Effect of Volatile Oils and / or Gamma Irradiation on the 4th Instar Larvae of Galleria Mellonella

Mohamed, H.F.; El-Naggar, S.E.M.; Ibrahim, A.A.; Elbarky, N.M. and Salama, M.S.M.

ABSTRACT

This study aimed at studying the effect of five volatile plant oils namely; Mentha piperita (Peppermint), Origanum marjorana (Marjoram), Plargonium graveolens (Geranium), Cymbopogon proximus (Lemon grass) and Ocimum basilicum (Basil), were used at different concentrations (0.625, 1.25, 2.50 and 5.00%) against the Greater wax moth, Galleria mellonella. Also, the combined effect of both gamma irradiations with the dose levels 0, 100 and 150 Gy and the volatile oils were investigated against this pest. The results obtained showed that the percentage of the population was significantly decreased by increasing the concentration at the most treatments. Adult emergence and sex ratio were fluctuated unmatched with the applied dose levels. The survival percentage was inversely correlated with the increase in concentration. In addition the average larval and pupal period significantly increased among all concentrations at all treatments compared with the control treatment. High bioactivity of the botanical oils and gamma-irradiation doses against the Greater wax moth, Galleria mellonella, such results may offer an opportunity for developing alternatives to rather expensive and environmentally hazardous organic insecticides.

KEYWORDS

Gamma irradiation; Essential oils; Galleria mellonella; Pupation; Emergence; Sex ratio; Survival; Larval and pupal period.
INTRODUCTION

The greater wax moth, Galleria mellonella L., is a major pest of the honey bee, Apis mellifera L. It feeds on wax and pollen stored in the combs of active honey bee colonies (Milan, 1970). The extensive use of the synthetic insecticides leads to the destruction of beneficial species such as parasites and predators as well as destruction of pollinating insects such as honey bees. Natural products are an excellent alternative to synthetic pesticides which reduced impacts to human health and the environment (Arnason et al., 1989; Kwon et al., 1996; Ahn et al., 1997; Koul et al., 2008). Several pest control measures have been applied to suppress the damage and keeping beehives clean. Fumigation was also used to wax combs before placed in storage (Caron, 1992). The extensive and continuous use of synthetic pesticides in controlling agricultural pests has created many problems, one of them is the incapability of toxic agents in controlling the target pests at the recommended doses (Prowse et al., 2006; Malarvannan and Subashini, 2007; Khalaf et al., 2009).

The use botanical insecticides to protect crops and stored products is as old as crop protection and considered as major weapons in the farmer’s arsenal against crop pests (Isman, 2008).

Irradiation techniques seem to offer solutions that are desirable in many aspects and prove to be cheaper, safer and more reliable than chemical control. Irradiation shortens life span of insects (Baxter and Blair, 1969) The use of gamma radiation and plant extract are the most promising for controlling Galleria mellonela. The combined effects of gamma irradiation and bioinsecticides on Lepidopterous insects have been studied by many authors (Mohamed, 2004; El-Nagar et al., 2004; El-Shall and Mohamed, 2005; Mohamed, 2006; Mohamed et al., 2014).

The objective of this work is to evaluate the effect of essential oils of the five plants namely; Peppermint, Marjoram, Geranium, Lemon grass, and Basil on some biological aspects, and larval and pupal period of the Greater wax moth, Galleria mellonella. Also, the combined effect of gamma radiation with the dose levels 0,100 and 150 Gy and the volatile oils on the biological aspects of the insect.

MATERIALS AND METHODS

Insect rearing

The strain of the greater wax moth, Galleria mellonella L. used in this study originated from eggs surface sterilized with formalin (5%) vapour treatment as suggested by David et al. (1972) and reared according to Hussein (2004).

The laboratory strain larvae of G. mellonella were reared under laboratory condition 30 ±2 ºC on a diet developed by Wiesner (1993) This media consists of: 22% corn groats (polenta), 22% wheat-flour (full com) or brushed-grain wheat, 11% milk powder (skim-milk), 11% honey, 11% glycerol, 5.5% yeast powder (“brewer’s yeast”, beer yeast).

Source of irradiation

The source of gamma radiation was a Cobalt 60 (60Co) irradiator, Nuclear Research Center, Abu Zaabal, Egypt; the dose rate of at the time of exposure was 1 Gray/ second.

Experimental technique

Volatile oils

Toxicological tests

The susceptibility of the 4th instar larvae of Galleria mellonella to the five plant oils were tested
by the use of contact technique as follows:

Ten 4th larval instar of *Galleria mellonella* (ten replications for each) were chosen for each treatment. The insecticidal activities of the essential oils against 4th instar larvae were evaluated using the contact method at four concentrations (5, 2.5, 1.25 and 0.625 %). The oil was diluted in 1 ml of acetone and applied to 5 cm diameter filter paper. The solvent was allowed to evaporate for 10 min. ten individuals of each test stage were placed in the Petri dish and then covered. In the control group, the filter paper was treated only with acetone. The (LC$_{50}$) were calculated according to the method of Finney (1971).

Insects resulting from such treatments were fed on fresh media and maintained under constant temperature and humidity to determine the following biological criteria: larval and pupal duration, adult emergence, survival, sex ratio and adult longevity.

**Irradiation process**

Full-grown pupae were irradiated with gamma rays by means of Co$^{60}$ source with two sterilizing doses (100 and 150 Gy).

**Toxicological tests**

The susceptibility of the 4th instar larvae of *Galleria mellonella* to plant oils was tested by the use of contact and fumigation technique.

The combined effect of gamma irradiation and different botanical oils on some biological aspects of *Galleria mellonella*

Only two dose levels of gamma irradiation were chosen (100 and 150 Gy) to study the combined effect of gamma irradiation with the (LC$_{50}$) of different botanical oils on some biological aspects. Two experimental groups were set up. The first group consisted of the F$_1$ progeny descendant of the irradiated parental males with 100 and 150 Gy. The second group consisted of the F$_1$ Progeny descendant of the irradiated parental females with 100 and 150 Gy. A parallel group of untreated insects was used as control. The larvae of F$_1$ progeny of each group, were fed on the media mentioned previously till 4th instar, ten replicates from the 4th instar larvae (10 larvae each) were put on 5 cm diameter filter paper treated with the (LC$_{50}$) of the tested oils, in a Petri dish, under laboratory conditions (30±2°C and 65 ±5% relative humidity). Treated insects were observed daily till 96h. Mortalities were recorded among the larval period. Insects resulting from such treatment were fed on fresh media (untreated) after 24h from treatment and maintained under constant temperature and humidity to determine: larval and pupal duration, adult emergence, sex ratio, the percentage survival and the adult longevity.

**Statistical analysis**

Data were analyzed using the Analysis of Variance (ANOVA) technique and the means were separated using Duncans multiple range test (P>0.05) (Steel and Torrie, 1980)
RESULTS

Effect of volatile oils on the 4th instar larvae of Galleria mellonella by using contact method

Biological effects of volatile oils

The biological activity of the tested botanical oils (Mentha piperita, Origanum majorana, Pelargonium graveolens, Cymbopogon proximus and Ocimum basilicum) at four different concentrations against the 4th instar larvae of Galleria mellonella has been studied. The biological activity of these oils included: the percentage of pupation, and the percentage of adult emergence, the percentage of survival, the average of sex ratio and the average of larval and pupal period, the results obtained show the following:

Data in Table (1) demonstrated the biological effects of four different concentrations of the five plant oils tested on the 4th instar larvae of Galleria mellonella. The percentage of pupation was significantly decreased by increasing the concentration at all treatments except, at the concentration 0.625% of the volatile oils M. piperita, P. graveolens, C. proximus and O. basilicum where there were no significant differences compared with the untreated control.

The highest percentage of pupation was 82% at the concentration 0.625% of the volatile oil O. basilicum while the lowest percentage of pupation was 0% at the concentrations 2.5% and 5% of the volatile oil O. basilicum at the concentration 5% of the volatile oil O. majorana and at 2.5% and 5% of the volatile oil O. basilicum compared to 97.89% in the control treatment.

Results in the same table show that the sex ratio was not significantly affected by the five plant oils at all concentrations compared with the control treatment where the highest ratio was 2.05:1 and 2.08:1 (males: females) at the concentrations (0.625% and 1.25%) among the volatile oil P. graveolens and the lowest ratio was 0:1 at the concentrations 5% among the volatile oil O. majorana and at 2.5% and 5% of the volatile oil O. basilicum.

Generally, the sex ratio was around 1.4:1 in control treatment. The data also revealed that the survival percentage was inversely correlated with the increase in concentration. It was significantly decreased by increasing the concentration at all treatments except at the concentration 0.625% of the volatile oils P. graveolens and O. basilicum there was no significant difference between them and the control treatment.

The biological activity of the tested LC50 of the botanical oils against the 4th instar larvae of G. mellonella has been studied (Table 1). The studied parameters included also: the percentage of pupation, the percentage of adult emergence, the percentage of survival, the average of sex ratio.

Data in Table (1) show the biological effects of (LC50) significantly reduced the percentage of pupation as compared with the control and acetone treatment groups. The percentage of pupation reduced to
Effect of Volatile Oils and / or Gamma Irradiation on the 4th Instar Larvae of *Galleria Mellonella*

45, 51, 49, 61 and 32, respectively, for the above-mentioned oils compared with the control treatment.

The percentage of adult emergence was significantly decreased at the LC$_{50}$ of *C. proximus* and *O. basilicum* to 78.29 and 88.17, respectively from the control treatment, but there were no significant differences between the other three oils and the control or acetone treatments.

**Table (1) :** Effect of some volatile oils on some biological aspects of the greater wax moth, *Galleria mellonella* (Contact method).

| Volatile oil          | % Concentration | %Pupation ±SE | %Emergence ±SE | Sex ratio         | %Survival ±SE |
|-----------------------|-----------------|---------------|----------------|------------------|---------------|
| **Mentha piperita**   | 0.625           | 71±5.471a     | 87.13±0.360ab  | 1.45±0.528a      | 62±5.739bc    |
|                       | 1.250           | 52±4.166cd    | 83.00±0.0699ab | 1.08±0.353a      | 45±4.285de    |
|                       | 2.500           | 26±3.715e     | 66.67±10.549b  | 1.15±0.472a      | 19±5.048f     |
|                       | 5.000           | 21±2.771e     | 73.33±11.715ab | 0.70±0.200a      | 17±3.008f     |
|                       | LC$_{50}$       | 45±2.24ed     | 92.50±0.534a   | 1.52±0.320a      | 42±3.590de    |
| **Origanum majorana** | 0.625           | 56±5.816c     | 97.50±0.250a   | 1.03±0.291a      | 56±5.816cd    |
|                       | 1.250           | 41±5.671d     | 95.14±0.305a   | 0.92±0.394a      | 40±5.967e     |
|                       | 2.500           | 11±4.336f     | 50.00±16.679b  | 0.25±0.201a      | 11±4.336g     |
|                       | 5.000           | 00±0.000c     | 00.00±0.000c   | 00.00±0.000c     | 00±0.000g     |
|                       | LC$_{50}$       | 51±4.07c      | 93.00±3.59a    | 1.65±0.340a      | 48±4.900cd    |
| **Pelargonium graveolens** | 0.625           | 78±2.496a     | 98.89±0.1112a  | 2.05±0.513a      | 77±2.136ab    |
|                       | 1.250           | 62±5.337bc    | 95.14±0.305a   | 2.08±0.719a      | 59±5.671cd    |
|                       | 2.500           | 48±6.115d     | 96.67±0.335a   | 0.87±0.165a      | 46±6.004de    |
|                       | 5.000           | 43±2.136d     | 97.50±0.250a   | 1.28±0.352a      | 42±2.496e     |
|                       | LC$_{50}$       | 49±2.34d      | 95.50±0.303a   | 1.40±0.30a       | 47±3.000de    |
| **Cymbopogon proximus** | 0.625           | 74±4.525ab    | 86.83±0.0442ab | 1.12±0.226a      | 64±4.525bc    |
|                       | 1.250           | 67±5.178bc    | 86.78±0.0476ab | 0.40±0.111a      | 60±5.967bc    |
|                       | 2.500           | 17±6.511e     | 50.00±16.679b  | 0.80±0.396a      | 17±6.511f     |
|                       | 5.000           | 07±2.136f     | 50.00±16.679b  | 0.10±0.100a      | 06±2.213g     |
|                       | LC$_{50}$       | 61±5.47bc     | 78.29±0.136b   | 1.40±0.300a      | 47±3.670de    |
| **Ocimum basilicum**  | 0.625           | 82±4.903a     | 89.45±0.3557ab | 1.81±0.481a      | 74±6.004ab    |
|                       | 1.250           | 23±5.787e     | 80.00±13.343ab | 1.15±0.381a      | 23±5.787f     |
|                       | 2.500           | 00±0.000f     | 00.00±0.000c   | 00.00±0.000a     | 00±0.000g     |
|                       | 5.000           | 00±0.000f     | 00.00±0.000c   | 00.00±0.000a     | 00±0.000g     |
|                       | LC$_{50}$       | 32±4.17de     | 88.17±0.5100ab | 1.37±0.230a      | 27.00±3.00f   |
| **Acetone**           | ----            | 79±3.789a     | 95.39±0.1897a  | 1.69±0.208a      | 75±3.075ab    |
| **Control**           | ----            | 86±3.715a     | 97.89±0.1411a  | 1.43±0.228a      | 84±3.402a     |
| **P**                 | 0.0489*         | 00.0000***    | 00.0000***     | 00.0177*         |
| **LSD 0.05**          | 11.3629         | 17.0904       | 8.2135         | 11.9443          |

Means followed by the same letter in each column (small letters) representation that are not significantly different at (p > 0.05)
Results in the same table showed no significant difference among the sex ratio of treated and untreated insects. The sex ratio seemed to be skewed to male side in treated insects, where the highest ratio was 1.65:1 and 1.60:1 (males: females) at the LC$_{50}$ of the two volatile oils O. majorana and C. proximus, respectively, while the lowest ratio was 1.37:1 at the LC$_{50}$ of the volatile oil O. basilicum. Generally, the sex ratio was around 1.01:1 in control treatment and around 1.31:1 in acetone treatment.

The data also revealed that the percentage of survival significantly decreased at all treatments when compared with the control and acetone treatments. The highest decrease in survival was 27% from the untreated control at the LC$_{50}$ of the volatile oil O. basilicum. Acetone treatment significantly decreased the percentage of survival than control treatment.

**Effect of volatile oils on the 4th instar larvae of Galleria mellonella by using fumigation method:**

**Biological effects of volatile oils**

The biological activity of the tested botanical oils (M. piperita, O. majorana, P. graveolens, C. proximus and O. basilicum) at different concentrations against the 4th instar larvae of G. mellonella has been studied. The biological activity of these oils included: the percentage of pupation, the percentage of adult emergence, the percentage of survival and the average of sex ratio. The results obtained can be illustrated as follows:

Data in Table (2) demonstrated the biological effects of different concentrations of the five plant oils tested on the 4th instar larvae of G. mellonella. The percentage of pupation significantly decreased by increasing the concentration at all treatments except, at the concentration 0.625% of the volatile oil M. piperita and the concentrations 0.625 and 1.25% of the volatile oil P. graveolens, where no significant differences was found compared with the control and acetone treatments. The highest percentage of pupation was 96 at the concentration 0.625% of the volatile oil P. graveolens while the lowest percentage of pupation was 52 from the untreated control in case of the concentrations 2.5% and 5% of the volatile oil C. proximus. The percentage of pupation in case of acetone treatment was 90 compared with 96 in control treatment.

As shown from data of the adult emergence percentage (Table, 2) not all viable pupae succeed to continue their development to produce viable adults. The percentage of adult emergence was significantly decreased among the four concentrations of the volatile oil M. piperita and among the two concentrations 2.5 and 5% of the volatile oil O. majorana. The highest percentage of adult emergence was 100 from the untreated control at the two concentrations 1.25 and 2.50 % of the volatile oil C. proximus and the lowest percentage of adult emergence were 56.55 and 56.76 from the untreated control at the concentration 5% of the two volatile oils O. majorana and M. piperita, respectively. While the percentage of adult emergence in case of acetone was 95.50 compared with 96 in control treatment.

Results in the same table showed that the sex ratio seemed to be skewed to male side in insects treated with the five plant oils at all concentrations where the highest ratio was 2.5:1 (males: females) at the concentration (2.5%) of the volatile oil M. piperita and the lowest ratio was 0.63:1 and 0.77:1 at the concentrations 1.25% of the volatile oil O. basilicum and 0.625% of the volatile oil O. majorana. Generally, the sex ratio was around 1.1:1 in control treatment and around 1.30:1 in acetone treatment.

The data also revealed that the percentage of survival significantly decreased by increasing the concentration at all treatments. The highest decrease
was 40% at the concentration 5% of the volatile oil *O. majorana*. The percentage of survival in the acetone treatment was 86% compared with 95.78% in the control treatment.

**Table (2): Effect of various volatile oils on some biological aspects of the greater wax moth, Galleria mellonella (Fumigation method).**

| Volatile oil         | % Concentration | % Pupation ±SE | % Emergence ±SE | Sex ratio | % Survival ±SE |
|----------------------|-----------------|----------------|-----------------|-----------|----------------|
|                      |                 |                |                 | Male ±SE  | Female         |
| Mentha piperita      | 0.625           | 92±2.65 a      | 070.03±4.24bc   | 1.62±0.45ab | 1 72.00±4.69bcdef |
|                      | 1.250           | 84±2.83 abc    | 068.81±6.47 c   | 1.72±0.58ab | 1 66.00±4.25cdefg |
|                      | 2.500           | 82±2.65 abcd   | 060.95±4.94 c   | 2.50±0.34a  | 1 60.00±3.16defgh |
|                      | 5.000           | 80±2.24 abcd   | 056.76±2.24 c   | 1.53±0.26ab | 1 56.00±4.79efgh  |
| Origanum majorana    | 0.625           | 86±4.79 ab     | 093.14±3.04 a   | 0.77±0.13 b | 1 80.00±5.00abcd  |
|                      | 1.250           | 86±3.61 ab     | 088.69±3.39 a   | 1.68±0.32ab | 1 76.00±3.61abcde |
|                      | 2.500           | 82±2.65 abcd   | 066.94±7.12 c   | 1.58±0.34ab | 1 54.00±4.97efgh  |
|                      | 5.000           | 70±3.16 bce    | 056.55±7.77 c   | 1.53±0.44ab | 1 40.00±5.92h     |
| Pelargonium graveolens| 0.625         | 96±1.73 a      | 095.78±1.83 a   | 1.37±0.19ab | 1 92.00±2.65ab    |
|                      | 1.250           | 90±3.16 a      | 097.50±1.77 a   | 1.37±0.39ab | 1 88.00±4.13ab    |
|                      | 2.500           | 68±5.66 ede    | 093.14±3.04 a   | 0.97±0.46ab | 1 64.00±6.56defg  |
|                      | 5.000           | 62±2.65 ef     | 084.75±4.93 ab  | 1.90±0.39ab | 1 52.00±2.65fgh   |
| Cymbopogon proximus  | 0.625           | 64±4.13 def    | 096.00±2.83 a   | 1.38±0.16ab | 1 60.00±5.00defgh |
|                      | 1.250           | 60±5.48 ef     | 100.00±0.00 a   | 1.60±0.25ab | 1 60.00±5.48defgh |
|                      | 2.500           | 52±2.65 f      | 100.00±0.00 a   | 1.65±0.61ab | 1 52.00±2.65fgh   |
|                      | 5.000           | 52±4.69 f      | 097.14±2.02 a   | 1.80±0.43ab | 1 50.00±3.88gh    |
| Ocimum basilicum     | 0.625           | 82±4.69 abcd   | 097.50±1.77 a   | 1.25±0.32ab | 1 80.00±5.00abcd  |
|                      | 1.250           | 82±2.65 abcd   | 092.06±3.96 a   | 0.63±0.23 b | 1 76.00±4.79abcde |
|                      | 2.500           | 80±3.16 abcd   | 088.92±3.76 a   | 1.52±0.24ab | 1 72.00±5.66bcdef |
|                      | 5.000           | 68±2.83 cde    | 096.67±2.36 a   | 1.43±0.22ab | 1 64.00±3.61defg  |
| Acetone              | ---             | 90±2.24 a      | 095.50±1.97 a   | 1.30±0.17ab | 1 86.00±2.83abc   |
| Control              | ---             | 96±1.73 a      | 096.00±1.73 a   | 1.10±0.19ab | 1 95.78±1.83a     |

**Means followed by the same letter in each column (small letters) representation that are not significantly different at (p > 0.05)**

Combined effect of the irradiated parental males and females of the greater wax moth, *Galleria mellonella* with 100 and 150 Gy of gamma radiation and treated the *F*₁ larvae with the LC₅₀ of some volatile oils on some biological aspects

The biological effects of LC₅₀ of the tested botanical oils (*M. piperita*, *P. graveolens* and *O. majorana*) against the 4th instar larvae of *Galleria mellonella* descendant of irradiated parental males mated with normal females are shown in Table (3).

The biological effects of these oils included: larval and pupal period, the percentage of pupation, the percentage of emergence, sex ratio and the percentage of Survival among F₁ larvae of *Galleria mellonella* descendant of the irradiated parental males.

The mean larval and pupal periods (Table 3) were significantly increased at all treatments when compared with the control and acetone treatments. It was higher at the combined treatments than the other treatments; it was 48.45, 43.73 and 47.48 days.
at the three volatile oils (M. piperita, P. graveolens and O. majorana) combined with the dose 100 Gy, respectively, compared with 35.10 and 38.51 days in control and acetone treatments, respectively. Also, significantly increased to 51.31, 48.56 and 50.81 days at the three volatile oils combined with the dose 150 Gy, respectively.

The results recorded in Table (3) show that the percentage of pupation was significantly decreased by increasing the dose rates of gamma radiation and in combined than the other treatments.

The percentage of pupation significantly decreased to 29, 32 and 43 % among the three volatile

| Treatment | Average larval and pupal period / day ±SE | %Pupation ±SE | %Emergence ±SE | Sex ratio | %Survival ±SE |
|-----------|------------------------------------------|---------------|----------------|-----------|---------------|
| control   | 35.1±0.38*                               | 93.00±1.53*   | 97.78±1.48*    | 1.02±0.08* | 91.0±2.34*    |
| acetone   | 38.51±0.64 t                             | 86.00±3.72*   | 95.39±1.89*    | 1.56±0.28* | 75.0±3.08 b   |
| 100 Gy    | 45.57±0.88 ed                            | 69.50±2.93 b  | 89.32±2.41 ab  | 1.23±0.25* | 62.0±2.81 c   |
| Mentha piperita | 43.19±0.68 e | 45.00±2.24 c | 92.50±5.34 ab  | 1.51±0.32 a | 42.0±3.59 de   |
| 100 Gy + Mentha piperita | 48.45±0.85 b | 29.00±3.48 de | 90.00±5.53 ab  | 1.12±0.30 a | 26.0±3.40 k   |
| Pelargonium graveolens | 39.69±0.69 f | 49.00±2.34 e | 95.50±3.03 a  | 1.40±0.29 a | 47.0±3.00 d   |
| 100 Gy + Pelargonium graveolens | 43.73±0.75 de | 32.00±4.17 a | 90.50±3.91 ab  | 1.15±0.19 a | 28.0±3.40 k   |
| Origanum majorana | 42.21±0.82 e | 51.00±4.07 c | 93.00±3.59 ab  | 1.65±0.34 a | 48.0±4.90 d   |
| 100 Gy + Origanum majorana | 47.48±0.85 bc | 43.00±2.14 c | 92.50±3.82 ab  | 1.62±0.35 a | 40.0±2.98 e   |
| 150 Gy    | 46.77±0.27 bc                            | 63.00±3.09 b  | 58.29±2.48 a   | 1.56±0.43 a | 36.5±1.83 ef  |
| 150 Gy + Mentha piperita | 51.31±0.64 b | 20.00±2.58 b | 80.00±6.94 b   | 1.10±0.23 a | 15.0±1.6 b    |
| 150 Gy + Pelargonium graveolens | 48.56±0.59 b | 27.00±3.35 de | 61.67±7.78 a  | 1.05±0.19 a | 17.0±2.61 b   |
| 150 Gy + Origanum majorana | 50.81±0.59 a | 32.00±3.27 d | 65.17±5.79 c  | 1.10±0.31 a | 21.0±2.77 oh  |
| LSD 0.05 | 1.9224 | 8.6432 | 12.7808 | 0.7901 | 8.5222 |
| P        | 0.1262 ns | 0.2264 ns | 0.000* | 0.0102* | 0.2064 |

Means followed by the same letter in each column (small letters) representation that are not significantly different at (p > 0.05)
Effect of Volatile Oils and/or Gamma Irradiation on the 4th Instar Larvae of Galleria Mellonella

oils (M. piperita, P. graveolens and O. majorana) combined with the dose 100 Gy, respectively, also, significantly decreased to 20, 27 and 32% at the three volatile oils combined with the dose 150 Gy, respectively, compared with 93% in the untreated control.

Statistical analysis of data shows that 150 Gy treatment and the tested botanical oils combined with 150 Gy significantly reduced the percentage of adult emergence as compared with that of untreated control and acetone treatments. It significantly reduced to 58.29, 80.00, 61.67 and 65.17% among the treatments 150 Gy, 150 Gy + M. piperita, 150 Gy + P. graveolens and 150 Gy + O. majorana, respectively, compared with 97.78% and 95.39% in the control and acetone treatment, respectively, while, the other treatments were not significantly reduced when compared with the control and acetone treatments.

Data in Table (3) also demonstrates that the sex ratio of F₁ generation was skewed in favour of males at all treatments.

The percentage of adult survival was significantly decreased at all treatments especially in case of the combined treatments as shown in Table (3).

Table (4) illustrates the combined effect of irradiated parental females of the greater wax moth, Galleria mellonella with 100 and 150 Gy of gamma radiation and treated the F₁ generation with the LC₅₀ of some volatile oils on some biological aspects.

The biological activity of the tested botanical oils (M. piperita, P. graveolens and O. majorana) with the concentration (LC₅₀) combined with 100 and 150 Gy of gamma radiation against the 4th instar larvae of G. mellonella resulted from irradiated parental females mated with normal males has been studied. The biological activity of these volatile oils combined with gamma radiation included: larval and pupal period, the percentage of pupation, the percentage of emergence, sex ratio and the percentage of survival.

The mean larval and pupal periods (Table, 4) were significantly prolonged at all treatments when compared with the untreated control and acetone treatments, especially, at the combined treatments as we mentioned previously in Table (3).

The results recorded in (Table, 4) shows also that the percentage of pupation was significantly reduced at all treatments when compared with the untreated control and acetone treatments. The highest reduction was at the combined treatments 150 Gy with the LC₅₀ of the three volatile oils, it reduced to 16, 21 and 20% among the three treatments 150 Gy + M. piperita, 150 Gy + P. graveolens and 150 Gy + O. majorana, respectively, compared with 93% in the control treatment.

The percentages of adult emergence were significantly reduced at many treatments (Table, 4). They were reduced to 63.69, 74.17, 70.33, 52.51, 59.17 and 60.83% among the treatments 100 Gy, 100 Gy + P. graveolens, 100 Gy + O. majorana, 150 Gy, 150 Gy + P. graveolens and 150 Gy + O. majorana, respectively, compared with 97.78% in the control treatment.

Data in (Table, 4) also demonstrates that the sex ratio of F₁ generation was tending in favor of males at all treatments, except, at the two treatments 150 Gy + M. piperita and 150 Gy + P. graveolens which was nearly the same as the control treatments (1.10) while the treatment 150 Gy + O. majorana, it reduced to 0.65 from the untreated control.

The data in (Table, 4) shows that the percentage of survival was decreased at all treatments. The highest decrease occurred was 13, 12 and 13% at the treatments 150 Gy + M. piperita, 150 Gy + P. graveolens and 150 Gy + O. majorana, respectively, compared with 91% in the untreated control.
DISCUSSION

Irradiation studies on several insects’ species indicated that, the pupal stage was generally the most suitable stage for treatment as it was easier to handle and leads to a reduction in reproductive potential (Proverbs and Newton, 1962a&b). Gamma-irradiation looks more effective and safer (Ahmed et al., 1985). Hallman (2003) suggested that normal growth, development or reproduction of the organ-

Table (4): Combined effect of the irradiated parental females of the greater wax moth, Galleria mellonella with 100 and 150 Gy of gamma radiation and treated the F₁ larvae with LC₅₀ of some volatile oils on some biological aspects.

| Treatment            | Average Larval And pupal Period / day ±SE | % Pupation ±SE | % Emergence ±SE | Sex ratio                      | % Survival ±SE |
|----------------------|------------------------------------------|----------------|-----------------|-------------------------------|----------------|
| control              | 35.1±0.38 i                             | 93.00±1.53a     | 97.78±1.48a     | 1.02±0.08 bc                  | 91.0±2.3a      |
| acetone              | 38.51±0.64 h                            | 86.00±3.72a     | 95.39±1.89a     | 1.56±0.28ab                   | 75.0±3.0 b     |
| 100 Gy               | 45.28±0.57 de                           | 50.50±3.29b     | 63.69±1.63 de   | 1.60±0.15ab                   | 32.0±2.0 d     |
| Mentha piperita      | 43.19±0.68 fg                           | 45.00±2.24bc    | 92.50±5.34ab    | 1.51±0.32ab                   | 42.0±3.5 c     |
| 100 Gy + Mentha piperita | 49.41±0.70 e                          | 28.00±2.91ef    | 87.50±5.16abc   | 1.30±0.26abc                  | 24.0±2.6 de    |
| Pelargonium graveolens | 39.69±0.69 h                            | 49.00±2.34b     | 95.50±3.03a     | 1.40±0.29abc                  | 47.0±3.0 c     |
| 100 Gy + Pelargonium graveolens | 44.15±0.97 ef                        | 32.00±2.49de    | 74.17±6.90bcd   | 1.23±0.22abc                  | 24.0±3.1 de    |
| Origanum majorana    | 42.21±0.82 g                            | 51.00±4.07b     | 93.00±3.59a     | 1.65±0.34ab                   | 48.0±4.9 c     |
| 100 Gy + Origanum majorana | 48.58±0.44 e                          | 38.00±3.27cd    | 70.33±4.80cde   | 1.85±0.33 a                    | 27.0±3.0 d     |
| 150 Gy               | 46.13±0.30 d                            | 32.00±3.00de    | 52.51±2.35 e    | 1.85±0.24 a                    | 16.5±1.5 ef    |
| 150 Gy + Mentha piperita | 52.53±0.69 a                          | 16.00±3.06 g    | 78.33±10.56abc  | 1.10±0.21bc                   | 13.0±2.1 f     |
| 150 Gy + Pelargonium graveolens | 49.88±0.75 bc                         | 21.00±3.79fg    | 59.17±10.87de   | 1.10±0.26 bc                   | 12.0±2.0 f     |
| 150 Gy + Origanum majorana | 51.67±0.64ab                          | 20.00±3.65fg    | 60.83±9.14de    | 0.65±0.15c                    | 13.0±2.6 f     |
| LSD 0.05             | 1.8437                                  | 8.6935         | 16.9379         | 0.6931                        | 8.0654         |

Means followed by the same letter in each column (small letters) representation that are not significantly different at (p > 0.05)
ism might be prevented by sub lethal doses of irradiation, while lethal doses of irradiation could kill insects immediately.

Lepidoptera’s insects require high doses of irradiation to achieve fully sterilized adults, these doses often render them less competitiveness than unpredicted, therefore, using sub sterilizing doses of radiation are increased competitiveness of released insects and possible integration with other non – polluting methods to control insects pests North & Holt (1968a&b).

The botanical oils and gamma-irradiation doses used in the present study are not toxic to vertebrates as well as they are cheap, the present study showed high bioactivity of the botanical oils and gamma-irradiation doses against the Greater wax moth, Galleria mellonella, such results may offer an opportunity for developing alternatives to rather expensive and environmentally hazardous organic insecticides.

The present study showed that the tested plant oils affected the adult longevity of males and females, the fecundity and fertility. Similar findings, were also obtained by many authors using different botanical oils Abdel El-Aziz et al. (2007) on A. ipsilon, Moawad and Ebadah, (2007) on Phthorimaea operculelle, El-Naggar et al. (2012) on Spodoptera littoralis, Yazdani et al. (2014) on the lesser mulberry pyralid, Glyphodes pyloalis. Karabörklü et al. (2010) on red flour beetle, Tribolium castaneum, Abd-El-Aziz (2011) on Ph. operculella, and Hamza et al. (2014) on Sitophilus granaries (L.) whom stated that the essential oils tested showed a significant fumigant insecticidal activity against different stages. The oils revealed weak fumigant activity against the pupal stage but both adult males and females showed high susceptibility to the fumigation.

El-Shall et al. (2005), in their study on S. littoralis, found that ethanol, petroleum either and chloroform extracts of Eucalyptus camaldulensis induced serious chronic effect on larvae, pupae and adult emergence when used alone or combined with gamma radiation. In addition, Sileem (2004) declared that, the effect of gamma irradiation and extracts from Malissa azedrach fruits or Schintis terebinthifdies leaves on Agrotis ipsilon, used alone or combined, reduced the development of larvae or pupae and inhibited adult emergence. The present study indicated that the combined effect of gamma irradiation and botanical oils induced more remarkable effects as compared to gamma irradiation or botanical oils each of them alone. These results are in accordance with that demonstrated by El-Shall and Mohamed (2005) using barnooof plant extract combined with gamma irradiation against A. ipsilon. As well, the present study indicated that treatment of both partners of mating pair induced more remarkable effects than did treatment of either sex separately.

CONCLUSION

Finally, in this work, we attempted to control Greater wax moth (GWM) with certain volatile plant oils which seem to be safer and less contaminant to bees and humans. Also, these materials are cheap, available to beekeepers, and could be used to control other hive infestations e.g. Varroa and acarine mites etc. Also, we conclude that the combined treatment with gamma irradiation and plant volatile oil were establish results more significantly affected than both gamma radiation and plant volatile oil each of them alone.

The sterilizing dose for male was 250 Gy, The sterilizing dose for female was 200 Gy., So we used the substerilizing doses 100 and 150 Gy at the combined treatment. The best oil was Ocimum basilicum, Mentha piperita, pelargonium graveolens, and Origanum majorana then cymbopogon proxi-
We recommended using the oil Mentha piperita with the dose 150 Gy for integrated pest management.

REFERENCES

- Abd El-Aziz, M.F. (2011): Bioactivities and Biochemical Effects of Marjoram Essential Oil used against Potato Tuber Moth, Phthorimaea operculella Zeller (Lepidoptera: Gelechiidae). Life Sci. J., 8(1): 288.

- Abdel-Aziz, S.E.; Elsayed, A.O. and Aly, S.S. (2007): Chemical composition of Ocimum americanum essential oil and its biological effects against, Agrotis ipsilon, (Lepidoptera: Noctuidae) Rese. J. Agric. Biol. Scie., 3(6): 740.

- Ahmed, M.Y.Y.; El-banby, M.A.; Salem Y.S. and Abdel-Baky, S.M. (1985): Ephestia kuehiella Z: Gamma Irradiation Effects on the Adult Stage and Mating Competitiveness of Sterile Males. Arab J. Nuc. Sci. Appl., 18(1):17.

- Ahn, Y.J.; Kwon, M.; Park, H.M. and Han, C.G. (1997): Potent insecticidal activity of Ginkgo biloba-derived trilactone terpenes against Nilaparvata lugens. In phytochemical pest control Agents; Hedin, P. Hollingworth, R., Miyamoto, J., Masler, E., Thompson, D., Eds.; Acs Symposium series 658; American Chemical society: Washington, DC, pp. 90-105.

- Aranson, J.T.; Philogne, B.J.R. and Morand, P. (1989): Insecticides of plant origin; ACS symposium series 387; Amer. Chem. Soc., Washington, DC, pp.164-172.

- Baxter, R.C. and Blair, H.A. (1969): Recovery and over recovery from acute radiation injury as a function of age in Drosophila. Radiat. Res., 39: 345.

- Caron, D.M. (1992): Wax Moth. American Bee Journal, Vol. 132 (10): 647-49, USA.

- David, W.A.L.; Elloby, S. and Taylor, G. (1972): The Fumigant action of formaldehyde incorporated in a semi synthetic diet on the granulosis of Pieris brassicae and its evaporation from the diet. J. Invertebr. Pathol., 19: 76.

- El-Nagar, T.F.K.; Abdel Fattah, H.M.; Khaled, A.S. and Aly, S.A. (2012): Efficiency of Peppermint oil Fumigant on Controlling Callosobruchus maculatus F. Infesting Cowpea Seeds. Life Sci. J., 9(2): 375.

- El-Naggar, S.E.M.; Ibrahim, S.M. and Mohamed, H.F. (2004): Combined and separate effects of gamma irradiation and barnoof plant extract on the dietary profile of the black cutworm, Agrotis ipsilon (Hufn.) I-Treatment of the eight days-old larvae. Seventh Arab Conference on the Peaceful Applications of Atomic Energy, Sanna, a,Yemen; 4–8 Dec.

- El-Shall, S.S.A. and Mohamed, H.F. (2005): The combined effect of gamma irradiation and plant extract (Barnoof) on the nutritional profile of the Black cutworm, Agrotis ipsilon (Hufn.) (Lepidoptera: Noctuidae), II- the effect on the F1 progeny during the 5th and 6th instars larvae. Arab J. Nucl. Sci. Appl., 38(2): 289.

- Finney, D.J. (1971): Probit Analysis, 3th Edition. Cambridge University, London. 333 pp.

- Hallman, G.J. (2003): Ionizing irradiation quarantine treatment against Plum curculio (Coleoptra: curculianidae). J. Econ. Ent., 95: 1399.

- Hamza, A.F.; El- Orabi, M.N.; Gharieb, O.H.; El-Saeady, A.A. and Hussein, A.E. (2014): Response of Sitophilus granaries (L.) to Fumigant Toxicity of some Plant Volatile Oils. 4th International Conference on Radiation Sciences and Applications, 13-17/10/2014, Taba, Egypt.

- Hussein, M.A. (2004): Utilization of entomopathogenic nematodes for the biological control of some lepidopterous pest Entomology (BioControl). Ph. D. Thesis. Faculty of Science, Ain Shams University, Egypt Pp.203

- Isman, M.B. (2008): Perspective Botanical insecticides: for richer, for poorer. Pest Manag. Sci., 64(1):8.

- Karabörklü, S.; Ayvaz, A. and Yilmaz, S. (2010): Bioactivities of Different Essential Oils against the Adults of Two Stored Product Insects. Pakistan J.
Effect of Volatile Oils and/or Gamma Irradiation on the 4th Instar Larvae of *Galleria Mellonella*

- **Khalaf, A.A.; Hussein, K.T. and Shoukry, K.K. (2009):** Biocidal activity of two botanical volatile oils against the larvae of *Synthesiomyia nudiseta* (Wulp) (Diptera: Muscidae). *Egypt. Acad. J. Biol. Sci.*, 2(1): 89.

- **Koul, O.; Walia, S. and Dhiwal, G.S. (2008):** Essential Oils as Green Pesticides: Potential and Constraints. *Biopestic. Int.*, 4(1): 63.

- **Kwon, M.; Ahn, Y.J.; Yoo, J.K. and Choi, B.R. (1996):** Potent insecticidal activity of extracts from *Ginkgo biloba* leaves against *Nilaparvata lugens* (Homoptera: Delphacidae). *Appl. Entomol., Zool.*, 31(L): 162.

- **Malarvannan, S. and Subashini, H.D. (2007):** Effect of *Dodonaea angustifolia* crude extract on biochemical profile of *Helicoverpa armigera* (Hubner) (Noctuidae: Lepidoptera). *Biochem. Cellular Arch.*, 7 (1): 1.

- **Milan, V.G. (1970):** Moth pests of honey bee combs. Glean. Bee Culture, 68: 424.

- **Moawad, S.S. and Ebadah, I.M.A. (2007):** Impact of some natural plant oils on some biological aspects of potato tuber moth *Phthorimaea operculella*, (Zeller) (Lepidoptera: Gelechiidae). *Rese. J. Agric. Biol. Sci.*, 3(2) 119.

- **Mohamed, H. F.; El-Naggar, S. E.; Elbarky, N. M.; Ibrahim, A. A. and Salama, M. S. (2014):** The impact of each of the essential oils of marjoram and lemon grass in conjunction with gamma irradiation against the Greater Wax Moth, *Galleria Mellonella*. *IOSR-JPBS*, 9(5):92.

- **Mohamed, H.F. (2006):** Effects of gamma irradiation and leaves extract of barnoof plant on larval development of *Agrotis ipsilon* (Hufngel). *Arab J. Nucl. Sci. Appl.*, 39 (2), 255.

- **Mohamed, H.F. (2004):** Inherited Sterility Induced by Gamma Irradiation and/or Barnoof Plant Extract on Reproductive Potential and Mating Ability of the Black Cutworm, *Agrotis ipsilon* (Hufngel). (Lepidoptera: Noctuidae), *Isotopes & Radiat. Res.*, 36 (4): 713.

- **North, D.T. and Holt, G.G. (1968a):** Inherited sterility in progeny of irradiated male cabbage loopers. *J. Econ. Entomol.*, 61: 928.

- **North, D.T. and Holt, G.G. (1968b):** Genetic and cytogenetic basis of radiation-induced sterility in the adult male cabbage looper, *Trichoplasia Ni*. Isotopes & Radi. In Entomol, 391.

- **Proverbs, M.D. and Newton, J.R. (1962 a):** Influence of gamma radiation on the development and fertility of the codling moth, *Carpocapsa pomonella*. *Canad. J. Zool.*, 40: 401.

- **Proverbs, M.D. and Newton, J.R. (1962 b):** Some effects of gamma radiation on the reproductive potential of the codling moth, *Carpocapsa pomonella*. *Canad. Entomol.*, 94:1162.

- **Prowse, G.M.; Galloway, T.S. and Foggo, A. (2006):** Insecticidal activity of garlic juice in two dipteran pests. *Agric. Forest. Entomol.* 8(1): 1.

- **Sileem, T. M. (2004):** Effect of Gamma Radiation and Some Plant Extracts on the Black Cutworm, *Agrotisipsilon*” M. Sc. Thesis, Plant Protection Dep., Faculty of Agric. Moshtohor Zagazig Univ. Banha Branch, Egypt.PP:175.

- **Steel, R.G.D and J.H. Torrrie. (1980):** Principle and procedures of 2nd ed., McGraw-Hill book Co., New York.

- **Wiesner, A. (1993):** Die Induktion der Immunabwehreines Insekts (*Galleria mellonella*, Lepidoptera). Durch Synthetische Materialien und Arteigen Haemolymphfaktoren, Berlin.

- **Yazdani,E.; Sendi,J. J. and Hajizadeh, J. (2014):** Effect of *Thymus vulgaris*L. And*Origanum vulgare*L. essential oils on toxicity,food consumption, and biochemical properties of lesser mulberry pyralid *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae). *J. Plant Prot. Res.*, 54(1): 54.
