ISSN NO. 2320-5407

*Journal homepage: http://www.journalijar.com*
*Journal DOI: 10.21474/IJAR01*

*INTERNATIONAL JOURNAL OF ADVANCED RESEARCH*

**RESEARCH ARTICLE**

# MAGNETIC FIELD EFFECT ON THE METABOLISM AND ENZYME ACTIVITY IN SOME HARMFUL INSECTS.

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## *Manuscript Info*

***Manuscript History:***

Received: 15 March 2016
Final Accepted: 20 April 2016
Published Online: May 2016

***Key words:***
Magnetism; Magnetic flux density; magnetic moments; Pest control; Metabolism; Enzyme; Protein; Carbohydrate; Lipids.

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## *Abstract*

Effect of the magnetic field on the physiological aspects of some insects was studied for larvae of three insects reared in laboratory under 25 ±2°C and 70 ±5% RH in suitable rearing boxes. An experimental set-up was designed to measure the magnetic flux density in the rearing box. The magnetic field was calibrated to obtain the point of the maximum gradient. Magnetic flux density was measured using a Tesla meter and recorded average values of 21.8, 249 and 863 Gauss (G) for the 3 insects respectively. The three insects were: cotton leaf warm, S. littoralis, red palm weevil, R. ferrugineus and the greater wax moth, Galleria mellonella. Rearing insects started with a suitable larval instar. Numbers of larvae (form 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. Results showed that each of body weight and growth rate as well as the physiological aspects was 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 ans 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.



## 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 entomopathagenic fungi (Serebrov et al. 2006), or heavy metals (Gong Qing and Yun Hong 2015) or chemical compounds e.g. boric acid (BA) (Hyrsl 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 G. 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 G. 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 R. ferrugiuneus 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 R. ferrugineus (coleopteran, curculiondae) 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.

The present work aimed to throw some lights on the effect of magnetic field (MF) on the physiological aspects of some insects. 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. The three investigated insects were: cotton leaf warm, Spodoptera littoralis (Lepidoptera), red palm weevil, Rhynchophorus ferrugineus (Curculionidae: Coleoptera) and the greater wax moth, Galleria mellonella (Pyralidae: Lepidoptera).

## Materials and Methods:-

The present is cooperation between the biology laboratory of the planet protection Research Institute, Eldokki, Giza and Physics laboratory of Engineering Basic Sciences Department, Faculty of Engineering, Menoufia University, Shebin El- Kom, Egypt.

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

To conduct the experiment, it was necessary to create a magnetic field to make the investigated larvae be exposed to the magnetic field and affects by the magnetic flux density. Creation of the magnetic field was mainly by fixing a certain number of permanent magnets around the plastic rearing boxes. Strength of the magnetic flux was measured in Gauss using Tesla-meter apparatus (Faculty of Engineering - Menoufiya University). The measured values of magnetic flux density were: 21.8, 249 and 863 Gauss (G) 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. (Table1).

**Table (1):** Actual measured values of magnetic flux density (B) in the rearing boxes of the investigated insects

| Point number | S. littoralis | R. ferrugineus | G. mellonella |
|---|---|---|---|
| **Values of magnetic flux density (G)** | | | |
| 1 | 30 | 277 | 677 |
| 2 | 130 | 385 | 1158 |
| 3 | 110 | 162 | 987 |
| 4 | 110 | 58 | 200 |
| 5 | 0.380 | 462 | 1312 |
| 6 | 210 | 560 | 399 |
| 7 | 320 | 285 | 1055 |
| 8 | 330 | 35 | 1280 |
| 9 | 320 | 142 | 970 |
| 10 | 260 | 291 | 253 |
| 11 | 130 | 145 | 946 |
| 12 | 170 | 85 | 64.9 |
| 13 | 30 | 317 | 134.2 |
| 14 | 50 | 162 | 1300 |
| 15 | 15 | 365 | 416 |
| **Average value** | **21.8** | **249** | **863** |

**Rearing insects in laboratory:-**

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 the control (without MF) which kept 2 meter far enough 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, Tables (2&3). Each of insect was reared in lab. condition room 25 ±2°C and 70 ±5% RH according to suitable own method and the appropriate own diet. S. littoralis reared on caster bean leaves while R. ferrugiueus 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. littaralis and R. ferrugineus was detected in milligrams.

**Preparation of insects for analysis:-**

The larvae from the control and treatments were prepared as described by Amin (1998). They were homogenized in distilled water (50 mg/1 ml). Homogenates were centrifuged at 8000 r.p.m. for 15 minute at 2°C in a refrigerated centrifuge.

**Bio-chemical analysis:-**

Five main components were determined in both (control) and (treated) specimens at the end of larval stage. Determination was conducted in fine bio-chemical analysis lab. in Plant Protection Research Institute. ARC, Cairo, Egypt.

The main 5 determinations were:

1. Total proteins which determined by the method of Bradford (1976).
2. Total carbohydrates which were estimated in acid extract of sample by the phenol-sulphuric acid reaction of Dubois et al, (1956). Total carbohydrates were extracted and prepared for assay according to Crompton and Birt (1967).
3. Total lipid, which were estimated by the method of Knight et al. (1972).
4. Alkaline phosphates enzyme was determined according to the method described by Powell and Smith (1954).
5. Invertase enzyme was determined according to the modifications of Amin (1998) to the method described by Ishaaya and Swirski (1976).

## Results and Discussion:-

**Calibration of the magnetic field:-**

The measurements is based on a search coil is connected to a flux meter, which directly gives accurate measurement of the magnetic flux density in millitesla, mT;(1 mT = 10 Gauss). To calibrate the magnetic field, the variation of the magnetic field as a function of the distance was measured and shown in **Fig. 1.**This figure shows that as distance increases the magnetic flux density (B) decreases. The point of maximum gradient of the magnetic flux density was determined by plotting the gradient of the magnetic field (dB/dx) as a function of distance **Fig. 2**. The obtained results show that the maximum gradient was obtained at x = 3cm.

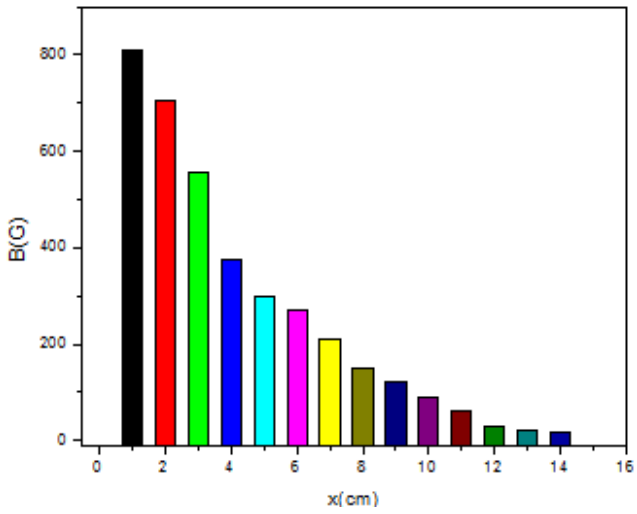


**Fig.1:** Magnetic flux density(B) as a function of the distance (x).

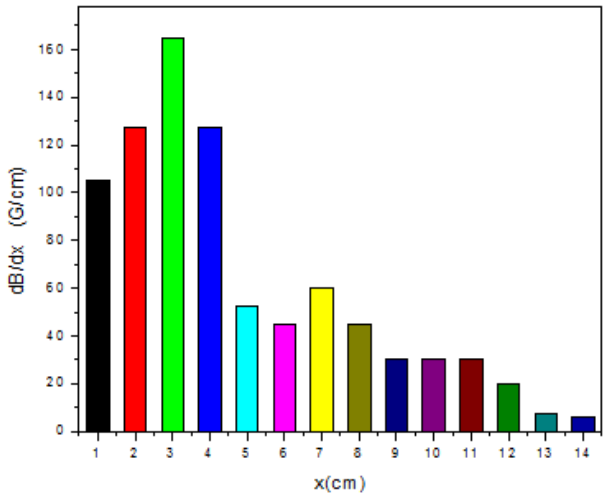


**Fig.2:** The magnitude of the magnetic field gradient (dB/dx) as a function of the distance(x)

The obtained results of the present study are tabulated in Tables (1-4). Table (1) indicated the actual measured values of the magnetic flux density in Gauss units were measured using Tesla meter. 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 magnetic flux density affected the insect under investigation. The value of magnetic flux recorded 21.8, 249 and 863 G for S. littoralis, R. ferrugineus and G. mellonella respectively.

Table (2) indicated the data of biochemical analysis for 5 parameters in the body of the 3 investigated insects. These parameters were: Total protein, total carbohydrates, total lipids, alkaline phosphates enzyme and invertase enzyme. Determination was conducted for samples from larvae of control (without MF) and from treatment (with MF). Values of the measured parameters indicated a clear disturbance in the metabolism and the physiological aspects for the 3 investigated insects, **Fig.3 (a, b& c)** and **Fig.4 (a & b).**

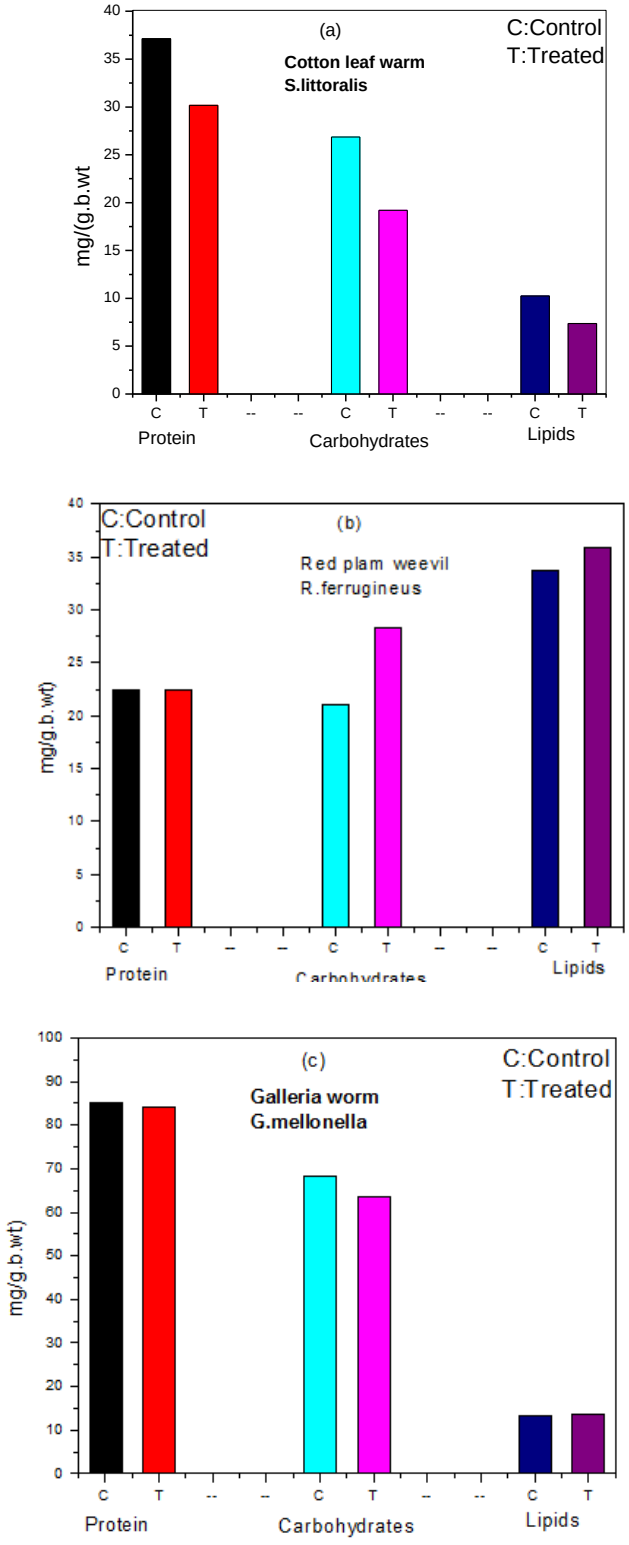


**Fig.3:** Bio-chemical analysis of the main body content in the investigated insects.

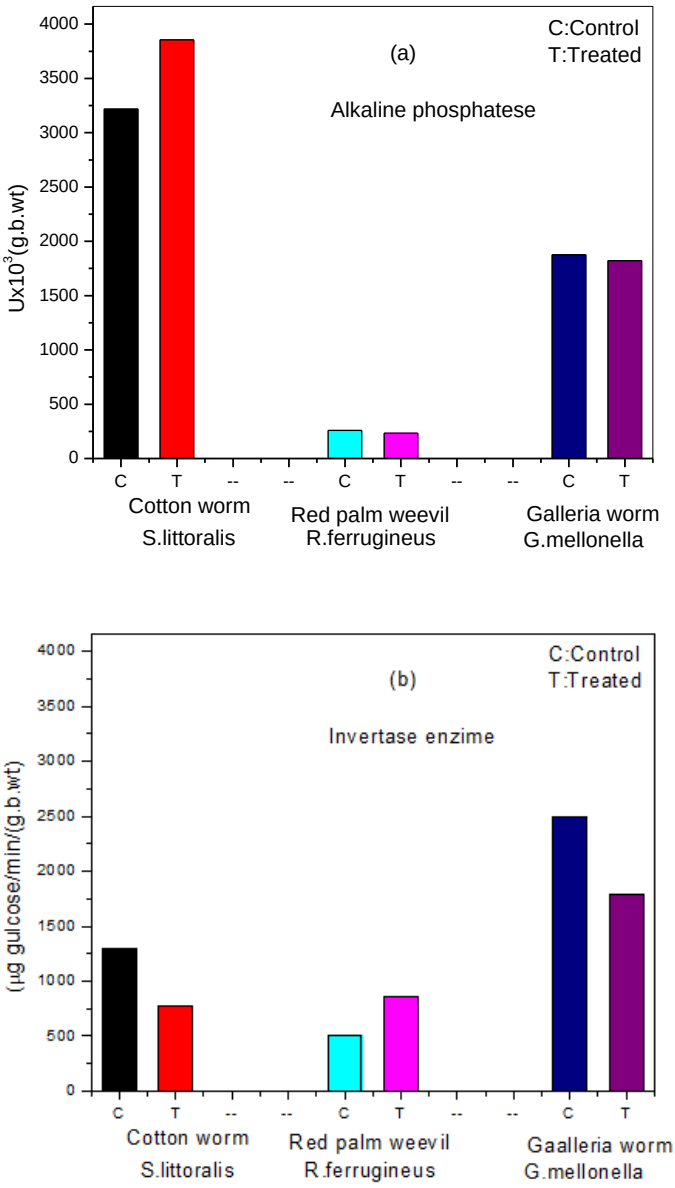


**Fig. 4:** Bio-chemical analysis of enzymes in the investigated insects.

**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 ($Ux10^3$/g.b.wt)** | **Invertase (µg glucose/min/g.b.wt)** |
| **Spodoptera littoralis** | | | | | |
| **C** | 37.13±1.42 | 26.87±1.72 | 10.24±0.74 | 2318±74.28 | 1300 ±74.83 |
| **T** | 30.8±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% |
| **Rhynchophorus ferrugineus** | | | | | |
| **C** | 85.13±1.42 | 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% |
| **Galleria mellonella** | | | | | |
| **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% |

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

In respect of treated S. littoralis larvae analysis showed that the total protein, carbohydrates and lipids recorded 30.80, 19.20 and 7.37 mg/g.b.wt, lower than those of the (control) as they recorded 37.13, 26.87 and 10.24 mg/g.b.wt respectively. About R. ferrugineus the same values 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. Also, 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 $x10^3$/ g.b.wt for treated larvae of S. littoralis against 2318 and 1300 units in control larvae (without MF).

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. About 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 the (control) respectively; **Fig.4 (a& b).**

Table (3) shows body weight in (mg) of 20 larvae of S. littoralis reared for 6 days (through $4^{th}$, $5^{th}$ and $6^{th}$ instars) under 21.8G 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 concentrated in the $5^{th}$ and $6^{th}$ instars.

Table (4) shows the body 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: $6^{th}$, $7^{th}$ and $8^{th}$ 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 | lnstar | 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 |
| May 10/2014 | 4th | 20 | 1.57 | 0.078 | --- | 2.12 | 0.106 | --- |
| 11 | 4th | 20 | 3.06 | 0.153 | 0.075 | 3.62 | 0.181 | 0.075 |
| 12 | 5th | 20 | 4.90 | 0.245 | 0.092 | 6.43 | 0.322 | 0.141 |
| 13 | 5th | 20 | 7.31 | 0.366 | 0.124 | 9.64 | 0.482 | 0.16 |
| 14 | 6th | 20 | 10.21 | 0.511 | 0.145 | 11.38 | 0.569 | 0.087 |
| 15 | 6th | 20 | 8.57 | 0.428 | -0.083 | 8.21 | 0.411 | -0.158 |
| Total (days) | 6 | | | | | | | |
| Gain weight/larva/6days experimental period(mg) | | | | 0.350 | | | 0.305 | |

The obtained results are in harmony with those of Vatanparast et al. (2014) 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. They reported also, presence of Ca, Mg and Na in larval midgut significantly increase 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. Adults weevils response to the broadband infrared radiation. It was reported also, the infrared radiation attract the weevils and lead them to worm please, where they will most likely to their eggs.

El-Shershaby et al. (2008) studied impact of bacillus thuringsensis on protein content and enzyme activity of S. littoralis. They stated that a negative changes in total protein content of treated larvae from 19.8-36.6% were evaluated. Also, they reported that, fluctuated changes in the enzymes activity of treated larvae were found. Shen et al. (1994) studied effects of sub-lethal dosages of Bacillus thuringiensis on the metabolism of G. mellonella larvae, in his results it was reported galleria protein was decreased compared with the normal larvae. The effect of the magnetic field on the studied insects is due to the absorption of energy from the field. also the magnetic moments which existed due to the orbital and spin rotations are aligned due to the applied field, this alignments being opposed by thermal agitation ( Smit et al.(1959)).

**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.

| 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) |
| 2th | 3 | 5 | 1.623 | 0.325 | 5 | 1.598 | 0.32 |
| 3th | 3 | 5 | 12.325 | 2.465 | 4 | 9.864 | 2.466 |
| 4th | 4 | 5 | 29.325 | 5.865 | 4 | 23.276 | 5.819 |
| 5th | 4 | 5 | 76.245 | 15.249 | 4 | 56.824 | 14.206 |
| 6th | 4 | 4 | 170.260 | 42.565 | 4 | 134.760 | 33.690 |
| 7th | 9 | 4 | 250.320 | 62.580 | 4 | 207.167 | 51.792 |
| 8th | 14 | 4 | 402.350 | 100.588 | 4 | 331.678 | 82.920 |
| Total (days) | 41 | | | | | | |
| Gain weight/larva/41days experimental period(mg) | | | | 100.263 | | | 82.600 |
| Gain weight/larva/day(mg) | | | | 2.445 | | | 2.015 |

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