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Susceptibility of Egg Stage of Potato Tuber Moth *Phthorimaea operculella* to Native Isolates of *Beauveria bassiana*

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Additional information is available at the end of the chapter

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Abstract

The pathogenicity of three local isolates of the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill was evaluated on eggs of potato tuber moth *P. operculella* (Zeller). The three isolates were coded as the following: B (isolate from Latakia), C (isolate from ICARDA) and D (isolate from Damascus). Three concentrations 10^4 , 10^5 , and 10^6 , respectively, conidia/ml were used for each isolate. Eggs in the control were sprayed by sterilized water. All tests were done under laboratory conditions of temperature $28 \pm 2^\circ\text{C}$ and relative humidity $40 \pm 5\%$. Susceptibility tests showed significant differences in averages of hatching rate between the control and both isolates B and C when 1×10^6 conidia/ml was applied, with averages 18.3 and 26.6% for previous isolates respectively, in contrast to 38.3 for isolate D and 66.6% for control. Findings indicated that eggs of *P. operculella* seemed sensible to local isolates of *B. bassiana* in varying degree, but further studies are required about the efficiency of effective isolates for controlling eggs of this pest in natural conditions.

Keywords: *Beauveria bassiana*, pathogenicity, *Phthorimaea operculella*, Syrian isolates

1. Introduction

Potato tuber moth (Gelechiidae: Lepidoptera) *Phthorimaea operculella* is one of worldwide spread pests on potato and Solanaceae [1, 2]. The female lay her eggs on the leaves and non-covered tubers near to eyes (buds), larvae dig tunnels during their nutrition causes damages that reach approximately 100% on cultivated and stored potato [3, 4]. Therefore, this moth

must be controlled in the field and in the store. There are many ways to control this pest starting by synthetic organic pesticides [5], natural origin insecticides like botanical extracts [6] and using genetically modified plants [5, 7, 8]. Natural parasitic enemies also successfully used like wasps from Braconidae, in addition to insect predators from Coccinellidae, Chrysopidae and Formicidae [9] and parasitic nematodes like *Steinernema carpocapsae*, *S. feltiae*, *S. glaseri*, and *Heterorhabditis bacteriophora* [10] which used successfully too. In last decade, biological origin insecticides like entomopathogenic viruses from group baculovirus [11] are used, as well as entomopathogenic fungi like *Beauveria bassiana* (Hypocreales: clavi-cipitaceae) [12, 13].

In this chapter, the pathogenicity of three native isolates of entomopathogenic fungus *Beauveria bassiana* was studied in different concentrations on eggs of potato tuber moth *Phthorimaea operculella* (Zeller), and it was determined the isolate which is the most pathogenicity on eggs, in vitro.

2. Materials and methods

2.1. Insect rearing

Tubers infected by potato tuber moth were collected from local markets, and laid on fine sand in aired glass cages (30 × 45 cm). Insects grew inside the cages in large numbers and fed by sugar solution (90%). Cages were supplied with fresh infected and noninfected tubers for activating insect rearing under laboratory conditions 28°C, R.H. 40 ± 5%.

2.2. Egg collecting

Eggs that are 1-day age were collected by using egg collecting chamber, which were prepared in the same manners of Maharjan [14], the chamber consists of plastic jar (7 × 15 cm) supplied with a cotton ball immersed in sugar solution. Five couples of moth adults (five males and five females) entered into the jar, and covered with gauze that was fixed by rubber, there is a piece of paper above the gauze. Eggs found on this paper were collected daily without opening the chamber [15]. Adults were fed by injecting the cotton ball (inside the jar) with sugar solution.

2.3. Infecting material and the spore suspension preparation

Three native isolates of the entomopathogenic fungus *B. bassiana* were used and were taken from different areas in Syria (Table 1).

Infecting material was prepared in safety bio-cabinet on Malt Extract Agar (MEA) medium in petri dishes of 9 cm in diameter, and dishes were placed inside a dark incubator at 25°C. Spores were harvested from dishes 2 weeks later, by adding 5 ml sterilized water for each dish then dish's contents were filtered across three layers of gauze. In addition, 5 ml of sterilized water was added over the gauze to assure collecting the maximum number of spores. The result liquid, which is considered as base solution 0.05% of tween 80%, was added to it.

Isolate name	Isolate code	Source	Isolate site
Latakia	B	Biological Enemies Center, Latakia	Soil of citrus orchard, Latakia
Icarda spt273	C	ICARDA, Aleppo	Isolated from dead <i>Eurygaster integriceps</i> , Aleppo
Damascus	D	Biotechnology Center, Damascus	Isolated from dead <i>Eurygaster integriceps</i> , Damascus

Table 1. Native isolates of entomopathogenic fungi *B. bassiana* and their sources and isolation sites.

Base solution concentration was determined by using a slide named Neubauer improved. The concentrations were adjusted for the three isolates to be: 10^4 , 10^5 , 10^6 conidia/ml. Control was treated with sterilized water and 0.05% of tween 80% was added to it.

2.4. Germination test

For testing the vitality of spores for each isolate, germination test was done in darkness under laboratory condition $28 \pm 2^\circ\text{C}$, R.H. $40 \pm 5\%$ where 5 μl from each isolate, at the concentration of 10^4 spore/ml, was distributed on three drops on small petri dish of 5 cm diameter that contained Agar-Agar medium. Every drop represents a replicate that was covered by a covering glass before it was closed and then the dish was placed in a dark chamber. Next day, germinated spores were counted from 100 spores under every covering glass; after it was colored by lactophenol Cotton Blue, and the average of germination ratio was calculated for every isolate from its own dish.

2.5. Egg infecting by the fungus spore

A total of 600 eggs of potato tuber moth, 1 day age, were distributed into 30 carton cups that equal to 20 eggs/cup. Nine cups for each isolate distributed into three cups for each studied concentration (10^4 , 10^5 , 10^6 spore/ml), as well as three cups for control. Replicate eggs were sprayed with 2 ml of every solution by "Perfume Water Spray Bottle." Eggs in control were sprayed with 2 ml of sterilized water with 0.05% of tween 80% were added. After eggs spraying, inside their cups, they were covered with fine gauze which is fixed by rubber. Cups were placed in chamber at $28 \pm 2^\circ\text{C}$, and R.H. $40 \pm 5\%$.

2.6. Readings

Hatching of treated eggs was observed to record the number of neonates, for 6 days period, in all treatments including the control. Hatched eggs ratio and dead eggs ratio were calculated when hatching was over, and nonhatched eggs were examined under $10\times$ to record their color changes.

2.7. Data analysis

Percentage of corrected mortality was calculated according to Abbott [16].

$$\% \text{Corrected mortality} = \frac{(\% \text{mortality in control} - \% \text{mortality in treatment}) \times 100}{(100 \% \text{ mortality in control})} \tag{1}$$

Data were analyzed by using SPSS program, where treatments were compared to test the significance of difference between averages by using LSD test at $p = 0.05$.

2.8. Scanning under electronic microscope

Eggs treated with local isolates of *B. bassiana* were observed under scanning electron microscope (SEM) and described in Science Faculty, Albaath University, according to its characteristics.

3. Results

3.1. Germination ratio

Averages of germination, after 24 h, were ranged between 47 and 67% (**Table 2**). Isolate B realized that has more germination ratio (67%) with significant difference from the isolate C which reached 48%, while isolate D reached to 55%. There is a significant difference in germination ratio between B and C.

3.2. Susceptibility of eggs

Reduction in hatchability rate was remarked in all treatments in comparison with control. Control realized a ratio of hatchability 66.6%, but this reduction in hatchability was not significant, between the control and the isolates, except in treatment with higher concentration (10^6 spore/ml) for the two isolates B and C (**Table 3**). Hatchability rate was 18.3% for isolate B and 26.6% for isolate C. There was no significant difference in hatchability between the isolate D and other treatments for the same previous concentration. Hatchability rates for the concentration 10^5 spore/ml were higher than 10^6 spore/ml, 33.3, 41.6 and 36.6% for isolates B, D and C, respectively.

Isolate name	Germination (average \pm SE)
B	67 \pm 5.77a
D	55 \pm 5.57ab
C	47 \pm 4.33b
LSD	14.46

Means with same small letters in the same column have no significant differences at $p = 0.05$.

Table 2. Means of germination rates of native isolates of *B. bassiana* fungus at temperature $28 \pm 2^\circ\text{C}$ and relative humidity $40 \pm 5\%$, 24 h after incubation.

Treatment-isolate/ concentration	Hatching rate of eggs (average \pm SE)	Mortality of eggs (average \pm SE)	Corrected mortality
Control	66.6 \pm 14.5a	33.3 \pm 14.5	—
Isolate B 10 ⁶ conidia/ml	18.3 \pm 1.6b	81.6 \pm 1.6	72.5
Isolate D 10 ⁶ conidia/ml	38.3 \pm 13ab	61.6 \pm 13	42.5
Isolate C 10 ⁶ conidia/ml	26.6 \pm 1.6b	73.3 \pm 1.6	60
LSD value	25.84	—	—
Isolate B 10 ⁵ conidia/ml	33.3 \pm 10.9a	66.6 \pm 10.9	50
Isolate D 10 ⁵ conidia/ml	41.6 \pm 6.6a	58.3 \pm 11.5	37.5
Isolate C 10 ⁵ conidia/ml	36.6 \pm 6.6a	63.3 \pm 6.6	45
LSD value	26.94	—	—
Isolate B 10 ⁴ conidia/ml	35 \pm 17.5a	65 \pm 17.5	47.5
Isolate D 10 ⁴ conidia/ml	45 \pm 13.2a	55 \pm 13.2	32.5
Isolate C 10 ⁴ conidia/ml	43.3 \pm 12.01a	56.6 \pm 6.16	35
LSD value	38	—	—

Means with same small letters in the same column have no significant differences at $p = 0.05$.

Table 3. Means of hatching rates of *P. operculella* eggs after the treatment by different isolates of the entomopathogenic fungus *B. bassiana* with different concentrations at $28 \pm 2^\circ\text{C}$ and relative humidity $40 \pm 5\%$.

Isolates in the concentration of 10⁴ spore/ml were realized with higher hatchability rates: 35, 45, 43 and 67% for B, D, C and control; respectively.

In concentration 10⁶ spore/ml, the isolate B reached the top corrected mortality rate on egg followed by C then D: 72.5, 60, 42.5%, respectively. These rates decreased to 50, 37.5 and 45% for the same previous isolates respectively, in concentration 10⁵ spore/ml. In contrast, the lowest corrected mortality rates were in concentration 10⁴ spore/ml: 47.5, 32.5 and 35% for B, D and C, respectively (Table 2).

3.3. Observing non-hatched egg

Number of dead eggs (nonhatched) resulted from the different treatments changed their color from transparent to yellowish or black. Black eggs percentage from dead eggs was 3, 7 and 20% for isolates D, C and B respectively in the concentration 10⁶ spore/ml, as well as they were 8, 8 and 20% for the same previous isolates in the concentration 10⁵ spore/ml and 5, 9 and 12% for the same isolates in the concentration 10⁴ spore/ml (Table 4).

Eggs of potato tuber moth were shown under scanning electron microscopes (SEM) (Image 1), on the left, smooth egg's shell with some sculptures were shown under 400 \times as well as a slot of larva emergence at the pole of egg. On the right, infected egg by *B. bassiana* was seen under 800 \times , where spores have distributed on the surface especially in sculptures, but the density of spores was not so high where egg has hatched so it was shown a slot of larva emergence.

Treatment-isolate/ concentration	Transparent dead eggs % (average \pm SE)	Yellow dead eggs % (average \pm SE)	Black dead eggs (average \pm SE)
Control	78 \pm 8.82	19 \pm 6.66	0 \pm 0
Isolate B 10 ⁶ conidia/ml	80 \pm 12.58	0 \pm 0	20.3 \pm 12.02
Isolate D 10 ⁶ conidia/ml	97 \pm 13.23	0 \pm 0	2.5 \pm 1.66
Isolate C 10 ⁶ conidia/ml	84 \pm 1.66	16 \pm 6	6.8 \pm 2.88
Isolate B 10 ⁵ conidia/ml	87 \pm 12	5 \pm 3.33	7.5 \pm 2.89
Isolate D 10 ⁵ conidia/ml	71 \pm 4.4	8.5 \pm 2.89	19.9 \pm 4.4
Isolate C 10 ⁵ conidia/ml	81 \pm 8.82	10.4 \pm 1.66	7.8 \pm 2.88
Isolate B 10 ⁴ conidia/ml	82 \pm 10.93	12.8 \pm 6	5 \pm 1.66
Isolate D 10 ⁴ conidia/ml	66.5 \pm 9.28	21 \pm 6	12 \pm 6.66
Isolate C 10 ⁴ conidia/ml	82 \pm 7.26	8.8 \pm 2.88	8.9 \pm 2.88

Table 4. Means of coloration rates of dead *P. operculella* eggs after treatment with different isolates of the fungus *B. bassiana* with different concentrations at 28 \pm 2°C and relative humidity 40 \pm 5%.

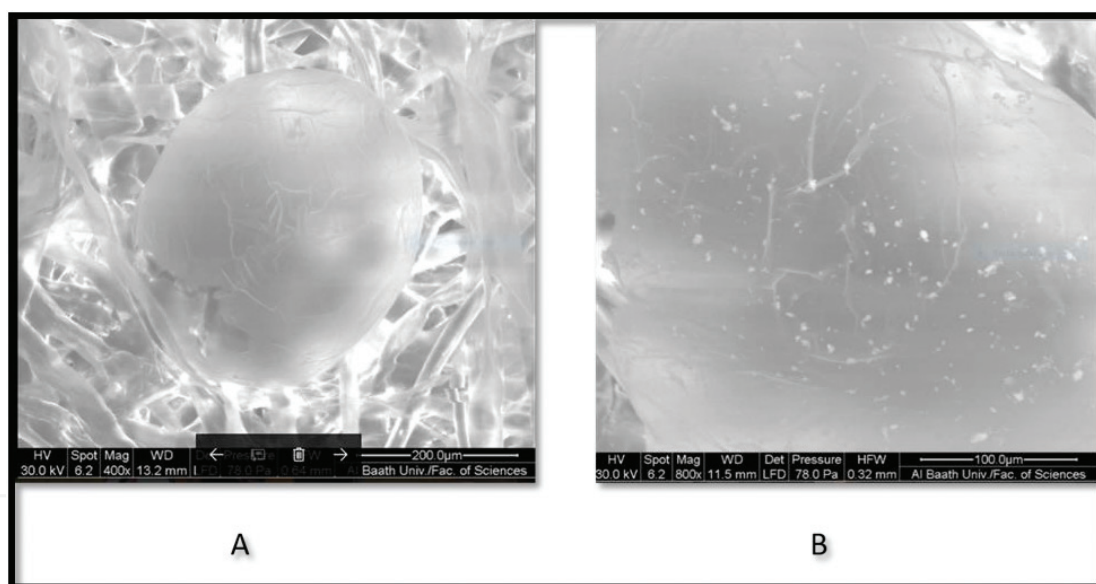


Image 1. Egg of *P. operculella* under SEM (A) noninfected under 400 \times (B) infected egg with *B. bassiana* under 800 \times .

4. Discussion

The results of this research showed the pathogenicity of the three native isolates of *B. bassiana* on egg stage of potato tuber moth, where they arrived at good death rates on eggs in different percentages between studied isolates. All results indicate to the ability of those isolates to infect the eggs stage of potato tuber moth, in spite of unsuitable condition of experiment especially the relative humidity (R.H.) was 40%, which was not optimum. That effected the germination of spores. In addition, the expression of virulence for those isolates affected negatively.

It is known that *B. bassiana* grows and germinates typically under 25–30°C and R.H. 100% [17]. Resulted death percentages from treatments with native isolates seem lower than results in similar study for the same fungus on the same moth, where egg death rate reached 76% after incubation at $25 \pm 1^\circ\text{C}$ and R.H. $80 \pm 5\%$ at the concentration 10^5 spore/ml of *B. bassiana* [12], while the rate did not exceed 67% in this research in the same concentration for the best isolate (B). That can be explained by the low relative humidity while this experiment.

On the other hand, the difference between the native isolates in pathogenicity on eggs, in this research may belong to the differences in vitality of spores and in germination rates. It is known that germinated spores have vitality and they can be active in control. Therefore, they penetrate the cuticle of insect [15, 18]. The results of germination rate showed that isolate B has higher rate of germination and with significant difference with D and C. However, it has the bigger chance to penetrate the host egg because the grand number of germinated spores on egg's surface. This issue may be the main reason for egg's infection was the greatest in B isolate in comparison with C and D isolates, that must has been a near percentage of infection depending on germination percentage.

In this study, the death of moth eggs may be to stop gas exchange between the egg and arounded air, where infected eggs by *B. bassiana* die and some of them became black because of fungal hyphae growth in the micropyles of egg shell [19]. Previous studies showed that eggs in most insects have sculptures that differ from one insect to another, where there is micropyle on front of egg pole which represents an entrance to sperm for fecundation operation. Also aeropyles aid in the exchange of oxygen and carbon dioxide and loss of some water. Woods [20] studied all those details delicately on eggs of *Manduca sexta* where he found that all aeropyles as well as the micropyle and egg shell in infected eggs were occupied by fungal hyphae. Therefore, gasses exchange operation decreased and the development of embryo died [20]. Therefore, infected eggs become sterile [19].

Shalaby et al. mentioned to coloration of *Tuta absoluta* eggs in black after its infection by *B. bassiana*. *T. absoluta* is Gelichiid [21] and black eggs resulted from infection by concentrations ranged between 10^7 and 10^{10} conidia/ml, death rate arrived 100%. Jaksch also mentioned [22] that eggs of *T. absoluta* showed spots in black as a result of direct infection with *B. bassiana*, eggs have dried clearly 4 days after incubation, white mycelium of fungus was observed on the pole of egg and a tissue of white spores have appeared on it.

Results of this research indicated that the most of nonhatching eggs had transparent color, and they represented nonfecunded eggs. Some of nonhatched eggs had yellowish color, and they represented fecunded eggs but they did not hatch for natural reasons, so they did not have a brown color as normal fecunded eggs before hatching [23–25]. The rest of dead eggs had a black color because of infection with *B. bassiana*, and it forms a percentage range from 0 to 20% of dead eggs. Low percentage of black eggs may belong to the low relative humidity and low concentrations in this research in comparison with another research on eggs of *Tuta absoluta*. Gottwald and Tedders [26] mentioned that decrease in fungus sporulation on dead host does not necessarily correlate to mortality that can be explained by several reasons like low temperature and relative humidity in its incubation climate or lose an essential substance for the development of fungus. For the same fungus, decrease in fungus sporulation on their dead hosts can be explained by

diversity of the virulence between strains. That attributed to their genetic diversity that supports strains in its specialization in certain host and in its geographical distribution. [27].

Eggshell's structure has an important role in spore ability to adhere on the egg surface and increases the chance to infection impact. Therefore, egg's sensibility differs between species, for example, eggs of Lepidoptera have a huge chance of death as a result of fungal infection which belongs to sculptures on eggshell [20, 28]. *Ceratitis capitata* eggs are considered insensitive because they have smooth shell, where adhesion of spores is so difficult and the probability of their infection with entomopathogenic fungi seems relatively weak [29].

The importance of controlling eggs stage belongs to one hand, egg stage is fixed stage and easier in controlling than larvae in family Gelechiidae. On the other hand, Gelechiidae larvae (Ex: *P. operculella*, *T. absoluta* and *Scrobipalpa ocellatella*) dig tunnels inside leaves, tubers or roots, so they are protected from entomopathogenic effect. All previous makes its control so complex. Therefore, controlling eggs existing on leaves, fruits, stems and tubers represent a solution, where eggs are more exposed to natural enemies that prevent the appearance of damaging larvae from the beginning [22].

5. Conclusion

The pathogenicity of three local isolates of the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill was evaluated on eggs of potato tuber moth *P. operculella* (Zeller). Isolates were taken from Latakia (isolate B), ICARDA spt273 (isolate C) and Damascus (isolate D). Three concentrations 10^4 , 10^5 , and 10^6 conidia/ml were used for each isolate; by spraying spore suspension on eggs. Eggs in the control were sprayed by sterilized water. The germination rate was evaluated after 24 h incubation in the dark. All tests were done under laboratory conditions of temperature $28 \pm 2^\circ\text{C}$ and relative humidity $40 \pm 5\%$. Results showed significant differences in germination rate, where the average of germination rate was 67, 55, and 47% for isolates B, D and C respectively. Susceptibility tests showed significant differences in averages of hatching rate between the control and both isolates B and C when 1×10^6 conidia/ml was applied, with averages 18.3 and 26.6% for previous isolates respectively, in contrast 38.3% for isolate D and 66.6% for control. Findings indicated that eggs of *P. operculella* seemed sensible to local isolates of *B. bassiana* in varying degree. Results encourage further studies about the efficiency of effective isolates for controlling eggs of this pest in natural conditions of store and field and testing the local isolates on the other stages (adults and larvae) under better condition than this research condition.

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