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

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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.

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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 ^{0}C 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 (^{60}Co) 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 substerilizing doses (100 and 150Gy).

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 latent effect of investigated botanical oils on some biological aspects of Galleria mellonella

According to the high mortality percentage of *Mentha piperita*, *Plargoniumgraveolens*, *Origanum marjorana, Ocimum basilicum* and *Cymbopogon proxiomus* were used in the determination of biological activity and biochemistry. Only one concentration (LC_{50}) was chosen to study the effect of botanical oils on some biological aspects of *Galleria mellonella*.

Insects resulting from such treatments were fed on fresh media (untreated), 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.

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^{0}Cand 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* compared to 86% in the control treatment.

As shown from data, adult emergence and sex ratio were unmatched with the dose increasing. The adult emergence percentage **(Table, 1)**, revealed that not all viable pupae succeed to continue their development to produce viable adults. The adult emergence percentage was significantly decreased among the concentration 2.5% of the volatile oil *M. piperita* also, among the concentrations 2.5% and 5% of the volatile oils *O. majorana*, *C. proximus* and *O. basilicum*. The highest percentage of adult emergence was 98.89 % at the concentration 0.625% of the volatile oil *P. graveolens* and the lowest percentage of adult emergence was 0% at the concentration 5% of the volatile oil *O.majorana* and at the concentrations 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 LC_{50} 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 (LC_{50}) significantly reduced the percentage of pupation as compared with the control and acetone treatment groups. The percentage of pupation reduced to

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
				Male±SE	Female	
Mentha piperita	**0.625**	71±5.471**ab**	87.13±03.360**ab**	1.45±0.528**a**	1	62±5.739**bc**
	1.250	52±4.166 **cd**	83.00±06.099**ab**	1.08±0.353**a**	1	45±4.285**de**
	2.500	26±3.715 **e**	66.67±10.549 **b**	1.15±0.472**a**	1	19±5.048 **f**
	5.000	21±2.771 **e**	73.33±11.715**ab**	0.70±0.200**a**	1	17±3.008 **f**
	LC_{50}	45±2.24 **cd**	92.50±05.340 **a**	1.52±0.320 **a**	1	42±3.590 **de**
Origanum majorana	**0.625**	56±5.816 **c**	97.50±02.502**a**	1.03±0.291**a**	1	56±5.816 **cd**
	1.250	41±5.671 **d**	96.67±03.335**a**	0.92±0.394**a**	1	40±5.967 **e**
	2.500	11±4.336 **f**	50.00±16.679 **b**	0.25±0.201**a**	1	11±4.336 **g**
	5.000	00±0.000 **f**	00.00±0.000 **c**	0.00±0.000**a**	1	00±0.000 **g**
	LC_{50}	51±4.07 **c**	93.00±3.59 **a**	1.65±0.340 **a**	1	48±4.900 **cd**
Pelargonium graveolens	**0.625**	78±2.496**a**	98.89±01.112**a**	2.05±0.513**a**	1	77±2.136**ab**
	1.250	62±5.337 **bc**	95.14±03.305**a**	2.08±0.719**a**	1	59±5.671 **cd**
	2.500	48±6.115 **d**	96.67±03.335**a**	0.87±0.165**a**	1	46±6.004 **de**
	5.000	43±2.136 **d**	97.50±02.502**a**	1.28±0.352**a**	1	42±2.496**e**
	LC_{50}	49±2.34 **d**	95.50±03.030 **a**	1.40±0.30 **a**	1	47±3.000 **de**
Cymbopogon proximus	**0.625**	74±4.525**ab**	86.83±04.042**ab**	1.12±0.226**a**	1	64±4.525**bc**
	1.250	67±5.178 **bc**	86.78±04.076**ab**	0.40±0.111**a**	1	60±5.967 **bc**
	2.500	17±6.511 **e**	50.00±16.679 **b**	0.80±0.396**a**	1	17±6.511 **f**
	5.000	07±2.136 **f**	50.00±16.679 **b**	0.10±0.100**a**	1	06±2.213 **g**
	LC_{50}	61±5.47 **bc**	78.29±03.160 **b**	1.40±0.300 **a**	1	47±3.670 **de**
Ocimum basilicum	**0.625**	82±4.903**a**	89.45±03.557**ab**	1.81±0.481**a**	1	74±6.004**ab**
	1.250	23±5.787 **e**	80.00±13.343**ab**	1.15±0.381**a**	1	23±5.787 **f**
	2.500	00±0.000 **f**	00.00±00.000 **c**	0.00±0.000**a**	1	00±0.000**g**
	5.000	00±0.000 **f**	00.00±00.000 **c**	0.00±0.000**a**	1	00±0.000 **g**
	LC_{50}	32±4.17 **de**	88.17±05.100**ab**	1.37±0.230 **a**	1	27.00±3.00 **f**
Acetone	**----**	**79±3.789a**	**95.39±01.897a**	**1.69±0.208a**	1	**75±3.075ab**
Control	**---**	86±3.715**a**	97.89±01.411**a**	1.43±0.228**a**	1	84±3.402**a**
P		**0.0489***	**00.0000*****	**0.0000*****		**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 96in 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±4.24**bc**	1.62±0.45**ab**	1	72.00±4.69**bcdef**
	1.250	84±2.83 **abc**	068.81±6.47 **c**	1.72±0.58**ab**	1	66.00±4.25**cdefg**
	2.500	82±2.65 **abcd**	060.95±4.94 **c**	2.50±0.34 **a**	1	60.00±3.16**defgh**
	5.000	80±2.24 **abcd**	056.76±2.24 **c**	1.53±0.26**ab**	1	56.00±4.79**efgh**
Origanum majorana	**0.625**	86±4.79 **ab**	093.14±3.04 **a**	0.77±0.13 **b**	1	80.00±5.00**abcd**
	1.250	86±3.61 **ab**	088.69±3.39 **a**	1.68±0.32**ab**	1	76.00±3.61**abcde**
	2.500	82±2.65 **abcd**	066.94±7.12 **c**	1.58±0.34**ab**	1	54.00±4.97**fgh**
	5.000	70±3.16 **bcde**	056.55±7.77 **c**	1.53±0.44**ab**	1	40.00±5.92**h**
Pelargonium graveolens	**0.625**	96±1.73 **a**	095.78±1.83 **a**	1.37±0.19**ab**	1	92.00±2.65 **ab**
	1.250	90±3.16 **a**	097.50±1.77 **a**	1.37±0.39**ab**	1	88.00±4.13 **ab**
	2.500	68±5.66 **cde**	093.14±3.04 **a**	0.97±0.46**ab**	1	64.00±6.56**defg**
	5.000	62±2.65 **ef**	084.75±4.93 **ab**	1.90±0.39**ab**	1	52.00±2.65**fgh**
Cymbopogon proximus	**0.625**	64±4.13 **def**	096.00±2.83 **a**	1.38±0.16**ab**	1	60.00±5.00**defgh**
	1.250	60±5.48 **ef**	100.00±0.00 **a**	1.60±0.25**ab**	1	60.00±5.48**defgh**
	2.500	52±2.65 **f**	100.00±0.00 **a**	1.65±0.61**ab**	1	52.00±2.65**fgh**
	5.000	52±4.69 **f**	097.14±2.02 **a**	1.80±0.43**ab**	1	50.00±3.88**gh**
Ocimum basilicum	**0.625**	82±4.69 **abcd**	097.50±1.77 **a**	1.25±0.32**ab**	1	80.00±5.00**abcd**
	1.250	82±2.65 **abcd**	092.06±3.96 **a**	0.63±0.23 **b**	1	76.00±4.79**abcde**
	2.500	80±3.16 **abcd**	088.92±3.76 **a**	1.52±0.24**ab**	1	72.00±5.66**bcdef**
	5.000	68±2.83 **cde**	096.67±2.36 **a**	1.43±0.22**ab**	1	64.00±3.61**defg**
Acetone	**----**	90±2.24 **a**	095.50±1.97 **a**	1.30±0.17**ab**	1	86.00±2.83**abc**
Control	**---**	96±1.73 **a**	096.00±1.73 **a**	1.10±0.19**ab**	1	95.78±1.83 **a**
LSD 0.05		**13.7422**	**15.1162**	**1.3865**		**17.4560**

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 LC_{50} of some volatile oils on some biological aspects

The biological effects of LC_{50} of the tested botanical oils (*M. piperita*, *P. graveolens* and *O. majorana*) against the 4thinstar 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 F_1 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

Table (3) : *Combined effect of irradiated parental males with 100 and 150 Gy and treated the F1 larvae with LC_{50} of some volatile oils on some biological aspects.*

Treatment	Average larval and pupal period / day ±SE	%Pupation ±SE	%Emergence ±SE	Sex ratio		%Survival ±SE
				Male ±SE	Female	
control	35.1±0.38^{g}	93.00±1.53^{a}	97.78±1.48^{a}	1.02±0.08^{a}	1	91.0±2.34^{a}
acetone	38.51±0.64 ^{f}	86.00±3.72^{a}	95.39±1.89^{a}	1.56±0.28^{a}	1	75.0±3.08^{b}
100 Gy	45.57±0.88^{cd}	69.50±2.93^{b}	89.32±2.41^{ab}	1.23±0.25^{a}	1	62.0±2.81^{c}
Menthapiperita	43.19±0.68^{e}	45.00±2.24**c**	92.50±5.34^{ab}	1.51±0.32**a**	1	42.0±3.59^{de}
100 Gy + *Menthapiperita*	48.45±0.^{8}5b	29.00±3.48^{de}	90.00±5.53^{ab}	1.12±0.30^{a}	1	26.0±3.40^{g}
Pelargonium graveolens	39.69±0.69^{f}	49.00±2.34^{c}	95.50±3.03^{a}	1.40±0.29^{a}	1	47.0±3.00^{d}
100 Gy + *Pelargonium graveolens*	43.73±0.75 ^{de}	32.00±4.17^{d}	90.50±3.91^{ab}	1.15±0.19^{a}	1	28.0±3.40^{fg}
Origanum majorana	42.21±0.82^{e}	51.00±4.07^{c}	93.00±3.59^{ab}	1.65±0.34^{a}	1	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}	1	40.0±2.98^{de}
150 Gy	46.77±0.27^{bc}	63.00±3.09 ^{b}	58.29±2.48^{c}	1.56±0.43^{a}	1	36.5±1.83^{ef}
150 Gy + *Mentha piperita*	51.31±0.64^{a}	20.00±2.58^{e}	80.00±6.94^{b}	1.10±0.23^{a}	1	15.0±1.6^{h}
150 Gy + *Pelargoniu mgraveolens*	48.56±0.59^{b}	27.00±3.35^{de}	61.67±7.78^{c}	1.05±0.19^{a}	1	17.0±2.61^{h}
150 Gy+ *Origanum majorana*	50.81±0.59^{a}	32.00±3.27^{d}	65.17±5.79^{c}	1.10±0.31^{a}	1	21.0±2.77^{gh}
LSD 0.05	**1.9224**	**8.6432**	**12.7808**	**0.7901**		**8.5222**
P	**0.1262ns**	**0.2264ns**	**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)

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*, 150Gy+*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 F_1 generation was sekwed 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 F_1 generation with the LC_{50} 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 (LC_{50}) 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 LC_{50} of the three volatile oils, it reduced to 16, 21 and 20% among the three treatments 150Gy +*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 F_1 generation was tending in favor of males at all treatments, except, at the two treatments 150Gy +*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.

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 F_1 larvae with LC_{50} of some volatile oils on some biological aspects.*

Treatment	Average Larval And pupal Period / day ±SE	% Pupation ±SE	% Emergence ±SE	Sex ratio		% Survival ±SE
				Male ±SE	Female	
control	35.1±0.38 **i**	93.00±1.53**a**	97.78±1.48 **a**	1.02±0.08 **bc**	1	91.0±2.3**a**
acetone	38.51±0.64 **h**	86.00±3.72**a**	95.39±1.89 **a**	1.56±0.28**ab**	1	75.0±3.0 **b**
100 Gy	45.28±0.57 **de**	50.50±3.29**b**	63.69±1.63 **de**	1.60±0.15**ab**	1	32.0±2.0 **d**
Mentha piperita	43.19±0.68 **fg**	45.00±2.24**bc**	92.50±5.34**ab**	1.51±0.32**ab**	1	42.0±3.5 **c**
100 Gy+ *Mentha piperita*	49.41±0.70 **c**	28.00±2.91**ef**	87.50±5.16**abc**	1.30±0.26**abc**	1	24.0±2.6 **de**
Pelargonium graveolens	39.69±0.69 **h**	49.00±2.34**b**	95.50±3.03**a**	1.40±0.29**abc**	1	47.0±3.0 **c**
100 Gy+ *Pelargonium graveolens*	44.15±0.97 **ef**	32.00±2.49**de**	74.17±6.90**bcd**	1.23±0.22**abc**	1	24.0±3.1 **de**
Origanum majorana	42.21±0.82 **g**	51.00±4.07**b**	93.00±3.59**a**	1.65±0.34**ab**	1	48.0±4.9 **c**
100 Gy+ *Origanum majorana*	48.58±0.44 **c**	38.00±3.27**cd**	70.33±4.80**cde**	1.85±0.33 **a**	1	27.0±3.0 **d**
150 Gy	46.13±0.30 **d**	32.00±3.00**de**	52.51±2.35 **e**	1.85±0.24 **a**	1	16.5±1.5 **ef**
150 Gy+ *Mentha piperita*	52.53±0.69 **a**	16.00±3.06 **g**	78.33±10.56**abc**	1.10±0.21**bc**	1	13.0±2.1 **f**
150 Gy+ *Pelargonium graveolens*	49.88±0.75 **bc**	21.00±3.79**fg**	59.17±10.87**de**	1.10±0.26 **bc**	1	12.0±2.0 **f**
150 Gy+ *Origanum majorana*	51.67±0.64**ab**	20.00±3.65**fg**	60.83±9.14 **de**	0.65±0.15**c**	1	13.0±2.6 **f**
LSD 0.05	**1.8437**	**8.6935**	**16.9379**	**0.6931**		**8.0654**
P	**0.1105ns**	**0.3856ns**	**0.0000*** **	**0.0119***		**0.0830ns**

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

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-

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 barnoof 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 *piperieta, pelargonium graveolens,* and *Origanum majorana then cymbopogon proxi-*

mus. We recommended using the oil *Mentha piperieta* with the dose 150 Gy for integrated pest management.

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