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Entomopathogenic Fungus, *Beauveria bassiana* (Bals.) and Gamma Irradiation Efficiency Against the Greater Wax Moth, *Galleria melonella* (L.)

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Abstract: Two concentrations of the entomopathogenic fungus, *B. bassiana*; 10^4 and 10^8 spores ml^{-1} against the fourth larval instar of the greater wax moth; *G. melonella*. There was a positive correlation between the fungal concentration and its lethality for the treated larvae. The larval mortality percentages increased significantly with 10^8 spores ml^{-1} as it reached 75.87%, after 96 h from the beginning of the treatment while it scored 44.83% with 10^4 spores ml^{-1} after 96 h as compared with 3.33% for the untreated control, when three different oses f gamma irradiation were exposed to the fourth larval instar of *G. melonella* (50, 100 and 150 Gy) combined with the fungal pathogenicity effect, the efficiency of *B. bassiana* increased especially when the gamma irradiation dose was increased no adults were produced with both fungal concentrations and 150 Gy gamma irradiation dose. Males were more tolerant than females in all examined treatments. Histological studies were carried out by transversal sections in the midgut of the treated larvae of *G. melonella*. Obtained micrographs showed great damage in the epithelial layer with increasing the number and size of vacuoles. Fungal spores, hyphal bodies, chlamydospores and blastospores were clearly marked in all treatments. Gamma irradiation combinations with the fungal concentrations increased the damage in the larval midgut.

Key words: *Beauveria bassiana*, gamma radiation. *Galleria melonella*

INTRODUCTION

Larvae of the greater wax moth, *Galleria melonella* (L.) Lepidoptera: Pyralidae) cause considerable damage to bees wax combs since it was discovered by Linnaeus in 1758, also attack weak or dead colonies and those placed in storage. Adult females wax moth fly at night and deposit masses of eggs on unprotected bees wax combs and in the cracks between hive bodies. After few days, these eggs hatch into caterpillars larvae crawl onto the comb and begin their feeding activity. Wax moth larvae damage or destroy the combs by chewing through the bees wax cells as they also feed on cocoons, bees cells and pollen. Chemical control lead to the development of high level resistance and to the negative impact on environment so there is a mass need to develop alternative means of control [1]. Entomopathogens as biological control agents are receiving increased attention because they provide environmentally safe insect control [2]. Most of entomopathogenic fungi belong to Deuteromycetes. *Beauveria bassiana* infected successfully larvae, pupae and adults of many insects and at the time of insect death nearly all of the internal organs of the insect are utilized by the fungus [3, 4]. The interactions that occur between the fungus and the insects are exceedingly complex and are dependent upon specific host-pathogen interaction [5-7].

Gamma irradiation reduced the viability of successive generations of certain insects. Effect of substerilizing doses of gamma radiation helped in increasing mortalities in the resulting progeny and to minimize the hazards of the irradiated insect [8, 9].

The present work deal with the combined effect of gamma irradiation doses (50, 100 and 150 Gy) and treatment of wax with two concentrations (10^4 and 10^8 spores ml^{-1}) of the entomopathogenic fungus *Beauveria bassiana* for feeding of the fourth larval instar of the greater wax moth, *Galleria melonella*. Some biological aspects e.g. larval surviving, pupation, emergence and sex ratio percentages were studied. Histological study was investigated by transversal sections in the midgut to evaluate the fungal attack and irradiation damage against the treated larvae.

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MATERIALS AND METHODS

Rearing insects: The greater wax moth, *G. melonella* (L.) larval were obtained from the infested hives and reared in the laboratory at a constant temperature of 27±1°C and 65±5% R.H [10].

Irradiation technique: Gamma cell irradiated unit 220 located at the National Center for Radiation Research and Technology (NCRRT) Atomic Energy Authority, Nasr City, Cairo. ^{60}Cobalt was the irradiation source of gamma rays used in the present study with a dose rate of 1.2 rad sec^{-1}. Three different gamma irradiation doses; 50, 100 and 150 Gy were applied to the fourth larval instar of *G. melonella*.

Fungal isolation and preparation: The fungal isolates were obtained from the Biological Control Unit, Department, of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University the fungal isolates were grown on autoclaved potato dextrose agar medium (PDA). The inoculated agar medium (PDA) with fungal spores was incubated for 2 weeks at 27°C in the Microbiology Laboratory at the National Center for Radiation Research and Technology, Atomic Energy Authority, Cairo. Spores were harvested by rising with sterilized distilled water, collected spores were filtered through chesse cloth to reduce clumping spores suspended in sterilized water were counted using a haemocytometer [11, 12]. Two different concentrations were prepared; 10^4 and 10^8 spores ml^{-1}, each concentration was used for wax treatment for the fourth larval instar of the greater wax moth, *G. melonella* feeding.

Larval mortality determination: The fourth larval instar of *G. melonella* which were fed on wax treated with both fungal concentrations and gamma irradiation different doses were daily observed to calculate the surviving larvae. Larval mortality percentage was recorded daily after treatment for four periods; 24, 48, 72 and 96 h and each treatment was triplicated. Mortality percentages were corrected with Abbott's formula [13].

Biological studies: After larval mortality calculation, the survived ones were transferred into small cages (25×25×15 cm) and kept under laboratory conditions to determine survived larvae, pupation, emergence and sex ratio percentages.

Histological studies: The combined effect of the entomopathogneic fungus, *B. bassiana* and gamma irradiation was investigated by transversal sections in the mid gut of the treated larvae. The fourth larval instar of *G. melonella* which were treated with both fungal concentrations and gamma irradiation

were dissected in saline solution (0.75% sodium chloride dissolved in distilled water). The mid gut were fixed in alcoholic Bouin for 24 h and then processed using the routine technique for paraffin embedding sections of 5Um thickness and stained with haematoxylin and eosin [14].

Statistical analysis: The data obtained form the present study were statistically analyzed, whenever the calculated "F" values were significant at 5% level [15].

RESULTS AND DISCUSSION

Data screened in Table 1: Registered the corrected larval mortality after treatment with fungal concentrations and gamma irradiation doses. Data was recorded for different four periods; 24, 48, 72 and 96 h. It was markedly noticed that larval mortality increased as the fungal concentration and gamma irradiation dose increased.

Table 1: The Combined effect between two concentrations of the fungus, *B. bassiana* of three doses of gamma radiation on the larval mortality of the greater wax moth, *G. melonella*

Periods (h)	Average larval mortality (%)			
Fungal concentrations (spores ml^{-1})	24	48	72	96
Fungal treatment only				
0	0.00	0.00	0.00	3.33
10^4	3.33	16.67	33.33	44.83
10^8	6.67	33.33	50.00	75.87
Fungi+gamma irradiation with 50 Gy				
0	0.00	3.33	6.67	13.34
10^4	6.67	20.00	40.00	51.72
10^8	10.00	43.33	60.00	86.21
Fungi+gamma irradiation with 100 Gy				
0	13.33	20.00	26.67	34.49
10^4	16.67	26.67	46.67	58.62
10^8	23.33	50.00	73.33	89.66
Fungi+gamma irradiation with 150 Gy				
0	16.67	26.67	40.00	51.72
10^4	20.00	33.33	56.67	72.41
10^8	20.00	56.67	80.00	96.56
LSD 0.05	7.20	7.80	10.00	12.20
0.01	9.40	9.80	14.30	16.10

Table 2: Biological aspects determinations due to the pathogenicity of different concentrations of the fungus *B.bassiana*, combined with gamma irradiation against the greater wax moth *G. melonella*

Fungal concentrations (Spores ml^{-1})	Biological aspects: Survived larvae (%)			Pupation (%)			Emergence (%)			Sex ratio (%): (♂)			(♀)		
	0	10^4	10^8	0	10^4	10^8	0	10^4	10^8	0	10^4	10^8	0	10^4	10^8
0	96.67	55.17	24.13	96.55	50.00	20.00	96.55	40.00	13.33	57.14	58.33	75.00	42.86	41.67	25.00
50	86.66	48.28	13.79	86.66	43.33	10.00	60.00	30.00	0.00	55.00	66.67	-	45.00	33.33	-
100	65.51	41.38	10.34	56.17	36.67	6.67	40.00	23.33	0.00	66.67	71.43	-	33.33	28.57	-
150	48.28	27.59	3.44	33.33	16.67	3.33	16.67	0.00	0.00	100.00	-	-	0.00	-	-
LSD 0.005	12.30			9.20			7.80								
0.01	16.70			14.10			9.70								

The combined effect between fungal concentrations and gamma irradiation doses gave better results in increasing the larval mortality percentage than each treatment applied separately. From data presented in Table 1: It could be concluded that there was a positive correlation between the larval mortality percentages and the fungal concentrations and gamma irradiation doses. The highest score of larval mortality was obtained when the fungal concentration was 10^8 spores ml^{-1} and combined with 150 Gy dose after 96 h exposure time which was 96.67% followed by the treatment of fungal concentration 10^4 spores ml^{-1} and combined with 150 Gy dose after 96 h exposure time being 72.41% as compared with 3.33% larval mortality percentage in untreated control. These findings are in agreement with those found by Brinkman *et al*. [16], El-Sinary [12] and Quesada-Moraga *et al*. [17] which explained that the efficiency of the entomopathogenic fungi began clearly after 48 h from inoculation and the hyphae penetrated the integument inside the trachea and the epithelial and epidermal cells, after 72 fours the fat tissues were damaged and lethality may release to 100% after 96 h.

Data presented in Table 2: Showed that there was a great reduction in the viability of different stages of *G. melonella* especially when fungal concentrations were combined with gamma irradiation. Highly significant reductions were reported when the fourth larval instars of *G. melonella* was treated with 10^8 spores ml^{-1} of fungi and 150 Gy dose of gamma irradiation where results were, 3.44, 3.33 and 0.00% for survived larvae, pupation and emergence percentages, respectively. Also no adults were produced from the treatment of 10^4 spores ml^{-1} and 150 Gy. The sex ratio was clearly affected, with all treatments the male ratio was greater than the female ratio which was ranged between (The first mentioned one is male); 57.14: 42.86% and 100.00: 0.00% with untreated control and treatment with 150 Gy of gamma irradiation dose. The previous obtained results are in harmony with results gained by Klingen *et al*. [18] and El-Sinary [1] who stated that the greatest the fungal concentration the highest the reduction in viability of tested insect. Also Prasad [19] and El-Sinary [20] stated that males are more tolerant than females for the effect of gamma irradiation.

Histological investigations explained and discussed the virulence and efficiency of fungal treatments and the effect of gamma irradiation doses as it screened the damages which happened to the midgut, epithelial layer, increasing in vacuoles numbers and size and the spread of fungal pathogen and its germination, penetration, growth and proliferation within the body of the infected host. Figure 1 showed untreated control transverse section of midgut without large vacuoles with complete epithelial layer. Figure 2 showed the fungal growth with 10^4 spores ml^{-1} treatment of the hyphal bodies, chlamydospores and spores were spread along the mid gut epithelial layer. Figure 3 showed the increasing of vacuoles and damage in epithelial layer with larger number of hyphal bodies with the treatment 10^4 spores ml^{-1} combined with 50 Gy of gamma irradiation dose. Figure 4 clearly presents the cavity and the wall of the mid gut filled with spores and hyphal bodies with 10^4 spores ml^{-1} combined with 100 Gy of gamma irragiation dose. Figure 5 screened a great vacuole clustered of spores and blastospores and spread of hyphal bodies along the cell wall with 10^4 spores ml^{-1} combined with 150 Gy of gamma irradiation dose. Figure 6 viewed spores of *B. bassiana*, hyhal bodies, chlamydospores of clusters of spores with the treatment of 10^8 spores ml^{-1}.

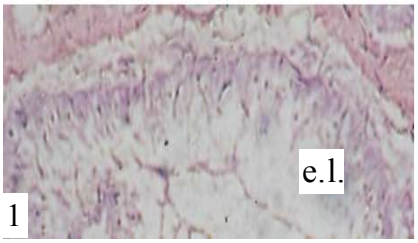

Fig. 1: Untreated control

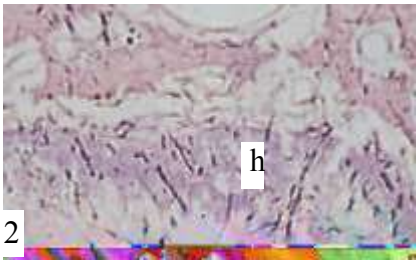

Fig. 2: 10^4

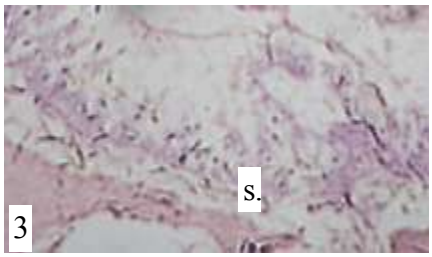

Fig. 3: 10^4+50 Gy

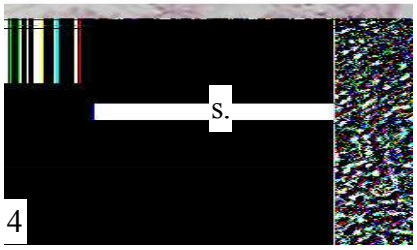

Fig. 4: 10^4+100 Gy

Fig. 5: 10^4+150 Gy

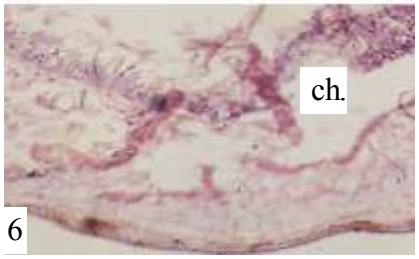

Fig. 6: 10^8

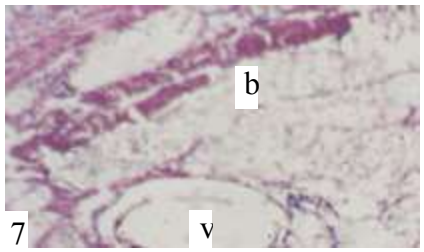

Fig. 7: 10^8+50 Gy

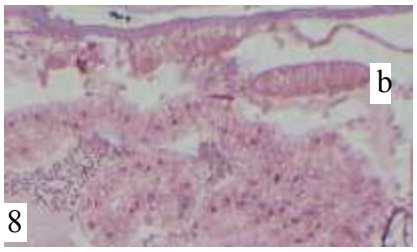

Fig. 8: 10^8+100 Gy

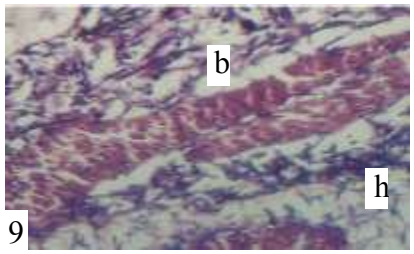

Fig. 9: 10^8+150 Gy

Micrograph of transversal sections of the *G. melonella* fourth larval instar midgut infected with *B. bassiana* combined with gamma irradiation doses (Gy)

#b: blastospore, ch: chlamydospore, e.l: epithelial layer, h: hyphal bodie, s: spores, v: vacuole

Figure 7 with 10^8 spores ml $^{-1}$ and 50 Gy dose of gamma irradiation screened increases in vacuoles and blastospores beside large numbers of hyphal bodies. Figure 8 recorded island of fungal growth in the mid gut cavity clustered beside blastspores with the treatment by 10^8 spores ml^{-1} and 100 Gy of gamma irradiation dose. Figure 9 viewed the lack of mid gut features which filled with enormous number of blastospores, hyphal bodies and chlamydospores resulted from treatment with 10^8 spores ml^{-1} and 150 Gy of gamma irradiation dose. These findings are in conformity with. Ramlee *et al*. [21] who stated that, after inoculation with *B. bassiana* fungal isolations, the hyphae penetrated the integument inside the whole body cavities then reached all cells like fat, neural and muscle tissues and damage it. Also, it reached Malpighian tubes and epithelial cells and finally colonized the gut lumen and the infected insects were already dead. After death by twenty four h. The. whitish mycelia begin to emerge from the cuticle of the dead body. These findings are in harmony with Sewify and Hashem [22] who stated that there were remarkable changes in the cellular response in the *G. melonella* when larvae were infected with the entomopathogenic fungus, *Metarhizium anispoliae*. Quesada-Maroga *et al.* [17] indicated that the death of *Spodoptera littorlaies* lurval instar by fungal species *B*. and *M. anispoliae* was due to the crude toxic protein extracted by fungi and that was dose related because in high doses mortality may reach 100% of treated larvae. Not only toxic protein extracts but also they observed a progressive bleeding of the midgut epithelium into the gut lumen with lyses of the e epithelium layer.

The previous results obtained from the treatment of the fourth larval instar of *G. melonella* by *B. bassiana* fungal isolations combined with gamma irradiation doses authenticate the important role which may entomopathogenic fungi play to reduce the risk of insect pests if could be safely applied to control pests in storage as a good bioagent and safe for environmental requirements. Combinations with gamma irradiation might magnify the virulence of fungal activity.

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