (1990) found that, applying of magnetic field in the biological system caused enhancement of metabolic activities. This finding was approved by (Shivpuje *et al.*, 2016) who recorded a noticeable enhancement in larval weight and suggested that increasing metabolic activities by activation of some enzymes as a result of exposure to the

magnetic field which resulted in more food consumed by larvae causing increase in larval weight.

**2- Biochemical parameters:**

**ALT activity:**

ALT activity as shown in Table (2) and Fig (6) cleared that, exposure of silkworm larvae during the third larval instar for eight, four and two minutes increased ALT activity recording 15.50, 15, 15.5 and 11.9 U/L, respectively as compared to 10.6 U/L for control. Also, the exposure to the same magnetic field through the fourth larval instar increased the activity of ALT enzyme recording 21.7, 14.2 and 11.00 U/L for exposing periods eight, four and two minutes, respectively.

**AST activity:**

The highest AST activities were recorded 13.6 and 13.0 U/L for haemolymph samples took from larvae exposed during the third larval instar to magnetic field 180 ml.t for four and eight min comparing to 9.4 U/L for the control group. As shown in Table (2) and Fig (7) the AST activity recorded 11.4 U/L for the samples took from larvae exposed to the same magnetic field for eight min during the fourth larval instar as compared to 9.4 U/L for the haemolymph samples taken from the larvae of the control group.

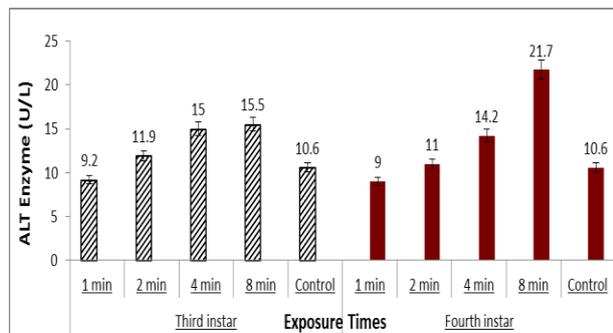
**Total protein content**

Total protein content manifests the highest values when the larvae exposed for eight, two and four minutes during the third larval instars to the tested magnetic field recording 3.26, 2.44 and 2.09 g/dl respectively, while the sample obtained from the control group recorded 1.64 g/dl as showed in Table(2) and Fig (8).

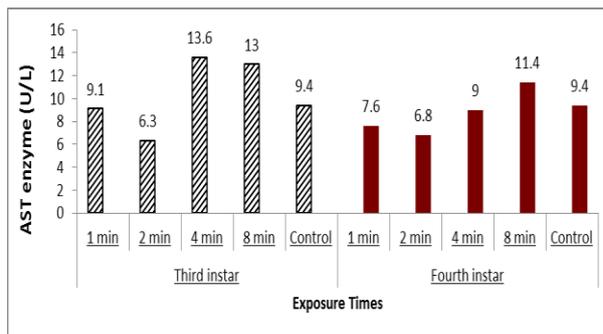
The total protein content recorded (3.02 g/dl) for the sample taken from larvae exposed to the tested magnetic field during the fourth larval instar for eight minutes, while the other exposing periods during the fourth larval instar decreased the content of total protein.

**Table 2. The effect of exposure the third larval instar or the fourth larval instar of silkworm, *B. mori* to the magnetic field 180 ml.t on some biochemical parameters.**

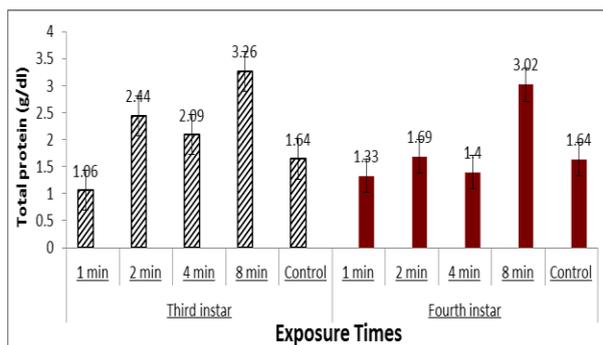
Treated instars	Exposing periods (min.)	ALT (U/L)	AST (U/L)	Total protein (g/dl)	Total lipid (mg/dl)	Glucose (mg/dl)	Amylase (U/L)
Third instar	1 min.	9.2	9.1	1.06	62	76	8
	2 min.	11.9	6.3	2.44	133	60	9
	4min.	15.0	13.6	2.09	203	121	13
	8 min.	15.5	13.0	3.26	137	84	18
Fourth instar	1 min.	9.0	7.6	1.33	89	71	10
	2 min.	11.0	6.8	1.69	115	216	16
	4min.	14.2	9.0	1.40	78	164	12
	8 min.	21.7	11.4	3.02	108	69	17
Control		10.6	9.4	1.64	81	76	9



**Fig. 6. Effect of exposure of 3<sup>rd</sup> and 4<sup>th</sup> larval instar to the magnetic field 180 ml.t on alanine amino transferase (ALT) activity in Silkworm, *B. mori* larval haemolymph.**



**Fig. 7. Effect of exposure of 3<sup>rd</sup> and 4<sup>th</sup> larval instar to the magnetic field 180 ml.t on aspartate amino transferase (AST) activity in Silkworm, *B. mori* larval haemolymph.**



**Fig. 8. Effect of exposure of 3<sup>rd</sup> and 4<sup>th</sup> larval instar to the magnetic field 180 ml.t on Total protein content in Silkworm, *B. mori* larval haemolymph.**

**Total lipid content:**

As shown in Table (2) and Fig (9) it was found that, exposure of the third larval instar for four, eight and two minutes to magnetic field 180 ml.t increased the total lipid content in the haemolymph of silkworm larvae and recorded 203, 137 and 133 g/dl, respectively as compared to 81 g/dl for control group.

The results for larvae exposed to the magnetic field during the fourth instar cleared that, the exposure periods for two and eight minutes manifest the highest lipid content recording 115 and 108 g/dl as compared to 81 g/dl for control group.

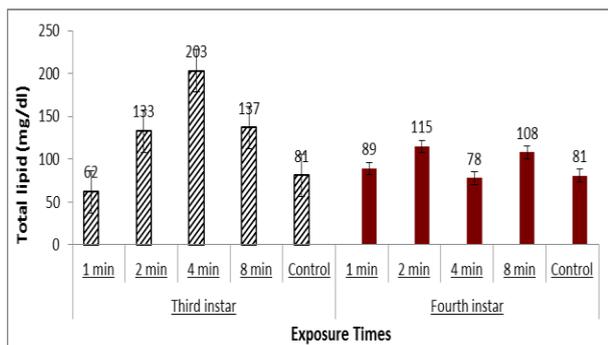
**Glucose content:**

As shown in Table (2) and Fig (10) all exposing periods during the third larval instar increased the glucose content and recorded 76, 60, 121 and 84 mg/dl for one, two, four and eight, respectively.

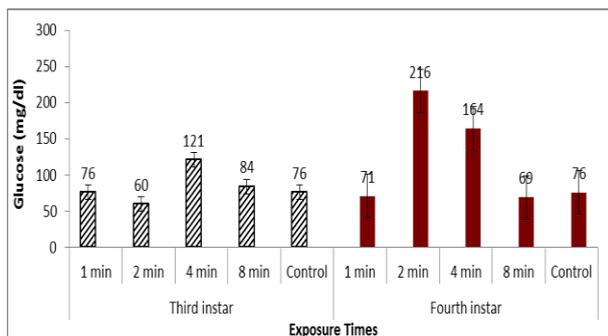
The same trend was happened for the exposing periods during the fourth larval instar since the glucose content recorded 71, 216 and 164 mg/dl for one, two and four, respectively as compared to 76 mg/dl for the haemolymph samples obtained from larvae of control group.

**Amylase activity:**

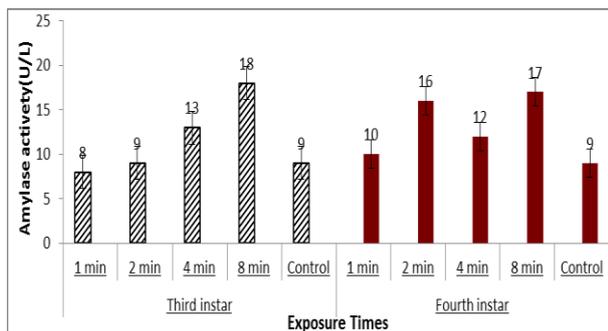
Data represent amylase activities showed in Table (2) and Fig (11). The highest activities of amylase enzyme were recorded from haemolymph samples taken from larvae magnetized once during the third larval instar for eight and four minutes recording 18 and 13 U/L, respectively. Treatment of larvae during the fourth instar by the magnetic field 108 ml.t for all tested exposing periods increased the activity of amylase enzyme recording 17.12.16 and 10 U/L for one, two, four and eight, respectively comparing to 9 U/L for haemolymph samples taken from control larvae.



**Fig. 9. Effect of exposure of 3<sup>rd</sup> and 4<sup>th</sup> larval instar to the magnetic field 180 ml.t on Total lipid content in Silk worm, *B. mori* larval haemolymph.**



**Fig. 10. Effect of exposure of 3<sup>rd</sup> and 4<sup>th</sup> larval instar to the magnetic field 180 ml.t on Glucose content in Silk worm, *B. mori* larval haemolymph.**



**Fig. 11. Effect of exposure of 3<sup>rd</sup> and 4<sup>th</sup> larval instar to the magnetic field 180 ml.t on Amylase activity in *B. mori* larval haemolymph.**

Madhuri *et al* (2016) reveal that magnetization of *B. mori* influence the level total protein in the larval haemolymph. The content of total protein increased with increasing the magnetic field strength from 1000 Gauss to 4000 Gauss. Tripathi, (2012) said that the total protein content in the larval haemolymph of fifth larval instar was increased due to the variation in the magnetic field strength and the content of total protein was increased gradually from control to 2000 Gauss but in the 3000 Gauss the level of total protein content for exposure of fifth instar *B. mori* larvae was maximum.

Also, various durations of exposure at 3500 gauss influenced the protein content in the larvae and pupae of *Bombyx mori* Mathur and Subba, (1987). Moreover, the magnetic field during egg stage influences the biosynthesis and utilization of free amino acids and protein metabolism Tripathi *et al.*, (2012) which responsible for these enhancements in larval and cocoon characters.

Prasad and Upadhyay, (2011) indicated that the glucose content in the fat body of *B. mori* larvae was

increased due to variation in the static magnetic field strength and raising exposure duration. They stated the glucose content enhanced with the increasing exposure duration of cocoon from 24 to 96 h in 0.1, 0.2 and 0.3 tesla magnetic field.

How exactly magnetic field influences the biological system is not cleared and efforts are being made in this direction but it was reported that, magnetization of larvae in the low magnetic field may cause increasing in general metabolic rate due to the activation of some enzymes as a result of more food consumed by the larvae which increased cellular activity in the silk gland, haemolymph and fat body, thus protein content in the tissues increased whereas, high strength of magnetic field caused stress responses Madhuri *et al.*, (2016).

Amino acids are raw substantial responsible for the production of silk protein. The amino acids like aspartic acid, methionine, hydroxyproline isoleucine, cystein and tryptophane are instantly utilized for the synthesis of silk (Hsu and Wang, 1964). The total free amino acid content of the haemolymph in *Bombyx mori* has also been noticed to be increase during 5<sup>th</sup> instar larval growth till the start of spinning (Chitra and Sridhar, 1972) and changes in the free amino acid content of the haemolymph in *Philosamia ricini* decreased till the start of spinning but further increased steadily to the highest level towards the end of cocoon formation (Pant and Morris, 1972). The increasing in free amino acids in different tissues with the aid of transaminases resulting in increasing protein content in haemolymph, fat bodies and silk glands consequently leading to increase silk yield. Since protein is chief components of natural silk thus, the magnetization of larvae may be base for sericulture industries to increase economic characters and silk yield of silkworm. Thirty per cent of the silk proteins were coming from the free amino acid and proteins of the haemolymph while the rest was synthesized by the salivary gland during the spinning process (Terra *et al.*, 1973).

It may be concluded that, the application of magnetic field 180 ml.t for 8 minutes on silkworm larvae stimulated the cytochrome system and improved the metabolic rate of larvae as a result of more food consumption by larvae and more free amino acids production in all body systems which consequently followed by increasing the proteins in silk gland then improving in cocoon yield. Thus, it will be recommended that, we have needed to increase the exposure duration over the larvae more than eight minutes to obtain more enhancements in silkworm economical and commercial traits.

## AKNOWLEDGMENT

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## تأثير المجال المغناطيسي على بعض الصفات البيولوجية والبيوكيميائية لدودة الحرير التوتية *Bomby mori*

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تم دراسة تطبيق المجال المغناطيسي ١٨٠ ملي تسلا على يرقات دودة الحرير (*Bombyx mori* Lepidoptera: Bombycidae) لتقييم تأثير فترات التعرض المختلفة ١ و ٢ و ٤ و ٨ دقائق على بعض الصفات البيولوجية ومكونات الهيموليمف البيوكيميائية. تم عمل مغنطة ليرقات لعمر الثالث والرابع مرة واحدة في اليوم الأول قبل التغذية الأولى. أي تعرض يرقات دودة الحرير خلال كلا من العمر البرقي الثالث والرابع للمجال المغناطيسي ١٨٠ ملي تسلا لمدة ثماني دقائق التي زيادة وزن اليرقات تليها التعرض للمغنطة لمدة أربع دقائق لليرقات خلال العمر البرقي الثالث والعمر البرقي الرابع. يرقات مجموعة الضابطة واليرقات المعرضة للمجال المغناطيسي لمدة ثماني دقائق خلال العمر البرقي الرابع والعمر البرقي الثالث حققت أعلى وزن لفشرة الشرقة للشرانق الناتجة. كانت نسب الحرير أعلى بصورة معنوية في الشرانق الناتجة من التعرض للمجال المغناطيسي خلال العمر البرقي الرابع لمدة دقيقتين متبوعًا بالتعرض لمدة دقيقة أثناء لعمر البرقي الثالث. أثر الاختلاف في مدة التعرض للمجال المغناطيسي بشكل ملحوظ على المكون البيوكيميائي للهيموليمف ليرقات دودة الحرير. نشاط الانزيم الناقل لحمض الألانين الأميني ومحتوى البروتين الكلي ونشاط إنزيم الأميليز سجل أعلى قيمه له في هيموليمف اليرقات المعرضة للمجال المغناطيسي الذي تم اختياره على يرقات العمر البرقي الرابع والثالث لمدة ثماني دقائق، في حين وجد أن نشاط الانزيم الناقل لحمض الأسبارتات الأميني ومحتوى الدهون الكلي هو الأعلى في عينات الهيموليمف المأخوذة من يرقات تعرضت لنفس المجال المغناطيسي خلال العمر البرقي الثالث لمدة أربع وثمانين دقائق على التوالي. كما أظهرت النتائج أن أعلى نسبة الجلوكوز سجلت في عينات الهيموليمف التي تم الحصول عليها من اليرقات التي عرضت مرة واحدة إلى المجال المغناطيسي لمختبر لمدة أربع دقائق أثناء العمر البرقي الثالث والعمر البرقي الرابع.