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Potency of Bio Magic (*Metarhizium anisopliae* fungus) and gamma radiation in the black cut worm, *Agrotis ipsilon* (Hufnagel) larvae

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Abstract

Background Integrated pest management is one of the ways to solve the problems of increased outbreaks of pests induced by the effects of climate change. Therefore, the present investigation was carried out to study the integrated effect of entomopathogenic fungus, *Metarhizium anisopliae* and gamma radiation to control 5th instar larvae the black cut worm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae), under laboratory condition. The leaf dipping technique was used to test the pathogenicity of different concentrations of the fungus against normal and F1 (resulted from irradiated male parent pupae with 100 Gy) larvae. As well as, the changes in the haemocytes were studied using LC₅₀ of *M. anisopliae* of both treatment (unirradiated and F1 larvae).

Results The results showed that larval mortality percentage was both *M. anisopliae* virulent and time dependent. Furthermore, F1 larvae were more susceptible to *M. anisopliae* where 100% mortality was recorded after 3 days of *M. anisopliae* treatment at 8×10^5 spores/ml concentration. While mortality of normal (unirradiated) larvae reached 40% at the same time and concentration. The hematological examination of larvae revealed significant reduces in total haemocyte counts, differential percentage and increase in morphological malformation of the haemocytes which was pronounced by F1 + *M. anisopliae* LC₅₀.

Conclusions According to the obtained results, the gamma irradiation increased the pathogenicity of the fungus against the 5th instar *A. ipsilon* larvae. So, the combination between the two control tools may provide a satisfactory integrated control program after further field trials.

Keywords *Agrotis ipsilon*, Gamma radiation, Entomopathogenic fungi, *Metarhizium anisopliae*, Mortality, Haematological studies

Background

The black cut worm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) is a devastating noctuid insect that is present worldwide, and is most prevalent in temperate or subtropical climates. This insect is multi-trophic and infests many harvests. Cotton, essential-oil cultures,

maize, tobacco, sunflower, tomatoes, sugar beet, and potato are the crops that have suffered the most harm. The larvae prefer to live underground and feeds exclusively on plant stems. It is hard to control *A. ipsilon* due to the hidden larvae life habitat (Amin et al. 2019).

As a result of the extreme randomly uses of chemical pesticides, pests develop a resistance to these chemicals. Besides, these pesticides led to residual toxicity in plant, negative impacts on beneficial insects and the environment pollution. Such issues have prompted research into the safety of insecticides, including microbiological agents like fungus, bacteria, and viruses, as well as the sterilisation of insects using gamma radiation.

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Gamma irradiation reduces the life duration and persuades a dominant mutation in genetic material of insects. Also, the sub-sterilizing doses of radiation cause reduction in the produced progeny (F1 generation) and become more susceptible to other control tools (Baxter and Blair 1969).

Scientists have given entomopathogenic fungi (EPF) a lot of attention because of their potential for biological pest control. *Metarhizium anisopliae* and *Beauveria bassiana* are the most famous EPF that have broad hosts (Amora et al. 2010).

The insect haemolymph and haemocytes are the main defence agents against forging bodies, so the insect pathogenic fungi reach the insect haemocoel infect after penetrating through the cuticle (Liu et al. 2009) and causing death by destroying the haemocytes (Dong et al. 2009).

Combination of gamma radiation and EPF was proved to be as an excellent integrated control program by many researchers on different pests like, El-Sinary and Rizk (2007) who reported that the pathogenicity of *B. bassiana* was in parallel correlation with 4th instar larvae of *Galleria mellonella* L. irradiation (50, 100 and 150 Gy). Moreover, Gabarty et al. (2013) stated that the activity of the humeral immune enzymes activity of the 6th instar larvae of *Spodoptera littoralis* (Boisd.) was reduced remarkably in larvae resulted from gamma irradiated male pupae (50, 100 and 150 Gy) and treated with *B. bassiana* or *M. anisopliae* compared to the control.

Subsequently, this study was designed to explore the efficacy of using the commercial biological control product; Bio Magic (*M. anisopliae*) in combination with gamma radiation to control 3rd instar larvae of *A. ipsilon* and their effects on the haemocytes count.

Methods

Agrotis ipsilon rearing

A laboratory strain was obtained from cotton leaf worm Department, Plant Protection Research Institute; Agricultural Research Centre, Giza, Egypt. Larvae were fed on fresh castor bean leaves and placed in a 20 ml plastic cups at 25 ± 2 °C and $70 \pm 5\%$ R.H. Once the pupae had matured, they were collected and retained in a plastic container inside a rearing cage (30 × 30 × 30 cm). Emerged adults were fed on 10% sugar solution in a rearing cage. Fresh plant leaves were soaked in water and placed inside the chamber for egg laying. When the larvae had reached the appropriate stages, they were transferred for the experiments.

Irradiation process

Full-grown male pupae of *A. ipsilon* were irradiated by the sub-sterilizing dose 100 Gy using Cobalt- 60 gamma cell, which located at the National Centre for Radiation

Research and Technology, Cairo (NCRRT) with the dose rate 0.766 KGy/h. The 5th larval instar of F1 generation was used in the experiment.

Tested the entomopathogenic fungus, *Metarhizium anisopliae*

The formulation commercially named by Bio Magic (*M. anisopliae* 1.15% WP) manufactured by T. Stanes and Company Limited, India and imported by Gaara Establishment for Export and Import, Cairo, Egypt. Four concentrations of 0.2, 0.4 and 0.8% (w/v) were prepared by diluting the powder in distilled sterilized water and adding 0.5% Tween 80, which equivalent the spores' number of 2×10^5 , 4×10^5 and 8×10^5 spores/ml, respectively.

Bioassay experiment

Normal 5th instar larvae resulted from irradiated male parent pupae with 100 Gy were used for the experiment. The leaf-dipping technique was used to determine the toxicity of different concentrations of *M. anisopliae* on the 5th instar larvae of *A. ipsilon*. Castor leaves were dipped for 1 min. in each concentration, and then the treated leaves were left for natural air-drying. Five larvae were placed in a small jars and allowed to feed on the treated leaves for 24 h., then fresh leaves were added to the replicates daily, five replicates were conducted for each concentration and control. The control group was fed on normal leaves (untreated). Larval mortality was recorded daily.

Haematological examination

The 5th instar larvae were fed on *M. anisopliae* treated leaves (with LC₅₀) for 24 h.; then the haemocytes were examined. The larvae were divided into 4 groups: normal (unirradiated), larvae fed on leaves treated with LC₅₀ *M. anisopliae*, F1 larvae resulted from irradiated male parent pupae with 100 Gy and F1 larvae fed on leaves treated with LC₅₀. Three replicates were prepared for each group (5 larvae/replicate).

For blood counting, the proleg on the abdominal segment was cut with a fine pair of scissors, and blood was allowed to ooze on a clean, grease-free, glass microscopic slide, the haemolymph was quickly drawn up to the 0.5 mark in a Thoma white blood cell dilution pipette and immediately diluted to 11 mark with Turek solution (1–2% glacial acetic acid, slightly colored with gentian violet). The first two or three drops of this solution were discarded; the haemocytes were counted in the four corners of the haemocytometer and multiplied by a factor of 50 to get the number of cells per cubic millimeter. If the cells were clumped or unevenly distributed in the chamber, the preparation was discarded, as described by (Rizk 1991).

To determine the differential haemocytes and the pathological changes resulted from the treatments, a drop of blood from a larva was spread into a thin film by the aid of a cover slip, air dried, fixed in absolute methanol for five min., stained by Giemsa stain for 35 min and the stain was flushed off with distilled water (Abdel-Rahman 1978).

Statistical analysis

Minitab program was used to adjust and analyse the obtained results using ANOVA, followed by Tukey Pairwise Comparisons test to examine the significant differences ($P \leq 0.05$) across the means of the treatments. LdP Line® software ([<http://www.ehabsoft.com/lpline>]) was used to determine the (LC₅₀) and (LC₉₀) values.

Results

Data in Fig. 1 indicates that the mortality percentage of the 5th instar *A. ipsilon* larvae was in parallel correlation with both *M. anisopliae* concentration and time increased. The results revealed there was no larval mortality after 1 day with concentration 2×10^5 spores/ml. In addition, the highest mortality percent (100%) was obtained after 4 days of the feeding on treated leaves with the highest concentration (8×10^5 spores/ml).

Figure 2 represents the mortality percentage of F1 5th instar larvae of *A. ipsilon* resulted from irradiated male parent pupae with 100 Gy, that fed on treated leaves with different concentration of *M. anisopliae* at different interval time. The data observed that F1 larvae were susceptible to *M. anisopliae* where the mortality was recorded after 1 day of treatment with the lowest concentration (2×10^5 spores/ml). Moreover, the accumulative daily

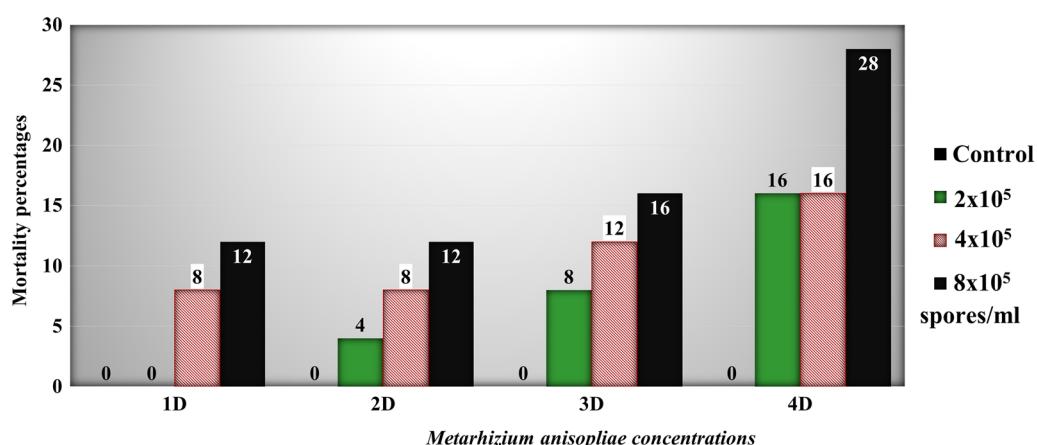


Fig. 1 Daily mortality of 5th instar *Agrotis ipsilon* larvae by different concentrations of *Metarhizium anisopliae*. Values represent the mean of 6 replicates each of 4 larvae

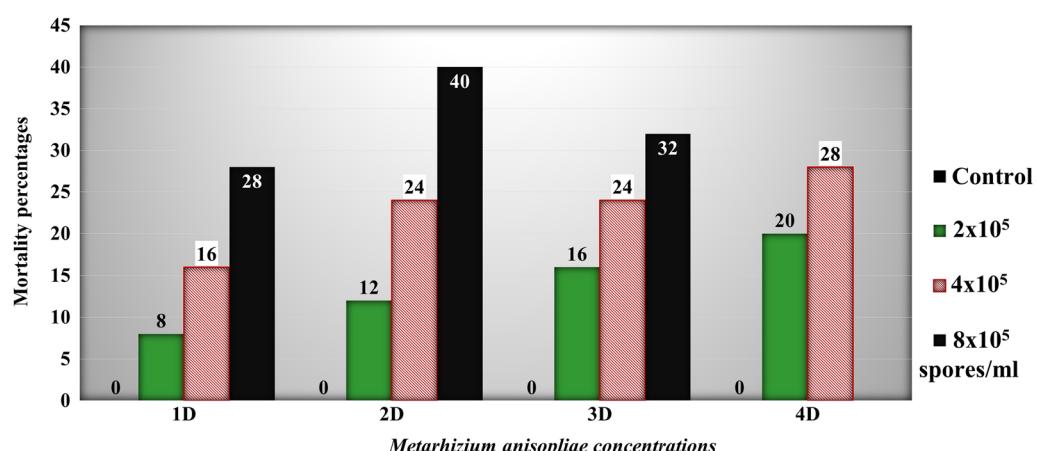


Fig. 2 Daily mortality of F1 5th instar *Agrotis ipsilon* larvae by different concentrations of *Metarhizium anisopliae*. Values represent the mean of 6 replicates each of 4 larvae

Table 1 Accumulative mortality of 5th instar *Agrotis ipsilon* larvae by different concentration of *Metarhizium anisopliae* after 4 days

| Conc. (spores/ml) | Larvae | |
|-------------------|-----------------|---|
| | Normal larvae | F1 larvae (from irradiated male parent pupae with 100 Gy) |
| Control | 0 ^C | 0 ^C |
| 2×10^8 | 28 ^B | 56 ^B |
| 4×10^8 | 44 ^B | 92 ^A |
| 8×10^8 | 68 ^A | 100 ^A |
| F-value | 31.33 | 207.47 |

Values represent the mean of 5 replicates each of 5 larvae

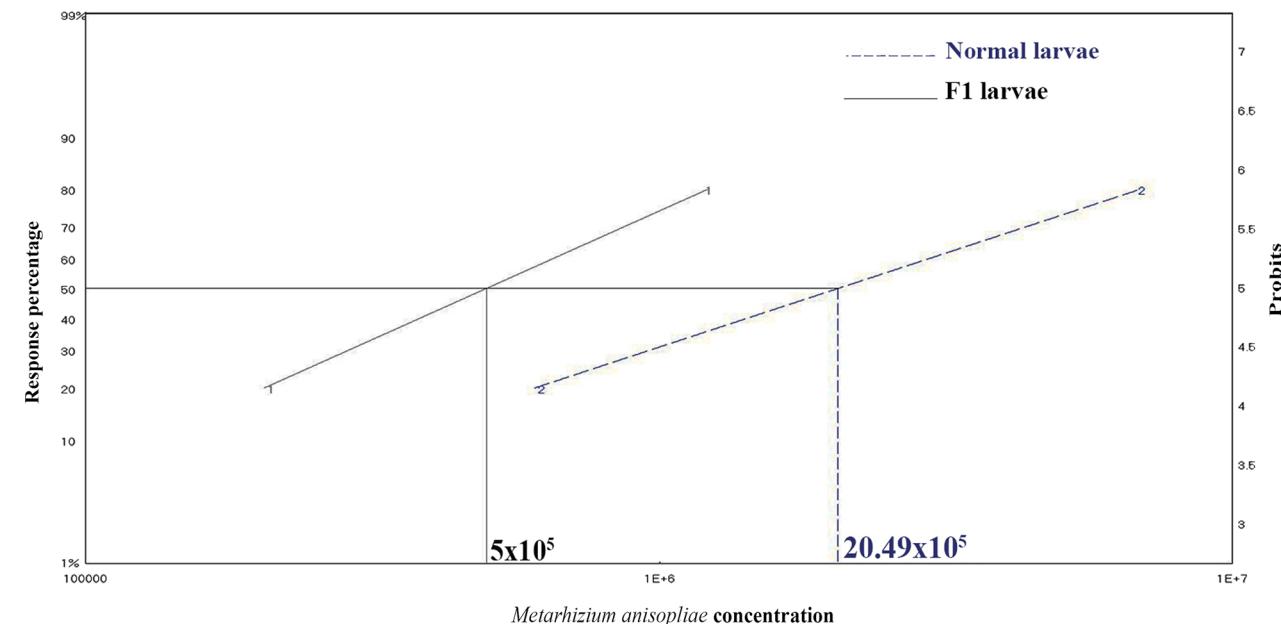
Means in the same column that do not share a letter are significantly different (Tukey Pairwise Comparisons)

mortality was raised by increasing the fungus concentration. The utmost mortality (100%) was recorded after 3 days of larvae feeding on treated leaves with the highest concentration (8×10^8 spores/ml).

Table 2 Median lethal (LC₅₀) and LC₉₀ values of *Metarhizium anisopliae* after 2 days of feeding normal and F1 5th instar *Agrotis ipsilon* larvae on treated leaves

| Treatment | LC ₅₀ (spores/ml) | LC ₉₀ (spores/ml) | Slope | Resistance ratio | Toxicity index |
|---------------------|---|------------------------------|-------|------------------|----------------|
| Unirradiated larvae | 20.49×10^5 (12.13 × 10 ⁵ –86.24 × 10 ⁵) | 130.57×10^5 | 1.59 | 4.09 | 24.4 |
| F1 larvae | 5×10^5 (4.26 × 10 ⁵ –6.05 × 10 ⁵) | 19.36×10^5 | 2.18 | 1.00 | 100 |

Toxicity Index and resistance ratio compared with accumulative mortality on F1 larvae

**Fig. 3** LC₅₀ values of *Metarhizium anisopliae* (Bio Magic) after 2D

The results in Table 1 show the accumulative mortalities of normal and F1 5th instar larvae of *A. ipsilon* fed on treated leaves with different concentration of *M. anisopliae* after 4 days. Data revealed that there were significant differences among the larval mortalities according to the different concentration of *M. anisopliae*.

Table 2 and Fig. 3 illustrate the LC₅₀/LC₉₀ values of *M. anisopliae* after 2 days of feeding normal and F1 5th instar larvae of *A. ipsilon* on treated leaves. The results revealed that F1 larvae was more susceptible than normal larvae and recorded low LC₅₀ 5×10^5 spores/ml with lower resistance ratio and toxicity index than those recorded by normal larvae.

Figure 4 demonstrates the total haemocytic count (THC) of normal and F1 5th instar larvae of *A. ipsilon* after 2 days feeding on treated leaves with LC₅₀ of *M. anisopliae* (Bio Magic). The recorded THC were 70.367×10^2 , 58.37×10^2 , 52.623×10^2 and 23.06×10^2 haemocytes in larvae fed on untreated leaves (control), normal larvae were fed on treated leaves with LC₅₀, F1 larvae and F1 larvae feed on treated leaves with LC₅₀ of *M. anisopliae* (F1 + LC₅₀), respectively. The results

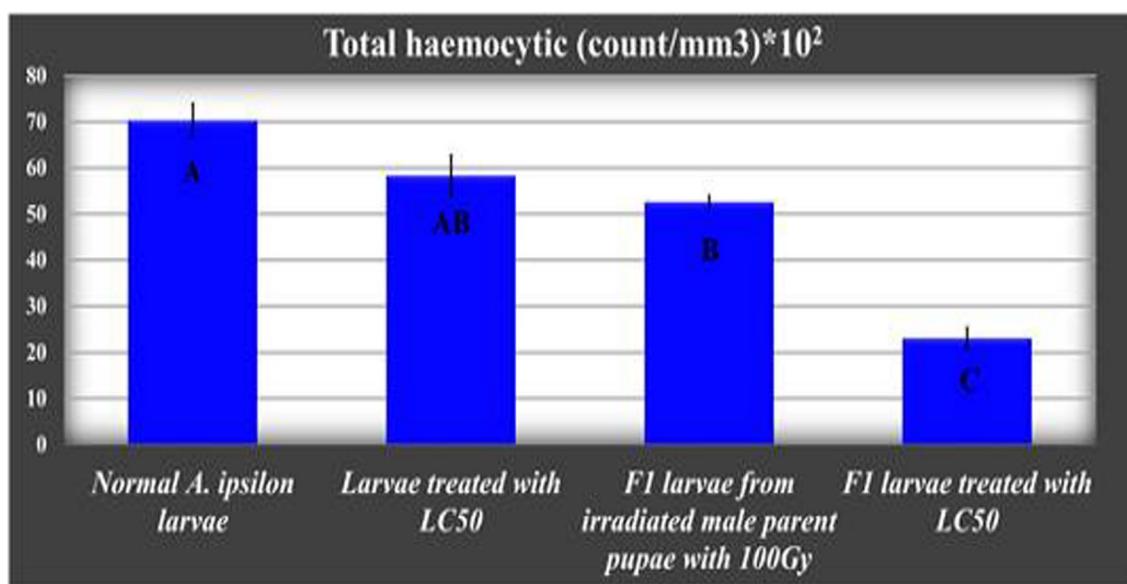


Fig. 4 Total haemocytic (count/mm³ × 10²) of normal and F1 5th instar resulted *Agrotis ipsilon* larvae after 2 days of treatment with LC₅₀ values of *Metarhizium anisopliae*. Values represent the mean ± SE of 3 replicates. Columns that do not share a same letter are significantly different (Tukey Pairwise Comparisons). LC₅₀ of normal (unirradiated) and F1 larvae 20.49×10^5 and 5×10^5 spores/ml, respectively

indicated a significant reduction in total haemocytes count in F1 larvae and F1 + LC₅₀ as compared to control. Meanwhile, the THC of normal larvae that fed on treated leaves with LC₅₀ was insignificantly changed when compared to the control or F1. From the aforementioned results, it is seemed that decrease in THC was more obvious in F1 + LC₅₀.

Haemocytes were morphologically examined based on staining similarity. Table 3 and Fig. 5 indicates that 8 types of the haemocytes had been recognized in 5th

instar larvae of *A. ipsilon*: **A-** Prohaemocytes: were small, round cells, with undifferentiated cytoplasm. The single, round nucleus with dense, homogenous chromatin is usually enlarged in size leading to formation of macro-nucleus, which in turn sparing the cellular cytoplasm. **B-** Plasmatocytes: characterized by their round to ovoid shape and variety of cytoplasmic texture ratio which was less than that of prohaemocytes. The nucleus with dark reddish purple stain, round with punctate or granular chromatin and usually central in position. **C-** Granulated

Table 3 Differential haemocytes percentage of normal (unirradiated) and F1 5th instar *Agrotis ipsilon* larvae after 2 days of feeding on treated leaves with the LC₅₀ of *Metarhizium anisopliae*

| Cell type | Treatments | | | |
|------------------|---------------------------------------|---|---|--|
| | Control (normal, unirradiated larvae) | Larvae feed on treated leaves with LC ₅₀ | F1 larvae (from irradiated male parent pupae with 100 Gy) | F1 larvae feed on treated leaves with LC ₅₀ |
| Prohaemocytes | 49.67 ^D | 77.3 ^B | 62.56 ^C | 87.7 ^A |
| Plasmatocytes | 24.76 ^A | 15.16 ^C | 21.16 ^B | 9.1 ^D |
| Granulated cells | 8.73 ^A | 5.36 ^A | 6.6 ^B | 3.2 ^C |
| Spindle cells | 7.17 ^A | 1.23 ^B | 5.6 ^A | 0 ^B |
| Oenocytoids | 4.2 ^A | 0 ^C | 2.7 ^B | 0 ^C |
| Spherulocytes | 1.5 ^A | 0.93 ^A | 1.3 ^A | 0 ^B |
| Adipohaemocytes | 2.63 ^A | 0 ^B | 0 ^B | 0 ^B |
| Cystocytes | 1.33 ^A | 0 ^B | 0 ^B | 0 ^B |

Values represent the mean of 3 replicates

Means in the same columns that do not share a same letter are significantly different (Tukey Pairwise Comparisons)

LC₅₀ of normal (unirradiated) and F1 larvae are 20.49×10^5 and 5×10^5 spores/ml, respectively

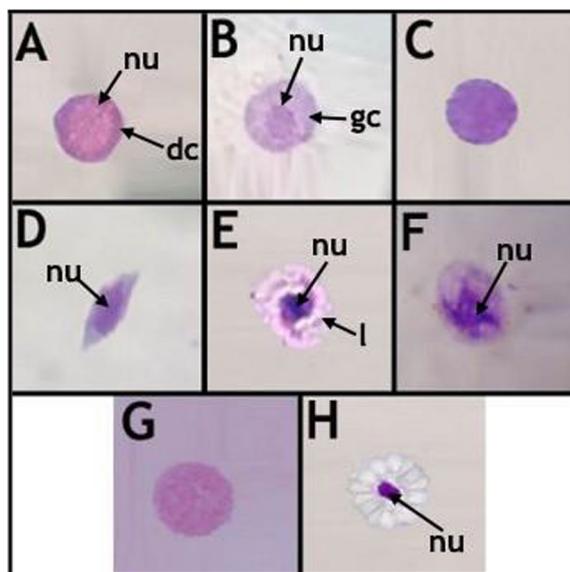


Fig. 5 Normal haemocytes of 5th instar larvae *Agrotis ipsilon* ($\times=1600$). **A** Prohaemocytes, **B** Plasmatocytes, **C** Granulated cells, **D** Spindle cells, **E** Adipohaemocytes, **F** Oenocytoids, **G** Spherule cells, **H** Cystocytes. nu: haemocyte nucleus, dc: dense chromatin in cytoplasm, gc: granular chromatin in cytoplasm, l: lipids droplets

cells: were round, characterized by the undifferentiated dark in colour with numerous granular inclusions cytoplasm and granulocyte nucleus. **D**- Spindle cells: spindle shaped cells distinguished by the presence of very large, distinct, usually spindle nucleus, mostly appearing as a solid purple structure. **E**- Oenocytoids: were slightly round characterized by round, large and approximately centric nucleus with homogenous chromatin. The cytoplasm contained numerous granular inclusions and filled with intricated canaliculi. **F**- Spherule cells: were round with undistinguished nucleus and appeared in dark pink colour. **G**- Adipohaemocytes: were rounds containing variable amounts of refringent fat droplets and several other non-lipid inclusions. **H**- Cystocytes: were extremely fragile cells with a single, small, irregular nucleus in envelope containing distinct, round, acidophilic inclusions. Furthermore, the results revealed that prohaemocytes were the most present type and cystocytes were the lowest type.

Table 3 and Figs. 6, 7, 8, 9, 10 and 11 show the morphological and percentage alterations in the haemocytes of F1 5th instar larvae of *A. ipsilon* and F1 larvae which fed on treated leaves with LC₅₀ of *M. anisopliae*. Data revealed that all treatment resulted in significant increase in prohaemocytes percentage than the control. Gamma irradiation of parent pupae caused enlargement in the prohaemocytes and become oval in shape. Meanwhile, LC₅₀ of *M. anisopliae* resulted in prohaemocytes

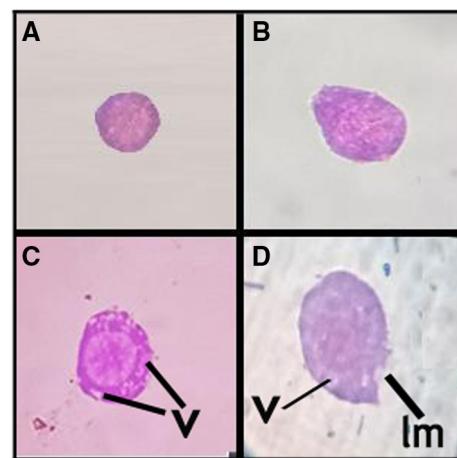


Fig. 6 Morphological changes in prohaemocytes ($\times=1600$). **A** Control (normal larvae), **B** F1 larvae (from irradiated male parent pupae with 100 Gy), **C** larvae feed on treated leaves with LC₅₀, **D** F1 larvae feed on treated leaves with LC₅₀. v: vacuole, lm: lysed membrane

enlargement and vacuolation of the cytoplasm. In addition the prohaemocyte membrane was lysed in F1 + LC₅₀ larvae.

Plasmatocytes percentage was significantly declined in all examined larvae (F1, LC₅₀ and F1 + LC₅₀) than the control. Parent irradiation with 100 Gy caused the cell to be enlarged or took slightly oval shape and the nucleus lost its central position and moved to the cell wall (Fig. 7B). *M. anisopliae* affect plasmatocytes in the same way of gamma radiation and inducing vacuolation of cytoplasm. The combined effect of *M. anisopliae* and radiation would be more destructive to cells than using

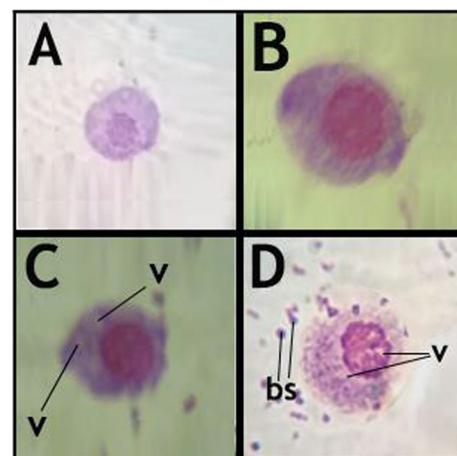


Fig. 7 Morphological changes in plasmatocytes ($\times=1600$). **A** Control (normal larvae), **B** F1 larvae (from irradiated male parent pupae with 100 Gy), **C** larvae feed on treated leaves with LC₅₀, **D** F1 larvae feed on treated leaves with LC₅₀. v: vacuole, bs: *M. anisopliae* blastospores

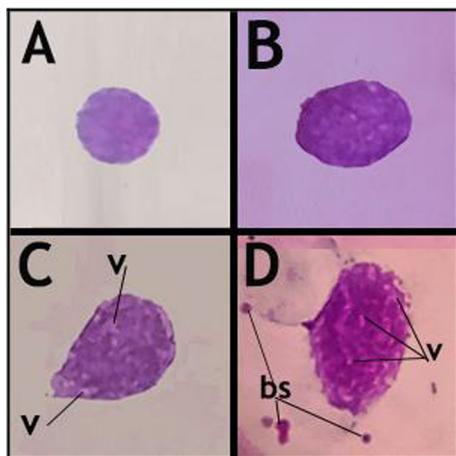


Fig. 8 Morphological changes in granulated cells ($\times=1600$). **A** Control (normal larvae), **B** F1 larvae (from irradiated male parent pupae with 100 Gy). **C** larvae feed on treated leaves with LC_{50} , **D** F1 larvae feed on treated leaves with LC_{50} . v: vacuole, bs: *M. anisopliae* blastospores

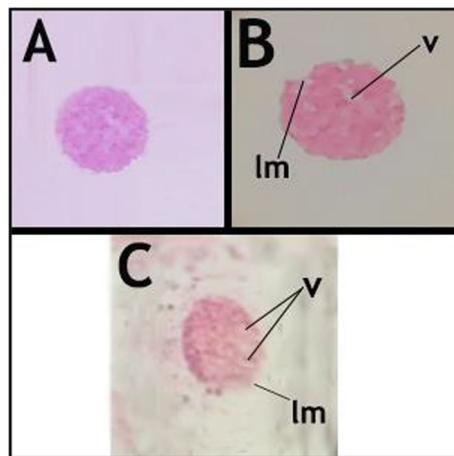


Fig. 11 Morphological changes in spherule cells ($\times=1600$). **A** Control (normal larvae), **B**: F1 larvae (from irradiated male parent pupae with 100 Gy). v: vacuole, lm: lysed membrane

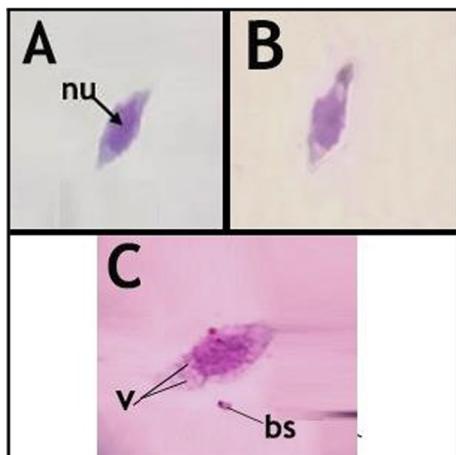


Fig. 9 Morphological changes in spindle cells ($\times=1600$). **A** Control (normal larvae), **B** F1 larvae (from irradiated male parent pupae with 100 Gy). **C** larvae feed on treated leaves with LC_{50} , nu: haemocyte nucleus, v: vacuole, bs: *M. anisopliae* blastospores

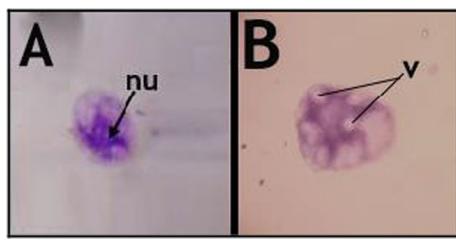


Fig. 10 Morphological changes in oenocytoids ($\times=1600$). **A** Control (normal larvae), **B** F1 larvae (from irradiated male parent pupae with 100 Gy). nu: haemocyte nucleus, v: vacuole

one of them only as the vacuoles were increased in the cell and the nucleus (Fig. 7D).

Granulated cells: Gamma irradiation and *M. anisopliae* caused significant reduction in granulated cells percentage which was more pronounced in the combination treatment. In contrast, the size of was enlarged as a response to the treatments and the cells became oval in shape (Fig. 8). Moreover, *M. anisopliae* treatments initiated presence of vacuoles in the cytoplasm on normal and F1 larvae (Fig. 8C, D).

The spindle cells were significantly reduced in F1 and normal LC_{50} treatments. Besides, F1 + LC_{50} treatment resulted in disappearance of spindle cell from haemoceal of the 5th instar larvae *A. ipsilon* (Table 3). Figure 9B, C shows that parent irradiation with 100 Gy or feeding normal larvae on LC_{50} treated leaves led to enlargement of spindle cells. Also, vacuoles were generated in the cytoplasm of the spindle cell of normal larvae feed on LC_{50} treated leaves with *M. anisopliae* (Fig. 9C).

The results in Table 3 exposed that feeding of normal and F1 larvae on treated *M. anisopliae* leaves owed to vanish of oenocytoids from the haemolymph and reduction of their percentage in F1 larvae. Gamma irradiation of parent pupae caused enlargement of oenocytoids cell, degranulation of the cytoplasm and appearance of vacuoles (Fig. 10B).

The haemocytic examination of haemolymph of the 5th instar larvae (normal, LC_{50} *M. anisopliae*, F1 and F1 + LC_{50}) indicated a non-significant decline in spherule cells percentages in LC_{50} and F1 larvae, in comparing to the normal larvae. Moreover, the combined effect of gamma radiation and *M. anisopliae* caused lyses of spherule cells from the haemolymph (Table 3). The

morphological alteration in the spherule cells resulted in lyses of the cell membrane and vacuoles appearance (Fig. 11).

Adipohaemocytes and cystocytes were completely disappeared from the haemolymph of the tested larvae (normal, LC₅₀ *M. anisopliae*, F1 and F1 + LC₅₀).

During the morphological examination of the larvae haemolymph, *M. anisopliae* blastospores of *M. anisopliae* in the body cavity (haemocoel) (Fig. 12A), blastospores attacking and entered the prohaemocytes cells were observed (Fig. 12B, C).

Discussion

As displayed from the achieved results, mortality percentages of 5th instar larvae *A. ipsilon* was concentration dependent that it raised as the concentration of the *M. anisopliae* increases. This in agreement with Gabarty (2011) found that mortality of 2nd instar larvae of *A. ipsilon* was increased with raising *M. anisopliae* concentration and days of the experiment. Also, Aamer et al. (2015) stated that the mortality of *G. mellonella* and *S. littoralis* larvae was in parallel correlation with increasing *M. anisopliae* concentration.

The effect of male parent pupae irradiation (100 Gy) on larval response to *M. anisopliae* revealed that the combined effect increased the F1 larval mortality and the mortality was increased with concentration increase. The same finding was obtained by El-Sinary and Rizk (2007) who reported that the pathogenicity of *B. bassiana* was in parallel correlation with 4th instar larvae of *G. mellonella* irradiation (50, 100 and 150 Gy). In addition, Gabarty (2011) announced that the combined effect of gamma radiation (LD₅₀) and *B. bassiana* or *M. anisopliae* increased the larval and pupal mortality and caused

remarkable disturbance of some biological aspects of the generation resulted from treated larvae.

The above calculated LC₅₀ values exposed that F1 larvae was more sensitive to *M. anisopliae* (Bio Magic) than the normal larvae. Same report was obtained by Mohamed et al. (2018) who stated that the pathogenicity of the *Paecilomyces lilacinus* and *B. bassiana* against *G. mellonella* larvae was increased by gamma irradiation of parent pupae. This is regarded to the effect of the sub-sterilizing doses of gamma radiation that causes genetic alteration in the produced progeny (F1 generation) which make it more susceptible to other control tools (Baxter and Blair 1969).

The above results indicated a significant decrease in total haemocytes count (THC) in larvae due to *M. anisopliae*, which was more remarkable when combined with gamma radiation. Obtained result was in accordance with that obtained by Gabarty (2011) who found a significant reduction in THC of 3rd, 4th and 6th larval instars of *A. ipsilon* resulted from 2nd instar larvae treated with LC₅₀ *B. bassiana* or *M. anisopliae*. Also, El-Sonbaty et al. (2016) documented that combination of gamma rays and *M. anisopliae* resulted in an obvious reduction in THC of 6th instar larvae of *S. littoralis* more than that induced by *M. anisopliae* or gamma radiation alone. The reduction in THC of F1 larvae was in accordance to that obtained by Rizk (1991) on *S. littoralis*, Sayed (2008) on *G. mellonella*, Gabarty (2011) on *A. ipsilon* and El-Sonbaty et al. (2016) on *S. littoralis*.

Insects have a difficult and efficient system of cellular defense against pathogens. Haemocytes are the main tool of that system that is responsible for phagocytosis, encapsulation, and clotting of the foreign (Irving et al. 2005). Therefore, the reduction in THC is regarded to the fungal metabolites (Vilcinskas et al. 1997).

In the present study, the differential haemocytes examination revealed presence of 8 types (prohaemocytes, plasmatocytes, granulocytes, adipohaemocytes, spindle cells, oenocytoids spherulocytes and cystocytes). The above data exposed that prohaemocytes are the abundant type. This is due to that prohaemocytes is the stem cell of haemocytes that can differentiate into other types (Pandey and Tiwari 2012). This report explain the significant increase in prohaemocytes percentage in all treatments (normal larvae feed on treated leaves with LC₅₀, F1 larvae and F1 larvae feed on treated leaves with LC₅₀ of *M. anisopliae* "F1 + LC₅₀"), as larvae need to produce more cells to cover the reduction in the other types.

Since plasmatocytes and granulocytes are identified as the defense haemocytes that responsible for phagocytosis and encapsulation (Vilcinskas et al. 1997). So, the reduction in their percentages of the present results confirm their defense role. Also, the results showed

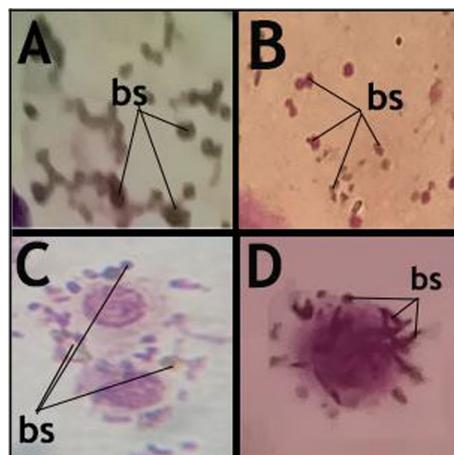


Fig. 12 Blastospores growth in haemocoel of examined *Agrotis ipsilon* larvae ($\times 1000$). bs: *Metarhizium anisopliae* blastospores

disappearance of spindle cells, adipohaemocytes, oenocytoids and cystocytes in response to *M. anisopliae*. The findings of prohaemocytes percentage increase and reduction of others haemocytes' percentages were in accordance with that of Salem et al. (2020). The reduction in the haemocytes percentages (plasmatoctyes and granulocytes) and lyses of others (adipohaemocytes, spindle cells oenocytoids and cystocytes) is regarded to the cytotoxic secreted from *M. anisopliae* to face the larvae immune defence. Moreover, the decline in the haemocytes percentages (plasmatoctyes, granulocytes, oenocytoids and spindle cells) and vanish of adipohaemocytes and cystocytes in F1 larvae could be owed to the effect of gamma radiation on the physiological condition of the body (Al Khalaf and AbdelBaki 2013).

Briefly, the results concluded that the combined effect of gamma irradiation (male parent pupae with 100 Gy) and *M. anisopliae* caused a rapid mortality of *A. ipsilon* larvae than using *M. anisopliae*, which was proved with significant decrease in total haemocytes count and deferential haemocytes percentages than induced by *M. anisopliae* or gamma radiation alone.

Conclusion

Agrotis ipsilon larvae resulted from irradiated parent (100 Gy) became more susceptible to the entomopathogenic fungus, *M. anisopliae*. So, it would be suggested that this integration can be used as an eco-friendly pest control choice.

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Author contributions

RMS, TSE and SAR designed the study, RMS and TSE carried out all experiments, recorded the data, analyzed and interpreted the results. SAR wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data and materials are available if requested.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors declared that there is no any issues relating to journal policies.

Competing interests

The authors declare that they have no conflict of interest.

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