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Synergistic effect of gamma radiation and entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* on the humoral immune enzyme response in cotton leaf worm *Spodoptera littolaris* (Boisd).

Gabarty, A.[1]; El-Sonbaty, S. M.[2] and Ibrahim, A. A.[3]

1- Department of Natural Products Research, National Center For Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt;E-mail:ahlamalgabarty@yahoo.com
2- Department of Microbiology Research, National Center For Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt; E-mail:sawsansonbaty@yahoo.com
3- Protection Research Institute, Agriculture Research Center, Egypt;
E-mail:omarmary123@yahoo.com

ABSTRACT

The present study was carried out to investigate the efficancy of substerilizing doses of gamma irradiation or/ and entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* on the humoral immune enzyme response of *Spodoptera littolaris* larvae. It was found that gamma irradiation of male pupae (50,100&150 Gy) decreases the humoral immune enzyme activity (phenoloxidase, prophenoloxidase, lysozyme) and protein concentration compared to the control of the 6th instar larvae *S. littolaris*. Injecting of Lc_{50} concentration of both *B. bassiana* ($0.4x10^5$sp/µl) and *M. anisopliae* ($8x10^8$sp/µl) to the last 5th instar larvae of *S. littolaris* were significantly increased phenoloxidase, prophenoloxidase, lysozym activities and protein concentration after one day, however, they were significantly declined on the second, third and fourth days and remained at a level lower than that of the controls. Both gamma irradiation and entomopathogenic fungi (*B. bassiana* and *M.anisopliae*) significantly decreased the immune enzyme activity and protein concentration ($p \leq 0.05$) after 2, 3 & 4 days. However, this decrease was remarkable in the larvae of irradiated males'pupae (100Gy) which were injected with entomopathogenic fungi. In conclusion, gamma radiation and tested entomopathogenic fungi synergistically inhibit the immune system of *Spodoptera littolaris* larvae.

Keywords: Gamma radiation, entomopathogenic fungi, immune system.

INTRODUCTION

Spodoptera littoralis (Boisduval,1833), the Egyptian cotton leaf worm (Noctuidae, Lepidoptera) is the serious ,polyphagous insect, which has a reproductive capacity and migration ability over distances made it an economically important pest of many agricultural crops. It was reported that it can attack 112 plant species belonging to 44 families, including 40 species known only from India (Mallik arjuna *et al.*, 2004).

Insects possess a highly efficient immune system. Insects immunity consists of both humoral and cellular defensive reactions (Gillespie *et al*., 1997; Lavine and Strand, 2002). Cellular immunity includes phagocytosis, encapsulation, and nodule formation (Lavine and Strand, 2002). However, antimicrobial peptides and phenoloxidase system both have major roles in humoral defense. Phenoloxidase is a key factor in the insect immunity system, with important roles in the processes of

coagulation, melanization and wound healing. It activation was carried out in the cuticle or in the haemolymph of many invertebrates in response to immune challenge or wounding , in addition its activation via the prophenoloxidase cascade, prophenoloxidase being the inactive zymogen of phenoloxidase. The prophenoloxidase activating enzyme is thought to be a clip domain serine protease that cleaves prophenoloxidase to phenoloxidase (Ashida, 1990). Phenoloxidase and an oxidoreductase catalyse the oxidation process of phenols present in the haemolymph to cytotoxic quinones (Ashida, 1990). These quinones polymerize non-enzymatically to melanin. Both quinones and melanin are toxic to microorganisms. The deposition of melanin causes parasites to become blackened in the haemolymph, and in the nodules due to the melanization of haemocytes encapsulate foreign bodies (Söderhall and Cerenius, 1998).

In insects, melanin and its precursors are used directly in the immune system phenoloxidase, which is the key enzyme in the synthesis of melanin that was found in the haemolymph, midgut and cuticle. It thought to be involved in the non-self-recognition, as well as the encapsulation of larger organisms and so, plays a crucial role in the insect immune response (Ashida and Brey, 1995, 1997; Wilson *et al*., 2001; Cotter *et al*., 2004). Melanin itself also has chemical properties that may inhibit fungal growth (Söderhall and Ajaxon, 1982; St. Leger *et al*., 1988).

The continuous use of chemical pesticides against pests dramatically leads to a resistance to the pesticides action. In addition, the extensive use of these chemicals gave rise to problems such as residual toxicity (pollution) and harmful effects on beneficial insects, which are natural enemies of target or non-target pest species. Such problems have become the cause of search for the safety of pesticides including microbial agents such as fungi, bacteria, viruses, and sterile the insect technique using gamma radiation.

The present study aimed to investigate the efficacy of substerilizing doses of gamma radiation or /and entomopathogenic fungi (*Beauveria bassiana* and *Metarhizium anisopliae*) on the enzymatic activity of phenoloxidase, prophenoloxidase and lysozyme as well as the protein content in the cotton leaf worm, *Spodoptera littoralis*.

MATERIALS AND METHODS

Rearing technique

S. littoralis strain used in the present study was taken from Natural control laboratory at the National Center for Radiation Research and Technology, (NCRRT), Nasr City, Cairo, Egypt. Castor bean leaves, *Ricinus communis* were used for larval feeding under laboratory constant conditions (25±2˚C and 65±5% relative humidity) and away from any intentional chemical pressure. Laboratory tests revealed its susceptibility.

Irradiation process

The irradiation process was performed using Gamma CO^{60}, at National Center for Radiation Research and Technology (NCRRT, Cairo). In this investigation, the dose rate was 2.7K Gy/hour. Full-grown pupae were irradiated with 3 sub-sterilizing doses50, 100 and150 Gy**.**Two crossing combination of each dose were set up as follows: 10 replicates of resulted irradiated ♂♂ X unirradiated ♀♀, 10 replicates of unirradiated ♂♂ X unirradiated ♀♀ was used as control.

Fungal cultures

Blastospores cultures of *Beauveria bassiana* and *Metarhizium anisopliae* produced by inoculating conidia into Sabouraud dextrose broth, which consists of glucose 40.0 g/L, peptone 10.0 g/L, yeast extract 2.0 g/L and up to 1000 ml distilled water. This medium was prepared and adjusted at pH (5.5-6.0). The culture was inoculated at 25±1^{0}C on gyratory shaker for 48h. The culture was filtered through sterilized glass wool to remove mycelia. Blastospores were collected from filtrates by centrifugation and washed twicy in a sterile solution of 0.85% saline. Fungal cells were counted on a hemacytometer and diluted in sterile saline solution to a concentration of (10^7,10^8,10^9&10^{10}) for *B. bassiana* and *M. anisopliae*. Five µl of each concentration 0.4x10^5sp/µl (Lc_{50}) for *B. bassiana* and 8x10^8sp/µl (Lc_{50}) for *M. anisopliae*were injected into the hemocoeles of late fifth instar of *S. littoralis* larvae. The control larvae injected with five µl of sterile 0.85% saline solution. While the treated and control larvae were placed in sterile batches containing cleaned castor bean leaves, then incubated at 25±1^{0}C and 75±5% RH. The injected larvae were surface sterilized with 70% ethanol and rinsed twicy with sterile distilled water. At 24, 48, 60& 72h after challenge representative sixth instar larvae were sacrificed and haemolymph was examined under phase contrast optics to insure that the fungus was replicating in challenged larvae (Hung and Boucias, 1992; Hung *et al*., 1993).

To evaluate the toxicity of the chosen entomopathogenic fungi, ten healthy 2nd instar larvae of *S. littoralis* (five replications for each) were left to feed on castor leaves treated with different concentrations of entomopathogenic fungi (10^7, 10^8, 10^9 and 10^{10}spores/ml) then put on treated straw with the same conc. in the plastic jars. Untreated leaves and straw used as control. The larvae fed on treated leaves for 5 days after that mortality counts recorded as percentage mortality. Mortality percentages were corrected using Abbott Formula (Abbott, 1925). The (Lc_{50}) calculated according to the method of Finney (1971).

Collection and treatment of haemolymph

The haemolymph for chemical analysis was obtained from the survived 6th instar larvae in each treatment. The haemolymph was obtained after 24, 48, 60&72 by cutting one or two of the prothoracic legs then the haemolymph was drown into a small test tube containing a few milligrams of phenylthiourea to prevent the inhibitory effect of tyrosinase (Schmidt and Williams, 1953; Abou El- Ghar*et al*., 1996).

Protein determination:-The protein concentration of the solutions in g/dl was measured by the method of Gornal *et al.,* (1949).

Enzyme assays:-

Phenoloxidase activity was monitored spectro-photometrically as the formation of dopachrome (Horowitz and Shen, 1952). Aliquots of 100µl of diluted haemolyph serum were incubated with freshly made L-Dopa (L-β-3,4-dihydroxyphenylalanine) (100µl; 3mg/ml in sodium cacodylate buffer, 10mM;pH 6.0) for 30 min at30 ℃. The mixture was made up to 1 ml with cacodylate buffer before measuring the absorbance at 490 nm in a spectrophotometer. The specific activity of phenoloxidase expressed as units of activity per g of protein. One unit of activity defined as the amount of enzyme that increases the absorbance by 0.001 units per min. To determine prophenoloxidase activity, 100 µl of diluted haemolymph serum were incubated with 100 µl of chymotrypsin (1 mg/ml in sodium cacodylate buffer) for 30 min prior to the addition of L-Dopa. Then 100 µl of the pre-incubated mixture were assayed as above. The value for phenoloxidase activity was subtracted from the value which was obtained for phenoloxidase activity after chemotrypsin activation to give the quantity of prophenoloxidase present in samples.

Lysozyme activity was determined according to the method of Gillespie *et al.*, (2000). The standard reaction medium contained 0.5 mg/ml of dried *Micrococcus lysodeikticus* cell walls in 10 mM sodium phosphate buffer (pH 5.5). 20 µl of the diluted haemolymph were added to 1.2 ml of the *M. lysodeikticus* suspension and the mixture was left to stand for 30 min at 30℃. 0.5 ml of 0.5 M NaOH was added before reading the turbidity of the suspension in a spectrophotometer at 450 nm. One unit of lysozyme was defined as the amount of enzyme that decreases the absorbance by 0.001 units per min. The specific activity of the lysozyme is defined as units of activity per g protein.

Statistical analysis

Experimental data were analyzed using one way analysis of variance (ANOVA) using SPSS (statistical package for social sciences, ver.15.0), and the significance among the samples was compared at P≤0.05. Results were represented as mean ±SE (n =3).

RESULTS

Effects of gamma radiation on enzymatic activities (phenoloxidase, prophenoloxidase, lysozyme) and protein concentration in *S. littolaris.*

The changes in the activity of haemolymph phenoloxidase, prophenoloxidase, lysozyme and protein concentration of F_1 progeny in the 6th instar larvae of *S. littolaris* resulted from irradiated males by (50,100&150 Gy) as demonstrated in Table (1). The results showed a significant decrease (p≤ 0.05) in the activity of phenoloxidase, prophenoloxidase, lysozyme and protein concentration compared to the control. The percentage decrease in the enzymatic activities of haemlymph in the 6th instar larvae of *S. littolaris* resulted from irradiated males by 50,100&150 Gy, and recorded as 11.6, 48.8 and 67.4, for phenoloxidase; 11, 22 and 31 for prophenoloxidase; 5.9, 17 and 69 for lysozyme and 23, 50 and 87 for total protein, respectively.

Table 1: Changes in the activity of haemolymph (phenoloxidase, prophenoloxidase, lysozyme) and protein concentration of F_1 progeny in the 6th instar larvae of *S.littolaris* resulted from irradiated males by (50,100&150 Gy).

Dose Gy	Phenoloxidase (Units/g protein)		prophenoloxidase (Units/g protein)		Lysozyme (Units/g protein)		Total protein (g/dl)	
	Mean ± SD	Red. %	Mean± SD	Red. %	Mean± SD	Red. %	Mean± SD	Red. %
0	43 ± 3^{bcd}	-	45 ± 3^{bcd}	-	305 ± 15^{bcd}	-	1.34 ± 0.02^{bcd}	-
50	38 ± 2.5^{acd}	11.6	40 ± 2^{acd}	11	287 ± 12^{acd}	5.9	1.027 ± 0.01^{acd}	23
100	22 ± 1^{abd}	48.8	35 ± 1.6^{abd}	22	253 ± 7.5^{abd}	17	0.67 ± 0.003^{abd}	50
150	14 ± 0.8^{abc}	67.4	31 ± 1.5^{abc}	31	94 ± 5^{abc}	69	0.174 ± 0.002^{abc}	87

[a] significant different for control at (p≤0.05). [b] significant different for irradiation by (50 Gy) at (p<0.05) .
[c] significant different for irradiation by(100) at (p≤0.05).[d]significant different for irradiation by(150) at (p≤0.05).
Values represent the mean. of 3replicates for each sample

Effects of *B. bassiana and M. anisopliae* on enzymatic activities (phenoloxidase, prophenoloxidase, lysozyme) and protein concentration in *S. littolaris.*

Changes in the activity of haemolymph phenoloxidase, prophenoloxidase, lysozyme and protein concentration in the last 5th instar larvae of *S.littolaris* injected by (Lc_{50}) of entomopathogenic fungi, *B.bassiana* and *M.anisopliae* after 1,2,3&4 days, Tables (2&3). The results indicated that there is a significant increased (p≤0.05) in the activities of phenoloxidase, prophenoloxidase, lysozyme and protein concentration after one day as compared with control, but after 2, 3& 4 days the activity of haemolymph phenoloxidase, prophenoloxidase, lysozyme and protein

concentration in experimental insects had declined significantly and remained at a level lower than that of the controls.

Table 2: Changes in the activity of haemolymph (phenoloxidase, prophenoloxidase, lysozyme) and protein concentration in the last 5th instar larvae of *S. littolaris* injected by (Lc_{50}) of entomopathogenic fungi, *B. bassiana*,after 1,2,3&4 days.

Days	Mean±SD											
	Phenoloxidase (Units/g protein)			Prophenoloxidase (Units/g protein)			Lysozyme (Units/g protein)			Total protein (g/dl)		
	Con.	Exp.	Red. %	Con.	Exp.	Red. %	Con.	Exp.	Red. %	Con.	Exp.	Red.%
1	28± 1.8^{d}	35± 2.4^{de}	+25	25± 1.4^{d}	30± 2.1^{de}	-20	302± 15^{d}	413± 20de	+37	1.03± 0.01^{d}	1.17± 0.001^{de}	+13
2	32± 1.3^{d}	25± 1.2^{de}	22	28± 1.9^{d}	20± 1.3^{de}	29	343± 14^{d}	224± 11de	35	1.88± 0.003^{d}	1.56± 0.01^{de}	17
3	41± 3.6^{d}	22± 0.8^{de}	46	32± 2^{d}	15± 1.3^{de}	53	365± 17^{d}	194± 9de	47	2.034± 0.01^{d}	1.18± 0.003^{de}	42
4	50± 3.2^{d}	13± 1.3^{de}	74	33± 1.8^{d}	10± 0.6^{de}	70	382± 10^{d}	135± 8de	65	2.25± 0.03^{d}	1.02± 0.004^{de}	55

1 .Con. =control, Red. = reduction, Exp. = experimental. Values represent the mean.of 3replicates for each sample.
2. ^{e}Significantly different from corresponding control value ($p \leq 0.05$).

Table 3: Changes in the activity of haemolymph phenoloxidase, prophenoloxidase, lysozyme and protein concentration in last 5th instar larvae of *S. littolaris* injected by (Lc_{50}) of entomopathogenic fungi, *M. anisopliae*, after 1, 2, 3&4 days.

Days	Mean±SD											
	Phenoloxidase (Units/ g protein)			Prophenoloxidase (Units/ g protein)			Lysozyme (Units/ g protein)			Total protein (g/dl)		
	Con.	Exp.	Red. %	Con.	Exp.	Red. %	Con.	Exp.	Red. %	Con.	Exp.	Red. %
1	34± 2.3^{d}	55± 3.2^{de}	+62	19± 1.4^{d}	31± 2.6^{de}	+63	294± 9^{d}	392± 15de	+33	0.853± 0.002^{d}	1.253± 0.02^{de}	+47
2	40± 2.6^{d}	32± 1.6^{de}	20	28± 1.5^{d}	24± 1de	14	300± 16^{d}	270± 12de	10	1.562± 0.003^{d}	1.053±. 001de	33
3	43± 3^{d}	31± 1.1^{de}	30	29± 1.7^{d}	20± 1.3^{de}	31	363± 11^{d}	254± 8de	30	1.635± 0.04^{d}	0.994± 0. 01de	39
4	45± 2.6^{d}	20± 1.4^{de}	56	35± 2.8^{d}	15± 0.8^{de}	57	395± 18^{d}	187± 6de	53	2.054± 0.002^{d}	0.736± 0. 03de	64

1 .Con. =control,Red. = reduction, Exp. = experimental. Values represent the mean. of 3replicates for each sample.
2.^{e} Significantly different from corresponding control value ($p \leq 0.05$).

Synergistic effects of gamma radiation and entomopathogenic fungi tested on immune enzyme and protein concentration in *S. littolaris.*

The changes in the activity of haemolymph phenoloxidase, prophenoloxidase, lysozyme and protein concentration of F_1 progeny in the last 5th instar larvae of *S. littolaris* resulted from irradiated males (100Gy) injected by (Lc_{50}) of *B. bassiana* and *M. anisopliae* after 1,2,3&4 days were indicated in Tables (4&5). The activities of phenoloxidase, prophenoloxidase, lysozyme and protein concentration were increased significantly ($p \leq 0.05$) after one day in comparison with the control. Meanwhile, the activities of these enzymes and protein concentration in haemolymph of irradiated and injected insects have decreased significantly after 2,3&4 days. Interestingly there was a significant correlation among phenoloxidase, prophenoloxidase, lysozyme activities and protein concentration when data from the treated and the control insects are were combined.

From aforementioned results, it is obvious that both gamma irradiation and entomopathogenic fungi (*B. bassiana* and *M.anisopliae*) tested caused a significant decrease after 2,3&4 days in the activity of phenoloxidase, prophenoloxidase, lysozyme and protein concentration. However, this decrease was greatly remarkable

in larvae resulted from mating the irradiated males (100Gy), that were treated with the (Lc_{50}) of the tested entomopathogenic fungi.

Table 4: Changes in the activity of haemolymph (phenoloxidase, prophenoloxidase, lysozyme) and protein concentration of F_1 progeny in last 5th instar larvae of S. *littolaris* resulted from irradiated males (100Gy) injected by (Lc_{50}) of *B. bassiana*, after 1,2,3&4days.

Days	Mean±SD											
	Phenoloxidase (Units/ g protein)			prophenoloxidase (Units/ g protein)			Lysozyme (Units/ g protein)			Total protein (g/dl)		
	Con.	Exp.	Red. %	Con.	Exp.	Red. %	Con.	Exp.	Red. %	Con.	Exp.	Red.%
1	41± 2.5 ^{d}	61± 3.6 ^{de}	+ 49	35± 1.1 ^{d}	51± 2.2 ^{de}	+ 46	105± 7.3 ^{d}	175± 6 ^{de}	+ 67	0.884± 0.002 ^{d}	1.314± 0.03 ^{de}	+49
2	58± 2.2 ^{d}	41± 1.3 ^{de}	29	37± 2 ^{d}	24± 1.2 ^{de}	35	221± 11 ^{d}	145± 7 ^{de}	34	2.04± 0.01 ^{d}	0.945± 0.002 ^{de}	54
3	75± 3.4 ^{d}	33± 2 ^{de}	56	42± 3 ^{d}	18± 0.9 ^{de}	57	238± 9 ^{d}	106± 4 ^{de}	55	2.263± 0.001 ^{d}	0.713± 0.01 ^{de}	68
4	143± 6 ^{d}	20± 1 ^{de}	86	100± 7 ^{d}	30± 1.4 ^{de}	70	237± 11 ^{d}	55± 2.1 ^{de}	77	2.763± 0.004 ^{d}	0.427± 0.03 ^{de}	84

1 .Con. =control, Red. = reduction, Exp.= experimental. Values represent the mean. of 3replicates for each sample.
2.^{e} Significantly different from corresponding control value ($p<0.05$).

Table 5: Changes in the activity of haemolymph (phenoloxidase, prophenoloxidase, lysozyme) and protein concentration of F_1 progeny in last 5th instar larvae of *S. littolaris* resulted from irradiated males (100Gy) injected by (Lc_{50}) of *M. anisopliae*, after 1,2,3 & 4 days.

Days	Mean±SD											
	Phenoloxidase (Units/ g protein)			Prophenoloxidase (Units/ g protein)			Lysozyme (Units/ g protein)			Total protein (g/dl)		
	Con.	Exp.	Red. %	Con.	Exp.	Red. %	Con.	Exp.	Red. %	Con.	Exp.	Red. %
1	34± 0.2 ^{d}	41± 0.3 ^{de}	+21	18± 0.4 ^{d}	21± 0.3 ^{de}	+17	291± 9 ^{d}	345± 7 ^{de}	+ 19	0.854± 0.001 ^{d}	0.963± 0.02 ^{de}	+13
2	40± 0.3 ^{d}	23± 0.3 ^{de}	43	27± 0.3 ^{d}	21± 1.2 ^{de}	22	324± 12 ^{d}	285± 9 ^{de}	12	1.564± 0.01 ^{d}	0.637± 0.04 ^{de}	59
3	44± 0.5 ^{d}	17± 0.1 ^{de}	61	30± 0.4 ^{d}	20± 0.9 ^{de}	33	384± 7 ^{d}	244± 6 ^{de}	36	1.634± 0.001 ^{d}	0.496± 0.01 ^{de}	69
4	48± 0.3 ^{d}	15± 0.3 ^{de}	69	35± 0.3 ^{d}	13± 0 .1 ^{de}	63	395± 8 ^{d}	142± 4 ^{de}	64	2.057± 0.01 ^{d}	0.392± 0.02 ^{de}	81

1 .Con. =control, Red. = reduction, Exp. = experimental.Values represent the mean. of 3replicates for each sample.
2.^{e} Significantly different from corresponding control value ($p<0.05$).

DISCUSSION

The results of the present study may have a great importance as the environmental safety of an insecticide now a day is considered to be of paramount importance. Fungi have a specific role in pest control with high persistence in the environment. Some insect pathogenic fungi have restricted host ranges while other fungal species have a wide host range for example, *M.anisopliae* and *B.bassiana.* In addition, gamma irradiation can reduce the viability of successive generations of certain insects, while the substerilizing doses of gamma radiation can increase mortalities in the resulting progeny and minimize the hazards of irradiated insects (Sutrisno *et al.,* 1991, Garcia and Gonzalez, 1993, and Gabarty, 2011).

The present study has focused on the changes in the enzyme activities induced by gamma radiation or the changes after 1,2,3&4 days resulted from injection of last 5thinstars larvae by (Lc_{50}) of *B. bassiana* and *M. anisopliae*. Moreover, the Synergistic effect of gamma radiation (100Gy) and the tested entomopathogenic fungi on the enzyme activities of F_1 progeny after 1, 2, 3&4 days.Results indicated that gamma irradiation caused a significant decrease in the enzyme activities of phenoloxidase, prophenoloxidase, lysozyme and protein concentration. However, this decrease was greatly remarkable in larvae resulted from irradiated males by (150 Gy).Gamma

irradiation of *Trogoderma granarium* larvae at 50 and 150 Gy has inhibited the phenoloxidase activity after 1 week in fourth instar of kaphra beetle (Lupa, 2000), and cause changes on each instar in phenoloxidase activity of cotton bollworm at 75 and 150 Gy (Saurian, 2004). The irradiated insects were found to have phenoloxidase activities significantly less than that of the control. Jakarpong *et al.,* (2005), recorded asimilar decrease in phenoloxidase activities of irradiated larvae of *Callosobruchus chinensis* at 100, 300 and 500 Gy.

Gamma radiation markedly reduced the protein content Gabarty (2008), who had recorded a significant decrease in the total content of haemolymph proteins of irradiated males (100Gy) F_1 progeny in the 6^{th} instar larvae of *A. ipsilon*.

In addition to gamma irradiation, our results indicated that entomopathogenic fungi, *B. bassiana and M. anisopliae* significantly increase the phenoloxidase, prophenoloxidase, lysozyme activities and protein concentration after one day. In the 2^{nd}, 3^{rd}& 4^{th} days enzyme activities and protein concentration were consistently lower than that of the control and declined over the period of infection. Trudeau *et al*., (2001) stated that the invasion of the insect body and haemolymph by fungi occurs once the fungus has passed through the cuticle of the external insect's skeleton and cause changes in the protein concentrations and enzyme activities. When conidia land on the cuticle of a suitable host, they attach and germinate, initiating cascades of recognition and enzyme activation reactions by both the host and the fungal parasite. The defensive responses to the fungi infection lead to activation of the phenoloxidase and other enzymes of cascade. The increase in phenoloxidase activity may be beneficial to *Bracon hebetor*, as increased levels of phenoloxidase may help to suppress microbial infection during the time interval, thus preserve the nutritional value of the paralyzed larva as a subsequent food source for the parasitoid (Hagstrum, 1983). The insect cuticle, fat body and insect protein were the barriers for insects to defend against microbes. *Metarhizium* in fection may results in declining hemolymph protein and phenoloxidase titers over the course of the infection until the death of *Schistocerca gregaria* (Gillespie *et al.,* 2000) and *Locusta migratoria* (Mullen and Goldsworthy 2006) whereas *Beauveria* infection increases the active phenoloxidase levels of *Melanoplus sanguinipes* (Gillespie and Khachatourians 1992). Lysozyme activity of *S. gregaria* was declined (Gillespie *et al.,* 2000) or remaine unchanged *as in Spodoptera exigua* (Boucias*et al.,* 1994). At the present study, *B. bassiana and M. anisopliae* severely deplete the proteins concentration.Insect infected with fungi was declined its total protein concentrations, it was dramatically declined in desert locust treated by 10^3, 10^6 and 10^{12} spores/ml of *M. anisopliae* (El banna *et al.,* 2012). Also Gabarty (2011), found that in the 3^{rd}, 4^{th}& 6^{th} instar larvae of *A. ipsilon* resulted from 2^{nd} instar larvae treated with Lc_{50} of the entomopathogenic fungi (*B. bassiana and M. anisopliae).* Reduction in the total protein content of the adult haemolymph of *S. gregaria* reported during the course of infection with the *M. anisopliae* (Gillespie *et al.,* 2000). It was reported in *G. mellonella* infected with *Pseudomonas aeurginosa* (Mckinstry and Steinaus, 1970). The reduction in the total protein and the loss of soluble protein from the host' haemolymph during parasitism may be due to proteolytic enzymes which were secreted into the haemocoel of the insect and hydrolyze the host protein (Gillespie *et al.,* 2000). Highnam and Hill (1969) stated that protein metabolism and excretion are all regulated hormonally. The disturbance of the host's endocrine balance by the parasite could be responsible for reduction in protein concentrations of host haemolymph. Decreasing the total protein content may lead to the death of treated insects and this may be one of the reasons of insect mortality. The reduction or the lack of protein leads to retardation of many

physiological processes in insects, adult insect requires protein to promote ovulation, and egg development supports this conclusion (House, 1963).

CONCLUSION

The present results indicated that both tested gamma radiation and entomopathogenic fungi caused a significant decrease in the immune system activity of phenoloxidase, prophenoloxidase, lysozyme and protein concentration over the period of infection. However, the combination of gamma irradiation (100Gy) and entomopathogenic fungi (Lc_{50}) used in the larval treatment have induced more remarkable in larvae resulted from irradiated males, and treated with (Lc_{50}) of *B. bassiana* after 3 days**.**

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ARABIC SUMMARY

التأثير المتكامل لاشعة جاما وفطرى *بيوفاريا بسيانا ومتاريزيم انزوبلى* على انزيمات جهاز المناعة فى يرقات دودة ورق القطن

أحلام جبرتى[1]، سوسن السنباطى[2] ، أحمد عدلى ابراهيم[3]

1- قسم بحوث المنتجات الطبيعية، المركز القومي لبحوث وتكنولوجيا الاشعاع، هيئة الطاقة الذرية، القاهرة

2- قسم بحوث الميكروبيولوجيا الاشعاعية، المركز القومي لبحوث وتكنولوجيا الاشعاع، هيئة الطاقة الذرية، القاهرة

3- معهد وقاية النباتات، مركز البحوث الزراعية ، وزارة الزراعة ،القاهرة

يهدف البحث بوجه عام لدراسة فاعلية الجرعات تحت المعقمة من اشعة جاما أو الفطريات الممرضة من فطرى *بيوفاريابسيانا ومتاريزيم انزوبلى* كلا على حدة او كليهما معا على انزيمات المناعة فى يرقات دودة ورق القطن .اظهرت النتائج المتحصل عليها إنخفاض ملحوظ فى انزيمات المناعة (phenoloxidase وprophenoloxidase وlysozyme) و المحتوى الكلى للبروتين فى يرقات العمر السادس الناتجة من

تشعيع العذارى الذكور كاملة النضج لدودة ورق القطن بالجرعات تحت المعقمة 50 و 100 و150 جراى بالإضافة إلى المجموعة الضابطة (الكنترول). وبحقن يرقات نهاية العمر الخامس بالتركيز نصف المميت Lc_{50} لفطرى *بيوفاريابسيانا* ($0.4x10^5$sp/µl) *ومتاريزيم انزوبلى* ($8x10^8$sp/µl) المختبرة أحدثت زيادة معنوية فى نشاط كلا من إنزيمات (phenoloxidase وprophenoloxidase وlysozyme) و المحتوى الكلى للبروتين بعد اليوم الاول مقارنة بالكنترول بينما بعد اليوم 2 ، 3 & 4 حدث انخفاض معنوى فى انزيمات المناعة المختبرة والمحتوى الكلى للبروتين واستمر هذا الانخفاض مقارنة بالكنترول.

كلا من اشعة جاما والفطريات المختبرة احدثت انخفاض معنوى ملحوظ بعد اليوم 2 ، 3 & 4 فى انزيمات المناعة المختبرة والمحتوى الكلى للبروتين ولقد بلغ هذا الإنخفاض أقصاه فى اليرقات الناتجة من تشعيع العذارى الذكور بالجرعة (100 جراى) والمعاملة بالتركيز نصف المميت Lc_{50} للفطريات المختبرة. بوجة عام هناك تأثير متكامل لاشعة جاما والفطريات الختبرة فى تثبيط انزيمات المناعة فى يرقات دودة ورق القطن *Spodoptera littolaris*.