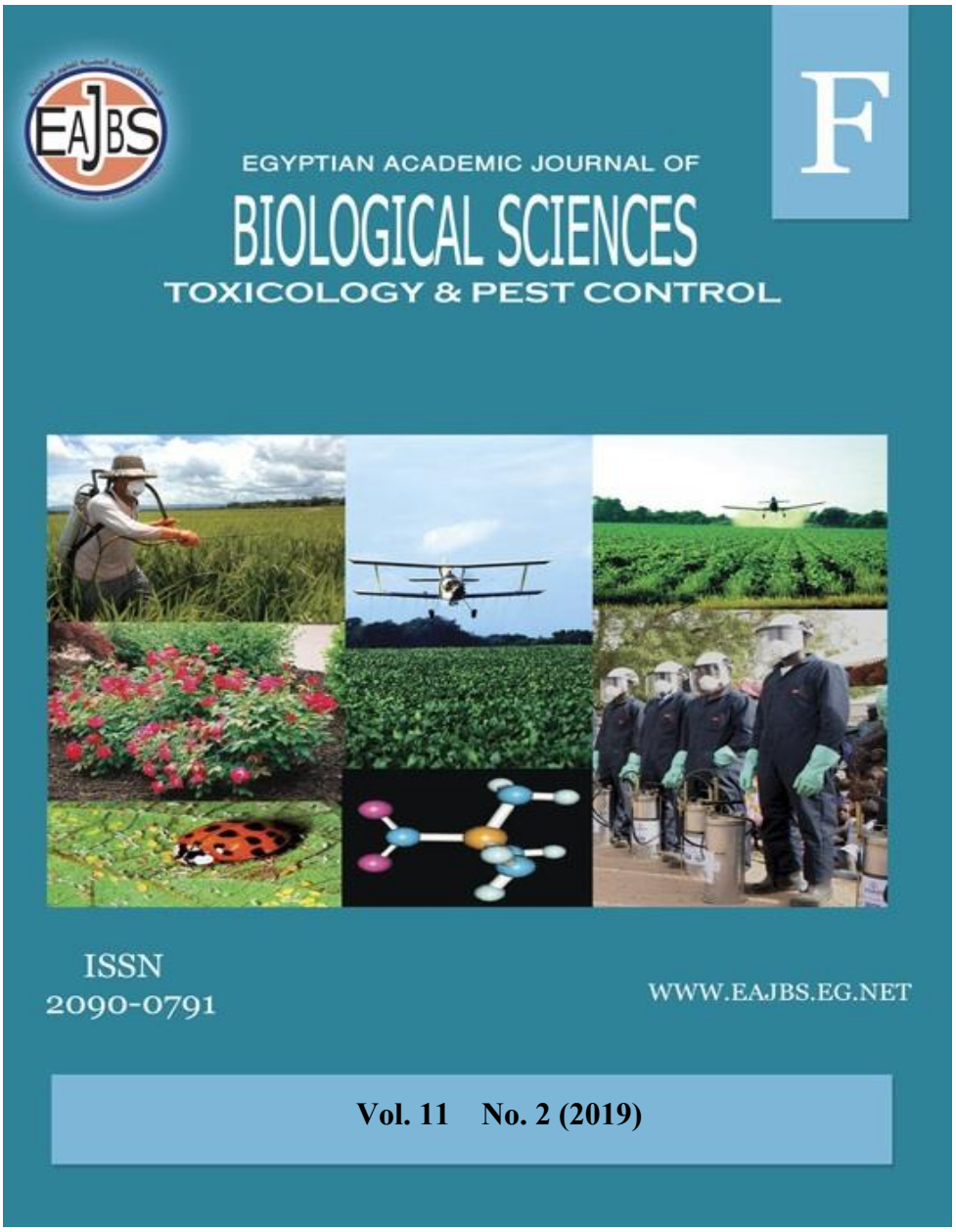

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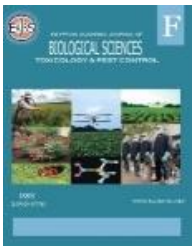

Laboratory Studies on the Efficacy of *Olea europaea* Plant Extract and Gamma Irradiation on Larvae of *Galleria mellonella* (Lepidoptera: Pyralidae)

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ABSTRACT

The present investigation was undertaken to study the effectiveness of *Olea europaea* leaves extract and gamma irradiation against the greater wax moth, *Galleria mellonella* larvae. Acetone *Olea europaea* leaves extract was prepared at (2, 1, 0.5, 0.25and 0.125%) to be examined for their efficacy against the 4th instar larvae. The effects of 5 different doses of gamma irradiation (50, 100, 150, 200 and 250 GY) was also assayed on the biology of greater wax moth, *G. mellonella*. Results revealed that larval mortality of *G. mellonella* reached (8.2%) and (57.3%) after1&10 days from treatment, with the lowest concentration of 0.125%. While, the highest concentration 2 % caused (31.0%) and (100 %) larval mortality after1&10 days post-treatment for plant extract. All doses of gamma irradiation prolonged the larval period and reduced both pupation and percentage of adult emergence. The number of eggs laid was decreased by increasing the irradiation doses at all treatments. The insects failed to lay any eggs especially, in the treatments (treated male × treated female) and (normal male × treated female) with doses 200 and 250 GY, respectively.

INTRODUCTION

The greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), is one of the most devastating and economically important pests of bee wax in the world (Haewoon *et al*., 1995)**.** The caterpillars of wax moth feed on combs, pollen, larval exuviae and other protein a ceous matter both in storage as well as in live honey bee colonies (Caron, 1999)**.** The use of chemical insecticides such as sulphur, para di chlorobenzene and calcium cyanide is harmful to the bee population (Grout, 1946; Whit-comb, 1967). The plant's based insecticides being the natural plant are safer and hence their use against pests has gained importance all over the world (Alkofahi *et al*., 1989).Some facts show that the use of synthetic chemicals to control insects and arthropods raises several obvious concerns related to the environment and human health. So, there is an alternative repellent or natural product. These products good efficacy and are environmentally friendly (Grad, 2010; Nerio *et al*., 2010).

Plant extracts are currently studied more and more because of their role in plant

protection as well as in urban entomology (Din *et al.*, 2001). Plant extract is safer for non-target organisms; therefore, plant-based formulations would be more feasible from an environmental perspective than synthetic larvicides (Bhat and kempraj, 2009).

The use of sub sterilizing doses of irradiation to produce sexually competitive moths has been suggested by many researchers. Furthermore, the F1 offspring of irradiated lepidopterous species are often partially or completely sterile. This phenomenon is known as F1 sterility or inherited sterility (Abd El-Hamid, 2004)**.** The use of irradiation technique as a physical control method is cheaper, safe and more reliable than chemical methods. However, the problem is to determine the minimum dosage of irradiation needed to control the insects while keeping the cost of treatment at an economical level before reaching the threshold of irradiation that will produce quality changes in some commodities of irradiated products.

The aim of the present study was to evaluate the effectiveness of plant extract (*Olea europaea* L.) and gamma irradiation against *Galleria mellonella* larvae under laboratory conditions.

MATERIALS AND METHODS

Insect Rearing Technique:

The strain of the greater wax moth, *Galleria mellonella* was obtained from the National Research Center (NRC) and reared according to (Hussein, 2004)**.**

The larvae of greater wax moth, *Galleria mellonella* were reared on a semi-synthetic diet composed of 22% wheat-flour, 22% corn groats, 11% honey, 11% milk powder, 11% glycerol, 5.5% yeast powder and 17.5% bee wax (Wiesner, 1993)**.** The larvae were obtained from beehives and transferred to transparent plastic rearing jars (17 ×17 × 27 cm); containing 250 gm. from the previous prepared the media, closed with a lid of muslin for aeration and incubated at 28 ± 2°C and 65 ± 5% R.H. when larvae grown to the pupal stages and then to the adult moths, apiece (15 ×15 cm) of paper tissue was folded and placed in the container to promote egg laying. Eggs were laid on the lid and a paper tissue. These eggs were gently removed and transferred to other rearing jar containing 250 gm. The media, closed tightly with a double muslin layer to prevent the escape of neonatal larvae, and incubated. Add fresh food frequently (1-2) times per week.

Preparation of Plant Extract:

The leaves of *Olea europaea* were dried at room temperature for 3 weeks and ground in an electric mill. Two hundred grams of the ground powder was taken and put inside one liter flask and the solvent was added and left for 72 hr. (Su, 1985; Abo-El-Ghar and El-Sheikh, 1987; Sharaf El-Din, 1998 and Barakat, 2012)**.** The flask was shaker for 30 min in a shaken then filtered. After that, the solvent was evaporated using a rotary evaporator at 50°C and reduced pressure. The solvent extract of crude gums was weighted and mixed again with the solvent to give a 20% (w/v) stock solution. Concentrations of 2, 1, 0.5, 0.25 and 0.125 % (w/w) were prepared for application by dilution of the 20 % stock.

Experimental Technique:

Pieces of old wax combs (5×5 cm) were treated with concentrations from plant extract and another piece was treated only by solvent as control. The comb pieces were dipped for 15 minutes and left for three hours to dry. The treated comb pieces were placed in a glass jar and incubated at 30°C and 65% R.H. Four replicates from each concentration and control (Acetone as control) were used, each containing 25 4th instar larvae of *G. mellonella*. Post-treatment numbers of dead and alive larvae were daily, counted for 10 days. The mortality percentages were corrected according to (Abbott,

1925). The lethal concentration (LC_{50}, LC_{90} and LC_{95}) values were assessed by probit analysis.

$$\% \text{ Mortality} = (a - b / a) \text{ X } 100$$

Where:-

a = the initial numbers of larvae placed in each jar (25 larvae)

b = Means numbers of larvae still alive per jar.

$$\text{Corrected Mortality} = (b\text{-}a /100 - a) \text{ X } 100$$

Where:-

b = % Mortality in treatment.

a = % mortality in control.

Irradiation:

1. Source of Irradiation:

Irradiation of *G. mellonella* pupae was carried out using gamma cell irradiation unit (CO 60 source) irradiator installed in the cyclotron project, Nuclear Research Center, Abu Zaabal, Egypt. The rate of irradiation source was 1Gray/ second.

2. Irradiation Process:

Full- grown pupae of *G. mellonella* were exposed to different gamma rays doses (50, 100, 150, 200 and 250 GY), to study the effect of irradiation on some biological aspects of *G mellonella*. Four mating combinations were set up as follows for each dose. Unirradiated male × unirradiated female (as control), unirradiated male × irradiated female, irradiated male × unirradiated female and irradiated male × irradiated female.

Every four combinations were kept in a separate jar, covered with pieces of thin cloth fixed in a rubber band, three replicates were performed for each one; the eggs laid by females were recorded and counted daily under a binocular microscope. Egg masses were transferred to clean jar (250 CC) capacity, washed with 0.15% formalin solution to avoid any contamination. Hatch larvae we counted daily and provided with a fresh diet. Percentage sterility was calculated according to Chamber Lain's formula as mentioned by (Guirguis, 1979).

$$\% \text{ sterility} = 100 - \left(\frac{a \times b}{A \times B} \text{ X } 100 \right)$$

Where:

a: No. of eggs / female in treatment

b: % hatching / female in treatment

A: No. of eggs / female in control

B: % hatching / female in control

Statistical Analysis:

The obtained mortality data were subjected to Probit analysis (Finney, 1971), using a computer Program of (Noack and Reichmuth, 1978). The statistical analysis was carried out using ANOVA with two factors under a significance level of 0.05 for the whole results using SPSS (ver.19) and data were treated as complete randomization design according to (Steel *et al.*, 1997).

RESULTS AND DISCUSSION

Effect of Acetone *Olea europaea* Leaves Extract on Larval Mortality of *Galleria mellonella* at 28 ± 2°C and 65± 5% R.H:

The effect of acetone *Olea europaea* leaves extract on larval mortality of *Galleria mellonella* are presented in Table **(**1**).** The results showed that mortality was increased by increasing the plant extract concentration. Plant extract of *Olea europaea* at the lowest concentration (0.125) caused 8.2% mortality after 1-day post-treatment compared with 57.3% accumulative mortality after 10 days of treatments. On the other hand, the highest

concentration (2%) caused 31.0% mortality after 1 day from treatment and reached to 100 % mortality after 10 days of treatments. According to the obtained LC_{50} values, data indicated 0.77 % as LC_{50} of *Olea europaea* extract after 7 days from treatment, but highest toxicity (LC_{50} = 0.47%) occurred 3 days later (10 days) after treatment by the same plant extract. The same trend of toxicity occurred for the LC_{90} and LC_{95} values (Table, 2).

These previous results are in agreement with many authors among them (Zaitoun, 2007) who studied the effect of ethanolic extracts of twenty-one medicinal and health plants on the development of the greater wax moth *Galleria mellonella*. The results indicated that feeding the moth larvae on most of the extracts, prolonged the larval stage duration 2-40 days more the control. Six extracts prolonged pupation period 2-5 days more than the control and also, had an insecticidal effect against the moth; they killed 100 or 95% of the tested wax moths without adverse effects on worker bees. In addition, (Asmaa *et al*., 2017) studied the effect of some natural substances (Egyptian and Chinese propolis ethanolic extract- cinnamon- clove- peppermint ethanolic extract) against the 4th larval instar of *G. mellonella*. Results indicated that larvae and pupae mortality increased with increasing concentration, while the adult stage was the more resistance stage. Also, these natural substances prolonged the larval duration compared to control. As well, (Surendra *et al.*, 2010) studied the effect of natural plant products (*Azadirachtin indica*, *Ocimum sanctum* and *Pongamia pinnata*) in the management of the greater wax moth, *G. mellonella*. Results indicated that the mortality was high with the extraction of *Azadirachta indica* is ranging from 84.81 to 93.65 % at different concentrations followed by *Ocimum sanctum* 65.36 to 73.41% while, *pongamia pinnata* caused the lowest mortality (19.85 to 52.1%) of three plant products tested. Neem performed better under all the experimental conditions.

Table 1: The effect of *Olea europaea* extract on the percentage larval mortality of the greater wax moth, *Galleria mellonella*.

Conc. (w/w) %	Accumulative larval mortality (%) after indicated days										
	1	2	3	4	5	6	7	8	9	10	Mean
Galleria mellonella											
2	31.0	38.1	42.0	47.7	50.0	53.7	60.0	69.1	90.2	100.0	58.18
1	27.6	33.0	35.9	38.8	41.3	49.2	55.4	61.0	85.2	95.6	52.3
0.5	22.9	24.4	27.1	30.2	32.6	34.3	40.0	43.3	85.2	88.3	42.83
0.25	10.0	12.5	15.7	18.0	20.2	27.3	30.0	37.0	75.5	78.6	32.48
0.125	8.2	10.8	17.5	20.3	25.1	30.0	33.4	38.6	45.3	57.3	28.65
Control	-	-	-	1.5	1.6	1.6	2.0	2.6	4.0	4.0	

Table (2): LC_{50}, LC_{90} and LC_{95} values of *Olea europaea* extract against the 4th instar larvae of *Galleria mellonella*.

Time after treatments (Days)	LC_{50} %	LC_{90} %	LC_{95} %	Slope ± SD	R.
Galleria mellonella					
7	0.77 (0.95-2.19)	6.54 (4.11-13.12)	11.98 (6.8-28.06)	1.38±0.14	0.98
10	0.47 (038-0.59)	2.60 (1.95-3.85)	4.19 (2.95-6.82)	1.75±0.15	0.99

R= Correlation coefficient of the regression line

SD= Standard deviation of the mortality regression line

Also, (Lalita and Sunita, 2018) studied the effectiveness of different plant extracts against *G. Mellonella* larvae. Acetone prepared extracts of leaves, stem, seeds and roots of thirteen medicinal and healthy plants. Results revealed that only six plant extracts with leaves cause mortality of wax moth. Larval mortality was highest (93.33%) with prepared extract of *p. psyllium* followed by leaf extract of *H. Sativum* (80%), *Raphanus sativus* (73.33%), *Linum usitatissimum* (66.66%), *Cucurbita moschata* (46.66%) and *Vicia sativa* (46.66%). Therefore, the plant extract of different plants was found effective against *Galleria mellonella* larvae.

Biological Effects of Gamma Irradiation on *Galleria mellonella:*

These experiments were carried out to study the effect of gamma irradiation doses (50, 100, 150, 200 and 250 GY) on the life cycle of the greater wax moth. The obtained results in Table (3) shows that the number of eggs laid per female decrease with the increasing irradiation doses being 550.0, 331.5, 55.0, 0 and 0, respectively. As compared to control (1900.07). The percent sterility significantly increased gradually with increase the does until reach 100 % sterility at the doses (150, 200 and 250 GY). Data also, showed that the F1 larval duration in case of irradiated female mated to normal male was increased with the dose increase. There were (35.5, 36.9 and 0 days) as compared with control (27.50) days.

A negative correlation was found between the percent of pupation and the increase of doses till reach 0 % at the doses (150, 200 and 250 GY). The percent of adult emergence of F1 generation was significantly affected especially with the highest doses (150, 200 and 250 GY) to reach 0 % as compared with control (99.6%). The sex ratio was in favor of males at does 50 and 100 GY. While in control, the number of female exceeds the number of males at the mating (irradiated female × normal male)

Table (3): Effect of irradiation on eggs, larvae, pupae and adults resulted from irradiated females mated with normal males of *Galleria mellonella*.

Irradiation Doses (GY)	Av. No. of eggs/ female	% Hatchability	% Sterility	Larvae duration (days)	% Pupation	% Emergence	Sex ratio	
							Male	Female
Control	1900.0.7±11.3	92.70±13.2	0.0	27.50±0.36	99.55	99.6	49.83	50.17
50	550.0±17.2	42.04±38.2	63.4	35.5±0.33	76.6	87.50	53.4	46.6
100	331.5±53.9	33.50±4.3	94.0	36.9±1.11	46.66	61.11	82.34	17.66
150	55.0±7.80	0.0	100.0	0.0	0.0	0.0	0.0	0.0
200	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0
250	0.0	0.0	100.0	.0.0	0.0	0.0	0.0	0.0

While, the results in Table (4) revealed that, there was no significant effect of the doses 50, 100, 150, 200 and 250 GY on the number of eggs laid per female in the treatment (irradiated male × normal female). On the other hand, the number of eggs hatch increased as dose increases, except the dose of 250 GY that they failed to hatch. Also, the percent of sterility was significantly increased as dose increases, excluding the dose of 250 GY reach to 100.0%. The larval longevity increased with the dose increases except at the dose 250 GY; it reached to zero. In contrast, the percent of pupation was negatively correlated to the doses. showing 83.4, 77.4, 67.0, 41.3 and 0 % at doses 50, 100, 150, 200 and 250 GY, respectively, as compared to control (99.55%). The percent of emergence was significantly decreased with increasing the doses. In the sex ratio the male ratio was increased with increasing the dose, the percentages were 56.3, 55.5, 85.7 and 88.88 and 0 % compared to control (49.83 %). While, the female ratio was decreased with increasing the dose to giving 43.7, 44.5, 14.3, 11.12 and 0 % as compared to control (50.17%).

Table (4): Effect of irradiation on eggs, larvae, pupae and adults resulted from normal females mated with irradiated males of *G. mellonella*.

Irradiation Doses (GY)	Av. No. of eggs/ female	% Hatchability	% Sterility	Larvae duration (days)	% Pupation	% Emergence	Sex ratio	
							Male	Female
Control	1900.0.7±11.3	92.70±13.2	0.0	27.50±0.36	99.55	99.6	49.83	50.17
50	793.0±18.5	69.33±3.1	55.8	35.2±0.33	83.4	87.38	56.3	43.7
100	477.6±48.0	57.30±1.9	61.2	36.7±0.15	77.4	84.5	55.5	44.5
150	304.3±19.9	54.02±1.7	76.8	38.2±0.34	67.0	51.7	85.7	14.3
200	183.7±15.50	33.7±0.4	83.5	38.5±0.73	41.3	50.6	88.88	11.12
250	120.6±10.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0

Data summarized in Table (5) indicated that there was a highly significant reduction in the number of eggs per female with the increase of doses at the mating (irradiated male × irradiated female). The numbers of eggs per female were 520.0, 312.0, 56.6, 0 and 0 at 50, 150, 200 and 250 GY, respectively, as compared to control (800. 6). The sterility was in accordance to the doses increased, showing the 100 % sterility at the doses, 50, 200 and 250 GY. Larvae showed a significant decline in longevity at the doses 100, 150, 200 and 250 GY. The percentages pupation was 41.03, 0, 0 and 0 % at 50, 100, 150, 200 and 250 GY, respectively, as compared to 99.55 % in the control. Adult emergence and sex ratio were unmatched with the dose increasing as they were zero at doses 100, 150, 200 and 250 GY.

Table (5): Effect of irradiation on eggs, larvae, pupae and adults resulted from irradiated females mated with irradiated males of *Galleria mellonella*.

Irradiation Doses (GY)	Av. No.of eggs/female	% Hatchability	% Sterility	Larvae duration (days)	% Pupation	% Emergence	Sex ratio	
							Male	Female
Control	1900.0.7±11.3	92.70±13.2	0.0	27.50±0.36	99.55	99.6	49.83	50.17
50	520.0±13.6	19.05±11.5	93.6	39.0±1.14	41.03	55.55	82.3	17.7
100	312.0±13.0	12.63±1.7	98.6	0.0	0.0	0.0	0.0	0.0
150	56.6±1.4	0.0	100	0.0	0.0	0.0	0.0	0.0
200	0.0	0.0	100	0.0	0.0	0.0	0.0	0.0
250	0.0	0.0	100	0.0	0.0	0.0	0.0	0.0

The present finding agrees with (Hussein *et al.*, 2018) who studied the effect of entomopathogenic fungi (EPF), *paecilomyces lilacinus* and *Beauveria bassiana*, on larval mortality of *G. mellonella* under laboratory conditions and also, study the effect of different doses of gamma irradiation (70,100, 125 and 150 GY) separately or combined with the LC_{50} of EPF, *B. bassiana* and *P. lilacinus* on the second instar larvae of *G. mellonella* larval mortality. Results indicated that the combined treatment of gamma irradiation and EPF increased the larval mortality rates than each treatment alone. The highest percentage of larval mortality was 78 and 84% with 125 GY + *B.bassiana*. According to the results, the gamma irradiation increased the pathogenicity of the fungi against *G. mellonella* larvae. (Salem *et al.*, 2014) Reported that the percentage of larval and pupal mortality of *Agrotis ipsilon* increased with the increase of the doses used. Similarly, (Abass *et al.*, 2017) reported that the percentage of larval and pupal mortality of *Spodoptera littoralis* increased significantly with increasing radiation doses. (El-Kholy and Abd El-Aziz, 2010) Study different doses of gamma irradiation on pupae of greater wax moth, *G. mellonella* of 100, 150, 300 and 400 GY. Results show decreased the average number of eggs per mated female, also, caused a reduction in the fecundity and the percentage of egg hatching**.** (El-barky *et al.*, 2015) Who studied the combined

treatment of 100 GY gamma radiation and LC_{50} of *Mentha piperita* and *Pelargonium graveolens* against fourth instar larvae of *G. mellonella*. Results show prolonged the larval-pupal periods and reduced both pupation and percentage of adult emergence.

Conclusion:

It may be concluded that the use of plant extract and gamma irradiation are the most promising new approaches for controlling the greater wax moth, *Galleria mellonella*. So, the treatments with *Olea europaea* plant extract and gamma irradiation are more efficient in the control of *G. mellonella* and also gamma irradiation had a high effect on biological development parameters of all life stages. Finally, in this work, we tried to control *G. mellonella* with materials seem to be safer, available to beekeepers and could be used to control other hive infestation such as Varroa and acarine mites.

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ARABIC SUMMARY

دراسات معملية على فاعلية مستخلص نبات الزيتون والتشعيع الجامي على يرقات دودة الشمع الكبرى

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تَم دراسة تأثير فاعلية مستخلص اوراق الزيتون والتشعيع الجامي على يرقات دودة الشمع الكبرى. وتَم تحضير تركيزات مختلفة من المستخلص الأسيتوني لنبات الزيتون وهى 1،2‘0.25،0.5,0.125%
لدراسة فاعليتها على يرقات العمر الرابع لدودة الشمع الكبرى. كما تم ايضاً إستخدام خمس جرعات مختلفة من التشعيع الجامي وهى 50 ،200،100،150, 250 جراي على التأثيرات البيولوجية لدودة الشمع الكبرى. اوضحت النتائج أن نسب الموت في اليرقات وصلت 8.2 % , 57.3 % بعد 1 إلى 10 أيام من المعاملة بالمستخلص وذلك مع التركيز المنخفض 0.125 % بينما وصلت نسب الموت إلى 31 % , 100% بعد 1 إلى 10 أيام من المعاملة بالتركيز العالي 2% لنفس المستخلص. احدثت معظم الجرعات المختلفة من التشعيع الجامي إطالة في مدة العمر اليرقي وسجلت إنخفاض في كلاً من النسب المئوية للتعذير وخروج الفرشات. كما اوضحت النتائج حدوث انخفاض في عدد البيضات الموضوعة بزيادة جرعات التشعيع في كل المعاملات وفشلت الحشرات في وضع أي بيض خاصة في معاملة الاناث المعاملة مع الذكور المعاملة، والذكور الغير معامله مع الاناث المعاملة، خاصة مع الجرعات (200، 250 جراي) على التوالي.