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

Hussein Farid Mohamed (✉ husseinmohamed29@yahoo.com)

Egyptian Atomic Energy Authority <https://orcid.org/0000-0002-3289-8383>

Samira Elsayed Mustafa El-Naggar

Egyptian Atomic Energy Authority

Mahmoud Abd El-Mohsen Sweilem

Benha University Faculty of Science

Adly Mohamed Ibrahim

ARC Plant Protection Research Institute

Ola Elsayed Abd Alrahman El-khawaga

EAEA: Egyptian Atomic Energy Authority

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Abstract

Background: the prevailing research become analyzing the impact of the one-of-a-kind doses of gamma irradiation

(70,100,125 and 150 Gy) one after the other or mixed with the LC₅₀ of certain entomopathogenic fungi (*Beauveria bassiana*, *Paecilomyces lilacinus*) on selected biological aspects of the greater wax moth, *Galleria mellonella* (larval–pupal period, The percentage pupation, The percentage adult emergence, sex ratio and The percentage adult survival) many of the first generations (F₁) of *G. mellonella*.

Results: The joint treatment of Gamma irradiation and LC₅₀ of *Beauveria bassiana* and *Paecilomyces lilacinus* increased the larval - pupal periods, while, pupation, adult emergence, survival percentage and the sex ratio were reduced in the combined treatments more than each treatment alone.

Conclusions: The second instar larvae of *Galleria mellonella* descending of the irradiated parental male or female pupae with 70,100 and 125 Gy then treated with the LC₅₀ of *Beauveria bassiana* and *Paecilomyces lilacinus* adversely affected on the different biological aspects. The combined treatment was greater than either, in the case of fungal or irradiation treatment alone.

Background

The greater wax moth (GWM), *Galleria mellonella* L. (Lepidoptera: Pyralidae) is one of the most disturbing and economically important pests of wax in the world (Chang and Hsieh **1992**, Haewoon et al. **1995**, Hussein et al. **2018**).

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 (Caron **1992**).

Many researchers have focused on the selection of virulent strains for target pests and their development as biological control agents (Abd EL-Wahed **2011**, Ibrahim et al. **2016**, Nazir et al. **2019**).

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 (Mohamed et al. **2018**).

The aim of the current work, study the effect of gamma irradiation in combination with LC₅₀ of entomopathogenic fungi on some biological aspects of the 2nd instar larvae of *Galleria mellonella* (L.)

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 an artificial diet at a constant temperature of 30°C and 65±5% relative humidity (R.H) according to (Metwally et al. **2012**). The irradiation process was performed using cobalt-60 gamma 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.

Isolation of the Entomopathogenic Fungi

The entomopathogenic fungal, *Paecilomyces lilacinus* isolates from soils and the entomopathogenic fungal, *Beauveria bassiana* isolates from dead insects (Ibrahim et al. 2016, El-khawaga 2017).

The Latent Effect of the Investigated Entomopathogenic Fungi on Some Biological Aspects of *G. Mellonella*

According to the high mortality percentage; *B. bassiana* and *P. lilacinus* were used in the determination of biological activity. The concentrations required to kill 50% of larvae during the observation period (LC₅₀) were chosen to study the effect of entomopathogenic fungi on some biological aspects (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 LC₅₀ of the tested entomopathogenic fungi (*B. bassiana* and *Plilacinus*) 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.

Table 1 Virulence of fungal isolates against 2nd instar larvae of *G. mellonella**

Fungal isolates	LC ₅₀ conidia/ml	LC ₉₅ conidia/ml	slope	χ ²	P value
<i>Beauveria bassiana</i>	1.2×10 ⁵	1.9×10 ⁸	0.2620±0.0305	13.15	0.001
<i>Paecilomyces lilacinus</i>	2.3×10 ⁵	4.3×10 ¹¹	0.2064±0.0299	04.30	0.120

* expressed as the LC₅₀, LC₉₅, and slope of toxicity regression lines after 10 days of dipping in different concentration

Combined Effect of Gamma Irradiation and Entomopathogenic Fungi on Some Biological Aspects of *G. mellonella*

Three dose levels of gamma irradiation (70,100,125) were chosen to study the combined effect of gamma irradiation with the (LC₅₀) of different entomopathogenic fungi (*B. bassiana* and *Plilacinus*) on some biological aspects.

For studying the effects on some biological aspects of *G. mellonella*, three experimental groups were set up. The first group consisted of the progeny of F₁ larvae descendants of the irradiated parental females as full-grown pupae with the three doses; 70,100 and 125Gy. The second group consisted of the progeny of F₁ larvae descendant of the irradiated

parental males as full-grown pupae with the three doses; 70,100 and 125Gy. The third group was used as a parallel group of unirradiated insects; males and females were used as a control.

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 LC₅₀ 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±2⁰C 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) (Steel and Torrie 1980)

Results

Table 1, Evaluated the Virulence of fungal isolates against 2nd instar larvae of *G. mellonella* expressed as the LC₅₀, LC₉₅, and slope of toxicity regression lines after 10 days of dipping in different concentration (**Methods**)

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

Table 2, shows the effect of gamma irradiation on the larval and pupal period, the percentage pupation, the percentage of emergence, the sex ratio and the percentage survival of *G. mellonella* among F₁ male descendants of irradiated parental males as full-grown pupae with four doses 70,100,125 and 150 gray. The average larval and pupal period significantly increases with the dose increase. It increases to 41.73, 44.72, 45.80 and 47 days at the four doses, respectively, compared to 35.25 days for the control treatment.

There was a negative correlation between the percentage of pupation and the increase of doses. The percentage of pupation significantly decreases to 78, 74, 68 and 60% at the four doses 70, 100, 125 and 150Gy respectively, compared to 92% for the control treatment (**Table 2**) while the percentage of the adult emergence of the F₁ generation was significantly decreased at all treatments. It decreases to 82.69, 80.71, 70.23 and 56.76 % at the four doses 70, 100, 125 and 150Gy respectively, compared to 93.55% for the control treatment (**Table 2**).

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

Doses (Gy)	Larval and pupal period/day ±SE	%pupation ±SE	% Emergence ±SE	Sex ratio ±SE		% survival ±SE
				male	female	
Control	35.25 ± 0.59 d	92 ± 3.75a	93.55 ± 2.64a	1.18 ± 0.13 a	1	86 ± 4.01a
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 ± 0.65ab	68 ± 3.75 cd	70.23 ± 1.60 c	1.40 ± 0.18 a	1	48 ± 3.75 c
150	47.00 ± 0.28a	60 ± 3.17 d	56.76 ± 3.17 d	1.60 ± 0.24 a	1	34 ± 2.45 d
LSD 0.05	1.73	9.69	8.44	0.47		10.38

*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 males of the greater wax moth, *Galleria mellonella* descending of the irradiated parental males as full-grown pupae

Table 2 also, displays that the average sex ratio was increased with the dose increase. It increases to 1.20, 1.33, 1.40 and 1.60 at the four doses 70, 100, 125 and 150Gy respectively, compared to 1.18 for the control treatment.

Additionally, the percentage of larvae that survived to the adult stage (**Table 2**) was significantly decreased with the dose increase. The percentage of survival was decreased to 66, 60, 48 and 34 at the four doses 70, 100, 125 and 150Gy respectively, compared to 86% for the control treatment.

Table 3 shows the P₁ females irradiated as full-grown pupae with 70, 100, 125 and 150 Gy then mated with a non-irradiated males. We observed that the parental females, which irradiated with the dose 150 Gy were, did not give any progeny.

The average larval and pupal period significantly increases with the dose increase. It increases to 42.27, 44.41 and 46.26 days at the three doses, respectively, compared to 35.25 days for the control treatment (**Table 3**).

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

Doses (Gy)	Larval and pupal period ± SE		%pupation ±SE	% Emergence ±SE	Sex ratio		% survival ±SE
					±SE		
					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
LSD 0.05	2.16		11.41	8.44	0.47		10.38

*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

There was a negative correlation between the percentage of pupation and the increase of doses. The percentage of pupation significantly decrease to 62, 54 and 38% at the three doses 70, 100 and 125 Gy respectively, compared to 92% for control treatment while, the parental females which irradiated with the dose 150 Gy did not give any progeny (**Table 3**).

The percentage of the adult emergence of the F₁ generation (F₁ females) was significantly decreased at all treatments. It decreases to 67.23, 62.00 and 58.66% of the three doses 70, 100 and 125 Gy, respectively, compared to 93.55% for the control treatment (**Table 3**).

Additionally, the percentage of larvae that survived to the adult stage (**Table 2**) was significantly decreased with the dose increase. The percentage of survival was decreased to 42, 34 and 22 % at the three doses 70, 100 and 125 Gy, respectively, compared to 86% for the control treatment.

Table 3 also, displays that the average sex ratio was increased with the dose increase. It was increased to 1.40, 1.60 and 1.80 at the three doses 70, 100 and 125 Gy, respectively, compared to 1.18 for the control treatment

The combined effect of gamma irradiation and the entomopathogenic fungi on some biological aspects of the Greater wax moth, *Galleria mellonella*

Table 4 demonstrates the effect of gamma irradiation combined with the LC₅₀ of the entomopathogenic fungi; *B. bassiana* and *M. anisopliae* on some biological aspects of *G. mellonella* descending of the irradiated parental males.

Table 4 Effect of gamma irradiation combined with the LC₅₀ of the entomopathogenic fungi on some biological aspects

Radiation Doses (Gy)	Fungi	Average Larval and pupal Period / day $\pm$ SE	% Pupation $\pm$ SE	% Emergence $\pm$ SE	Sex ratio		%Survival $\pm$ SE
					Male $\pm$ SE	Female	
Control	-	36.22 $\pm$ 0.58 d	80 $\pm$ 3.17 a	95.00 $\pm$ 03.07 a	1.13 $\pm$ 0.11 a	1 1	76 $\pm$ 4.01 a
70 Gy	<i>B.bassiana</i>	43.55 $\pm$ 0.29 c	42 $\pm$ 2.00 bc	71.00 $\pm$ 05.33 b	1.40 $\pm$ 0.48 a	1	30 $\pm$ 3.17 bc
	<i>P.lilacinus</i>	42.14 $\pm$ 0.41 d	50 $\pm$ 3.17 b	75.66 $\pm$ 04.14 b	1.33 $\pm$ 0.27 a	1	38 $\pm$ 3.75 b
100 Gy	<i>B.bassiana</i>	46.33 $\pm$ 0.39 b	30 $\pm$ 4.48 de	63.33 $\pm$ 05.66 b	1.60 $\pm$ 0.24 a	1	20 $\pm$ 4.48 c
	<i>P.lilacinus</i>	45.21 $\pm$ 0.44 b	38 $\pm$ 2.00 cd	68.33 $\pm$ 04.87 b	1.30 $\pm$ 0.30 a	1	26 $\pm$ 2.45 bc
125 Gy	<i>B.bassiana</i>	48.10 $\pm$ 0.34 a	22 $\pm$ 2.00 e	70.00 $\pm$ 12.28 b	1.00 $\pm$ 0.31 a	1	16 $\pm$ 2.45 d
	<i>P.lilacinus</i>	46.63 $\pm$ 0.32 b	28 $\pm$ 2.00 e	63.33 $\pm$ 02.98 b	1.20 $\pm$ 0.20 a	1	18 $\pm$ 2.00 c
LSD		1.20	8.19	18.04	0.904		10.15

*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

Also, Table 4 shows that the mean of larval and pupal periods were significantly increased with increasing the irradiation dose. It highly significantly increased to 43.55, 46.33 and 48.10 days for *B. bassiana* combined with the doses 70, 100 and 125 Gy respectively, compared to 36.22 days in the control treatment. Also, it significantly increased to 42.14, 45.21 and 46.63 days for *P. lilacinus* combined with the same previous irradiation doses, respectively.

As Shown from the previous data given in Table 4, the percentage of pupation was significantly decreased with increasing the irradiation dose and more decreases at *B. bassiana* than at *P. lilacinus* treatments. It significantly decreased to 42, 30 and 22% for *B. bassiana* when combined with the doses 70, 100 and 125 Gy respectively, compared to 80% in the control treatment while, it significantly decreases to 50, 38 and 28% for *P. lilacinus* combined with the same previous irradiation doses, respectively.

The percentage of adult emergence was significantly reduced to 71, 63.33 and 70 % for *B. bassiana* combined with the doses 70, 100 and 125 Gy respectively, compared to 95% in the control treatment Also, it significantly reduced to 75, 70 and 63.33 % for *P. lilacinus* combined with the same previous irradiation doses, respectively (Table 4).

Also, data in Table 4 shows that the sex ratio was in favour of males for *B. bassiana* combined with the doses 70 and, 100 Gy (1.4 and 1.6, respectively), but was 1:1 at the dose rate 125 Gy compared to 1.1:1.0 at the

control treatment. Also, it increased to 1.33 and 1.30 and 1.20 for *P. lilacinus* treatment combined with the same previous irradiation doses, respectively.

Additionally, **Table 4** demonstrates that the percentage of survival decreased with increasing the irradiation dose. It was significantly decreased to 30, 20 and 16 % for *B. bassiana* combined with the doses 70, 100 and 125 Gy respectively, compared to 76% in the control treatment. Also, it significantly reduced to 38, 26 and 18% for *P. lilacinus* treatment combined with the same previous irradiation doses, respectively.

Table 5 indicates that the effect of gamma irradiation combined with the (LC₅₀) of the entomopathogenic fungi; *B. bassiana* and *P. lilacinus* on some biological aspects of *G. mellonella* descending of the irradiated parental females.

The mean larval and pupal periods were significantly increased with the increasing irradiation dose. It highly significantly increased to 44.73, 47.56 and 48.70 days at 70, 100 and 125 Gy doses combined with *B. bassiana* compared to 36.22 days in the control treatment. Also, it significantly increased to 43.27, 45.16 and 47.40 days at 70, 100 and 125 Gy, respectively, combined with *P. lilacinus* compared to 36.22 days in the control treatment.

Table 5 Effect of gamma irradiation combined with the (LC₅₀) of the entomopathogenic fungi on some biological aspects

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

*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

Table 5 shows that 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.

Also, data in **Table 5** shows that the sex ratio was in favour of males at the two doses 70 and 100 Gy combined with *B. bassiana* (1.5 and 1.0, respectively). While highly reduced to 0.6 at the dose 125 Gy compared to 1.1 in the control treatment. Also, it increased to 1.7 and 1.2 at the two doses 70 and 100 Gy combined with *P. lilaceous*, respectively. While, at the dose 125 Gy it reduced to 0.8.

Furthermore, **Table 5** demonstrates that the percentage of adult survival decreases with the increasing the irradiation dose and more decreases in *B. bassiana* than in *P. lilacinus*. It was significantly decreased to 24, 16 and 10 % at the three doses 70, 100 and 125 Gy combined with *B. bassiana*, respectively, compared to 76% in the control treatment. While, it significantly reduces to 34, 18 and 14 % at the three doses 70, 100 and 125 Gy combined with *P. lilacinus*, respectively.

Discussion

The biological activity (larval and pupal period, pupation, adult emergence, sex ratio and survival) of 2nd instar larvae of *G. mellonella* treated with LC₅₀ of *B. bassiana* and *P. lilacinus* has been studied. The results obtained indicated that the LC₅₀ of entomopathogenic fungi caused a significant increase in the larval and pupal period as compared to the control. These results agree with **Abd El- Ghany et al. 2012** 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 2011** and **Ibrahim et al. 2016** who found that the LC₅₀ (10⁸ conidia /ml) of the entomopathogenic fungi, *B. bassiana* and *M. anisopliae* prolonged the larval and pupal period of *A. ipsilon*. Likewise, **Kaur et al. 2011** found 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 LC₅₀ of *B. bassiana* and *P. lilacinus* reduced pupation, adult emergence and the percent survival of *G. mellonella*. Similarly, the present investigation also, agreement with **El-Sinary and Rizk 2007** 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. **Kannan et al. 2008** found that *Metarhizium anisopliae* at the highest conidial concentration (10⁸) significantly reduced the emergence of *Anopheles stephensi* to 40 %. In addition, **Malarvannan et al. 2010** found that the *B. bassiana* at spore concentration 2.4×10^7 and 2.4×10^4 conidia/ml caused the least pupation and adult emergence of *S. litura*, respectively. While, **Abd EL-Wahed 2011** found that the LC₅₀ (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. 2011** 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 with **El-Sinary and Rizk 2007** 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. 1973** 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. 1983 also, found that the adult emergence was reduced as the radiation dose increased. Similarly, Carpenter et al. 1987 reported on fall armyworm when irradiated P₁ adults with the dose 100 Gy. Besides, Makee and Saour 1997 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 Ramesh et al. 2002 on *Spodoptera litura* and Hofmeyr et al. 2005 on *Cryptophlebia leucotreta* reported that sex ratio skewed in favor of males. On the other hand, Carpenter et al. 2001 did not observe a skewed sex ratio in favor of male offspring for the *Cactoblastis cactorum* F₁ adults.

The effect of different doses of gamma irradiation (70,100 and 125Gy) in combination with the LC₅₀ 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 unirradiated females or unirradiated 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.

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 El-Sinary and Rizk 2007 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 2011 also, showed that the LC₅₀ (10⁸) of the two entomopathogenic fungi; *B. bassiana* and *M. anisopliae* in combined with the LD₅₀ (72Gy) 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.2018 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.

Conclusions

In general, the results indicated that females were more radiosensitive to gamma irradiation than males. The F₁ progeny resulting from the irradiated full-grown male pupae was more sterile than the irradiated P₁ males and the F₁ males were usually more sterile than the F₁ females. The entomopathogenic fungi, *B. bassiana* and *P. lilacinus* were the most virulent isolates causing the highest adult longevity against 2nd instar larvae of *Galleria mellonella* and had the lowest LC₅₀ values. Full-grown male and female pupae irradiated with different doses of gamma irradiation (70,100,125 and 150 Gy) showed delayed effects on the different biological aspects of *G. mellonella* females were more radiosensitive than males. The sterilizing dose for males was above 150 Gy, while the sterilizing dose for females was 150 Gy.

The second instar larvae of *Galleria mellonella* descending of the irradiated parental male or female pupae with 70,100 and 125 Gy then treated with the LC₅₀ of *B. bassiana* and *P. lilacinus* adversely affected on the different biological aspects. The combined treatment was greater than either, in the case of fungal or irradiation treatment alone.

Declarations

Ethics approval and consent to participate

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

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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.

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Not applicable

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