BIOLOGICAL EFFECT OF THE ENTOMOPATHOGENIC FUNGUS, *Beauveria Bassiana* (BALSAMO) VUILLEMIN ON THE POTATO TUBER MOTH, *Phthorimaea Operculella* (SELLER)

Maan Abdul Aziz Shefik
Department of Biology, College of Sciences, University of Al- Mustansiriyah. Baghdad-Iraq.

Abstract

This study has been carried to evaluate the effect of the entomopathogenic fungi *Beauveria bassiana* on the various developmental stages of *Phthorimaea operculella*. Both first and second instars larvae were more susceptible than the third and fourth instars. The infected prepupae and pupae resulted in marked decreases in the emergence and longevity of moths, deposited eggs and egg hatchability. An obvious increase in the pupal duration was observed and malformed adults were also recorded. The latent was markedly obvious, especially in high doses of *B. bassiana.*

التأثير البايولوجي للمسبب المرضي الفطر *Beauveria Bassiana* (BALSAMO) VUILLEMIN على عثة درنات البطاطا *Phthorimaea Operculella* (SELLER)

معـن عبد العزيز شفيق
قسم علوم الحياة، كلية العلوم، الجامعة المستنصرية. بغداد-العراق.

الخـلاصـــة

تم أجراء هذه الدراسة لمعرفة تأثير الفطر *Beauveria bassiana* كمسبب مرضي للحشرات على تطور المراحل المختلفة لعثة درنات البطاطا *Phthorimaea operculella*، اظهرت النتائج أن كل مـن الطورين اليرقي الأول والثاني لعثة درنات البطاطا كانا أكثر حساسية للفطر من الطورين الثالث والرابع. أما الأطوار قبل العذراء والعذراء المصابة قد سجلا أنخفاض ملحوظ في ظهور البالغات وكذلك نتج عنهما فترة حياة قصيرة للبالغات، وأنخفاض في عدد البيض التي وضعتها الاناث، وأنخفاض في نسبة الفقس. وتبين أن هناك زيادة واضحة في فترة الطور العذري، والعذارى المعاملة بالفطر نتج عنها زيـادة نسـبة التشوه في البالغات. ولوحظت الأصابة الكامنة بشكل واضح خاصة فـي التراكيـز العاليـة للفطـر *B. bassiana*.

Introduction

Potato plant *Solanum tuberosum* (L.) was considered as a major export crop in Egypt [1, 2]. It's economic importance comes after cotton and rice crops. The potato tuber moth *P. operculella* (Lepidoptera:Gelechiidae) is a major pest of potato. It cause sever damages during harvest and in the store [3]. Fungi belongs to several major groups and ranged from obligate host specific parasites to omnivorous facultative saprophytic. The most successful fungus for pest control was belonging to genus *Beauveria* (Vuill.) and has been subjected to extensive studies and developed as commercial products to control specific insect on agricultural crops. The ubiquitous fungus

Beauveria bassiana (Balsamo) Vuillemin causes common diseas associated with dead moribund insects in nature [4]. This fungus has been scrutinized world wide as microbial control agent of many insect pests [5, 6]. *P. operculella* is considered as important economic pest because its larvae cause severe damage to crops specially potato, tomato, egg plant and some Solanaceae plants [1, 7]. Using of pathogens, as biological control agents of some insect species, has been increased during the last few years. *Beauverai bassiana*, as an entomopathogenic fungus, has been used to suppress the population of the European corn borer, *Ostrinia nubilalis* (Hubner) [8, 9, 10, 11, 12 and 13]. More than 30 entomopathogenic fungi have been tested as biological control preparation of different insect pests [14, 15, 16, 17, 18, 19, 20 and 21]. The present work was carried out to study the effect of *B. bassiana* on the different larval development stages of the potato tuber moth *Phthorimaea operculella*.

Materials and Methods

Pathogen: A commercial formulation (Naturalis-L, Troy- Bioscience -Arizona) based on the fungus *Beauveria bassiana* with potency of 23 x 10^7 conidia/ ml.

Insect: Standard laboratory colony of potato tuber moth *P. oprculella* was reared on potato tuber under laboratory condition of about 26±2 ^oC and 70±5 % RH. Eggs were obtained from the stock culture and kept in Petridish until hatching. Pupae were individually kept in specimen tuber (1x3cm) till adult emergence. Adults moth were kept in ovipositor cages that consist of chimney glass, about 8 cm in diameter and 16 cm high, the lower rim of which rested on the bottom of Petridish lined with a disk of filter paper and the upper rim covered with muslin. Each cage was provided with a small plastic cover containing a piece of cotton soake d in 5% sugar solution. Eggs were obtained from the stock culture and kept in Petridish till hatching. Groups containing fresh pieces of potato. Larval development was allowed to continue until adult emergence.

Bioassay of *B.bassiana:* *B. bassiana* fungus was assay against newly molted or hatched 1st, 2nd, 3rd and 4th instars larvae of *P. operculella* by dipping potato tuber in suspension containing the prescribed dose of the pathogen in distilled water to which, one drop of tween-80 was added as wetting agent and infected by spraying 10 ml of an aqueous suspension of *Beauveria* conidia/spore containing 16.5x10^7, 8.25x10^7, 2.06x10^7, 1.03x10^7 and 0.5x10^7 conidia/ml on the inner surface of sterile plastic Petridish and plastic cups. Excess liquid was removed from the tuber when allowed to dry at room temperature 26±2 ^oC. The tubers were then placed in plastic cups 15x4 cm, for each concentration, ten replicates were used. Each replicate comprised of 10 larvae placed on the tuber by means of camel's hairbrush and reared at 26±2 ^oC and 70± 5% RH, for seven days. Control larvae were fed on potato tuber treated only with water and the wetting agent Tween-80. Prepupae and pupae were treated with conidial inoculums. Mortalitie s were corrected according to [22].

Results and Discussion

Mortalities of *P. operculella* larvae exposed to a concentration of 16.5x10^7 of *B. bassiana* indicated that these larvae were susceptible to the pathogen, the calculated LC 50's for *B. bassiana* were 4.7 x 10^7 conidia/ml, for the 1st, 2nd, 3rd and 4th instars larvae, respectively (Table 1). The duration of the treated pupae was significantly prolonged concentrations ranged from 6.5 x10^7 to 2.06 x 10^7 conidia/ml as compared with the control, while at Low concentrations of 1.03x10^7 to 0.26x10^7 conidia/ml, there was no obvious effect as compared to the control. The percentage of moth emergence showed a highly progressive decrease with the increase of concentration of *B. bassiana*. Thus emergence decreased from 100% in the control to 0% at 16.5x10^7 conidia/ml. An obvious malformation was observed among the emerged moth after treatment of the prepupae with any of the used concentration. The longevity of the emerged adults was significantly affected being shorter after exposure to *B.bassiana*. The egg production of the female progresssively decreeased from 90.83 ± 10.93 to 32.00 ± 1.12 eggs/female, with the increase in the concentration of *B.bassiana* from 0.26 to 4.12 (x10^7) conidia/ml as compared to 179.93 ± 23.99 eggs/female, in the control. At concentrations of 16.5x10^7 and 8.25x10^7conidia/ml, no eggs were obtained (Table 2). The duration of treated pupae was significantly prolonged at concentration of 16.5x10^7, 2.06x10^7 conidia/ml, as compared to control (Table 3). The percenttage of moth emergence showed a highly progressive decrease with the increase in the concentration of *B.bassiana*. Thus emergence decreased from

96.7 % in the control to 10% at 16.5 x 10^7 conidia/ml. An obvious malformation was observed among the emerged moth after treatment of the pupae with any of the used concentration, also the longevity of emerged adults was signifycantly affected being shorter. The egg production of the resulted female progressively decreased with increase in the concentration of *B.bassiana* (Table 3). An obvious malformation was observed among the emerged moth after treating the prepupae with concentration varied from 16.5x10^7 to 2.06x10^7. Data in (Table 4) Show the effect of *B. bassiana* on the adults *p. operculella* at different concentrations. The longevity of adult males was shortened to 9.3$\pm$ 0.4 days at 16.5x10^7 conidia/ml as compared with 12.9$\pm$0.11 days in the control. At the lowest concentration of 0.26x10^7 conidia/ml, the male longevity was 12.2$\pm$0.57 days. At concentration of 16.5 x 10^7 conidia/ml, the longevity of adult females was 11.4$\pm$0.24 days. Treated adult females of *P. operculella* showed delayed effect on the egg production. The produced egg was 103.3$\pm$15.8 at the concentration of 16.5x10^7 conidia/ml, increased to 138.9 $\pm$ 3.35 eggs / female at 0.2 x10^7 conidia / ml.

In the control, the egg production averaged 146$\pm$11.43 eggs/female. Fungi reach the heamocoel through the cuticle or the mouth parts. Infection therefore resulted from contact between a virulent infection inoculum and a susceptible insect cuticle, germination, penetration of the germ tubes through the integument and finally spread of the pathogen through the host tissues. Entomopathogenic fungi produce mycotoxine and cuticledegrading enzymes that permit infection like lipase, protease and chitinase, which kill the host by inducing progressive degeneration of host tissues, due to loss of structural integrity of membranes followed by dehydration of cells as a result of fluid loss [9]. During the present study, the treatment of prepupae and pupae with different concentration of *B. bassiana* preparation resulted in decreasing the number of emerged adults of *P.operculella* which showed a high percent of malformation. Resulted adult males and female lived shorter time and laid low number of eggs. The 1st and 2nd larval instars were more susceptible than the 3rd or 4th instar. also, feeding adult female of *Phthorimaea* with contaminated diet resulted in a marked decrease in the deposited eggs. *B.bassiana* provide a great amount of *Ostrinia nubilalis* and *Chilo partellus* [11]. When applied as a spray to infested crops, the entomopathgens invades and immobile the insect with tow days, symptoms appeared within seven days, the mycoses tissues (a tissueinvasive mycelial phase) of the insects remain adhered to the crop and additional spores are released to maintain to maintain a high level of infective on the crop [23, 24]. The entomopathological activity of the fungus against insect seem to be driven from several cuticle dissolving enzymes chitinase and protease, *B. bassiana* produce a multiple extra cellular chitinase isozymes [25]. The entomopathogenic fungus *B. bassiana* infects the insect by direct penetration through the insect cuticle, members included both host specific and generalist strain, which have the potential for use as biocontrol agents are oxalic and citric acids which act as fungus metabolites [26]. Pathogenic fungi posses an intriguing array of mechanisms that permit them to break down and assimilate host materials while most part, the fungal metabolites assist the pathogen with physical aspects of ingress, cuticle-degrading enzymes that destroy activity or modify the structural integrity of the host, inhibition of selective processes or enzymes of the host and interference with the regulatory system of the host such damage, associated with disease symptoms may be produce both by the pathogen enzymes and by its low molecular weight metabolites (toxin). Undoubtedly many pathogens enzymes are important determinates of virulence because they enable the pathogen to coexist with the changing metabolic processes associated with the host's diseased state, once fungi invade the heamocoel, the host may be killed by some combination of mechanical damage produced by fungal growth, nutrient exhaustion and toxicosis, the relative important of these mechanisms varies with the specific fungal isolate or host, many entomopathogenic fungi produce toxins but although some toxins are fully described chemically. Dustruxins and other toxins include the cyclic depsipeptides beauvericin and bassianolid which may function principally as endocellular ionophores which may paralyze host cells [27]. Bidochka and khaehatourious [26] Revealed that the relationship between oxalic acid and citric acid together with *B. bassiana* conidia in grasshopper mortality was marked by synergistic, they suggested that acid metabolites produced by *B. bassiana* might play an important role in cuticle solubilization and subsequent hyphal penetration.

Table 1: Effect of *Beauveria bassiana* on different larval instars of *Phthorimaea operculella*

Larva Instars	LC 50 Conidia/ml	Slope	Variance	Confidence Limits (95 %)
First	$1.98x10^7$	1.209	0.0065	$1.26x10^7$ - $2.75x10^7$
Second	$2.13x10^7$	1.070	0.0077	$1.30x10^7$ - $3.09x10^7$
Third	$4.08x10^7$	0.906	0.0121	$1.97x10^7$ - $6.19x10^7$
Fourth	$4.71x10^7$	1.242	0.0073	$2.85x10^7$ - $6.56x10^7$

Table 2: Effect of different concentrations of *Beauveria bassiana* as suspension on the prepupae of *Phthorimaea operculella*.

Conc. Conidia / ml		16.5	8.25	4.12	2.06	1.03	0.52	0.26	Control
		($X10^7$)							
Pupal duration		8.9±0.47	8.66 ± 0.33	7.5 ± 0.22	7.75 ± 0.25	7.44± 0.17	7.2± 0.13	6.75 ± 0.25	6.25 ± 0.25
% of emergence		26.6	45	63.5	70	77	80	90	100
% of malformation		60	50	33.3	22.7	20	10	5	_____
Average longevity in days	males	3	4.66 ± 0.33	4.25 ± 0.47	5.6 ± 0.37	6.6 ± 0.24	7.5 ± 0.42	9.1 ± 0.3	12.6 ± 1.68
	females	4	4.5 ± 0.5	5.5 ± 0.5	7.2 ± 0.37	7.8 ± 0.37	8.6 ± 0.24	9.3 ± 0.3	3.8 ± 1.8
No of eggs / female		0	0	32 ± 1.1	42.2 ± 10.4	53.8 ± 17.2	71.8 ± 14.5	90.8 ± 10.9	179.9 ± 23.9
% of hatching		0	0	10	22	45	80	85	100

Table 3: Effect of different concentrations of *Beauveria bassiana* as suspension on the pupae of *Phthorimaea operculella*

Conc. Conidia / ml		16.5	8.25	4.12	2.06	1.03	0.52	0.26	Control
		($X\ 10^7$)							
Pupa duration in days		15	8.99 + 0.47	8.66 + 0.33	7.77 + 0.22	7.44 + 0.17	6.75 + 0.21	6.75 + 0.21	6.75 + 0.21
% of emergence		10	16	23.3	٣٠	36.6	40	53.3	96.7
% of malforming adult		100	35	30	20	17	15	12	0
Adult longevity	male	١	3	3 + 0.18	4.2 + 0.47	5 + 0.63	6.5 + 0.42	8.2 + 0.47	12.26 + 1.6
	female	__	٤	3.66 + 0.33	4.6 + 0.5	6.2 + 0.37	7.83 + 0.47	9.6 + 0.37	13.1 + 1.7
No of eggs female		0	0	0	66 + 13.5	80.2 + 14.8	101 + 19	138 + 34.5	189 + 57.2
% of hatching		0	0	0	70	75	85	88.8	95

Table 4: Effect of different concentrations of *Beauveria bassiana* on adult *Phthorimaea operculella*.

Conc. Conidia /ml	Male longevity in days mean ± S.E.	Female longevity in days mean ± S.E.	Deposited eggs / female mean ± S.E.
16.5×10^7	9.3 ± 0.4	11.4 ± 0.24	103.3 ± 15.8
8.25×10^7	10.2 ± 0.37	11.8 ± 0.37	105.2 ± 23.6
4.12×10^7	10.6 ± 0.5	12.2 ± 0.3	112.7 ± 15.9
2.06×10^7	11.4 ± 0.5	12.4 ± 0.4	112.8 ± 14.19
1.03×10^7	11.5 ± 0.3	12.3 ± 0.66	116.1 ± 15.34
0.52×10^7	12.1 ± 0.23	13.0 ± 0.77	135.8 ± 13.43

0.26×10^7	12.2 ± 0.57	13.3 ± 0.29	138.9 ± 3.35
Control (Untreated)	12.9 ± 0.11	13.6 ± 0.4	146.3 ± 11.43

References

1. Abul-Naser, S. M.; Fahmy, S. M. and El-Sheriff, A. **1971**. Studies on the potato tuber worm Phthorimaea operculella Zeller: *Bull. Soc. Ent. Egypt* **5**:157-191.
2. Mary, F.A.; Doaoud, M.A.; El- Saadany, G.B. and Ibrahim, M.Y., **1999**. Biological studies on potato tuber moth Phthorimaea operculella Zeller: *Annals. Agric. Ain Shams Univ.* **44**: 363-378.
3. Hemeide, E. A. **1976**. Biological and Physiological studies on Phthorimaea operculella Zeller (Lepidoptera: Gelechii-dae): M. Sc. Thesis Ain Shams Univ. Egypt P.104.
4. McCoy, C. W.; Samaom, R. A. and Boucias, D. G. **1988**. *Entomogenous fungi in hand book of natural pesticides,* Ignoffo, C. M. and Mandava, N. B. (Eds.). Vol.5 Microbial pesticides part A Entomophagus protozoa and fungi. Boca. Fl. pp.151-236.
5. Hoffman, M.P.; Thurston, H. D. and Smith, M. **1993**. *Breeding for resistance to insect and plant pathogens, In: Crop improvement for sustainable agricultural system*. Galway, M.B. and Francis, C.A. (Eds.). University of Nebraska press. pp.79-99.
6. Hoffman, M.P. **1997**. *Biological control of insect pests of vegetables.* In: Proceedings of 1997 Pennsylvania vegetable conferences and trade show. Jan. 28-30, Hershey, PA. P.56.
7. Salam, H. S.; Dimetry, N. Z. and Sharaby, A. M., **1972**. Contribution to the biology of the potato tube moth Phthorimaea operculella Zell. *Bull. Soc. Entomol.* **56**:61-67.
8. Bartlett, K. A. and Lfebure, C. L., **1934**. Field experiment with Beauveria bassiana (Balsamo) Vuillimen a fungus attacking the European corn borer. *J. Econ. Entomol.* **27**:1147-1157.
9. Ferron, P. **1981**. *Pest control by the fungi Beauveria bassiana and Metarhizium*: *In: microbial control pests and plant pests and plant diseases*, (Ed.) by HD Burges, Academic press, 1970-1980, New York and London.
10. Hussey, N. W. and Tinsely, T. W. **1981**. *Impression of insect pathology in the peoples' Republic of China*: *In: microbial control pests and plant diseases*, (Ed.) by HD Burges, Academic press, 1970-1980, pp.785-795, New York and London.
11. Lewis, L.C. and Bing, L. A. **1991**. Bacillus thuringiensis Berl. and Beauveria bassiana (Balsamo) Vuillimen for European corn borer control: program for immediate and season-long suppression: *Can. Entomol.* **123**: 387-393.
12. Riba, G. **1984**. Field plot test using artificial mutant of the entomopathogenic fungus Beauveria bassiana against the European corn borer, Ostrinia nubilalis (Lepidoptera: Pyralidae): *Entomophaga*, **29**: 41-48.
13. York, G.T. **1958**. Field tests with the fungus Beauveria sp. For control of the European corn borer low state: *J. Sci.* **33**: 123-129.
14. Ferron, P. and Robert, P. H. **1975**. Virulence of entomopathogenic (Fungi imperfect) for the adults of Aconthocelides obtectus (Coleoptera: Bruchidae): *J. Invert Pathol.* **25**: 279-283.
15. Ferron**,** P.; Robert, P. H. and Deotte, A., **1975.** Susceptibility of Orycts rhinoceror adults to Metarhizium anisopliae: *J. Invert. Pathol.* **25**: 313-319.
16. Gustelena, L. A., **1980.** Results of the testing of microbial preparations against the large bark beetle: *Ssp. Biol.* **15**: 49-54.
17. Ignofo, G. M.; Garcia, C.; Ayashina, O. A. and Lappa, N. V. **1979.** Laboratory and field studies with Beauveria: a mycoinse ticidal preparation of beauveria bassiana product in the Soviet Union: *J. Econ. Entomol.* **72**:562-565.
18. Lecuona, R. C. and Elves, S. B. **1988.** Efficiency of Beauveria bassiana (Balsamo) Vuilliumen, Blbrongnienientii (Sace) Rech and granulose virus on Deatraea saccharalis at different temperature: *J. Apple. Entomol.* **105**: 223-228.
19. Moore, K. C. and Erlandson, M. A. **1988**. Isolation of Aspergillus parasiticus spores and Beauveria bassiana (Balsamo) Vuilliumen from Melanopline grasshopper (Orthoptera: Acrid-idae) and demonstration of their pathogenicety Melanophis senguim (Fabricus): *Cont. Entomol.* **120**: 989-991.
20. Tong-Kwee, L.; Mohammed, R.; Fee, C. G. and Lan, C. C., **1989**. Studies on Beauveria bassiana isolated from the Cocoa mired Heloptis theobromae: *Crop Protec.* **8**:358-362.

21. West, E. J. and Briggs, J. D., **1968**. In vitro toxin production by the fungus Beauveria bassiana and bioassay in greater wax moth. *J. Econ. Entomol.* **61**: 661-684.
22. Abbott, W. S. **1925.** A method of computing the effectiveness of an insecticide: *J. Econ. Entomol.* **18**: 265-267.
23. Andersen, S. O.; Hojrup, P. and Roepstorff, P. **1995**. Bisect cuticular proteins: *Insect Biochem. Mol. Bio*. **25**: 153-176.
24. Samson, R. A.; Evans, H. C. and Latge, J. P. **1988**. Atlas of entomopathogenic fungi Springey-Verlag, New York, Shaplay, A. W. and Bradly, T. J. **1982.** A comparative study of magnesium sulphate tolerance in saline water mosquito larvae. *J. Insect Physiol.* **28**: 641-664.
25. Havuakkala, I.; Mitamura, C.; Hara, S.; Hirayae, K.; Nishizawa, Y. and Hibi, T. **1993.** Induction and purification of Beauveria bassiana chitinolytic enzymes, *J. Invert. Pathol.* **61**: 97-102.
26. Bidochka, M. J. and Khachatourians, G. K. **1990.** Identification of Beauveria bassiana extracellular protease as a virulence facror in pathogencity toward the migratory grasshopper Melanoplus sanquinipes: *J. Invert. Pathol.* **56**: 362-370.
27. Sabbour, M.M. **2002.** The role of chemical additives in enhancing the efficacy of Beauveria bassiana and Metarhizium anisopliae against the potato tuber moth Phthorimaea operculella (Zeller) (Lepidoptera: Gelechiidae): *Pakistan J. Bio. Scie.* **5**(11): 155-159.