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Biological effects of gamma radiation on *Galleria mellonella* (L.)

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ABSTRACT

The efficiency of gamma radiation doses was tested against some biological aspects of the progeny of *Galleria mellonella*. Male and/or female full-grown pupae were irradiated with 40, 100, 160, 200 and 260Gy. The results showed that the fecundity, % egg hatchability, % pupation and adult emergence were negatively correlated with the dose increase. On the other hand, % sterility, egg incubation period and the larval duration were increased significantly ($p<0.05$) with the increase of doses used. In general, the results indicated that the biological activity of gamma irradiation against *G. mellonella* larvae was more remarkable when both crossed females and males were irradiated followed by irradiated females crossed with non-irradiated males.

Introduction and methodology

The greater wax moth, *Galleria mellonella* (L.), is a lepidopterous insect; its larval stage, feeds on wax and pollen stored in combs of active honeybee colonies **(Milam, 1970)**. It does not attack adult bees but destructs combs of a weak colony by chewing the comb; spinning silk-lined tunnels through the cell wall and over the face of the comb, which prevent the bees to emerge by their abdomen from their cell, so they die by starvation as they unable to escape from their cell. They also eat out a place to spin their cocoons in the soft wood of the beehive. *G. mellonella* can also destroy stored honeycombs. Therefore, it is considered a major pest of the honeybee. Damage will vary with the level of infestation and the time that has elapsed since the infestation first began. In time, stored combs may be completely destroyed, and the frames and combs become filled with a mass of tough, silky web. Moreover, combs that are apparently free of wax moth may contain eggs that will hatch later **(Charrière and Imdorf, 2004)**.

Fumigation is the recommended control tool used to wax combs before placed in storage **(Caron, 1992)**. Regarding to the chemical pollutions and the resistance developed for them, it is necessary to find new safe control method. Irradiation technique offers a solution for these problems as it is cheaper, safe and more reliable than chemical methods. Irradiation shortens life span of insects **(Baxter and Blair, 1969)** possibly by accelerating senescence **(Aly *et al.*, 1996)**. Irradiation may also cause protein denaturation, which may impair enzyme activity **(Tribe**

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and Webb, 1979). According to Kinipling **(1955),** insect exposure to ionizing radiation causes sterilization through induction of dominants lethal mutation in the genetic structure of organisms.

The present study aimed to investigate the effectiveness of gamma irradiation on the development and reproduction of the greater wax moth, *G. mellonella***.**

Materials and Methods

Insect rearing technique:-

The greater wax moth, *Galleria mellonella* (L.) larvae were obtained from the infested hives and reared in the laboratory at 28± 2°C and a relative humidity of 65± 5% on artificial media as described by Hussein **(2004)** and away from any intentional chemical pressure**.**

Irradiation process:-

The irradiation process was performed using Gamma cell (Co^{60} source), at National Center for Radiation Research and Technology (NCRRT, Cairo). The dose rate was 6.6 kGy/h. at the time of the present investigation.

Bioassay Experiments:-

Six day-old pupae of *G. mellonella* were exposed to different gamma rays doses (40,100,160,200 and 260 Gy), to study the effect of irradiation on some biological aspects of *G. mellonella*. Four mating combinations were made:

Unirradiated ♂	×	Unirrdiated ♀ (as control)
Unirradiated ♂	×	Irradiated ♀
Irradiated ♂	×	Unirradiated ♀
Irradiated ♂	×	Irradiated ♀

Each 5 combinations were kept in a separate jar, covered with pieces of thin cloth fixed in with a rubber band, three replicates were performed for each one; the fecundity (No. of eggs laid/♀), were recorded and counted daily under a binocular microscope. Egg masses were transferred to clean jar 250 c.c capacity, washed with 0.15% formalin solution to avoid any contamination. Hatched larvae were counted daily and provided with fresh diet. % of sterility were calculated as mentioned by Guirguis and Manweiler **(1994)**. Larval duration, % Pupation, % adult emergence and sex ratio were also recorded.

$$\% \text{ Sterility} = 100 - \frac{a \times b}{A \times B} \times 100$$

Where:

a: No. of eggs/female treatment

b: % hatching/ female treatment

A: No. of eggs/female control

B: % hatching/ female control

Statistical Analysis:-

The data were statistically evaluated by analysis of variance (F) followed by Duncan's multiple range test to examine the significant differences between treatment. The 5% level of probability was used in all statistical tests. The statistical software program CoStat **(1995)** was used for all analyses.

RESULTS

Irradiated female mated with normal male

The obtained results in Table (1) and Figures (1 & 2) show that when full grown pupae were irradiated with 40, 100, 160, 200 and 260 Gy, the number of eggs laid per female decrease with the increasing radiation doses being 522, 331.7, 57, 0 and 0, respectively; as compared to control (799.7). The incubation period of the eggs was positively correlated to the dose increase, except at the high dose (160 Gy) the eggs failed to hatch. The percent sterility significantly increased gradually with the increased dose until reach 100% sterility at the doses (160, 200 and 260 Gy).

Data in Table (1) also showed that the F_1 larval duration in case of irradiated female mated to normal male were significantly increased with the dose increase. There were (35.3, 36.8 and 0) as compared with control (28.47). A negative correlation was found between the percent pupation and the increase of doses till reach 0% at the doses 160, 200 and 260 Gy, and a significant difference was found between treatments.

When full grown pupae were irradiated with the doses 40, 100, 160, 200 and 260 Gy, the percent of adult emergence and sex ratio of F1 generation were significantly affected; the lower the dose, the higher the percentage of adult emergence. The sex ratio was in favor of males at dose levels 100 and 160 Gy. While in control, the number of females exceeds the number of males.

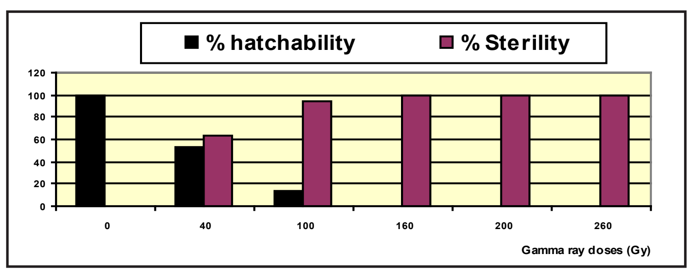

Figure (1): Number of eggs resulted from irradiated female mated with normal male

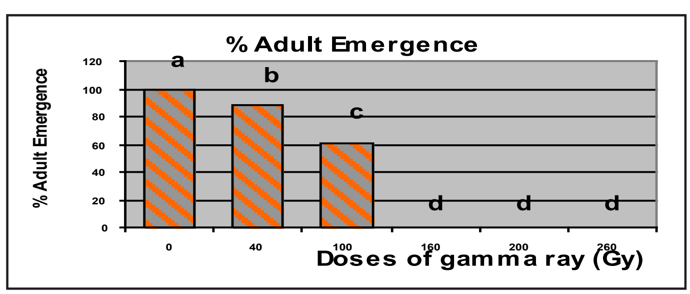

Figure (2): Percent of adult emergence of F1 generation resulted from irradiated female mated with normal male

Table (1): Effect of irradiation on eggs, larvae, pupae and adults resulted from irradiated females mated with normal males of *Galleria mellonella*

Radiation Doses (Gy)	Eggs		Larvae	Pupae		Adults	
	Fecundity (No. of eggs / female)	Incubation period (days)	Duration	No.	% Pupation	Sex ratio	
						Male	Female
Control	799.7 ± 11.3[a]	7.4 ± 0.365[b]	28.47±0.37[c]	783.33±13.3[a]	99.57[a]	49.89	50.1
40	552 ± 17.2[b]	9.4 ± 0.433[a]	35.3±0.33[b]	41.66±2.72[b]	77.6[b]	53.4	46.6
100	331.7 ± 54.9[c]	9.6 ± 0.361[a]	36.8 ±1.01[a]	2.33 ±0.33[c]	46.66[c]	83.33	16.66
160	57 ± 7.81[d]	0[c]	0[d]	0[c]	0[d]	0	0
200	0[d]	0[c]	0[d]	0[c]	0[d]	0	0
260	0[d]	0[c]	0[d]	0[c]	0[d]	0	0

- Letters indicate the variance between the means (Duncan›s multiple range test).
- Values represent the mean ±S.E. of 3 replicates for each group.

Irradiated male mated with normal female

The results recorded in Table (2) and Figures (3 & 4) reveal that there was no significant effect of the doses 40, 100, 160, 200 and 260 Gy on the number of eggs laid per female when irradiated male mated with untreated virgin female. On the other hand, the incubation period of the eggs was significantly increased as dose increases, excluding the dose of 260 Gy that failed to hatch.

The larval longevity gradually increased with the dose increase except at the dose 260 Gy; it was zero. In contrast, the percent pupation was negatively correlated to the doses. Showing 83.5, 77.4, 68, 41.4 and 0 at doses 0, 40, 100, 160, 200 and 260 Gy, respectively, as compared to the control (99.57).

Irradiation of full grown pupae significantly decreases the percent of adult emergence of F_1 generation. The male ratio was increased with increasing dose; the percents were 56.3, 56.5, 86.7 and 88.88 as compared to the control (49.89). While, the female ratio was decreased with increasing dose giving 43.7, 43.5, 13.3, 11.11 and 0% as compared to the control (50.1).

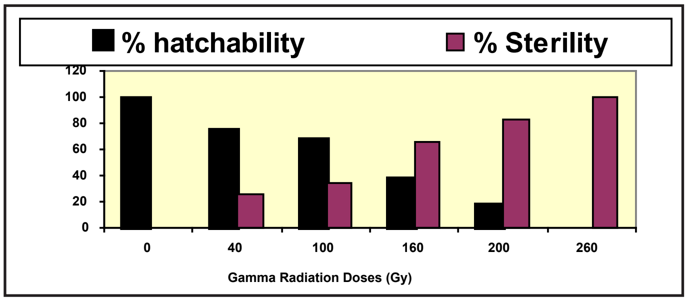

Figure (3): Number of eggs resulted from normal female mated with irradiated male parent pupae

Table (2): Effect of irradiation on eggs, larvae, pupae and adults resulted from normal females mated with irradiated males of *Galleria mellonella*

Radiation Doses (Gy)	Eggs		Larvae	Pupae		Adults	
	Fecundity (No. of eggs / female)	Incubation period (days)	Duration	No.	% Pupation	Sex ratio	
						Male	Female
Control	799.7±11.3[a]	7.48±0.36[c]	28.47±0.37[c]	783.3±14.5[a]	99.57	49.89	50.1
40	794±19.5[a]	9.1±0.1[b]	36.3±0.33[b]	271.6±28.3[b]	83.5	56.3	43.7
100	771.3±48[a]	9.5±0.23[ab]	37.7±0.15[a]	69.3±28.9[c]	77.4	56.5	43.5
160	756±27.1[a]	9.8±0.10[a]	38.3±0.35[a]	6.6±1.7[d]	68	86.7	13.3
200	751.33±16.58[a]	10±0.22[a]	38.5±0.74[a]	6.6±0.8[d]	41.4	88.88	11.11
260	743±10[a]	0[d]	0[d]	0[d]	0	0	0

- Legends as in table (1).

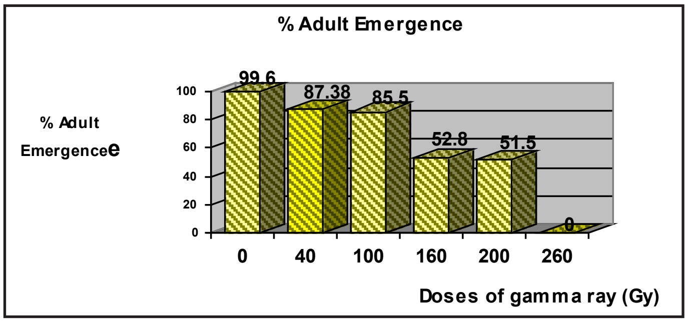

Figure (4): Percentage of adult emergence of F1 generation resulted from normal female mated with irradiated male of *Galleria mellonella*

Irradiated male mated with irradiated female

Data in table (3) and Figures (5 & 6) indicate. that there was a highly significant reduction in the number of eggs per female with the increase of the doses when irradiated male mated with irradiated female. The numbers of resulted eggs per female were 545, 314, 55.6, 0 and 0 at 40, 100, 160, 200 and 260 Gy, respectively, as compared to the control (799.7).

The incubation period of the eggs was in correspondence to the doses increased, it was 10 days at 100 Gy and 0 days at the dose 160 Gy as compared with 7.5 days in the control. Similarly, the sterility was in accordance with the doses increased, showing the 100% sterility at the doses 160, 200 and 260 Gy. Larvae showed a significant decline in longevity at the doses above 100Gy.

The percent of pupation was inversely correlated to the dose increase. The percentages pupation was 42.03, 0, 0, 0 and 0 at, 40, 100, 160, 200 and 260 Gy, respectively, as compared with 99.6 in the control.

Adult emergence and sex ratio were unmatched with the dose increasing as they were zero at 100, 160, 200 and 260 Gy.

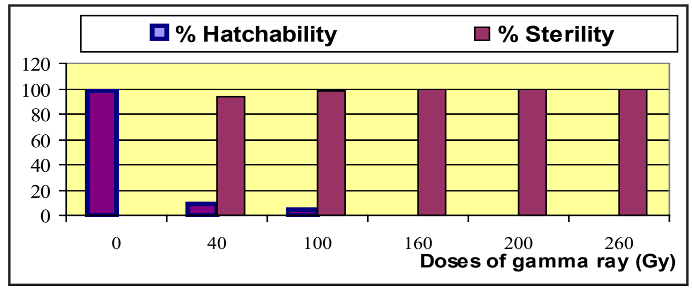

Figure (5): Number of eggs per irradiated female mated with irradiated male of wax moth

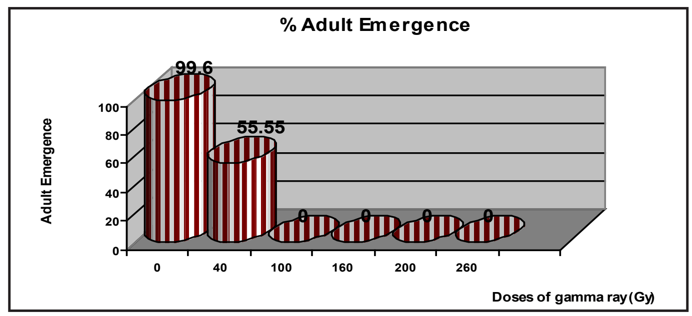

Figure (6): Adult emergence of F_1 generation of irradiated female mated with irradiated male of wax moth

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

Radiation Doses (Gy)	Eggs		Larvae	Pupae		Adults	
	Fecundity (No. of eggs / female)	Incubation period (days)	Duration	No.	% Pupation	Sex ratio: Male	Sex ratio: Female
Control	799.7 ± 11.3[a]	7.4 ± 0.36[b]	28.47 ± 0.37[b]	783.33 ± 14.5[a]	99.57	49.8	50.1
40	545 ± 13.7[b]	9.7 ± 0.11[a]	39 ± 1.15[a]	2.33 ± 0.33[b]	42.03	83.3	16.7
100	314 ± 13.7[c]	10 ± 0.15[a]	0[c]	0[b]	0	0	0
160	55.6 ± 1.4[d]	0[c]	0[c]	0[b]	0	0	0
200	0[e]	0[c]	0[c]	0[b]	0	0	0
260	0[e]	0c	0[c]	0[b]	0	0	0

- Legends as in table (1).

DISCUSSION

The present study showed that the fecundity and fertility of *G. mellonella* were inversely proportional with the dose of radiation, which was more pronounced when both irradiated sexes mated together and female *G. Mellonella* was more sensitive to radiation doses rather than male. Our results were in accordance with those of **Al-Taweel *et al.* (2002)**, they conveyed that the percentage of egg hatch reached 0% for *G. mellonella* F_1 progenies produced from normal females mated with irradiated males and mated as follows: F_1 female x normal male and F_1 male x F_1 female, for doses of 0.15 and 0.20 kGy, respectively. **Milcheva (2005)** finding was in accordance with the obtained result, he reported that the cross of 2 irradiated parents' pupae of *Galleria Mellonella* results in sterility at 150 Gy. Also, **Mansour and Ismaeil (2024)**, who reported that as the irradiation dose increased, female fecundity suffered and both sexes' fertility declined. Additionally, at 400Gy irradiation the survival rate of the offspring was. Furthermore, Similar results were obtained by **Bloem *et al.* (2003)** on female *Cryptophlebia leucotreta;* **Mohamed (2004)** on male *Agrotis ipsilon*; **Aye *et al.* (2008)** on *Plodia interpunctella*, **Salem *et al.* (2014)** on *Agrotis ipsilon* and **Sayed and Zahran (2017)** on *Helicoverpa armigera*. **Ibrahim and Sayed (2023)** on *Spodoptera frugiperda*.

The reasons of fecundity and fertility reduction may relate to dominant lethal mutation as reported by **Marec *et al.* (2001)** on *Ephestia Kuehniella* and **Milcheva (2005)** on *Galleria mellonella* or lack of eupyrene sperm transferred by irradiated male during mating as suggested by **Bloem *et al.* (2003)** and **Alm El- Din (2001)** on *Spodoptera littoralis*. Also, the loss of sperm bundles, degeneration and malformation in the resulting sperm, which were too short and clumped in damaged testis were the way to explain the deleterious effects occurred in the fecundity and fertility of treated male pupae of *E. Kuehniella* **(Sawires, 2005)**.

In addition, **Haiba (1990)** reported sever effect of gamma irradiation on the germanium and vitellarium of the overioles in the resulted females of *Phethoremia Operculella*.

On the other hand, larval duration of larvae resulted from irradiated parents' pupae, was increased in accordance to the dose increased at all different combinations this result agree with **Bloem *et al.* (2003)** on false codling moth; *Cryptophelebia leucotreta*, **Sawires (2005)** on the mediterranean flour moth; *E. kuehniella*, **Salem *et al.* (2014)** on *A. ipsilon* and **Sayed and Zahran (2017)** on *H. armigera*.

Our results indicated that the percent pupation and percent of adult emergence in F_1 progeny were suppressed by increasing the dose of gamma radiation given to the parental males or females or both. Similar results obtained by **Sallam *et al.* (2000)** on *Earias insulana*, **Salem *et al.* (2014)** on *A. ipsilon* and **Sayed and Zahran (2017)** on *H. armigera*. They reported that the rate of pupation and percent adult eclosion were decreased and correlated with irradiation dose used.

Male bases of sex ratio had been obtained in the first generation of all mattings at the different doses used (40 – 260 Gy), this result goes parallel to that of **Hoe and Tien (25)** on *Plutella xylostella*; **Abd-Elwahed (2004)** on *P. operculella*, **Sawires (2005)** on *Ephestia kuehniella* and **Sayed and Zahran (2017)** on *Helicoverpa armigera*.

Generally, As shown from the obtained results, gamma irradiation affected the different biological parameters studied (fecundity, egg-hatchability, larval and pupal mortality, larval and pupal duration, adult emergence, malformation and sex ratio) for *G. mellonella*. The magnitude of this effect was found to differ according to the crossed sexes i.e. irradiated females x irradiated males > non-irradiated females x irradiated males, irradiated females x non-irradiated males) and according to the irradiation dose.

Conclusion

From the aforementioned discussion, it could be recommended that substerilizing doses of gamma irradiation may contribute to reduction in the application of synthetic insecticides in controlling *Galleria mellonella* in stores.

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