

EFFECT OF MAGNETIC FIELD ON METABOLISM AND ENZYME ACTIVITY IN SOME HARMFUL INSECTS

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ABSTRACT: To investigate the effect of magnetic field on the physiological aspects of some insects, larvae of three insects were reared in laboratory under $22 \pm 2^\circ\text{C}$ and $60 \pm 5\%$ RH in a suitable rearing boxes. The three insects were: cotton leaf worm, *Spodoptera littoralis*, red palm weevil, *Rhynchophorus ferrugineus* and the greater wax moth, *Galleria mellonella*. Rearing insects started with a suitable larval instar. Numbers of larvae (from each insect) divided into 2 similar and equal groups, the first reared as a control (without magnetic field), while the second group was exposed to the magnetic field, (MF) along rearing periods. An appropriate numbers of larvae from the control and the treated ones were taken for Bio-chemical analysis and the main parameters were recorded. The measured physiological parameters were: total protein, total carbohydrates, total lipids, invertase enzyme and alkaline phosphatase. Magnetic flux in around the center of the rearing plastic containers for the three insects were prepared and adjusted with similar magnates. Strength of the magnetic flux was measured using millitesta apparatus. It was recorded an average of 0.218, 2.487 and 8.629 ml.t. for the 3 insects respectively. Results showed that each of body weight and growth rate as well as the physiological aspects were affected with the magnetic field. Rate of growth was negatively affected as it decreased in cotton leaf worm and red palm weevil. Results also demonstrated that invertase enzyme decreased in the treated larvae of *S. littoralis* and *G. mellonella* with 40.15% and 28.33% respectively lower than the control. In the same time, magnetic field led to increase the invertase enzyme in *R. ferrugineus* with 71.6% higher than the control samples. In spite of the strong magnetic flux around the larvae of *G. mellonella* their influence was limited. This may be due to the special behaviour of the worms as they of internal presence and entrance inside the rearing media for these larvae.

Key words: Magnetism, Pest control, Metabolism, Enzyme, Protein, Carbohydrate, Lipids.

INTRODUCTION

Magnetism and using magnetic field seems to be promising physical method in pest control (Hussein *et al.*, 2014). Recently we have to focus some lights on magnetism and the effect of electro-magnetic waves on the different biological aspects of insects. In normal conditions of rearing insect cultures, it is logic to say the proportion of total protein, total carbohydrates and total lipids are remained relatively steady in body content. Also, the same similar steady pattern of enzyme activity occurred in insects under the normal condition. Changes and alteration of the main components (Protein, Carbohydrates and Lipids) as well as the enzyme activity only appears under such stresses that insects exposed to.

Stresses may be micro-organisms e.g. bacteria or nematodes (Hussaini *et al.*, 2002) or entomopathogenic fungi (Serebrov *et al.*, 2006), or heavy metals (Gong Qing and Yun Hong 2015) or chemical compounds e.g. boric acid (BA) (Hyrsi *et al.*, 2007), Venous (Sak *et al.*, 2011), Antibiotics in artificial diets (Buyukguzel and Kalender 2008).

Stresses also, may be physical factors e.g. temperature, different types of waves e.g. gamma rays (Hussein *et al.*, 1999) and electro-magnetic waves (Hussein *et al.*, 2014).

Sak *et al.* (2011) in their studies on *Galleria mellonella* larvae stated that total protein increase in cases of injury and

wounding or exposure to high doses of venoms. Buyukguzel and Kalender (2008) in their studies on the same insect stated that high concentrations of antibiotics (fluconazole) in the artificial diet of the larvae markedly decreased the total protein content.

Hyrsl *et al.* (2007) stated that lowest boric acid (BA) in diets affected enzyme activity in *Galleria mellonella* larvae and led to significantly increased larval and pupal mortality and prolonged development.

Importance of the red palm weevil was strongly documented as El-Mergawy *et al.* (2011) stated that red palm weevil *Rhynchophorus ferrugineus* is an invasive pest of palm trees and it has invaded Middle East and several countries of the Mediterranean Basin during the last three decades.

Shi *et al.* (2014) reported that *Rhynchophorus ferrugineus* (Coleoptera, Curculionidae) is the most destructive pest of palm trees worldwide containing it invasive areas, such as the southern of China. Tagliavia *et al.* (2014) reported that the larvae bore into palm trunk and feed on the palm tender tissue and sap, leading the host tree to death.

This current study aimed to throw some lights on the effect of magnetic field (MF) on the physiological aspects of cotton leaf worm, *Spodoptera littoralis* (Lepidoptera), red palm weevil, *Rhynchophorus ferrugineus* (Curculionidae: Coleoptera) and the greater wax moth, *Galleria mellonella* (Pyralidae: Lepidoptera). The investigated parameters were: total protein, total carbohydrates and total lipids, as well as the enzyme activity e.g. alkaline phosphates enzyme and invertase enzyme in both normal and treated larval stage of three harmful insects.

MATERIALS AND METHODS

This research was conducted at the biology laboratory of the Plant Protection Research Institute, Eldokki, Giza, in

cooperation with physics laboratory of the Engineering Faculty of Menoufia University.

1- Creating and adjusting the magnetic field (MF):

To conduct the experiment, the investigated larvae were exposed to a magnetic field (MF) to study the biological effects of the electro-magnetic waves and strength of magnetic flux. Creation of the magnetic field was mainly produced by fixing certain number of similar magnet pieces around the plastic rearing boxes. Strength of the magnetic flux was measured in milli-tesla using Teslameter apparatus (Faculty of Engineering – Menoufiya University). The measured values of magnetic flux strength were: 0.218 ± 0.099 , 2.49 ± 1.48 and 8.63 ± 3.88 milli-tesla for *S. littoralis*, *R. ferrugineus* and *G. mellonella* respectively.

Each value of the pre-mentioned strength was a statistical average for 15 actual value measured around the center of the rearing box. (Table 1).

2- Rearing insects :

Rearing process was concentrated on the suitable larval stage in appropriate rearing boxes. Rearing was conducted for 2 similar groups of larvae, the first was served as control (without MF) which kept 2 meter far from the second group which exposed to (MF). Rearing period were 6, 41 and 8 days for *S. littoralis*, *R. ferrugineus* and *G. mellonella*, respectively. Each of insect species was reared under laboratory conditions of $22 \pm 2^\circ\text{C}$ and $60 \pm 5\%$ RH according to suitable own method and the appropriate own diet.

Spodoptera littoralis reared on caster bean leaves while *R. ferrugineus* reared on sugar cane stems and *G. mellonella* reared on artificial diet mainly consists of flower and yeast with glucose. Daily and / or periodically regular bio-weight of *S. littoralis* and *R. ferrugineus* was determined in milligrams.

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Table (1): Actual measured values of magnetic flux (mlt) in the rearing boxes of the investigated insects.

Point number	<i>S. littoralis</i>	<i>R. ferrugineus</i>	<i>G. mellonella</i>
	values of magnetic flux (mlt)		
1	0.30	2.77	6.77
2	0.13	3.85	11.58
3	0.11	1.62	9.87
4	0.11	0.58	2.00
5	0.38	4.62	13.12
6	0.21	5.60	3.99
7	0.32	2.85	10.55
8	0.33	0.35	12.80
9	0.32	1.42	9.70
10	0.26	2.91	2.53
11	0.13	1.45	9.46
12	0.17	0.85	6.49
13	0.30	3.17	13.42
14	0.05	1.62	13.00
15	0.15	3.65	4.16
$\bar{x} \pm SD.$	0.218 ± 0.099	2.49 ± 1.48	8.63 ± 3.88

3- Preparation of insects for analysis:

The larvae from the control and treatments were prepared as described by Amin (1998), where it was homogenized in distilled water (50 mg/1 ml). Homogenates were centrifuged at 8000 rpm for 15 minutes at 2°C in a refrigerated centrifuge.

4- Bio-chemical analysis:

Five main components were determined in control and treatment specimens at the end of larval stage. Determination was conducted in fine bio-chemical analysis

laboratory of the Plant Protection Research Institute. ARC, Cairo, Egypt :

- a- Total proteins determined by the method of Bradford (1976).
- b- Total carbohydrates were extracted and prepared for assay according to Crompton and Birt (1967) and estimated in acid extract of sample by the phenol-sulphuric acid reaction of Dubois *et al.* (1956).
- c- Total lipids were estimated by the method of Knight *et al.* (1972).
- d- Alkaline phosphatase enzyme was determined according to the method described by Powell and Smith (1954).

e- Invertase enzyme was determined according to the modifications of Amin (1998) to the method described by Ishaaya and Swirski (1976).

RESULTS AND DISCUSSION

Results in Table (1) show the actual measured values of the magnetic flux in milli-tesla units (mt), which were measured by millitesla apparatus. Fifteen sites in the center of each rearing box were chosen to

measure the magnetic flux for each insect. Average of the 15 values of the rearing box represented the final strength of magnetic flux affected the insect under investigation. The value of magnetic flux recorded were : 0.218 ± 0.099 , 2.49 ± 1.48 and 8.63 ± 3.88 mlt for *S. littoralis*, *R. ferrugineus* and *G. mellonella*, respectively.

Results in Table (2) show the biochemical analysis for 5 parameters in the body of the 3 investigated insects.

Table (2): Biochemical analysis for larvae specimens without magnetic field (MF) and the treated of the three investigated insects.

Parameter	Main body component			Enzymes	
	Total protein (mg/g.b.wt)	Total carbohydrates (mg/g.b.wt)	Total lipids (mg/g.b.wt)	Alkaline phosphates (U x 10 ³ /g.b.wt)	Invertase (µg glucose/min/g.b.wt)
<i>Spodoptera littoralis</i>					
C	37.13 ± 1.42	26.87 ± 1.72	10.24 ± 0.74	2318 ± 74.28	1300 ± 74.83
T	30.80 ± 2.71	19.20 ± 0.98	7.37 ± 0.31	3855 ± 113.72	778 ± 50.18
T.ch.	-	-	-	+	-
V.ch.	6.33	7.67	2.87	1537	522
% ch.	17.05%	28.54%	28.03%	66.31%	40.15%
<i>Rhynchophorus ferrugineus</i>					
C	85.13 ± 5.03	68.23 ± 4.95	13.40 ± 0.64	1876 ± 88.06	2499 ± 118.55
T	84.00 ± 3.15	63.67 ± 3.24	13.67 ± 0.69	1821 ± 54.73	1791 ± 88.54
T.ch.	-	-	+	-	-
V.ch.	1.13	4.56	0.27	55	708
% ch.	1.33%	6.68%	2.01%	2.93%	28.33%
<i>Galleria mellonella</i>					
C	22.42 ± 1.68	21.00 ± 0.93	33.70 ± 2.41	259 ± 34.88	500 ± 24.08
T	22.43 ± 1.03	28.27 ± 1.55	35.97 ± 0.97	232 ± 30.47	858 ± 53.22
T.ch.	+	+	-	-	+
V.ch.	0.01	7.27	2.27	27	358
% ch.	0.04%	34.62%	6.74%	10.42%	71.60%

sC = Control, T = Treatment, T.ch. = Trend of change, V.ch = Value of change, % ch = % of change

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These parameters were: total protein, total carbohydrates, total lipids, alkaline phosphates enzyme and invertase enzyme. Determination was conducted for samples from control larvae (without MF) and from treatment larvae (with MF). Values of the measured parameters indicated a clear disturbance in the metabolism and the physiological aspects for the 3 investigated insects, Fig. (1 a, b & c) and Fig. (2 a & b).

Regarding to the analysis of treated *S. littoralis* larvae results showed that the total protein, carbohydrates and lipids recorded 30.80, 19.20 and 7.37 mg/g b.wt, lower than those of control as they were : 37.13, 26.87 and 10.24 mg/g b.wt , respectively. *Rhynchophorus ferrugineus* treatments recorded 22.43, 28.27 and 35.97 in the treated larvae against 22.42, 21.00 and 33.70 mg/g b.wt for the control, respectively. In addition, protein, carbohydrates and lipids in treated larvae of *G. mellonella* recorded : 84.00, 63.67 and 13.67 while it was 85.13, 68.23 and 13.40 mg/g b.wt for control, respectively. The disturbance extended also to presence of enzymes in body of the 3 investigated insects. Alkaline phosphatase and invertase

recorded 3855 and 778 U x 10³/ g b.wt for treated larvae of *S. littoralis* against 2318 and 1300 units in control larvae (without MF), respectively. Also, alkaline phosphates and invertase enzymes decreased in the treated larvae of *G. mellonella* as their values recorded 1821 and 1791 units against 1876 and 2499 units for control, respectively. As for treated larvae of *R. ferrugineus*, the biochemical analysis showed that alkaline phosphatase and invertase recorded 232 and 858 units against 259 and 500 units for control, respectively. (Fig.2 a&b).

Results in Table (3) show the body weight in (mg) of 20 larvae of *S. littoralis* reared for 6 days (through 4th, 5th and 6th instars) under 0.218 mT of magnetic flux in comparison with another group with the same number reared without (MF) magnetic field. Rate of growth / larva / day was recorded. Results indicated that the gain weight / larva was 0.350 mg in control, which considerably decreased in the treatment (with MF) to only 0.305 mg. The main decrease in weight was noticed in the 5th and 6th instars.

Table (3): Body weight of *S. littoralis* in control (without magnetic field) and under treatment (with magnetic field) in (mg) during 6 days of experimentation for different instars.

Date	Instar	No. of larvae	Control (without MF)			Treatment with (MF)		
			larvae weight (mg)	weight / larva (mg)	growth rate / larva / day	larvae weight (mg)	weight / larva (mg)	growth rate / larva / day
May10, 2014	4 th	20	1.57	0.078	--	2.12	0.106	--
11	4 th	20	3.06	0.153	0.075	3.62	0.181	0.075
12	5 th	20	4.90	0.245	0.092	6.43	0.322	0.141
13	5 th	20	7.31	0.366	0.121	9.64	0.482	0.160
14	6 th	20	10.21	0.511	0.145	11.38	0.569	0.087
15	6 th	20	8.57	0.428	-0.083	8.21	0.411	-0.158
Total (days)	6							
Gain weight / larva / 6 days (experimentation period (mg))				0.350			0.305	

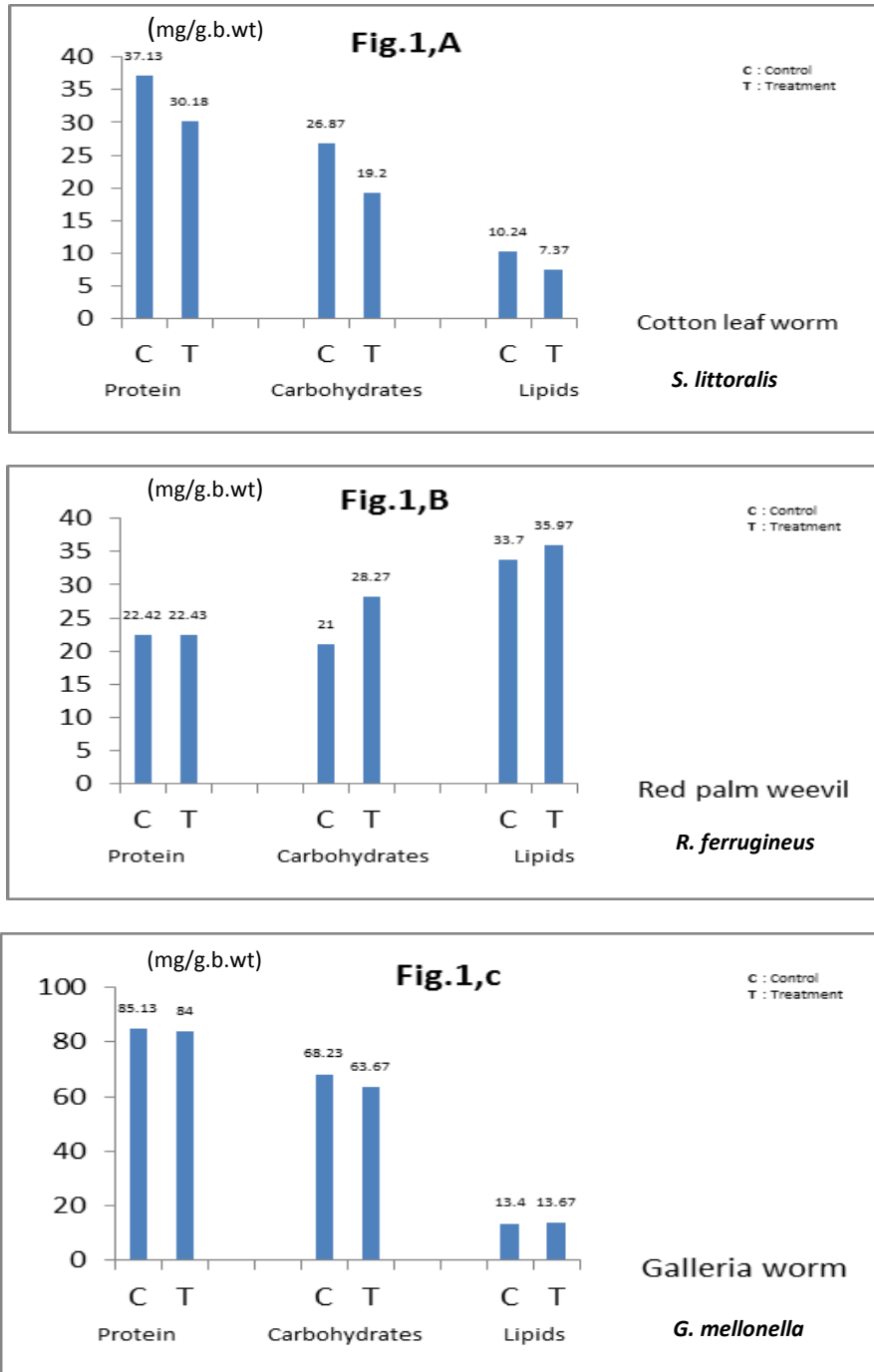


Figure (1): Bio-chemical analysis of the main body content in the investigated insects.

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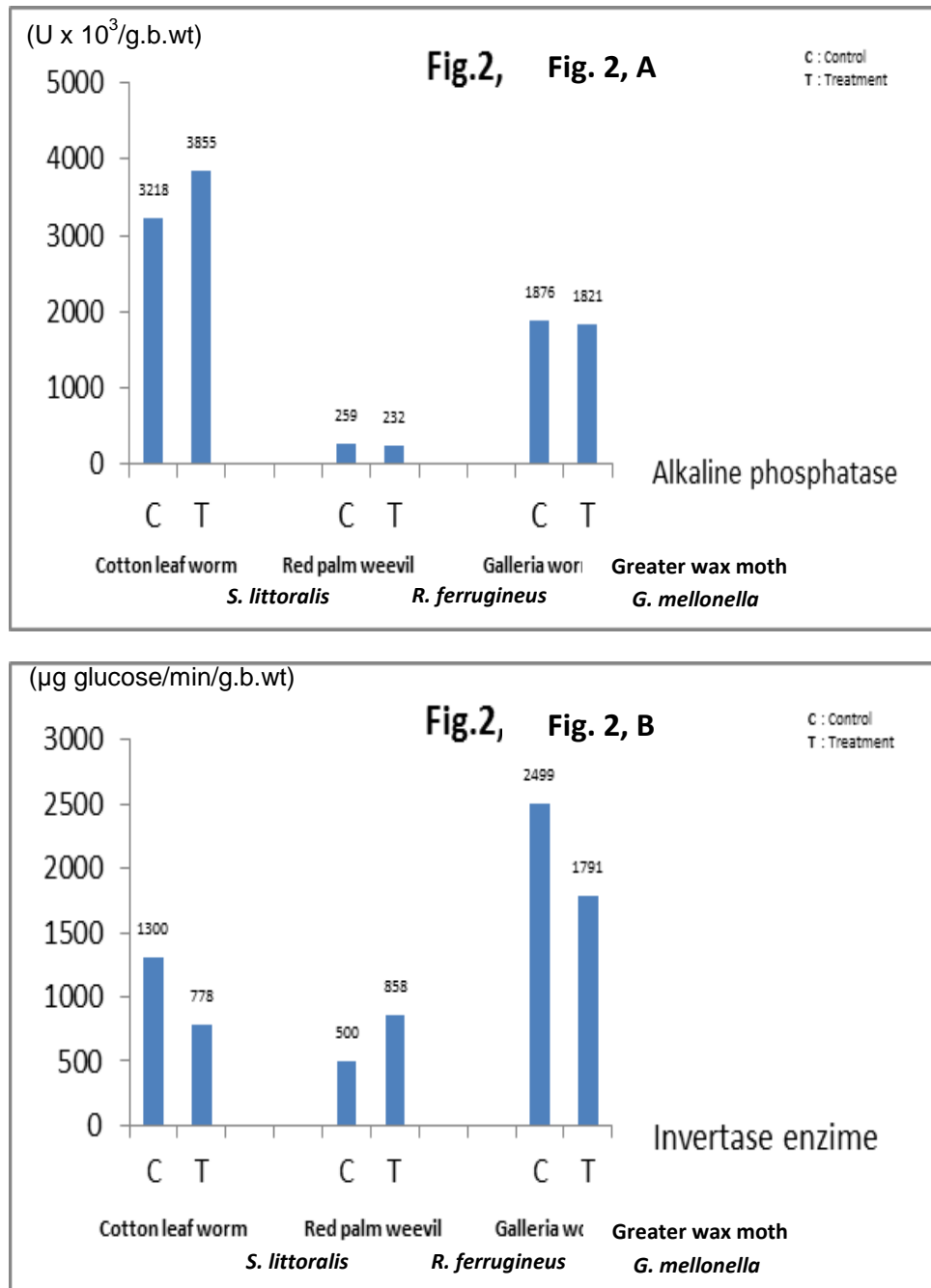


Figure (2): Bio-chemical analysis of enzymes in the investigated insects.

Results in Table (4) show the weight of *R. ferrugineus* larvae along 41 days of experimentation with MF and without MF control. Results indicated that the gain body weight / larva / day along the rearing period was 2.445 mg in case of the control (without MF), while it was only 2.015 mg for the treated larvae. Decrease in this gain weight concentrated in late period of rearing, (pre-pupa) period: 6th, 7th and 8th instars.

The obtained results are in harmony with those of Vatanparast *et al.* (2014) who found in their studies on enzyme activity reported that the red palm weevil has been adapted to overcome the plant cell wall barrier, specially lignocellulosic and pectic compounds, by producing cellulose and pectinase enzymes, as well as, they reported the presence of Ca, Mg and Na in larval midgut which significantly increased pectinase activity, while K did not affect the enzyme activity. Furthermore, Ragaei and

Sabry (2013) reported that, red palm weevil have infrared receptors (called sensilla) on their bodies, on the cuticle of larvae, pupae and on the adult's wings. These receptors have absorbance area which uptake the heat of infrared radiation, and found that adults weevils affected by the broadband infrared radiation. It was reported also, that the infrared radiation attract the weevils. El-Shershaby *et al.* (2008) studied the impact of *Bacillus thuringiensis* on protein content and enzyme activity of *Spodoptera littoralis*, and stated that a negative changes in total protein content of treated larvae from 19.8-36.6% were evaluated, also, they found fluctuated changes in the enzymes activity of treated larvae. Shen *et al.* (1994) studied effects of sub-lethal dosages of *Bacillus thuringiensis* on the metabolism of *Galleria mellonella* larvae, and reported that *Galleria* protein was decreased compared with the normal larvae.

Table (4): Body weight of *R. ferrugineus* larvae in control (without magnetic field) and under treatment (with magnetic field) in mg. along 41 days of experimentation for different instars.

Instar	instar duration (day)	Body weight of control (without MF)			Body weight of treatment (with MF)		
		No. of larvae	larvae weight (mg)	Weight / larva (mg)	No. of larvae	larvae weight (mg)	Weight / larva (mg)
2 nd	3	5	1.623	0.325	5	1.598	0.32
3 rd	3	5	12.325	2.465	4	9.864	2.466
4 th	4	5	29.325	5.865	4	23.276	5.819
5 th	4	5	76.245	15.249	4	56.824	14.206
6 th	4	4	170.260	42.565	4	134.760	33.690
7 th	9	4	250.320	62.580	4	207.167	51.792
8 th	14	4	402.350	100.588	4	331.678	82.920
Total (days)	41						
Gain weight / larvae / 41 days experimentation period (mg)				100.263			82.600
Gain weight / larva / day (mg)				2.445			2.015

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تأثير المغناطيسية على الأيض الغذائي والنشاط الإنزيمي لبعض الحشرات الضارة

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الملخص العربي

لدراسة تأثير المجال المغناطيسي على أيض وفسولوجيا بعض الحشرات تم اختيار ثلاثة أنواع من الحشرات ذات الأهمية الاقتصادية وهي: دودة ورق القطن الكبرى وسوسة النخيل الحمراء ودودة الشمع الكبرى (الجاليريا) ، وتم تربية يرقات كل حشرة في قسمين:- الأول: للمقارنة (بدون مجال مغناطيسي) والثاني: (معاملة) تحت تأثير مجال مغناطيسي طوال فترة الطور اليرقي وعلى درجة حرارة $22 \pm 2^\circ\text{C}$ ورطوبة نسبية $60 \pm 5\%$. وقد تم إيجاد المجال المغناطيسي عن طريق تثبيت وحدات مغناطيس متماثلة القوة حول صناديق التربية ليرقات (المعاملة)، كما تم قياس شدة الفيض المغناطيسي في الصناديق الثلاث بجهاز تسلامتر (كلية الهندسة - جامعة المنوفية) والذي سجل ٠.٢١٨، ٢.٤٨٧، و ٨.٦٢٩ مللي تسلا للحشرات الثلاث على الترتيب.

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- خلال مدة التربية تم وزن الجسم الحي ليرقات دودة ورق القطن وسوسة النخيل دورياً لحساب معدل النمو / لليرقة / يوم وتأثير المجال المغناطيسي على معدل النمو.
 - في نهاية فترة التطور اليرقي تم أخذ عينات من يرقات الحشرات الثلاث لتحليلها كيميائياً لتقدير: البروتين الكلي - والكربوهيدرات الكلية - والدهون الكلية وكذلك تقدير إنزيم Alkaline phosphatase وإنزيم invertase وذلك لدراسة تأثير المجال المغناطيسي على عمليات الأيض الغذائي metabolism ومكونات الجسم الأساسية وكذلك قياس النشاط الإنزيمي به.
- وقد أظهرت النتائج اختلال في نسب المكونات الأساسية الثلاث في المعاملة عن الكنترول إذ أنه بالنسبة لدودة ورق القطن الكبرى فقد قلت النسبة المئوية للبروتين والكربوهيدرات والدهون بـ ١٧,٥٪، ٢٨,٥٤٪ وأخيراً ٢٨,٠٣٪ على الترتيب. وفي حالة سوسة النخيل الحمراء فقد زادت نسبة الكربوهيدرات الكلية بنسبة ٣٤,٦٢٪ عنها في الكنترول. وبخصوص دودة الشمع الكبرى (الجاليريا) فقد قلت نسبة البروتين والكربوهيدرات بـ ١٠,٣٠، ٦,٦٨٪ بينما كانت الزيادة في الدهون بـ ٢,٠١٪ فقط وقد يرجع قلة تأثير الجاليريا بالمجال المغناطيسي إلى طبيعة معيشة هذه الحشرة حيث أنها داخلية المعيشة internal إذ تعيش يرقاتها داخل البيئة التي تتغذى عليها.
- وبالنسبة للنشاط الإنزيمي فقد أوضحت النتائج زيادة إنزيم Alkaline phosphatase بنسب كبيرة في دودة ورق القطن الكبرى ١٩,٧٩٪ بينما قل إنزيم invertase في المعاملة عن الكنترول في دودة ورق القطن والجاليريا بنسبة ٤٠,١٥، ٢٨,٣٣٪ على الترتيب بينما زاد في سوسة النخيل الحمراء بنسبة ٧١,٦٠٪.

