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INFLUENCE OF THE ENTOMOPATHOGENIC FUNGUS, *Beauveria bassiana* (BALSMO) ON THE MATURE LARVAE OF THE POTATO TUBERMOTH, *Phthorimaea operculella* (ZELLER) UNDER DIFFERENT DEGREES OF TEMPERATURE AND RELATIVE HUMIDITY

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ABSTRACT

This study was concerned with the efficiency of the entomopathogenic fungus *Beauveria bassiana* (Balsmo) on the mature larvae (4th instar) of the potato tubermoth *Phthorimaea operculella* (Zeller) with two different environmental factors under laboratory conditions, e. g. temperature regimes (18, 21, 24, 27 and 30°C) and relative humidity regimes (50, 60, 70, 80 and 90% R. H.). Four concentra-tions of conidial powder were prepared ($1x10^8$, 10^7, 10^6, 10^5) conidia/ml. Some biological aspects were determined, e.g. Survived larvae, pupation and emergence percentages. Results indicated that there were a positive relation between fungal concentrations and reduction in percentages of larval development.

Different regimes in temperature and relative humidity have great influence on the fungal pathogenicity. Highly significant reduction in the fourth larval instar development was obtained with 27 and 30°C temperature regimes and with 80 and 90% relative humidity regimes. Percentage of larval mortality was scored with the four fungal concentrations for 8 days. It was clearly noticed that 10^8 fungal concentra- tion gave 60.67 and 81.67 percentage of mortality after 3 and 4 days, respectively. When the fourth larval instar was reared on gamma irradiated potato tubers (40, 80 and 120 Gy) the larval mortality was significantly increased especially with 120 Gy. Larval duration was increased as the dose of gamma irradiation increased.

Keywords: *Phthorimaea operculella*, *Beauveria bassiana*, gamma radiation, environmental factors, larval mortality.

INTRODUCTION

The potato tubermoth, *Phthorimaea operculella*, (Zeller) (PTM) causes serious damage to potato plant leaves and to tubers either in the field or in storage, also it attacks in addition to potatoes many of solanaceous crops either in fields or in stores (Kares, 1991, Doss *et al.*, 1994 and El-Sinary, 1995). The use of traditional agrochemicals for such pest control is undesirable because of the resistance problems and other harmful effects on beneficial insects, fish and wildlife. So, there is an urgent need for safe but effective, biodegradable pesticides with no toxic effects on non-target organisms. This has created a world wide interest in the development of alternative strategies including the search for new types of insecticides (El-Sinary and Rizk, 2001). The biocontrol element is much needed to control this pest *Bacillus thurigiensis* is now registered and recommended as a control against *P. operculella* in Egypt. Recently, the granulosis virus (GV) is one of the most promising agents for controlling PTM in Egypt (Abol-Ela *et*

al., 1996). It is an evident that the entomopathogenic fungi constitute one of the most important mortality factors among insect pests (Sewify *et al.*, 2000). Several studies have been conducted using entomopathogenic fungi for control of stored-products pests (Ferron, 1977 ; Fernandes *et al.*, 1983; Serale and Doberski, 1984 ; Rodrigues and Pratissoli, 1990 ; Adane *et al.*, 1996 ; Moino, and Alves, 1997). The entomopathogenic fungus *B. bassiana* has a well developed chitinolytic system which has been considered to be important for causing pathogenicity in Lepidopteran pests (Selman, *et al.*, 1997).

The present study was carried out to investigate the pathogenicity of the fungus *B. bassiana* against the potato tubermoth *P. operculella* under different environmental factors in laboratory and to determine larval mortality and combined effect of the fungus *B.bassiana* and gamma irradiation on the PTM larval mortality.

MATERIALS AND METHODS

Rearing Technique:

Larvae of the potato tubermoth (PTM) *P. operculalla* were mainly fed on potato tubers, which were cleaned from dust and parasites by washing and drying with clean towels or tissue papers. A thin layer of clean sand (exposed to high temperature in oven to kill other insects or parasites) was distributed on the bottom of the rearing cages to allow pupation (Hemeida, 1976). Stock culture was reared in laboratory at a constant temperature of 27 ± 2°C and 60 ± 5 R. H % and photoperiod (12 L: 12 D) (Mariy *et al.*, 1999).

Fungal isolation:

The fungal isolates were obtained from the biological control, 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. Spores were harvested by rinsing with sterilized distilled water. Collected spores were filtered through cheese cloth to reduce clumping. Spores suspended in sterilized water were counted using a haemocytometer.

Fungal preparation:

Four concentrations were prepared (1×10^8, 10^7, 10^6, and 10^5 conidia/ml), each concentration was used to inoculate the fourth larval instar of the potato tubermoth. Treatments were daily observed to calculate the surviving larvae, pupation and emergence percentages. Each treatment was triplicated with 50 larvae for each.

Effect of some environmental factors on the pathogenecity of *B. bassiana* on the viability of the 4th larval instar of PTM:

The fourth larval instar (which get out the potato tubers searching for the suitable media for pupation) was examined for the pathogenicity of *B. bassiana*. Different four concentrations of *B. bassiana* were mixed with the sand which larvae pupate in it to lit the fungi to penetrate the larval tissues

1- Temperature:

Five degrees of temperature namely; 18, 21, 24, 27 and 30°C were examined for its effect on the pathonegisity of the fungus *B. bassiana* on the fourth larval instar of the PTM.

2- Relative humidity:

Five degrees of R. H.; namely 50, 60, 70, 80 and 90% were examined for its effect on the pathogenecity of the fungus *B. bassiana* on the fourth larval instar of the P.T.M. Different salts were used to reach that degrees of R. H.; Ca NO_3 , $(NH_4)_2$ NO_3 , NaCl and KOH to obtain 50, 60, 70 and 80% R. H., respectively, Relative humidity of 90% was prepared by adding water at the bottom of the dissicator. Small cages contained the larvae and fungus were kept in dissicators containing saturated salt solutions (El-Sinary, 1995).

Larval mortality determination:

The fourth instar larvae of PTM were placed in small cages (25 x 25 x 15cm) (50 larvae / cage) furnished with treated sand with the fungus *B. bassiana* four concentrations and kept at laboratory conditions with temperature of 27 ± 2°C and 60 ± 5 R.H % and a photoperiod (12L : 12D). Larval mortality was recorded daily after treatment and each treatment was triplicated. Mortality percentages were corrected with Abbott's formula (Abbott, 1925)..

Irradiation experiments:

Potato tubers were irradiated with three different doses of gamma rays 40, 80, and 120 Gy, then infested with first larval instar of PTM, when the fourth instar larvae get out the tubers for pupation they were placed in small cages furnished with sand mixed with different concentrations of *B.bassiana*. The mortality of larval were calculated daily and corrected with Abbott's formula.

The irradiation source used in the present study was the Gamma Cell Irradiation Unit (^{60}Co source) located at the National Center for Radiation Research and Technology, Atomic Energy Authority, with a dose rate of 4.1 rad/sec.

Statistical analysis:

The data obtained from the present study were statistically analysed, whenever, the calculated "F" values were significant at 5% level (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

The data presented in table (1) clearly indicated that there was a reverse relation between fungal concentrations and the survived larvae, pupation and emergence percentages. Concentrations (1×10^8 and 10^7) gave the most promising results and succeeded to reduce the survived larvae, pupation and emergence percentages to reach 0.00 with some temperature regimes which were suitable to fungi to make its virulent effect.

Obtained data showed that the pathogenicity of the fungus differed from one degree of temperature to the other. Degrees of 27 and 30°C showed the highest virulent effect of *B. bassiana* especially with the higher concentrations of fungus; survived larval percentages scored (at 27°C) 15.67, 30.00, 40.67 and 57.33, pupation percentages scored 0.00, 0.00, 27.87 and 40.69 and emergence percentage recorded 0.00, 0.00, 11.76 and 17.14 with 1×10^8, 10^7, 10^6 and 10^5 concentrations of *B. bassiana,* respectively. At temperature 30°C, survived larval percentages were 0.0, 18.67, 31.33 and 54.00, pupation percentages were 0.00, 0.00, 21.28 and 34.57 and the emergence percentages were 0.00, 0.00, 10.0 and 14.29 with 1×10^8, 10^7, 10^6 and 10^5 concentrations of *B. bassiana,* respectively. Flactuations in fungal virulent effect were observed with changes in temperature regimes, the previous biological aspects (survived larvae, pupation and emergence percentage) differed from one degree of temperature to another i. e., 18°C showed higher reduction in PTM. viability than 21°C. That was due to the unsuitable degree for the larval instar itself, as mentioned by El-Sinary, 1995 who stated that the longest average of PTM larval period of 30.9 days was scored at 19°C against 11.3 days at 31°C. Also Mariy *et al.,* 1999 mentioned that the larval duration of PTM was 35.41 days at 15°C and 7.85 days at 35°C. The biological aspects percentages gradually decreased to reach maximum reduction with 27 and 30°C. These findings were in agreements with Busoli *et al.,* 1989 who stated that the suitable temperature regimes for *B. bassiana* was 28±3°C and also were confirmed with Dayer (1993) who mentioned that environmental factors (variable temperatures) may account for variation because the growth, sporulation and enzymatic activities of fungal isolates were greatly affected by these factors. They also in agreement with results obtained by Yasuda *et al.*, (1997) and Martin *et al.*, (2000).

The pathogenicity of *B. bassiana* on different biological aspects of PTM fourth larval instar under five different relative humidity degrees (50, 60, 70, 80 and 90%) was expressed in table (2), which showed that different R.H % played an important role in this experiment. Obtained data showed that biological aspects (percentages of survived larvae, pupation and emergence) of PTM fourth larval instar decreased as the relative humidity degree increased. Also, when the concentrations of *B. bassiana* increased the previous biological aspects decreased. Concentrations of 1×10^8 and 10^7 were completely lethal to larvae of PTM at 90% R.H, no resulted pupae at the same R.H. degree with 1×10^8, 10^7 and 10^6 concentrations of the fungi and no emerged adults were obtained at 90% R.H. with the four different concentra- tions. The lowest effect of the fungi pathogenicity was obtained at

Table (1): The influence of the temperature degrees on the pathogenicity of different concentrations of the fungus *B. bassiana* on the fourth larval instar of the potato tubermoth, *P. operculella*.

Biological aspects / Fungal concentrations	Survived larvae (%)					Pupation (%)					Emergence (%)				
Temperature (°C) degree	0	10^8	10^7	10^6	10^5	0	10^8	10^7	10^6	10^5	0	10^8	10^7	10^6	10^5
18	89.33	38.00	40.67	42.67	60.67	88.06	21.05	31.15	43.75	56.04	91.53	11.67	17.37	35.71	39.22
21	91.33	43.33	45.33	48.00	63.67	91.04	26.15	38.24	50.00	61.05	93.60	17.65	23.26	38.89	39.66
24	94.00	35.67	39.67	49.33	68.67	95.04	11.32	18.64	32.43	44.66	95.52	0.00	9.09	16.67	26.09
27	94.67	15.67	30.00	40.67	57.33	96.48	0.00	0.00	27.87	40.69	95.62	--	--	11.76	17.14
30	93.33	0.00	18.67	31.33	54.00	95.00	0.00	0.00	21.28	34.57	96.24	--	--	10.00	14.29
L.S.D. 0.05	9.3					8.1					6.2				
0.01	14.4					12.5					9.7				

Table (2): The influence of the relative humidity degrees on the pathogenicity of different concentrations of the fungus *B. bassiana* on the fourth larval instar of the potato tubermoth, *P. operculella*.

Biological aspects / Fungal concentrations	Survived larvae (%)					Pupation (%)					Emergence (%)				
Relative humidity (%)	0	10^8	10^7	10^6	10^5	0	10^8	10^7	10^6	10^5	0	10^8	10^7	10^6	10^5
50	91.33	50.67	55.33	63.33	70.00	89.05	38.46	44.12	70.91	74.29	90.16	15.38	30.00	38.46	49.15
60	94.67	43.33	45.33	60.00	65.33	92.25	53.53	38.55	50.00	63.27	95.42	11.11	21.88	33.33	39.74
70	95.33	20.67	38.00	46.67	66.67	94.41	28.95	40.35	45.71	55.00	95.56	0.00	3.04	19.51	27.42
80	94.0	15.33	20.67	38.33	62.00	93.62	20.00	29.03	33.90	50.54	93.18	0.00	0.00	7.41	12.77
90	90.67	0.00	0.00	15.33	38.67	91.91	0.00	0.00	0.00	8.97	88.80	0.00	0.00	0.00	0.00
L.S.D. 0.05	12.2					9.8					7.2				
0.01	16.3					14.2					9.3				

50% R.H. in all PTM biological parameters followed by 60 and 70% R.H., Relative humidity 80 and 90% gave the highest pathogenic effect of fungi *B. bassiana.* The highest concentra- tion of the fungi (1×10^8) gave the best results, as it registered 50.67, 43.33, 20.67, 15.33 and 0.00 percentages of survived larvae with 50, 60, 70, 80 and 90% R.H, respectively. Percentages of pupation were 55.33, 45.33, 38.00, 20.67 and 0.00 with 50, 60, 70, 80 and 90% R.H., respectively at fungi concentration of 1×10^7. High reduction in emergence percentage which make the pathogenic role of the fungi *B. bassiana* succeeded in reducing PTM current generations, (with 1×10^8 Conc.) it scored 15.38, 11.11, 0.00, 0.00 and 0.00 with 50, 60, 70, 80 and 90% R.H. respectively. These findings were confirmed with those obtained by Anderson *et al.,* (1988), when *B. bassiana* gave 98 and 64% mortality to the 3rd and 4th larval instars of *Leptinotarsa decemlineata* when held at relative humidity greater than 90%. Similar results were obtained by Busoli *et al.,* (1989), Yasuda *et al.,* (1997) ; Geden *et al.,* ; Vandenberg *et al.,*(1998) and Devi, (2001).

Average larval mortality was recorded and calculated (Table, 3) after 24, 48, 72, 96, 120 and 144, 168 and 192 hrs for each treatment (Larval mortality was corrected by Abbott's formula). From data represented in table (3) it could be clearly noticed that there was a positive relation between the fungus *B. bassiana* concentra- tions and the larval mortality, i.g. The mortality increased when the concentration increased. The two highest concentrations have highly significant pathogenic effect on the 4th instar larvae, after 72 hrs mortality percentage was 59.32 and reached 80.00 at 96 hrs with 1×10^8 concentration and it was 46.67 at 72 hrs and reached 76.67 at 96 hrs with 10^7 concentration.

It was also noticed that when the concentration increased the time (hrs) for larval mortality decreased, e. g. it required 96, 96, 120, 144 and 120 hrs for the time (hrs) required to score percentages of larval mortality of 81.67, 76.67, 69.33, 39.33 and 3.33 for 1×10^8, 10^7, 10^6 (fungal concentrations) and control (no treatment), respectively. These findings were in harmony with those found by Brinkman *et al.,* (1997) ; Zurek and Keddie, (2000) and Devi *et al.,* (2001). Combined effect between *B. bassiana* (4 concentrations) and gamma irradiation (40, 80 and 120 Gy) were also represented in table (3). It could be concluded that gamma irradiation significantly increased the effect of *B. bassiana,* so the mortality percentages increased especially with higher concentrations of fungus and higher doses of gamma rays. Larval mortality recorded 100.0 with 80 and 120 Gy after 96 and 120 hrs with 1×10^8 and 10^7 concentrations of the fungus, respectively. The time required to reach complete 4th larval instar mortality increased when gamma irradiation dose increased, this was in agreement with El-Sinary, 1995 who mentioned that gamma rays treatments caused longer larval duration than untreated larvae. It was clearly noticed that larval mortality in most treatments began after 48 hrs except with 1×10^8 and 10^7 of fungus concentration when combined with gamma radiation (40, 80 and 120 Gy). This was explained by Ramlee *et al.,* 1996 who stated that at 48 hrs after inoculation, fungal hyphae penetrated the integument inside the trachea and via the nuclei of epidermal cells. Fungal hyphae infiltrated the fat bodies

Table (3): The combined effect of different concentrations of the fungus *B. bassiana* and the doses of gamma radiation on the mortality of the fourth larval instar of the potato tubermoth, *P. operculella*.

Treatment \ Days	Average mortality (%)							
	After one day	2	3	4	5	6	7	8
Fungal treatment only								
10^8	0.00	0.00	60.67	81.67	--	--	--	--
10^7	0.00	0.00	46.67	76.67	--	--	--	--
10^6	0.00	0.00	52.33	51.33	69.33	--	--	--
10^5	0.00	0.00	0.00	12.67	20.67	39.33	--	--
No treatment	0.0	0.00	0.00	0.00	3.33	--	--	--
Irradiation with 40 Gy + fungi								
10^8	8.67	20.67	67.33	88.67	--	--	--	--
10^7	5.00	11.33	62.67	80.67	--	--	--	--
10^6	0.00	0.00	28.67	55.33	72.33	--	--	--
10^5	0.00	0.00	0.00	20.67	36.00	41.33	--	--
(Irradiation only	0.00	0.00	0.00	0.00	4.00	7.33	15.33	--
Irradiation with 80 Gy + fungi								
10^8	10.67	39.33	73.33	100.0	--	--	--	--
10^7	6.67	15.33	65.33	88.67	100.0	--	--	--
10^6	0.00	0.00	42.00	58.67	77.33	89.33	--	--
10^5	0.00	0.00	0.00	25.33	40.67	50.00	59.33	--
Irradiation only	0.00	0.00	0.00	0.00	0.00	5.33	18.67	28.67
Irradiation with 120 Gy + fungi								
10^8	12.67	50.67	95.33	100.0	--	--	--	--
10^7	9.33	38.67	80.67	92.67	100.0	--	--	--
10^6	0.00	11.33	42.67	61.33	82.67	93.33	--	--
10^5	0.00	0.00	0.00	33.33	45.33	58.67	62.67	65.33
Irradiation only	0.00	0.00	4.00	7.33	18.00	23.33	27.33	53.33
L. S. D. 0.05	6.7	7.4	9.1	12.5	11.5	12.7	11.4	13.8
0.01	9.8	10.0	11.3	17.6	17.1	18.2	16.9	20.1

underlying the integument. At 72 h after inoculation, the fat tissues were damaged by progressive colonization with hyphae. Subsequently, the hyphae invaded the muscle tissue, neural tissues, germ cavities, Malpighian tubules, gut musculature and epithelial cells and finally colonized the gut lumen. Between 96 and 120 hrs post-inoculation, all internal organs were heavily colonized with hyphae, and the infected insects were already dead. Twenty-four hours after death, whitish mycelia begin to emerge from the cuticle of the dead body.

From the previous results it could be concluded that the fungus *B. bassiana* could be an effective agent to reduce the risk of PTM attack and could be safetly applied to control this pest in storage as a good bioagent, with understanding of the environmental requirements of fungal isolation. Combination with gamma irradiation to potato tubers increase the virulent effect of fungus *B. bassiana*. Additional studies are needed to determine the pathogenicity of *B. bassiana* on PTM in field conditions.

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تأثير كفاءة فطر *Beauveria bassiana* (Balsmo) الممرضة للحشرات على يرقات العمر الرابع لفراشة درنات البطاطس *Phthorimaea operculella* (Zeller) تحت درجات مختلفة من الحرارة والرطوبة

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تم فى هذا البحث تقدير كفاءة الفطر *Beauveria bassiana* (Balsmo) الممـــرض للحشرات على يرقات العمر الرابع لفراشة درنات البطاطس *P.operculella* معمليا تحت تـــأثير عاملين مختلفين من العوامل البيئية (الحرارة والرطوبة) فقد أستخدمت خمس درجات مختلفـة (١٨ ، ٢١ ، ٢٤ ، ٢٧ و ٣٠ °م) وخمس درجات مختلفة للرطوبة النسـبية (٥٠ ، ٦٠ ، ٧٠ ، ٨٠ ، ٩٠ %) وأستخدمت أربعة تركيزات مختلفة لجراثيم الفطـــر (X١ ١٠ ^٨ ، ١٠ ^٧ ، ١٠ ^٦ ، ١٠ ^٥ كونيديا / مل) حيث يتم خلط تركيزات الفطر مع الرمل الذى يتم فيه تعذير يرقات العمر الرابع ثـم تقدر بعض المعايير البيولوجية مثل النسب المئوية لليرقات الحية والتعذيـــر وخـــروج الفراشـات الكاملة. وقد أثبتت النتائج أن هناك علاقة طردية بين تركيزات الفطر وبين حيوية اليرقات المعاملة به. وكان للتغيرات فى درجات الحرارة والرطوبة النسبية عظيم الأثر على سمية الفطر لليرقـــات حيث كان هناك إنخفاض معنوى فى حيوية اليرقات عند درجات حرارة ٢٧ ، ٣٠ °م وكذلك عنـد رطوبة نسبية ٨٠ ، ٩٠%. تم تقدير نسبة الموت ليرقات العمر الرابع خـــلال ٨ أيـــام مـــن بـــدء المعاملة بتركيزات الفطر الأربعة المختلفة. تركيز ١٠ ^٨ كان أكثر التركيزات فاعليـــة فـــى قتـــل اليرقات بنسبة ٦٠,٦٧ ، ٨١,٦٧% بعد فترة ٧٢ ، ٩٦ ساعة. عند تعريض درنـــات البطـــاطس لثلاث جرعات من أشعة جاما (٤٠ ، ٨٠ ، ١٢٠ جراى) تمت إصابتها بيرقـــات حديثـــة الفقـــس فراشة درنات البطاطس وعند بلوغها للعمر الرابع تخرج من الدرنات للتعذير فى الرمل المخلـــوط بتركيزات الفطر المختلفة فكانت أفضل النتائج عند خروج اليرقات من البطاطس المعامل بـــ ١٢٠ جراى والتى تعذر فى الرمل المخلوط بالتركيزات الأعلى للفطر.