Mohamed et al. Egyptian Journal of Biological Pest Control (2018) 28:95
https://doi.org/10.1186/s41938-018-0099-z

Egyptian Journal of
Biological Pest Control

RESEARCH Open Access

# Effect of gamma irradiation and/or certain entomopathogenic fungi on the larval Mortality of *Galleria mellonella* L

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## Abstract

The present investigation was carried out to study the effect of $LC_{50}$ of the entomopathogenic fungi (EPF), *Paecilomyces lilacinus* and *Beauveria bassiana*, on larval mortality of the greater wax moth (GWM), *Galleria mellonella* L., under laboratory conditions and also to study the effect of different doses of gamma irradiation (70, 100, 125, and 150 Gy), separately or combined with the $LC_{50}$ of the isolates of the EPF, *B. bassiana*, and *P. lilacinus*, on the second-instar larvae of *G. mellonella* larval mortality. The combined treatment of gamma irradiation and EPF increased the larval mortality rates than that at each treatment alone. The highest percentage of larval mortality was 78 and 84%, with 125 Gy + *B. bassiana* in the case of $F_1$ male and $F_1$ female, respectively. According to the obtained results, 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.

**Keywords:** Gamma irradiation, Entomopathogenic fungi, *Galleria mellonella*, Control

## Background

The greater wax moth (GWM), *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 larvae of the wax moth cause considerable damage to combs left unattended by bees. Combs in weak or dead colonies and in storage areas are subject to attack (Caron 1992).Chemical pesticides have been the practical method used by growers for the control of economically insect pests, but their negative side-effects on non-target organisms, groundwater contamination, residues on food crops, and the development of insect resistance to chemicals have forced the industry and scientists to focus on developing alternative control measures. This pest species has received more attention as a model organism for toxicological investigations involving entomopathogens than as a honeybee pest, with more focus on proven (demonstrated) control measures (Ramarao et al. 2012). Even though evidence for a successful and sustainable biological control agent of GWM is still lacking, previous researchers have explored various biological agents and bio-products, including *Bacillus thuringiensis* Berliner (H-serotypeV) (Bt), *Bracon hebetor* (Say), *Trichogramma* species, the red imported fire ant (RIFA) (*Solenopsis invicta* Buren and *Solenopsis germinita* Fabricius), and the use of the male sterile technique (MST). The EPF are the most important ones among all the microbial biocontrol agents (MBCAs) due to their broad host range and route of pathogenicity. Studies of Jafari et al. (2010) revealed that male sterilization was most effective when the wax moth pupae were partially sterilized (using 350 Gy of gamma radiation). However, the release of irradiated pupae ended prematurely because the pupae were fragile and required a high input cost. In an effort to substitute irradiated pupae, irradiated eggs were released, but a similar experiment has never been performed on wax moths. In addition, Bloem et al. (2005) showed that the emerging larvae were more destructive, raising fears that use of

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irradiated $F_1$ eggs of GWM could exacerbate economic losses.

The aim of the present study was to evaluate the effect of the gamma irradiation and/or EPF, separately or combined in controlling *G. mellonella* under laboratory conditions.

## Materials and methods

### Insect rearing and irradiation process

The greater wax moth, *G. mellonella* larvae, were obtained from infested hives that reared in the Nuclear Research Center (NRC), Egyptian Atomic Energy Authority (EAEA), Anshas, Egypt, and in the bio-insecticide Production Unit, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt. *G. mellonella* larvae were reared on an artificial diet at a constant temperature of 30 °C and 65 ± 5% (R.H.) according to (Metwally et al. 2012). The irradiation process was performed, using a cobalt-60 gamma cell 220, located at Cyclotron project, Nuclear Research Center, Atomic Energy Authority (Anshas). The dose rate at the experiment was 0.926 kGy/hour.

### Effect of gamma irradiation on larval mortality

To determine the effect of gamma irradiation on mortality rate of treated larvae, full-grown pupae of *G. mellonella* were exposed to four doses (70, 100, 125, and 150 Gy). Ten larvae resulting from irradiated parental males or females were transferred to clean small plastic containers and allowed to feed on artificial diet; each treatment was replicated 5 times. Dead larvae were counted. Mortality percentages were calculated and corrected using Abbott' Formula (Abbott 1925).

### Preparation of spore suspension

Spore suspension was prepared from 15-day-old culture of fungal isolates of; *Paecilomyces lilacinus* and *Beauveria bassiana* on Sabouraud Dextrose Agar (CDA) media. The fungal surface was scraped, using a sterile loop with 10 ml of sterile distilled water having 0.02% Triton X-100, as a wetting agent (Rombach et al. 1986). The suspension was then filtered through sterile muslin cloth to eliminate the medium (Sasidharan and Varma 2005). Spore concentration of the filtrate was determined, using a Neubauer Hemocytometer. This served as the stock suspension. Different spore concentration was prepared by adding sterile 0.02% Triton X-100 in distilled water. Spore suspensions of fungal isolates at four different concentrations, ($1 \times 10^6$, $1 \times 10^7$, $1 \times 10^8$, and $1 \times 10^9$ spores/ml) were prepared and preserved at 5 °C until used in bioassay.

### Impact of the entomopathogenic fungi against the second-instar larvae of *G. mellonella*

In order to determine the pathogenicity of the tested isolate of fungi against G. *mellonella* larvae, the second-instar larvae were immersed individually for 30 s in 9 ml of different spore concentrations ($1 \times 10^6$, $1 \times 10^7$, $1 \times 10^8$, and $1 \times 10^9$ spores/ml) of the fungal isolates. For the control treatment, larvae were dipped into 0.02% Triton X-100 solution (Hafez et al. 1994). Then, the treated larvae were placed individually in small plastic containers and allowed to feed on a semi-synthetic diet. All treated larvae were incubated at 30 °C and 65 ± 5% R.H and photo phase of 12 h. Each treatment had a batch of 10 larvae and was replicated five times. Dead larvae were counted daily. Mortality percentages were calculated and corrected, using Abbott' Formula (Abbott 1925). The median lethal concentration and time were calculated according to the method of Finney (1971)**.**

### Combined effect of gamma irradiation and entomopathogenic fungi on mortality of *G. mellonella* larvae

Three doses of gamma irradiation (70, 100, and 125) were chosen to study the combined effect of gamma irradiation with the ($LC_{50}$) of the most virulent isolates on the mortality rate and some biological aspects of *G. mellonella*. Three experimental groups were set up. The first consisted of the progeny of $F_1$ larvae descendant of the irradiated parental males as full-grown pupae with three doses: 70, 100, and 125 Gy. The second consisted of the progeny of $F_1$ larvae descendant of the irradiated parental females as full-grown pupae with the three doses: 70, 100, and 125 Gy. The third one used as a control (unirradiated insects; males and females). The progeny of $F_1$ larvae of each group was fed on artificial diet till the second-instar larvae; five replicates from the second-instar larvae (10 larvae each) were dipped into 9 ml of $LC_{50}$ of the most virulent isolates for 30 s in clean small plastic containers, fitted with moist filter paper, and allowed to feed on artificial diet under laboratory conditions (28 ± 2 °C and 65 ± 5% R.H). Dead larvae were counted daily. The percentage mortality was calculated and corrected using Abbott' Formula (Abbott 1925).

### Synergistic/antagonistic action

These tests were conducted to identify the synergistic/antagonistic actions that occurred due to combated of gamma irradiation at the doses (70, 100, and 125 Gy) with the $Lc_{50}$ of *B. bassiana* and *P. lilacinus.* The combined (joint) action of the combinations was presented as co-toxicity factor, based on (Sun and Johnson 1960) to distinguish among potentiation, antagonism and additive, by applying the formula given below:

$$\text{Co-toxicity factor} = \frac{\text{Observed\%mortality} - \text{Expected\%mortality} \times 100.}{\text{Expected\%mortality.}}$$

A positive factor of ≥ 20 indicates potentiation, a negative factor of ≤ − 20 indicates antagonism, and the intermediate values of > − 20 to < 20 indicate an additive effect. The expected percentage mortality was summited for each treatment in the mixture in every test by treating larvae by each one alone.

### Statistical analysis

The average percent mortality of the tested larvae was calculated and corrected, using Abbott's formula (Abbott 1925). The corrected percentages of mortality were statistically computed according to the method of Finney (1971). Computed percentage of mortality was plotted versus the corresponding concentrations on logarithmic probability paper to obtain the corresponding Log-concentration probit lines. The lethal concentration 50 was determined for established regression lines. All data obtained were analyzed, using the analysis of variance (ANOVA), and the means were separated, using Duncan's multiple range test ($P > 0.05$) (Steel and Torrie 1980).

## Results and discussion

### Latent effect of gamma irradiation on the mortality of $F_1$ GWM, larvae descendant from irradiated parents

Data presented in Table 1 show the effect of gamma irradiation on the percentage larval mortality in the $F_1$ progeny of *G. mellonella* descendant of the irradiated parental males and females, as full-grown pupae, with the four doses 70, 100, 125, and 150 Gy. At the four doses, the percentages of larval mortality among the $F_1$ progeny, descendant of the irradiated parental males and females, significantly increased as the dose increases. They increased to 22, 26, 32, and 40% in case of males and to 38, 46, and 62% in case of females, at the four doses 70, 100, 125, and 150 Gy, respectively, compared to 8% in the control treatment (Table 1). The parental females, irradiated with the dose 150 Gy, did not give any progeny.

**Table 1** Effect of gamma irradiation on mortality percentage of the greater wax moth $F_1$ progeny descending from the irradiated parental males and females as full-grown pupae

| Doses (Gy) | The percentage larval mortality among $F_1$ progeny ± SE | |
|---|---|---|
| | Descending from the irradiated parental males | Descending from the irradiated parental females |
| Control | 08 ± 3.75 c | 08 ± 3.75 c |
| 70 | 22 ± 3.75 b | 38 ± 3.75 b |
| 100 | 26 ± 2.45 b | 46 ± 4.01 b |
| 125 | 32 ± 3.75 ab | 62 ± 3.75 a |
| 150 | 40 ± 3.17 a | – |
| LSD 0.05 | 10.04 | 11.41 |

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

Hallman (2003) suggested that normal growth, development, or reproduction of the organism might be prevented by sub lethal doses of irradiation, while lethal doses of irradiation could kill insects immediately. Lepidopterous insects require high doses of irradiation to achieve fully sterilized adults; these doses often render them less competitiveness than unpredicted; therefore, using sub sterilizing doses of radiation are increased competitiveness of released insects and possible integration with other non-polluting methods to control insect pests (North and Holt 1968). The severity of gamma irradiation effect differed according to the gender irradiated and irradiation doses used. These results are in agreement with Makee and Saour (1997) who exposed the adult male *Phthorimaea operculella* to different doses of gamma irradiation and found that the mean developmental time and the percentage mortality of the $F_1$ progeny increased at each examined dose. Salem et al. (2014) reported that the percentages 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 the increasing radiation doses.

### Effect of $LC_{50}$ of *entomopathogenic* fungi on the percentage larval mortality of *G. mellonella*

The data presented in Table 2 show the lethal concentration (LC50 and LC95) values of the two fungal species, *B. bassiana* and *P. lilacinus*. The median lethal values ($LC_{50}$) were 1.2 × 105 and 2.3 × 105 conidia/ml, while LC95 values were 1.9 × 108 and 4.3 × 1011 conidia/ml, for the two tested fungi, respectively (Figs. 1 and 2).

Data illustrated in Fig. 3 show the effect of $LC_{50}$ of the EPF, *B. bassiana* and *P. lilacinus* on mortality of the second-instar larvae of *G. mellonella* at different time intervals. The percentage of the larval mortality increased horizontally with the time increase after treatment. *B. bassiana* and *P. lilacinus* caused the highest larval mortality 51.25 and 43.75%, respectively, after 96 h from treatment, compared to 10% in the control. The accumulative percentage's mortality reached to 56.25 and 46.25%, respectively, at the end of the larval period, which was significantly different at all both EPF tested, compared to 21.25% in the control treatment.

**Table 2** Virulence of fungal isolates against second-instar larvae of *Galleria mellonella* expressed as the $LC_{50}$, $LC_{95}$, and slope of toxicity regression lines after 10 days of dipping in different concentration

| Fungal isolates | $Lc_{50}$ conidia/ml | $Lc_{95}$ conidia/ml | slope | $\chi^2$ | *P* value |
|---|---|---|---|---|---|
| *Beauveria bassiana* | $1.2 \times 10^5$ | $1.9 \times 10^8$ | 0.2620 ± 0.0305 | 13.15 | 0.001 |
| *Paecilomyces lilacinus* | $2.3 \times 10^5$ | $4.3 \times 10^{11}$ | 0.2064 ± 0.0299 | 04.30 | 0.120 |

By the end of larval period, the total larval mortality deviation from control was 264.70% (< twice and half) and 217.64% (> twice) than the control treatment after treatment with *B. bassiana* and *P. lilacinus*, respectively.

Several studies of El-Sinary and Rizk (2007), Abd El-Ghany et al. (2012), and Ibrahim et al. (2016) reported the potential of B. *bassiana* on *G. mellonella*. There are no studies reporting *P. lilacinus* as an endophytic fungus causing negative effects on insect herbivores, but there are some reports of it being pathogenic to a number of insects, including *Ceratitis capitata*, *Setora nitens*, *Aphis gossypii*, and *Triatoma infestans* (Fiedler and Sosnowska 2007). The results of the present study showed that the $LC_{50}$ values of the second-instar larvae of *G. mellonella* varied between the two isolated fungi. Based on the $LC_{50}$ values, *B. bassiana* ($1.2 \times 10^5$ conidia/ml) was the most virulent than *P. lilacinus* ($2.3 \times 10^5$ conidia/ml). Hussein et al. (2012) found that the $LC_{50}$ and $LC_{95}$ values of fourth-instar larvae of *G. mellonella* were ($1.43 \times 10^3$, $4.71 \times 10^5$, and $1.04 \times 10^5$, $1.01 \times 10^8$ conidia/ml) for *B. bassiana* isolates, BbaAUMC3263 and BbaAUMC3076, respectively.

### Effect of the combination of gamma irradiation and the $LC_{50}$ of *B. bassiana* and *P. lilacinus* on the percentage larval mortality of G. *mellonella* descending from the irradiated parental males and females

The percentage larval mortality of *B. bassiana* and *M. anisopliae* at ($LC_{50}$) against the second-instar *G. mellonella* larvae descendant of the irradiated parental males and females, with the three doses 70, 100, and 125 Gy crossed with non-irradiated males and females, was studied (Table 3).

Those with doses 70, 100, and 125 Gy combined with *B. bassiana* achieved the highest larval mortality (50, 62,

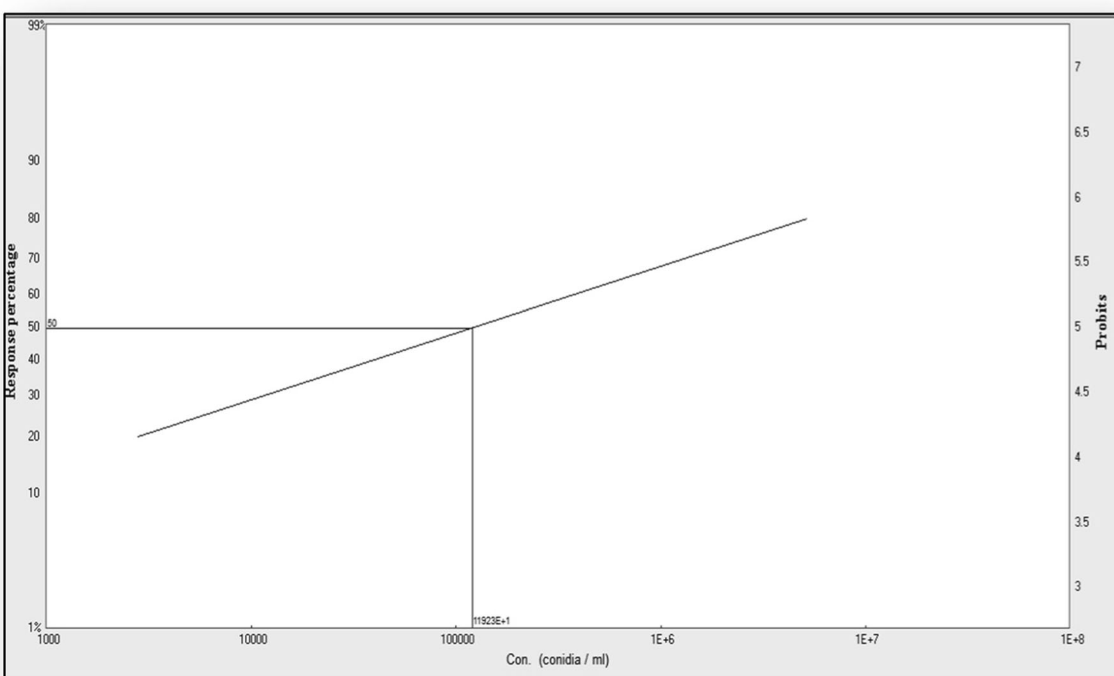


**Fig. 1** Concentration-mortality probit lines of *Beauveria bassiana* against the second-instar larvae of *Galleria mellonella*

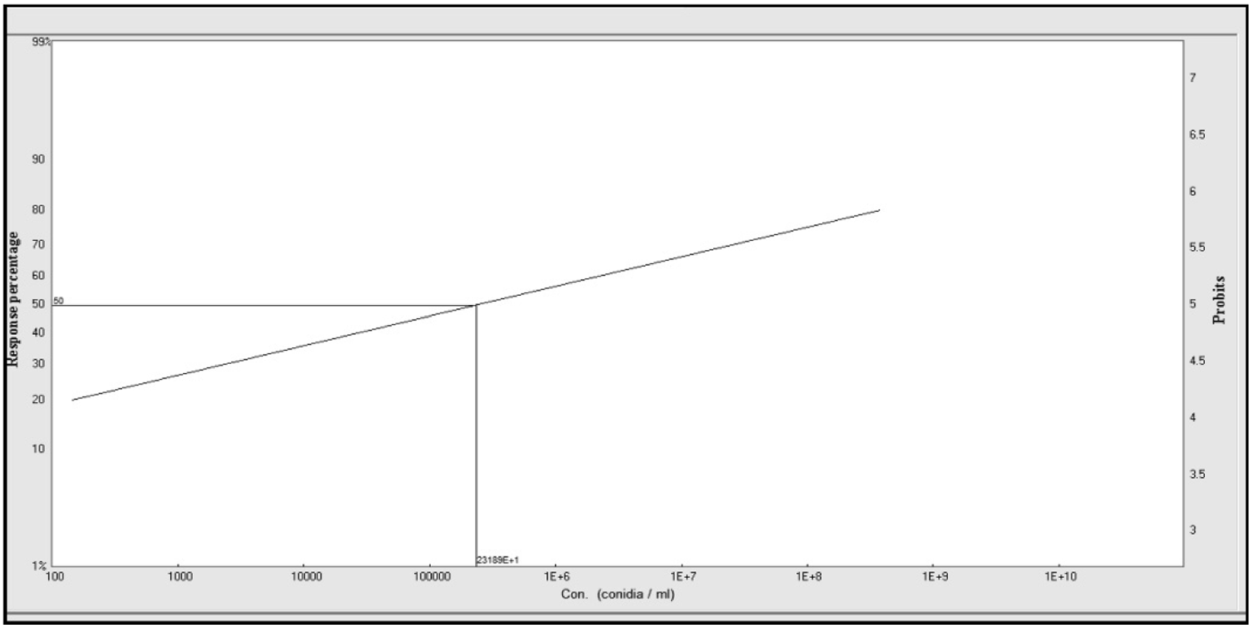


**Fig. 2** Concentration-mortality probit lines of *Paecilomyces lilacinus* against the second-instar larvae of *Galleria mellonella*

and 64%), respectively, after 96 h from treatment compared to 12% those in the control treatment. They increased to 42, 48, and 56%)for *P. lilacinus* combined with the same previous irradiation doses, respectively. Accumulative larval mortality at the end of larval period (which is calculated at the end of the larval period until the beginning of pupation) significantly increased to 58, 70, and 78% at the same previous doses when combined with *B. bassiana*, respectively, and 50, 62, and 72% when combined with *P. lilacinus*, compared to 20% in the control treatment (Table 3).

*B. bassiana* achieved the highest larval mortality after 96 h. among all treatments. It increased to 54, 68, and 74%, when combined with 70, 100, and 125 Gy, respectively, while it increased to 46, 54, and 66% for *P. lilacinus* treatment when combined with 70, 100, and 125 Gy, respectively, compared to 12% in the control treatment.

The expected accumulative larval mortality at the end of larval period was significantly increased to 64, 78, and 84% (320, 390, and 420% than the control treatment) using the same previous three doses, respectively, when combined with *B. bassiana*, compared to 12% in the control, while it increased to 54, 74, and 80% (270, 370, and 400% than the control, respectively) for *P. lilacinus*.

The effect of the tested doses of gamma irradiation in combination with the $LC_{50}$ of *B. bassiana* and *P.*

**Table 3** Effect of gamma irradiation combined with the $LC_{50}$ of the entomopathogenic fungi on the percentage larval mortality of *Galleria mellonella* descending from the irradiated parental males

| Radiation dose (Gy) | Fungi | % Larval mortality after/h ± SE | | | | | |
|---|---|---|---|---|---|---|---|
| | | 24 h | 48 h | 72 h | 96 h | Accumulative larval mortality | Accumulative larval mortality deviation from control |
| Control | – | 02 ± 2.45 | 06 ± 2.01 | 10 ± 2.01 | 12 ± 2.45 | 20 ± 2.45 e | 100 |
| 70 Gy | *B. bassiana* | 16 ± 2.46 | 32 ± 2.01 | 48 ± 2.00 | 50 ± 3.17 | 58 ± 2.00 cd | 290 |
| | *P. lilacinus* | 10 ± 0.00 | 22 ± 2.00 | 34 ± 4.01 | 42 ± 3.75 | 50 ± 3.75 d | 250 |
| 100 Gy | *B. bassiana* | 22 ± 2.00 | 36 ± 2.45 | 50 ± 0.00 | 62 ± 2.00 | 70 ± 3.17 ab | 350 |
| | *P. lilacinus* | 12 ± 2.00 | 26 ± 2.00 | 38 ± 2.45 | 48 ± 2.00 | 62 ± 2.00 bc | 310 |
| 125 Gy | *B. bassiana* | 32 ± 2.00 | 42 ± 2.00 | 54 ± 2.45 | 64 ± 2.45 | 78 ± 2.00 a | 390 |
| | *P. lilacinus* | 24 ± 2.45 | 32 ± 2.00 | 46 ± 2.45 | 56 ± 2.45 | 72 ± 2.00 a | 360 |
| LSD | 8.19 | | | | | | |

Means followed by the same letter in each column represent those that are not significantly different at $p > 0.05$

**Table 4** Potency of interaction between of gamma irradiation and $LC_{50}$ of the entomopathogenic fungi, *Beauveria bassiana* and *Paecilomyces lilacinus* against *Galleria Mellonella* larvae descending from the irradiated parental males

| Radiation doses (Gy) | Fungi | Observed % mortality* | Expected % mortality** | Co-toxicity factor | Joint effect type |
|---|---|---|---|---|---|
| Control | – | 08 ± 3.75 | – | – | – |
| 70 Gy | *B. bassiana* | 58 ± 2.00 | 78.3 | – 25.9 | Antagonism |
| | *P. lilacinus* | 50 ± 3.75 | 68.3 | – 26.8 | Antagonism |
| 100 Gy | *B. bassiana* | 70 ± 3.17 | 82.3 | – 14.9 | Additive |
| | *P. lilacinus* | 62 ± 2.00 | 72.3 | – 14.2 | Additive |
| 125 Gy | *B. bassiana* | 78 ± 2.00 | 88.3 | – 11.7 | Additive |
| | *P. lilacinus* | 72 ± 2.00 | 78.3 | – 8.0 | Additive |

*The observed mortality for the mixture of two treatments was the percentage of the mortalities of each combination
**The expected mortality was summited for each treatment in the mixture in every test by treating larvae by each one alone

*lilacinus* against larvae of *G. mellonella* descending from the irradiated parental males crossed with unirradiated females or unirradiated males crossed with the irradiated females on some biological aspects was examined. Obtained results showed that the combined effect increased the larval mortality than each of them alone. The results are in accordance with El-Sinary and Rizk (2007) who used *B. bassiana* at ($10^4$ and $10^8$ spores/ml) combined with different doses of gamma irradiation (50, 100, and 150) against the fourth-instar larvae of *G. melonella* and found that 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.

Data presented in Tables 4 and 5 summarized the expected percentage mortality, observed percentage mortality, and co-toxicity factor of tested treatments and their binary mixtures. Concerning the $F_1$, resulted from irradiated males, tested combinations had additive effects (co-toxicity factors > – 20) (Table 4), except at the dose of 70 Gy + $LC_{50}$ for both fungi that exhibited antagonistic effects (co-toxicity factors were < – 20).

In the case of progeny of treated females (Table 5), only the dose of 100 Gy and $LC_{50}$ of *P. lilacinus* mixture of six binary tested combinations showed a slight additive effect. The majority of the combinations exhibited antagonistic effects. The results suggested that the synergistic effects of gamma radiation in combination with *B. bassiana* and *P. lilacinu*s were higher in the $F_1$ progeny of the irradiated males than in the $F_1$ progeny of the irradiated females (Tables 4 and 6).

In the present study, the efficiency of the integration of inherited sterile technique (IST) with EPF, *B. bassiana*, and *P. lilacinus* for controlling *G. mellonella* larvae was determined. *B. bassiana* and *P. lilacinu*s had a clear effect on the mortality of $F_1$ progeny of *G. mellonella* larvae, whether they were produced from irradiated male or irradiated females. Ahmadi et al. (2013) recorded a higher mortality for adults exposed to gamma radiation and essential oils together than that for adults exposed to gamma radiation or essential oils alone.

## Conclusion

Finally, in this work, we tried to control *G. mellonella* with certain EPF which seem to be safer and less contaminant to bees and humans. Also, these

**Table 5** Potency of interaction between of gamma irradiation and $LC_{50}$ of the entomopathogenic fungi, *Beauveria bassiana* and *Paecilomyces lilacinus* against *Galleria mellonella* larvae descending from the irradiated parental females

| Radiation doses (Gy) | Fungi | Observed % mortality* | Expected % mortality** | Co-toxicity factor | Joint effect type |
|---|---|---|---|---|---|
| control | – | 08 ± 3.75 | – | – | – |
| 70 Gy | *B. bassiana* | 64 ± 4.01 | 94.3 | – 31.9 | Antagonism |
| | *P. lilacinus* | 54 ± 2.45 | 84.3 | – 35.9 | Antagonism |
| 100 Gy | *B. bassiana* | 78 ± 2.01 | 102.3 | – 23.7 | Antagonism |
| | *P. lilacinus* | 74 ± 4.01 | 92.3 | – 19.8 | Additive |
| 125 Gy | *B. bassiana* | 84 ± 2.45 | 118.3 | – 29.0 | Antagonism |
| | *P. lilacinus* | 80 ± 3.17 | 108.3 | – 26.1 | Antagonism |

*The observed mortality for the mixture of two treatments was the percentage of the mortalities of each combination
**The expected mortality was summited for each treatment in the mixture in every test by treating larvae by each one alone

**Table 6** Effect of gamma irradiation combined with the $LC_{50}$ of the entomopathogenic fungi; *Beauveria bassiana* and *Paecilomyces lilacinus* on the percentage larval mortality of *Galleria mellonella* descending from the irradiated parental females as full-grown pupae

| Radiation doses (Gy) | Fungi | % Larval mortality after/h ± SE | | | | | |
|---|---|---|---|---|---|---|---|
| | | 24 h | 48 h | 72 h | 96 h | Accumulative larval mortality | Accumulative larval mortality deviation from control |
| Control | – | 02 ± 2.45 | 06 ± 2.01 | 10 ± 2.01 | 12 ± 2.45 | 20 ± 2.45 e | 100 |
| 70 Gy | *B. bassiana* | 20 ± 3.17 | 34 ± 2.45 | 50 ± 0.00 | 54 ± 2.45 | 64 ± 4.01 b | 320 |
| | *P. lilacinus* | 14 ± 2.46 | 24 ± 2.45 | 38 ± 3.75 | 46 ± 2.45 | 54 ± 2.45 c | 270 |
| 100 Gy | *B. bassiana* | 24 ± 2.45 | 44 ± 2.45 | 58 ± 2.00 | 68 ± 2.00 | 78 ± 2.01 a | 390 |
| | *P. lilacinus* | 18 ± 2.00 | 28 ± 2.00 | 40 ± 0.00 | 54 ± 2.45 | 74 ± 4.01 a | 370 |
| 125 Gy | *B. bassiana* | 36 ± 2.45 | 48 ± 2.00 | 62 ± 2.00 | 74 ± 2.45 | 84 ± 2.45 a | 420 |
| | *P. lilacinus* | 28 ± 2.00 | 36 ± 2.45 | 54 ± 2.45 | 66 ± 2.45 | 80 ± 3.17 a | 400 |
| LSD | 9.02 | | | | | | |

Means followed by the same letter in each column represent those that are not significantly different at $p > 0.05$

materials are cheap, are available to beekeepers, and could be used to control other hive infestations, e.g., *Varroa* and acarine mites. Also, we conclude that the combined treatment with gamma irradiation and EPF were establishing results that more significantly affects than both gamma radiation and EPF each of them alone.

**Acknowledgements**
Not applicable

**Funding**
No funding.

**Availability of data and materials**
Data supporting the conclusions of this article are presented in the main Manuscript.

**Authors' contributions**
HFM: Has written the manuscript, Tables and Statistical review; TMS: helped in the Practical Part; SEM EL-N and MAMS: completed the final review; AAMI: Participated in the practical part of the Entomopathogenic Fungi OAAARE: Has done the practical part of the manuscript. All authors read and approved the final manuscript.

**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.

**Competing interests**
The authors declare that they have no competing interests.

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Received: 10 June 2018 Accepted: 13 November 2018
Published online: 03 December 2018

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