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Research Article

Effect of Gamma Irradiation Combined with Certain Entomopathogenic Fungi on some Biological Aspects of *Galleria mellonella*

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ABSTRACT

A popular study is to analyze the effects of single doses of gamma radiation (70, 100, 125 and 150 Gy) sequentially or mixed with the Lethal Concentration 50 (LC_{50}) of certain entomopathogenic fungi (*Beauveria bassiana*, *Paecilomyces lilacinus*) against the Greater Wax Moth (GWM), *Galleria mellonella* selected biological aspects of the impact. Larval pupal period, percent pupation, percent adult pupation, sex ratio and percent adult survival of *G. mellonella* First Generation (F1).

Gamma irradiation and LC_{50} of combined treatments of *Beauveria bassiana* and *Paecilomyces lilacinus* prolonged the larval-pupal period, while pupation, adult emergence, survival and sex ratio were lower in the combined treatment than in either treatment alone get more. Second-instar the wax moth larvae from male or female pupae irradiated to 70, 100 and 125 Gy were then treated with the LC_{50} of *Beauveria bassiana* and *Paecilomyces lilacinus*, adversely affecting various biological aspects. The combined treatment was greater than either, in the case of fungal or irradiation treatment alone.

Keywords: Entomopathogenic fungi; *Galleria mellonella*; Gamma irradiation

INTRODUCTION

The greater wax moth, *Galleria mellonella* L. (Lepidoptera: *Pyralidae*) is one of the most disturbing and economically important pests of wax in the world [1-3].

This insect feeds on comb wax in the larval stage and damages it severely. The larvae of the wax moth, cause extensive damage to combs left unattended by bees. Combs in weak or dead colonies and storage areas are subject to attack [4,5]. Many researchers have focused on the selection of virulent strains for target pests and their development as biological control agents [6-8].

Entomopathogenic fungi that infect insects have received considerable attention from scientists for their potential for biological control of pests. Some insect pathogenic fungi have restricted host ranges while other fungal species have a wide host range, for example, *Beauveria bassiana* and *Paecilomyces lilacinus*.

Gamma irradiation increased the pathogenicity of the fungi against the tested larvae. The combination of the two control tools may provide satisfactory control of the insect pest, especially, in the storage [9].

The aim of the current work, study the effect of gamma irradiation in combination with LC_{50} of entomopathogenic fungi on some biological aspects of the 2nd instar larvae of *Galleria mellonella* (L.)

MATERIALS AND METHODS

Insect rearing and irradiation process

The greater wax moth, *G. mellonella* larvae were obtained from infested hives and reared in the Nuclear Research Center (NRC), Egyptian Atomic Energy Authority (EAEA), Egypt, Anshas area, the bio-insecticide Production Unit, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt. *G. mellonella* larvae reared on the artificial diet at a constant temperature of 30°C and 65 ± 5% Relative Humidity (RH) according to [10]. The irradiation process was performed using cobalt-60 gamma

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cell 220 located at cyclotron project, Nuclear Research Centre, Atomic Energy Authority (Anshas). The dose rate was 0.926 kGy/hour during the experiment. Full-grown pupae of *G. mellonella* were exposed to four doses 70, 100, 125 and 150 Gy to study the effect of irradiation on some biological aspects of F1 progeny descendant of irradiated parental male and female pupae.

Ten larvae resulting from irradiated parental males and females and transferred to clean small plastic containers and allowed to feed on an artificial diet, each treatment was replicated five times. Dead larvae were counted. The larval and pupal period, pupation, adult emergence, sex ratio, Adult survival percentage and the resulting progeny (F1) were determined as well.

The experimental media

Culture media were adjusted by the addition of:

- 10 µg/ml Dodine, 100 µg/ml Chloramphenicol, 50 µg/ml Streptomycin and 32.5 g Sabouraud Dextrose Agar (SDA) in 500 ml distilled water "Dodine media"[11].
- Chloramphenicol, Thiabendazole and Cycloheximide (CTC) medium consisting of CDA supplemented with 0.5 g/l chloramphenicol and 0.25 g/l cycloheximide and 0.002 g/l thiabendazole [12].

Isolation of the entomopathogenic fungi

The entomopathogenic fungal, *Paecilomyces lilacinus* isolates from soils and the entomopathogenic fungal, *Beauveria bassiana* isolates from dead insects.

Identification of the fungal isolates

The fungal colonies arising on the plates were purified. The purified cultures grown on CDA and SDA media were identified at Mycological Center (AUMC), Assiut University, Egypt.

The strain numbers for the *B. bassiana* was (AUMC 9894) and strain numbers for the *Paecilomyces lilacinus* was (AUMC 9884).

Bioefficacy of entomopathogenic fungi against *G. mellonella* 2nd instar larvae

To ascertain whether the most virulent isolate of fungi is pathogenic to *G. mellonella* larvae, according to the second instar larvae were each submerged for 30 seconds in nine ml of various spore concentrations from the fungal isolates. Larvae were dipped into a 0.02% Triton X-100 solution as the control treatment [13]. After that, the treated larvae were put one by one in little plastic containers and given an artificial food to consume. All treated larvae were incubated for 12 hours during the photo phase at 30°C, 65 ± 5% relative humidity. Five times each of each treatment's batches of 10 larvae were reproduced. Daily counts of dead larvae were made. According to Finney's approach, the median fatal concentration and time were determined [14].

The investigated entomopathogenic fungi's latent effect on a few biological features of *G. mellonella*

B. bassiana and *P. lilacinus* were utilized to estimate biological activity in light of the high mortality rate. To investigate how entomopathogenic fungi affect several biological characteristics, the concentrations necessary to kill 50% of larvae during the observation time (LC_{50}) were selected (Table 1).

To evaluate the biological activity of *G. mellonella* under laboratory conditions, four replicates of the 2nd instar larvae (20 larvae for each) were dipped into 9 ml of LC_{50} of the tested entomopathogenic fungi (*B. bassiana* and *P. lilacinus*) for 30 seconds. Then treated larvae were placed individually in small plastic containers and allowed to feed on the semi-synthetic diet. For the control treatment, larvae were dipped into 0.02% Triton X-100 solution. Dead larvae were counted daily. Growth parameters, namely larval-pupal period, pupation, adult emergence, sex ratio and survival (%) were recorded.

Effect of entomopathogenic fungi and gamma irradiation on some biological aspects of *G. mellonella*

To investigate the combined effect of gamma irradiation with the (LC_{50}) of various entomopathogenic fungi (*B. bassiana* and *P. lilacinus*), three dose levels of gamma irradiation (70,100,125) were used. Three experimental groups were set up to explore the influence on some biological characteristics of *G. mellonella*. The first group was made up of the 70, 100 and 125 Gy-irradiated F1 larvae offspring of the irradiated parental females as fully formed pupae. The second group was made up of the 70, 100 and 125 Gy-irradiated full-grown pupae of F1 larvae descended from irradiated parental males. Insect males and females were utilized as a control in the third group, which served as a parallel group of un-irradiated insects.

The progeny of F1 larvae of each group were fed on an artificial diet till the 2nd instar larvae, five replicates from the 2nd instar larvae (10 larvae each) were dipped into 9 ml of LC_{50} of the tested entomopathogenic fungi (*B. bassiana* and *P. lilacinus* for 30 seconds) in clean small plastic containers fitted with moist filter paper and allowed to feed on an artificial diet under laboratory condition (28 ± 20°C and 65 ± 5% relative humidity). Larval and pupal period, the percentage pupation, the percentage of adult emergence, sex ratio and the percentage of adult survival were determined.

Statistical analysis

The lethal concentration 50 was determined for established regression lines. All data obtained were analyzed using the Analysis of Variance (ANOVA) technique and the means were separated using Duncan's multiple range test (P>0.05) [15].

RESULTS

Table 1, evaluated the virulence of fungal isolates against 2nd instar larvae of *G. mellonella* expressed as the LC_{50}, LC_{95} and slope of toxicity regression lines after 10 days of dipping in different concentrations (Table 1).

The latent effect of gamma irradiation on some biological aspects of F1 progeny descendant of irradiated parental males as full-grown pupae.

Table 2, demonstrates how gamma radiation affected the larval and pupal periods, as well as the sex ratio, of the greater wax moth, *Galleria mellonella* in F1 male offspring of the irradiated paternal males as full-grown pupae with four doses of 70, 100, 125 and 150 gray with an increase in dose, the average larval and pupal period lengthens noticeably. In comparison to 35.25 days for the control treatment, it rises to 41.73, 44.72, 45.80 and 47 days for the four doses, respectively. With an increase in dose, the average sex ratio rose. At the four doses of 70, 100, 125 and 150 Gy, it rises to 1.20, 1.33, 1.40 and 1.60, respectively, as opposed to 1.18 for the control treatment (Table 2).

Figure 1, shows the percentages of pupation, emergence and survival of *G. mellonella* in F1 male descendants of irradiated parental males at four different doses: 70, 100, 125 and 150 gray. The percentage of pupation and the rise in dosages were negatively correlated. When compared to 92% for the control treatment, the percentage of pupation significantly drops to 78, 74, 68 and 60% at the four doses of 70, 100, 125 and 150 Gy, respectively. Meanwhile, the percentage of the F1 generation's adult emergence greatly drops for all treatments. It drops from 93.55% for the control treatment to 82.69, 80.71, 70.23 and 56.76% at the four doses of 70, 100, 125 and 150Gy, respectively (Figure 1). Additionally, Figure 1 shows that as the dose was increased, the proportion of larvae that reached the adult stage considerably dropped. At the four doses of 70, 100, 125 and 150Gy, the survival percentage fell to 66, 60, 48 and 34, respectively, from 86% for the control treatment.

The average larval and pupal period dramatically lengthens with an increase in dose (Table 3). Compared to 35.25 days for the control treatment, it rises to 42.27, 44.41 and 46.26 days for the three doses, respectively. With the dose increase, the average sex ratio went up. At the three doses of 70, 100 and 125 Gy, it rose from 1.18 for the control treatment to 1.40, 1.60 and 1.80, respectively (Table 3).

Figure 2 demonstrates a negative association between the increase in dosages and the percentage of pupation.

At the three dosages of 70, 100 and 125 Gy, respectively, the percentage of pupation drastically decreased to 62, 54 and 38% compared to 92% for the control treatment, while the parental females that received the dose of 150 Gy did not produce any offspring.

At all treatments, there was a considerable reduction in the F1 generation's (F1 females) adult emergence percentage. It drops from 93.55% for the control treatment to 67.23, 62.00 and 58.66% for the three dosages of 70, 100 and 125 Gy, respectively (Figure 2). Additionally, a significant decrease in the percentage of larvae that reached the adult stage was seen as the dose was increased (Figure 2). In comparison to the control treatment, the percentage of survivorship reduced to 42, 34 and 22% at the three doses of 70, 100 and 125 Gy, respectively.

Table 1: Virulence of fungal isolates against 2^{nd} instar larvae of *G. mellonella**.

Fungal isolates	LC_{50} conidia/ml	LC_{95} conidia/ml	slope	X^2	P value
Beauveria bassiana	1.2×10^5	1.9×10^8	0.2620 ± 0.0305	13.15	0.001
Paecilomyces lilacinus	2.3×10^5	4.3×10^{11}	0.2064 ± 0.0299	4.3	0.12

Note: (*): Expressed as the Lethal Concentration 50 (LC_{50}) , LC_{95} and slope of toxicity regression lines after 10 days of dipping in different concentration.

Table 2: Effect of gamma irradiation on the progeny of the greater wax moth, *Galleria mellonella*.

Doses (Gy)	Larval and pupal period/ day ± standard error	% Pupation ± standard error	% Emergence ± standard error	Sex ratio ± standard error		% Survival ± standard error
				male	female	
Control	35.25 ± 0.59^{d}	92 ± 3.75^{a}	93.55 ± 2.64^{a}	1.18 ± 0.13^{a}	1	86 ± 4.01^{a}
70	41.73 ± 0.71^{c}	78 ± 3.17^{b}	82.69 ± 2.64^{b}	1.20 ± 0.08^{a}	1	66 ± 2.45^{b}
100	44.72 ± 0.26^{b}	74 ± 2.45^{bc}	80.71 ± 3.81^{b}	1.33 ± 0.09^{a}	1	60 ± 4.48^{b}
125	45.80 ± 0.65^{ab}	68 ± 3.75^{cd}	70.23 ± 1.60^{c}	1.40 ± 0.18^{a}	1	48 ± 3.75^{c}
150	47.00 ± 0.28^{a}	60 ± 3.17^{d}	56.76 ± 3.17^{d}	$1.6 0 \pm 0.24^{a}$	1	34 ± 2.45^{d}
Least significant difference 0.05	1.73	9.69	8.44	0.47		10.38

Note: ($^{a-d}$): Means followed by the same letter in each column (small letters) represent that are not significantly different at ($p < 0.05$).

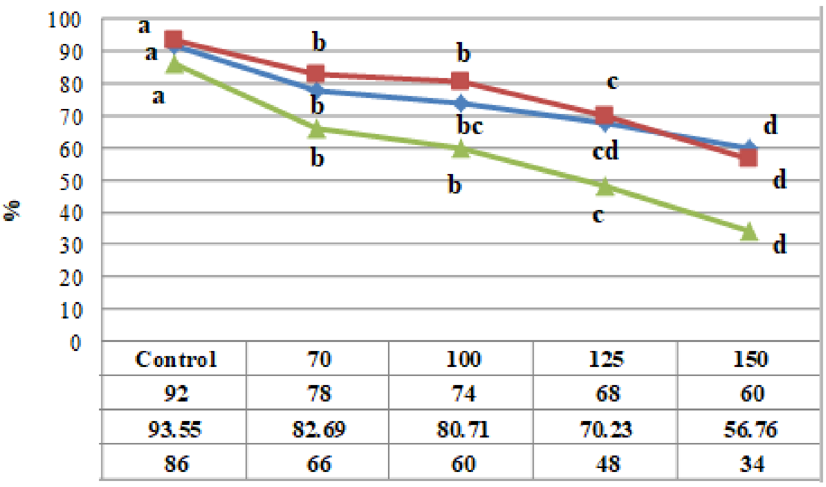

Figure 1: Effect of gamma irradiation on the progeny of the greater wax moth, *Galleria mellonella* among F1 males descendant of the irradiated parental males as fullgrown pupae. **Note:** ( ): % Pupation; ( ): % Emergence; ( ): % Survival.

Table 3: Effect of gamma irradiation on the progeny among F1 females of the greater wax moth, *Galleria mellonella*

Doses (Gy)	Larval and pupal period ± standard error	% Pupation ± standard error	% Emergence ± standard error	Sex ratio ± standard error		% Survival ± standard error
				male	female	
Control	35.25 ± 0.59^{c}	92 ± 3.75^{a}	93.55 ± 2.64^{a}	1.18 ± 0.13^{a}	1	86 ± 4.01^{a}
70	42.27 ± 0.84^{b}	62 ± 3.75^{b}	67.23 ± 2.10^{b}	1.4 ± 0.08^{a}	1	42 ± 3.75^{b}
100	44.41 ± 0.50^{ab}	54 ± 4.01^{bc}	62.00 ± 3.27^{b}	1.6 ± 0.09^{a}	1	34 ± 4.01^{b}
125	46.26 ± 0.88^{a}	38 ± 3.75^{c}	58.66 ± 3.75^{b}	1.8 ± 0.18^{a}	1	22 ± 2.00^{c}
Least significant difference 0.05	2.16	11.41	8.44	0.47		10.38

Note: ($^{a\text{-}d}$): Means followed by the same letter in each column (small letters) represent that are not significantly different at ($p < 0.05$). The progeny among F1 females of the greater wax moth, *Galleria mellonella* descending of the irradiated parental females as full-grown pupae.

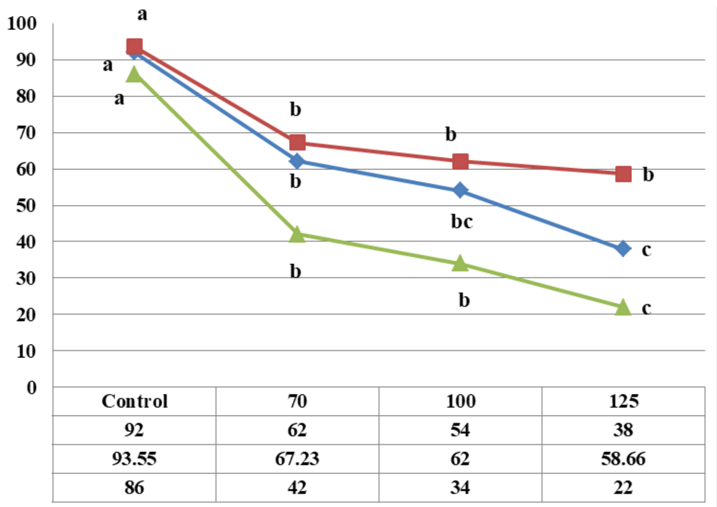

Figure 2: Effect of gamma irradiation on the percentage pupation, emergence and survival of the greater wax moth, *Galleria mellonella*in F1 male progeny of the irradiated parental females as full- grown pupae. **Note:** ( ): % Pupation; ( ): % Emergence; ( ): % Survival.

The combined impact of entomopathogenic fungi and gamma irradiation on some biological characteristics of the greater wax moth, *Galleria mellonella*

Table 4 shows the impact of gamma irradiation in conjunction with the LC_{50} of the entomopathogenic fungi, *B. bassiana* and *P. lilacinus*, on the average larval and pupal period per day and the sex ratio of G. *mellonella* descending from the irradiated parental males.

As the irradiation dose was increased, there was a substantial increase in the mean larval and pupal durations (Table 4). It considerably increased from 36.22 days in the control treatment to 43.55, 46.33 and 48.10 days for *B. bassiana* combined with the doses of 70, 100 and 125 Gy, respectively. Additionally, for *P. lilacinus* paired with the identical prior irradiation dosages, it dramatically rose to 42.14, 45.21 and 46.63 days, respectively.

Table 4 data also reveals that the sex ratio was 1.4 and 1.6 in favor of males for *B. bassiana* paired with doses of 70 and 100 Gy, respectively, but was 1:1 at the dosage rate of 125 Gy as opposed to 1.1:1.0 at the control treatment. Additionally, it rose to 1.33, 1.30 and 1.20 following *P. lilacinus* treatment in conjunction with the identical dosages of prior irradiation, respectively.

Figure 3 illustrates how gamma radiation and the entomopathogenic fungi's LC_{50} affect the proportion of pupation, emergence and survival. With increasing irradiation dose, the percentage of pupation was dramatically reduced and treatments on *B. bassiana* showed greater declines than those on *P. lilacinus*. When combined with doses of 70, 100 and 125 Gy respectively, it significantly decreased for *B. bassiana* to 42, 30 and 22%, respectively, compared to 80% in the control treatment, while it significantly decreased for *P. lilacinus* to 50, 38 and 28%, respectively, when combined with the same previous irradiation doses.

In comparison to the control treatment, the percentage of adult emergence for *B. bassiana* combined with doses of 70, 100 and 125 Gy was considerably reduced to 71, 63.33 and 70%, respectively. Additionally, for *P. lilacinus* paired with the identical prior irradiation dosages, it significantly decreased to 75, 70 and 63.33%, respectively (Figure 3). Figure 3 also shows that as the irradiation dose was increased, the proportion of survival fell. In comparison to the control treatment, it was significantly reduced, dropping to 30, 20 and 16% for *B. bassiana* paired with dosages of 70, 100 and 125 Gy respectively. Additionally, for *P. lilacinus* treatment paired with the same prior irradiation dosages, it significantly decreased to 38, 26 and 18%, respectively.

The effect of gamma irradiation in combination with the (LC_{50}) of the entomopathogenic fungi, *B. bassiana* and *P. lilacinus*, is shown with respect to the mean larval and pupal period as well as the sex ratio of G. *mellonella* descending from the irradiated parental females (Table 5).

With increasing irradiation dose, the mean larval and pupal period increased considerably. It considerably increased from 36.22 days in the control treatment to 44.73, 47.56 and 48.70 days at 70, 100 and 125 Gy dosages combined with *B. bassiana*. Additionally, it considerably increased from 36.22 days in the control treatment to 43.27, 45.16 and 47.40 days at 70, 100 and 125 Gy, respectively, when coupled with *P. lilacinus*.

Table 5 data also reveals that at the two doses of 70 Gy and 100 Gy mixed with *B. bassiana*, the sex ratio was in favor of males (1.5 and 1.0, respectively). However, it was significantly decreased from 1.1 in the control treatment to 0.6 with a dose of 125 Gy. At the two doses of 70 Gy and 100 Gy mixed with *P. lilaceous*, it also climbed to 1.7 and 1.2, respectively. At 125 Gy, however, it decreased to 0.8.

The percentage of pupation, emergence and adult survival of G. *mellonella* descended from irradiated parental females is shown in Figure 4 to be affected by gamma irradiation and the entomopathogenic fungi's (LC_{50}). The percentage of pupation was significantly decreased with the increasing irradiation dose. It significantly decreases to 36, 22 and 16% with the three doses 70, 100 and 125 Gy combined with *B. bassiana* respectively, compared to 80% in the control treatment. Also, it significantly decreased to 46, 26 and 20% with the three doses 70, 100 and 125 Gy combined with *P. lilacinus* respectively. Additionally, Figure 4 shows that for all treatments in *B. bassiana* and *P. lilacinus*, the percentage of emergence decreases when the irradiation dose is increased. In comparison to the control treatment, it considerably dropped from 95% to 64.33, 73.33 and 60.00% at the three doses of 70, 100 and 125 Gy coupled with *B. bassiana*, respectively. While it considerably decreases from 95% in the control treatment to 74.00, 65.00 and 53.000% at the three dosages of 70, 100 and 125 Gy coupled with *P. lilacinus*, respectively (Figure 4).

Also, Figure 4 shows that as the irradiation dose are increased, the percentage of adult survival decreases and it decrease more in *B. bassiana* than in *P. lilacinus*. When joined with *B. bassiana* at the three doses of 70, 100 and 125 Gy, it was significantly reduced from 76% in the control treatment to 24, 16 and 10%, respectively. While at the three doses of 70, 100 and 125 Gy joined with *P. lilacinus*, it drastically decreases to 34, 18 and 14%, respectively.

Table 4: Effect of gamma irradiation combined with the LC_{50} of the entomopathogenic fungi on some biological aspects.

Radiation doses (Gy)	Fungi	Average larval and pupal period / day ± standard error	% Pupation ± standard error	% Emergence ± standard error	Sex ratio		%Survival ± standard error
					Male	Female	
Control	-	36.22 ± 0.58^{d}	80 ± 3.17^{a}	95.00 ± 03.07^{a}	1.13 ± 0.11^{a}	1	76 ± 4.01^{a}
70 Gy	*B.bassiana*	43.55 ± 0.29^{c}	42 ± 2.00^{bc}	71.00 ± 05.33^{b}	1.40 ± 0.48^{a}	1	30 ± 3.17^{bc}
	P.lilacinus	42.14 ± 0.41^{d}	50 ± 3.17^{b}	75.66 ± 04.14^{b}	1.33 ± 0.27^{a}	1	38 ± 3.75^{b}
100 Gy	*B.bassiana*	46.33 ± 0.39^{b}	30 ± 4.48^{de}	63.33 ± 05.66^{b}	1.60 ± 0.24^{a}	1	20 ± 4.48^{c}
	P.lilacinus	45.21 ± 0.44^{b}	38 ± 2.00^{cd}	68.33 ± 04.87^{b}	1.30 ± 0.30^{a}	1	26 ± 2.45^{bc}

125 Gy	B.bassiana	48.10 ± 0.34ᵃ	22 ± 2.00ᵉ	70.00 ± 12.28ᵇ	1.00 ± 0.31ᵃ	1	16 ± 2.45ᵈ
	P.lilacinus	46.63 ± 0.32ᵇ	28 ± 2.00ᵉ	63.33 ± 02.98ᵇ	1.20 ± 0.20ᵃ	1	18 ± 2.00ᶜ
Least significant difference		1.2	8.19	18.04	0.904		10.15

Note: (a-e): Means followed by the same letter in each column represent that are not significantly different at ($p < 0.05$). The insects of *G. mellonella* resulted from irradiated parental males.

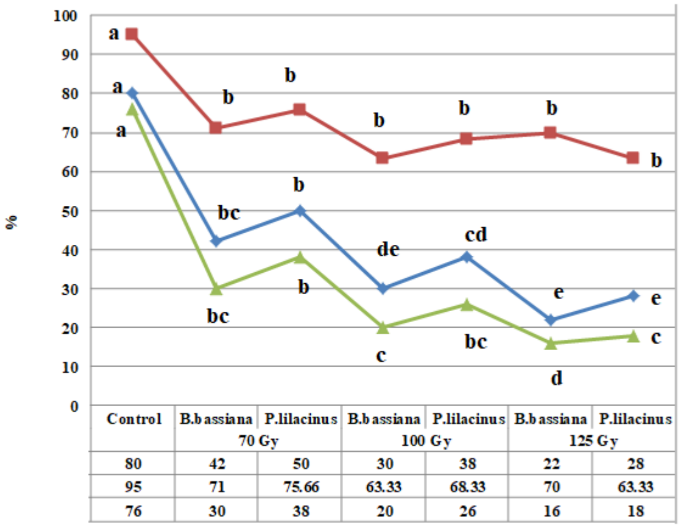

Figure 3: Effect of gamma irradiation combined with the LC_{50} of the entomopathnogenic fungi on the percentage pupation, emergence and survival. **Note:** ( ): % Pupation; ( ): % Emergence; ( ): % Survival.

Table 5: Effects of entomopathogenic fungus (LC50) in combination with gamma irradiation on certain biological aspects.

Radiation dose (Gy)	Fungi	Mean of larval and pupal period / day ± standard error	% Pupation ± standard error	% Emergence ± standard error	Sex ratio		% Survival ± standard error
					Male	Female	
Control (0)	-	36.22 ± 0.58ᵈ	80 ± 3.17ᵃ	95.00 ± 03.07ᵃ	1.1 ± 0.11ᵃ	1	76 ± 4.01ᵃ
70 Gy	B.bassiana	44.73 ± 0.43ᵇ	36 ± 4.01ᶜ	64.33 ± 08.18ᵃ	1.5 ± 0.44ᵃ	1	24 ± 5.11ᵇᶜ
	P.lilacinus	43.27 ± 0.62ᶜ	46 ± 2.45ᵇ	74.00 ± 03.68ᵃ	1.7 ± 0.43ᵃ	1	34 ± 2.45ᵇ
100 Gy	B.bassiana	47.56 ± 0.37ᵃ	22 ± 2.00ᵈ	73.33 ± 11.33ᵃ	1.0 ± 0.31ᵃ	1	16 ± 2.45ᶜ
	P.lilacinus	45.16 ± 0.73ᵇ	26 ± 4.01ᵈ	65.00 ± 10.02ᵃ	1.2 ± 0.37ᵃ	1	18 ± 4.91ᶜ
125 Gy	B.bassiana	48.70 ± 0.34ᵃ	16 ± 2.45ᵈ	60.00 ± 18.75ᵃ	0.6 ± 0.40ᵃ	1	10 ± 3.17ᶜ
	P.lilacinus	47.40 ± 0.18ᵃ	20 ± 3.17ᵈ	53.00 ± 16.20ᵃ	0.8 ±0.37ᵃ	1	14 ± 3.75ᶜ
Least significant difference		1.43	9.02	33.36	1.06		11.05

Note: (a-d): Means followed by the same letter in each column represent that are not significantly different at ($p < 0.05$). The insects of *G. mellonella* in this table descending of the irradiated parental females.

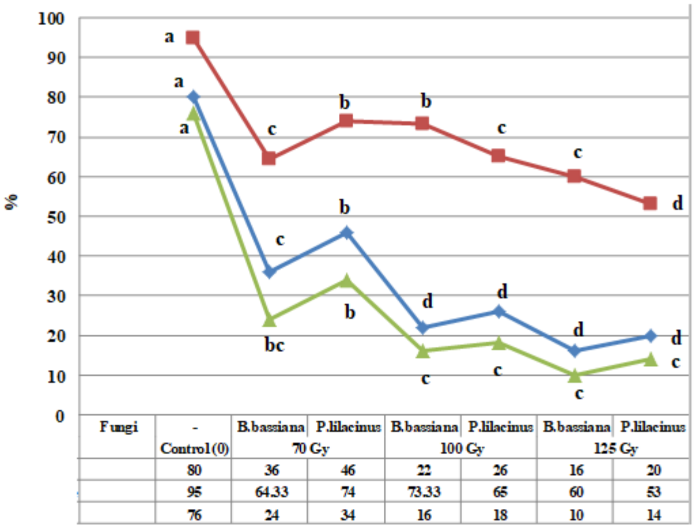

Figure 4: The percentage of pupation, emergence and adult survival of *G. mellonella* descended from irradiated parental females. **Note:** ( ): % Pupation; ( ): % Emergence; ( ): % Survival.

DISCUSSION

The biological activity (larval and pupal period, pupation, adult emergence, sex ratio and survival) of 2nd instar larvae of *G. mellonella* treated with LC_{50} of *B. bassiana* and *P. lilacinus* has been studied. The results obtained indicated that the LC_{50} of entomopathogenic fungi caused a significant increase in the larval and pupal period as compared to the control. These results recognized that the crude extracts of isolated fungi were screened against *G. mellonella* applied different fungal crude extracts of isolates achieved variable results on both larval and adult mortality percentages. The effect of crude extracts of *Beauveria bassiana* (Siwa and El- Farafra isolate) giving total death percentages (82.48 and 74.22 % respectively) to *G. mellonella* larvae comparing with the control treatment 0%. The results exhibited significant effects of *B. bassiana* (El-Farafra and Siwa Oasis isolates) against *G. mellonella* (larval and pupal weights). The % of larvae death increased with increasing the concentration of crude extract of *B. bassiana* (El-Farafra and Siwa isolates). Also pupal death percentage of *G. mellonella* increased while adult emergence decreased with increasing concentration of crude extract of El-Farafra and Siwa isolates of *B. bassiana*. Abd EL-Wahed, et al. [6]; Ibrahim, et al. [16] who found that the LC_{50} (108 conidia/ml) of the entomopathogenic fungi, *B. bassiana* and *M. anisopliae* prolonged the larval and pupal period of *A. ipsilon*. Likewise founded that the *B. bassiana* significantly reduced the larval period of *S. litura* and this reduction was recorded in second and fourth instar treated larvae. Moreover, the LC_{50} of *B. bassiana* and *P. lilacinus* reduced pupation, adult emergence and the percent survival of *G. mellonella*. Similarly, the present investigation also, agreement with 17 who found *B. bassiana* at conidial concentration 108 conidia/ml reduce the pupation and the adult emergence of *G. mellonella* to 20 and 13.33%, respectively [17].Kannan, et al., [18] found that *Metarhizium anisopliae* at the highest conidial concentration (108) significantly reduced the emergence of *Anopheles stephensi* to 40 %. In addition, the *B. bassiana* at spore concentration 2.4 × 107 and 2.4 × 104 conidia/ml caused the least pupation and adult emergence of *S. litura*, respectively [19]. While, Abd EL-Wahed, et al. [6] found that the LC_{50} (108 conidia /ml) of the entomopathogenic fungi, *B. bassiana* and *M. anisopliae* reduce the adult emergence of *A. ipsilon* to 78.7 and 80.9%, respectively. Kaur, et al. [16] also, observed a fewer number of adults (15.56 - 72.02%) emerged when larvae of *S. litura* treated with the entomopathogenic fungi, *B. bassiana*. Our results showed that the sex ratio was affected, with the fungal treatments, which was in favor of the male. These findings are in agreement with those found by Rizk, et al., [17] who observed male ratio was greater than the female ratio when the 4th instar larvae of *G. mellonella* treated with *B. bassiana*.

These results were in agreement with LaChance, et al., [20] who reported that the survival to the adult stage of F1 larvae of pink bollworm were significantly reduced at all doses and the sex ratio among emerging F1 adults was also skewed in favor of males and more males were obtained when P1 males were treated with the dose of 50 Gy and above. El- Kady, et al., [21] also, found that the adult emergence was reduced as the radiation dose increased. Similarly, Carpenter, et al., [22] reported on fall armyworm when irradiated P1 adults with the dose 100 Gy. Besides, Saour, et al., [23] exposed the adult male *Phthorimaea operculella* to different doses of gamma irradiation (100, 150 and 200 Gy) he found that the mean developmental time and the percentage mortality of the F1 progeny increased at each examined dose. Moreover, the sex ratio of the F1 progeny was skewed in favor of the males. Both on *Spodoptera litura* and on *Cryptophlebia leucotreta* reported that sex ratio skewed in favor of males [24,25]. On the other hand, no observation was found on a skewed sex ratio in favor of male offspring for the *Cactoblastis cactorum* F1 adults [26].

The effect of different doses of gamma irradiation (70,100 and 125 Gy) in combination with the LC_{50} of the entomopathogenic fungi tested, *B. bassiana* and *P. lilacinus* against the 2nd instar larvae of *G. mellonella* descending of the irradiated parental males mated with un-irradiated females or un-irradiated males mated with the irradiated females on some biological aspects were examined. Our results showed that the combined effect prolonged the larval and pupal duration; reduced the percentage of pupation, adult emergence and survival. The sex ratio of the F1 generation also declined more in favor of males.

CONCLUSION

The present study indicated that the combined effect of the entomopathogenic fungi and gamma irradiation-induced more remarkable effects as compared to the entomopathogenic fungi or gamma irradiation of each them alone. Our results are following Rizk, et al., [17] using *B. bassiana* at 104 and 108 spores / ml combined with different doses of gamma irradiation (50,100 and 150) against the 4th instar larvae of the greater wax moth, *G. melonella*, the efficiency of *B. bassiana* increased, especially when the gamma irradiation dose was increased, where no adults were produced with both the fungal concentrations and 150 Gy gamma irradiation dose. Abd EL- Wahed, et al. [6] also, showed that the LC_{50} (108) of the two entomopathogenic fungi; *B. bassiana* and *M. anisopliae* in combined with the LD_{50} (72 Gy) of gamma irradiation against the 2nd instar larvae of *A. ipsilon* significantly increased the larval and pupal period and decreased the percentage adult emergence, of *A. ipsilon*. Hussein, et al., [10] indicated that the gamma irradiation increased the pathogenicity of the fungi against the tested larvae. The combination between the two control tools may provide satisfactory control of the insect-pest, especially, in the storage.

AUTHORS' CONTRIBUTIONS

The Manuscript writing was done by all authors. HF contributed writing and final review, S M set the basic ideas for the manuscript, MAS revised and corrected, AAMI developed and designed the biological control experiments and OEAE designed, wrote and analyzed the tables statistically, reference settings were done by all authors. Then, final manuscript was read and approved by all authors. All authors read and approved the final manuscript.

ETHICS APPROVAL

The authors declare that they have ethics approval and consent to participate.

CONSENT FOR PUBLICATION

The authors consent for publication.

AVAILABILITY OF DATA AND MATERIALS

Data supporting the conclusions of this article are presented in the main Manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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