

## COMBINED EFFECT OF *BEAUVERIA BASSIANA* (BALS.) AND GAMMA IRRADIATION ON POTATO TUBER MOTH *PHTHORIMAEA OPERCULELLA* (ZELLER)

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### Abstract

The objective of this study was to show the susceptibility of different immature stages (eggs, larvae and pupae) of potato tuber moth *Phthorimaea operculella* (Zeller) (Lepidoptera: Glechiidae) to the entomopathogenic fungus (EPF) *Beauveria bassiana*, and the effect of irradiating full-grown male pupae of *ph. operculella* at sub-sterilizing doses 100 and 200 Gy of gamma irradiation (alone and in combination with LC<sub>50</sub> of *B. bassiana*) on some biological aspects of resulting F<sub>1</sub> larvae, the results showed that, the EPF *B. bassiana* was pathogenic to all immature stages with LC<sub>50</sub> values of 5.5x10<sup>3</sup>, 28.56 and 1.1x10<sup>5</sup> conidia/ml, respectively, larvae were more susceptible to infection by *B. bassiana* than eggs and pupae. The percents of larval and pupal mortality were increased as the radiation doses increased while the percents of pupation and adult emergence were decreased as the radiation dose increased. Combination of gamma radiation and *B. bassiana* was more effective than either treatment of them alone.

**Keywords:** *Beauveria bassiana*, gamma-irradiation, pathogenicity, biological aspects, *phthorimaea operculella*.

### INTRODUCTION

Potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Glechiidae) is considered from the most serious pests of potatoes in Egypt as well as many other countries. It causes severe damage to potato tubers in the field and in the storage. Its main hosts are potato, tomato, and pepper.

The use of chemical insecticides to control potato tuber moth is associated with many problems of creating more resistance strains of the insect, the treated tubers become invalid for human consumption due to persistence of residues in the tubers. This resulted in attempts to develop an integrated control program for potato tuber moth in the field and storage.

Entomopathogenic fungi such as *Beauveria bassiana* (Bals.) belong to the class of Hyphomycetes, are naturally occurring environmentally safe organisms, have a broad spectrum of hosts, can be mass produced easily, rapidly and economically (Rumbos and Athanassiou, 2017). They offer promise in the microbial control of certain economic crop pests (Jaronski, 2010). The use of irradiation techniques as a

physical control method is cheap, safe and more reliable than chemical methods. Application of singular methods in insect control have limited success, so combining two or more control strategies with additive effects may increase program efficiency (Carpenter and Young, 1991). Recently, laboratory studies have revealed that semi sterility is compatible with other methods of pest control.

The objective of this study is to investigate the susceptibility of different immature stages (eggs, larvae and pupae) of *Ph. operculella* to entomopathogenic fungus *B. bassiana* and the effects of sub-sterilizing doses of gamma-irradiation alone and combined with LC<sub>50</sub> concentration of the fungus *B. bassiana* on some biological aspects of F<sub>1</sub> larvae resulting from irradiated parental males.

## **MATERIALS AND METHODS**

### **Insect rearing:**

The tested insect, *Ph. operculella* (Lepidoptera: Gelechiidae) was obtained from the laboratory strain which reared (for five successive years) in the unit of stored product pests, Nuclear Research Center (NRC) Atomic Energy Authority, Egypt, as adults of both sexes were taken to mate and oviposit in chimney glass. The rearing technique was done according to Hemida (1976).

### **Entomopathogenic fungus:**

Entomopathogenic fungus (EPF) used in the present study was *B. bassiana* (Bals.) conidia, obtained from fungal culture produced at the Bio-Insecticide Production Unit Plant Protection Research Institute, Dokki, Giza, Egypt.

### **Susceptibility of immature stages of *Ph. operculella* to *B. bassiana*:**

Pathogenicity of the entomopathogenic fungus *B. bassiana* to immature stages (eggs, (larvae and pupae)males) of *Ph. operculella* was carried out in Petri dishes (9 cm diameter) containing filter paper according to the method of Dal Bello *et al*, 2001) different conidial concentrations ranged from 10<sup>4</sup> to 10<sup>10</sup> conidia/ml of *B. bassiana* were used separately for each stage and applied to the filter paper by mini pump sprayer. Three replicates for each stage (10 individuals for each replicate) were carried out for each conidial concentration and three replicates without conidia as control. males of larvae and pupae were differentiated from females by method of Hemida (1976). The experiment was maintained at room temperature 25±2°C and relative humidity 60±5 RH. Dead individuals were recorded daily and infection confirmation by the fungus was done by collecting dead individuals and then transferred to sterilized Petri dish with moistened piece of cotton and incubated at 25°C until fungal sporulation on the cadavers appear.

### **Effect of gamma-irradiation on some biological studies:**

#### **Experiment (1):**

This experiment was aimed to show, the effects of mating normal females with parental males ( $P_1$ ) which irradiated as full grown pupae for 24-48hrs at two sub-sterilizing doses (100 and 200 Gy) using (3500 Gamma cell irradiation units cobalt 60 source) located at the Middle Eastern Regional Radioisotope Centre for the Arab countries, Cairo (Egypt) with dose rate of 3.75 Gy/min, on some biological aspects. The eggs laid by each female were collected, counted and incubated at  $25 \pm 2^\circ\text{C}$  and  $60 \pm 5\%$  RH. Percentage of, hatchability, pupation and adult emergence were studied. Three replicates were carried out for each dose level and control.

#### **Experiment (2):**

Adult males irradiated at 100 and 200 Gy and normal were put with normal female in separate cages for mating and oviposition. Five replicates each contain one hundred eggs collected from each treatment were placed into small boxes containing seven potato tubers. The boxes were covered with tissue fixed with rubber band. Mature larvae ( $F_1$  larvae) which hatched from the eggs were treated with  $LC_{50}$  concentration of the EPF *B. bassiana*. Mortality percent of larvae, pupae and percent of adult emergence were calculated.

#### **Statistical analysis:**

Mortality percentages of all experiments were adjusted against that of control using (Abbott, 1925). Mortality data for concentrations ( $LC_{50}$  and  $LC_{90}$ ), times ( $LT_{50}$  and  $LT_{90}$ ) and the slope of regression lines were calculated using probit analysis (Finney, 1971). Data from biological studies were subjected to analysis of variance (ANOVA).

## **RESULTS AND DISCUSSION**

### **Susceptibility of immature stages of *Ph. operculella* to entomopathogenic fungus *B. bassiana*:**

Mortality percentages of different immature stages (eggs, larvae and pupae) of potato tuber moth *Ph. operculella* after treatment with different concentrations of the entomopathogenic fungus *B. bassiana* are shown in table (1). The obtained results showed that all concentrations tested of *B. bassiana* caused mortality to different immature stages of *Ph. operculella*. The obtained data agree with Oliveria et al., (2010) who reported a high mortality of larvae and pupae of *Ceratitis capitata* at all concentrations of conidia of *Metarhizium anisopliae* and *B. bassiana*. The present results also show that mortality percentages of all tested stages seemed to be concentration dependent where the highest mortality percentage value recorded for the eggs, larvae and pupae were 96.7, 99.98 and 100%, respectively at the highest concentrations  $10^{10}$ ,  $10^7$ , and  $10^9$  conidia/ml, respectively. The obtained results are in

full agreement with (Li and Zhang, 2005) who concluded that, seven strains of *B. bassiana* were isolated from the cadavers of larvae of potato (*Ph. operculella*) and all of them were virulent to the larvae and the strain Bb 8001 caused cumulative mortality of 70 and 86.7% as inoculated at  $10^5$  and  $10^7$  conidia /ml. the obtained results are in agree with El-Sinary and Rizk (2007) who tested two concentrations of the entomopathogenic fungus *B. bassiana*  $10^4$  and  $10^8$  spore/ml against the 4<sup>th</sup> larval instar of the greater wax moth *Galleria mellonella* and found positive correlation between the fungal concentration and its lethality for the treated larvae. The obtained results also agree with Zheng *et al.*, (2016) who reported that larval mortality of potato tuber mouth caused by infection of *M. anisopliae* (KMa0107strain) increased with increase of the concentration of conidial suspension.

**Table 1. Mortality percentages of different immature stages (eggs, (larvae and pupae) males) of *Phthorimaea operculella* (Zeller) after treatment with different concentrations of *Beauveria bassiana*:**

Con. Conidia/ml	Immature stages		
	Mean% of eggs mortality	Mean% of larval mortality	Mean% of pupal mortality
$10^4$	--	88	--
$10^5$	--	95	--
$10^6$	70	96.5	71.4
$10^7$	83.3	99.98	85.6
$10^8$	90	--	93.3
$10^9$	93.3	--	100
$10^{10}$	96.7	--	100

The results also show that the larval stage of *Ph. operculella* was more susceptible to fungal infection than eggs and pupae, this agree with Hafez *et al.* (1997) who reported that both first-and second instar larvae of *Ph. operculella* were susceptible to *B. bassiana* than larvae in the third and fourth instar, and infection of pre-pupae and pupae resulted in marked decrease in the infection.

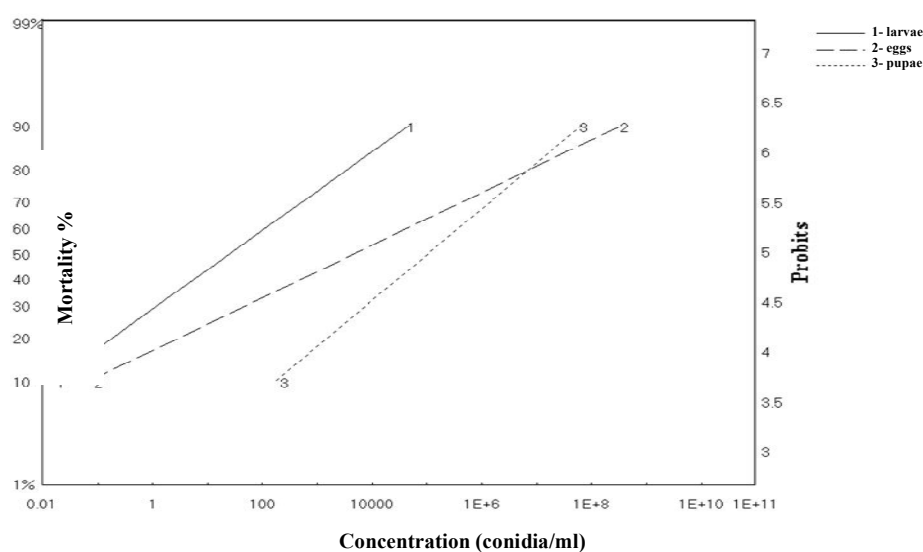
The obtained results also supported by Yuan *et al.*, (2017) who evaluated six *B. bassiana* strains with potential for control of potato tuber worm *Ph. operculella* (Zeller) larvae and found that strain SD was the most virulent causing up to 98% mortality.

Regarding the toxicity values of EPF *B. bassiana* (Bals.) on different immature stages of *Ph. operculella* based on  $LC_{50}$ , are indicated in table (2) and Fig (1). The data in the table indicated that, when larvae were treated with *B. bassiana*  $LC_{50}$  and  $LC_{90}$  values were equal to 28.56 and  $5.1 \times 10^4$  conidia/ml, which are lower than those obtained for eggs  $5.5 \times 10^3$  and  $3.9 \times 10^8$  conidia/ml and those obtained for pupae  $1.1 \times 10^5$  and  $7.1 \times 10^7$  conidia /ml respectively, so larvae are more susceptible to infection with *B. bassiana* than eggs and pupae, this may be due to nature of the

cuticle and degree of sclerotization of the body surface among the three stages or may be due to mobility of larvae which may give them a great chance to be in contact with the fungus which is the main reason for infection. The results are in full agreement with Hajeck and St-leger (1994) who showed that, low pathogenicity of the fungi against many insect species was due to the nature of the cuticle in terms of its density, thickness and degree of sclerotization.

**Table 2. Toxicity values of entomopathogenic fungus *Beauveria bassiana* against immature stages of potato tuber moth *Phthorimaea operculella*.**

Insect stage	(LC <sub>50</sub> ) in conidia/ml	(LC <sub>90</sub> ) in conidia/ml	Slope±S.E
Eggs	$5.5 \times 10^3$	$3.9 \times 10^8$	0.264±0.057
Larvae (males)	28.56	$5.1 \times 10^4$	0.394±0.114
Pupae (males)	$1.1 \times 10^5$	$7.1 \times 10^7$	0.459±0.071



**Fig. 1. LD-P lines of entomopathogenic fungus *Beauveria bassiana* against immature stages of potato tuber moth *Phthorimaea operculella*.**

Regarding the median lethal time of *B. bassiana* to different immature stages of *Ph. operculella* data in table (3) show that, both LT<sub>50</sub> and LT<sub>90</sub> of the three stages (eggs, larvae and pupae) decrease as the initial concentration increases and the shortest LT<sub>50</sub> value for larvae was, 1.2d at the highest concentration  $10^7$  conidial/ml, while the shortest LT<sub>50</sub> value for eggs and pupae were, 1.13 and 1.48d, respectively at the highest concentration  $10^{10}$  conidia/ml. these results agree with Zheng *et al.*, (2016) who showed that the median lethal time of *M.anisopliae* to the potato tuber

moth larvae differed with different concentrations and the shortest LT<sub>50</sub> values was for the 2nd instar, and the longest LT<sub>50</sub> was for the 4<sup>th</sup> instar.

**Table 3. Lethal time (days) of different immature stages of *Phthorimaea operculella* after treatment with entomopathogenic fungus, *Beauveria bassiana* (Bals.) at different concentration under laboratory conditions.**

Eggs			
Con.(conidia/ml)	LT <sub>50</sub>	LT <sub>90</sub>	Slope ± S.E
10 <sup>6</sup>	2.45	4.83	4.358±0.5255
10 <sup>7</sup>	2.07	3.67	5.1829±0.5324
10 <sup>8</sup>	1.82	3.19	5.2579±0.5004
10 <sup>9</sup>	1.79	3.04	5.585±0.5177
10 <sup>10</sup>	1.48	2.46	5.781±0.5188
larvae (males)			
Con.(conidia/ml)	LT <sub>50</sub>	LT <sub>90</sub>	Slope ± S.E
10 <sup>4</sup>	1.51	2.88	4.571±0.455
10 <sup>5</sup>	1.43	2.74	4.544±0.393
10 <sup>6</sup>	1.30	2.44	4.708±0.479
10 <sup>7</sup>	1.21	2.05	5.607±0.534
Pupae (males)			
Con.(conidia/ml)	LT <sub>50</sub>	LT <sub>90</sub>	Slope ± S.E
10 <sup>6</sup>	3.26	7.8	3.38±0.392
10 <sup>7</sup>	2.91	6.1	3.989±0.415
10 <sup>8</sup>	2.28	4.31	4.618±0.405
10 <sup>9</sup>	1.49	2.72	4.947±0.412
10 <sup>10</sup>	1.13	2.71	3.59±0.360

**Effect of gamma irradiation:**

**Table 4. Effects of irradiating parental adult males of *Phthorimaea operculella* (resulting from irradiated full grown male pupae at sub-sterilizing doses) on percentage of adult emergence, pupal mortality, fecundity and hatchability**

Dose (Gy)	%adult emergenc	%pupal mortality	average number of eggs/ female±S.E	%hatchability
0	98.0 <sup>a</sup>	2.0 <sup>b</sup>	88.68±3.64 <sup>a</sup>	89.89
100	96.0 <sup>a</sup>	4.0 <sup>b</sup>	79.10±3.61 <sup>a</sup>	77.68
200	89.0 <sup>a</sup>	11.0 <sup>a</sup>	62.90±3.86 <sup>b</sup>	60.99

Data in table (4) Regarding, the irradiating full grown male pupae at 100 and 200 Gy, there were slight decrease in the percent of adult emergence compared to control subsequently, the percentage of pupal mortality was slightly affected it was 4.0 and 11.0% respectively, compared to 2.0% in the control group. Concerning the effects on percent of fecundity and egg hatchability, the dose level 200Gy caused a

significant decrease in both aspects, it was 62.90 and 60.99% respectively, compared to 88.68 egg and 89.89% in the control. This reduction in laid eggs per female probably occurred because of the lack of eupyrene transferred by irradiated males during mating. Similar reduction in the case of *Pectinophora gossypiella* was reported by Qureshi *et al* (1993), Al-Taweel and Ahmed (1997) on *Ephestia figulilella*, and Abass *et al.*, (2017) on *Spodoptera. littoralis*, all reported that the egg laying and hatchability percentage decreased with increasing radiation doses. However Abd El-Hamid (2004) exposed full grown male pupae of *Agrotis ipsilon* to three doses of gamma-irradiation (50, 100 and 150Gy), and found that the fecundity did not significantly differ from control at doses 50 and 100 Gy, while it was significantly reduced at 150Gy. The reason for the reduction of fecundity and fertility was that the irradiated males were frequently failed to transfer sperm to females spermatheca and this failure resulted to reduced fecundity.

**Table 5. Effect of irradiating parental adult males of *Phthorimaea operculella* on some biological aspects of (F<sub>1</sub> larvae resulting from irradiated full grown male pupae) at sub-sterilizing dose.**

<b>F<sub>1</sub> larvae treatments</b>	<b>% pupation</b>	<b>%larval mortality</b>	<b>%adult emergence</b>	<b>%pupal mortality</b>
F <sub>1</sub> N $\sigma$ xN $\varphi$ control	91.0 <sup>a</sup>	9.0 <sup>c</sup>	84.3 <sup>a</sup>	6.7 <sup>b</sup>
F <sub>1</sub> T $\sigma$ (100Gy) x N $\varphi$	50.0 <sup>b</sup>	50.0 <sup>b</sup>	41.1 <sup>b</sup>	8.9 <sup>a</sup> <sup>b</sup>
F <sub>1</sub> T $\sigma$ (200Gy)x N $\varphi$	24.0 <sup>c</sup>	76.0 <sup>a</sup>	11.0 <sup>c</sup>	13.0 <sup>a</sup>

**Data were obtained from 100 eggs which replicated ten times.**

Date in table (5) summarized that larval and pupal mortalities were significantly increased as the radiation doses increased, while pupation and adult emergence were decreased as the radiation dose increased, it was 50 and 24% for pupation and 41.4 and 11% for adult emergence these mean that F<sub>1</sub> progeny were more sterile than parents at 100 and 200 Gy, respectively compared to 84.3% in control. Our data are in agreement with El-Nagar *et al.*, (1984) who reported that, irradiating full grown pupae of *A. ipsilon* at 5 or 10 krad gamma radiation and crossed with unirradiated female. The F<sub>1</sub> progeny were more sterile than parents, and mortality among F<sub>1</sub> larvae was high and dose dependent, Qureshi *et al.* (1993) on *Pectinophora gossypiella* reported that, gamma radiation doses 50 and 200 Gy resulted in decreased adult emergence by increasing radiation doses and more deformed moths were recorded at dose 200 Gy.

**Table 6. Effects of Gamma-irradiation (100 and 200Gy), with LC<sub>50</sub> of the entomopathogenic fungus, *Beauveria bassiana* and joint action on some biological aspects of F<sub>1</sub> larvae of *Phthorimaea operculella*.**

*F <sub>1</sub> Larvae treatments	** % pupation	%larval mortality	*** %adult emergence	%pupal mortality
*F <sub>1</sub> (N♂xN♀)	91.0 <sup>a</sup>	9.0 <sup>d</sup>	84.3 <sup>a</sup>	6.7 <sup>b</sup>
F <sub>1</sub> L(N♂xN♀) +LC <sub>50</sub> <i>B.bassiana</i>	55.0 <sup>b</sup>	45.0 <sup>c</sup>	46.45 <sup>b</sup>	8.55 <sup>b</sup>
F <sub>1</sub> L(T♂100Gy xN♀)+LC <sub>50</sub> <i>B.bassiana</i>	29.1 <sup>c</sup>	70.9 <sup>b</sup>	18.89 <sup>c</sup>	10.21 <sup>ab</sup>
F <sub>1</sub> L(T♂200Gy xN♀)+LC <sub>50</sub> <i>B.bassiana</i>	15.12 <sup>d</sup>	84.88 <sup>a</sup>	00.00	15.12 <sup>a</sup>

\*F<sub>1</sub> larvae hatched from 100egg for each treatment, replicated 10 times.

\*\*from hatched larvae

\*\*\*from hatched pupae

This study aimed to determine the susceptibility of F<sub>1</sub> larvae of *Ph. operculella* developed from irradiated full grown male pupae at 100 and 200Gy and crossed with normal female to LC<sub>50</sub> concentration of *B. bassiana*. Table (6) illustrated the percent of resulting larvae transferred into pupae and adult emergence were 29.1 and 18.89% respectively at 100Gy, while in control treated with LC<sub>50</sub> *B. bassiana*, these percentages were 55.0 and 46.45%, respectively. The joint action effect of 200Gy and *B.bassiana* showed that, the percentage of larvae that reached the pupal stage was 15.12%, and no adult emerged from the pupae (all pupae died or prevented). While these percentage was 91.0% for pupae and 84.3% adult emergence in control treatment. These study was found to impair the development of *Ph.operculella*, i.e reduced percent larval that reached to pupal stage and adult emergence. Our data indicated that combination of gamma radiation and *B.bassiana* was more effective than either treatment alone. These results agree with El-Sinary and Rizk (2007) using *B. bassiana* at 10<sup>4</sup> and 10<sup>8</sup> spores/ml combined with gamma irradiation (50, 100 and 150Gy) against the 4<sup>th</sup> instar larvae of *Galleria melonella*, the efficiency of *B.bassiana* increased, especially when the gamma-irradiation dose was increased, where no adults were produced with both of the fungal concentration and 150Gy gamma dose. Abd El-wahed (2011) also showed that the LC<sub>50</sub>(10<sup>8</sup>) of *B.bassiana* and *M.anisopliae* in combined with the LD<sub>50</sub> (75Gy) of gamma irradiation against the 2<sup>nd</sup> instar larvae of *A.ipsilon* descending of irradiated parental males pupae, significantly increased the larval mortality and decreased the percentage of adult emergence, fecundity and fertility of *A.ipsilon*, El-Khawaga (2018) reported that the combined effect of gamma-irradiation and *B.bassiana* against the 2<sup>nd</sup> instar larvae of *G.mellonella*, drastically decreased the number of eggs per mated female and percentage of egg hatchability



and reported that the combined effect of the entomopathogenic fungi, and gamma-irradiation induced more remarkable effects as compared to each of them alone.

### CONCLUSION

In order to complete the pest control management we can use the LC<sub>90</sub> of *B. bassiana* to reduce infestation by *Ph. operculella* in the field and irradiate the potato bulk with the sub-sterilize dose 200Gy which has an effect on the insect and no effect on germination of potato crop.

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## التأثير المشترك لفطر *Beauveria bassiana* وأشعة جاما على فراشة درنات البطاطس

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أجرى البحث بهدف دراسة مدى حساسية الأطوار الغير ناضجة (البويض، اليرقات، العذارى) لفراشة درنات البطاطس *Phthorimaea operculella* للفطر الممرض للحشرات *Beauveria bassiana* وأيضا دراسة تأثير التشعيع بالجرعات تحت المعقمة 100، 200 جراى للعذارى الذكور (سواء مذفردة أو متحدة مع الجرعة النصف المميئة للفطر) على بعض الأطوار البيولوجية ليرقات الجيل الأول الناتجة من تشعيع العذارى الآباء، وأوضحت الدراسة أن البويض واليرقات والعذارى كانت قابلة للإصابة بالفطر بجرعات نصف مميئة هي  $5.5 \times 10^3$ , 28.56,  $1.1 \times 10^5$  كونيديا لكل ملي علي الترتيب وأن اليرقات كانت أكثر حساسية للإصابة بالفطر عن كل من البويض والعذارى. وأن نسب موت كل من اليرقات والعذارى كانت تزيد بزيادة جرعة أشعة جاما وأن نسب التعذير وخروج الطور الكامل كانت تقل مع زيادة جرعة أشعة جاما وأن استخدام الجرعة تحت المعقمة مع الجرعة النصف المميئة للفطر كانت أكثر تأثيرا عن استخدام كل معاملة منهم علي حدة.

