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Susceptibilities of certain insect pests to *Beauveria bassiana* (Bals.) as Bio-control agent

Alyaa Abdel- Mottaleb Ali Gazzy [1]

[1] Assistant Professor of Entomology, Zoology Department, Faculty of Science, Kafrel-sheikh University, Egypt

Abstract

The Egyptian strain of the fungus, *Beauveria bassiana* (Bals.) Vill. is one of the bio control agents to several economic insect species. This fungus grows naturally in soils throughout the world and acts a parasite on various arthropod species. It belongs to the entomopathogenic fungi. Biological control is very important to replace the harmful of chemical pesticides and environmental pollution as the dangerous of it continue for several years.

This study is to evaluate the susceptibilities of four pests to the fungus under laboratory in 2013 at zoology department, Faculty of science, Kafrel-sheikh University.

Therefore, in this research the fungus is used as a biological insecticide, to control some pests, which are the cowpea seed beetle, the clover seed beetle, larvae of *Seasamia cretica* and the Egyptian cotton leaf worm in 2013.

In this laboratory study, six groups of 20 adults (120) insects of the two insect pests, the cowpea seed beetle and the clover seeds beetle were treated by the fungus conidia with five concentrations. Five groups were treated by the fungus and one control group. An isolate collected from *Sesamia cretica* (Led.) larvae in Kafrel-sheikh region and cultured on Potato Dextrose Agar medium (P D A) at 25^0C and 75 ± 5% RH. Spore suspensions at different concentrations were prepared in distilled water containing 0.1 % Tween 80. The insects were individually treated by dipping method in the fungus suspension and bio assayed against each species, adults of cowpea seed beetle, *Callosobruchus maculates* (L) were treated with different fungus concentrations.

The results indicated that all concentrations of *B. bassiana* caused mortalities in the insects except $1X10^7$ conidia/ ml. The highest number of dead *C. maculates* adults for all concentrations was recorded between the 6^{th} and 8^{th} day after treatment.

The results revealed that conidia concentration of $5X10^7$ of *B. bassiana* caused a mortality of 85% of clover seed beetle, *Bruchidius trifolii* (Moits), increased to reach 100% by $7.5X\ 10^7$ and $1X\ 10^8$ conidia/ ml. These results revealed that, *B. bassiana* is highly virulent to adult beetle of *B. trifolii*.

The results also illustrated that the total larval mortality of *S. cretica* increased as conidia concentration increased of *B. bassiana*.

So, the results showed that each of *C. maculates*, *B. trifolii* and *S. cretica* insects are sensitive to *B. bassiana* in contrast to *S. littoralis* larvae were insensitive to the fungus.

Key words: *Beauveria bassiana*, Bio control agent, *Spodoptera littoralis*

1. Introduction

This study provides a method to control the beetles (The cowpea seed beetle, the clover seeds beetle, larvae of *Sesamia cretica* and the Egyptian cotton leaf worm) using different concentration of *Beauveria bassiana*, to prevent the use of chemical insecticides which cause the environmental pollution. Cotton is the most important product in kafer el-shiekh, Egypt. Cotton leaf worm causes a lot of damage in the leaves of the corps.Therefore an increased interest in the development of biological control agents as substitutes for, or supplements to, chemical insecticides.

Entomopathogenic fungi that infect insects have received attention by scientists for their potential for biological control of pests. Some pathogenic fungi have restricted host ranges while other fungal species have a wide host range for example, *Beauveria bassiana*. Many researchers have focused on the development of biological control agents by selection of virulent fungal strains as targets for pests[1-4].

The origins of microbial pest control date back to the early nineteenth century, when the Italian scientist Agostino Basse spent more than 50 years studying white muscadines disease in silkworms (*Bombyxmori* L.). He identified *Beauveria bassiana* (Bals. Criv.). Viull, named in his honor, as the cause of the disease.

This discovery not only laid the foundation for microbial pest control, also significantly influenced the work Louis Pasteur, Robert Kock and other pioneers of microbiology [1,2,5]. Today, over 100years later, there are no known reports of significant adverse effects that can be attributed to use these organisms in bio control *Beauveria bassiana* (Balsamo)is a registered bio pesticide with abroad host range of approximately 700 insect species used for management of several crop insect pests [6-12].

In agricultural fields, the entomopathogenic fungal species have been investigated for their role as natural enemies for insects as Conidia adhere to the surface of the host, release extracellular enzymes, including lipases, proteases, and chitinase. These enzymes help them breach the host's chitinous exoskeleton[13].

The cowpea seed beetle, *Callosobruchus maculates* L. (Coleoptera: Bruchidae) is common in Egypt. The adults are major pest of economically important leguminous grains, such as cowpeas, lentils, green gram, and black gram in the field and stores. The females lay eggs which are cemented singly to the pods in the field and on the seeds in the storage, the larvae then bore into the pods which become unsuitable for human consumption, viability for replanting, or to produce sprouts[14].

The clover seeds beetle, *Bruchidus trifolii* Mots (Coleoptera: Bruchidae). The adult attacks the clover seeds pods in the field and females attack their single eggs on the seed coats. The hatching larvae bore and feed inside the seeds causing great damage[15].

The pink borer, *Sesamia cretica* Led. (Lepidoptera: Noctuidae) is one of the most important corn borers in Egypt.

The geographical range of *S. cretica* includes most of the countries and islands of the Mediterranean basin and extends through the Middle East and northern Africa. Insecticides were extensively used to control the pest in maize fields[16,17].

Spodoptera littoralis Boisd (Lepidoptera: Noctuidae), also referred to asthe Egyptian cotton leaf worm, causes considerable damage to many field crops in Egypt. The devastating impacts caused by these pests have led to the need for development of both biological and chemical control methods[18-19]. In the present study a number of these insect pests were treated with the Egyptian strain of *B. bassiana* recovered from *Sesamia cretica* larvae on artificial medium to evaluate their susceptibility to the fungus in five different spore concentrations. All experiments were carried out under laboratory conditions.

2. Material and Methods

2.1-Entompathogenic fungus:

B. bassiana in this laboratory study in an isolate collected from *Sesamia cretica* larvae in Kafrel-Sheikh region, Faculty of science, Egypt (2016) according to Sewify 1997[19].

Conidia were cultured on autoclaved potato dextrose agar medium (PDO) Cantwell, 1975 and Pandit & Som 1988. The medium is composed of 200 g potato, 20 g dextrose, 20 g agar and 1000 ml distilled water[7,20].

After preparing the medium and poured into Petri dishes (9 cm diam.). The plates were left for cooling, incubated with the fungus conidia and at 25±1^{o}c& 75± 5% RH.

Each plate was incubated with 1 ml of a fungus suspension containing 5 X 10^{7} conidia in one ml. The fungus grew and sporulated on the medium within 10-15 days.

After sporulation the plates were kept in a moist chamber for a week. The conidia production was harvested by scraping method in ten plates of the medium.

From each plate 1 cm was cut and transferred into a glass tube containing 10 ml of distilled water. The mean number of conidia on 1 cm was determined and the production of the conidia on the medium was calculated for each plate. To assess the weight of produced spores per plate, 10 mg of fungus spores were added into 10 ml distilled water and the number of spores in one ml. was assessed using a haemo-cytometer.

2.2-The tested insect pests:

Several economic insect pests were treated with the Egyptian strain of *B. bassiana* to evaluate their susceptibility to the fungus. All experiments were carried out under laboratory conditions at the faculty of science, zoology department, Egypt of 25± 1^{0}c and 75± 5% R. H.

2.2.1 The cowpea seed beetle and the clover seeds beetle:

Adults of the two insect pests were obtained from Department of Stored Product Insect Research at Sakha Agric. Res. Stations.

Six groups of each species 20 adults (120) insects in this experiment were used. Five groups were treated by the fungus conidia. Experimented concentrations were, 1X10^{7}, 2.5X10^{7}, 5X10^{7}, 7.5X$10^{7}$and 1X10^{8} conidia /ml. All conidia concentrations contained 0.1 % Tween 80. The sixth group was treated with distilled water contained 1% Tween 80 and used as a control. The adults of each species were individually dipped in the fungus *B. bassiana* suspension for 3 sec. Each adults group was placed into a Petri dish containing the insects and daily examined. Number of dead adults were recorded.

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2.2.2 Larvae of *Sesamia cretica*:

Egg-clusters of the borer were collected from maize fields during May 2013 and reared in the laboratory. The newly hatched larvae were reared at 25± 1^0c and 75± 5 % R.H. on corn ear silk bundles in glass containers 5X8 cm furnished with filter paper (5 larvae / container) and covered with perforated polyethylene sheet fitted in place by rubber band[21]. The food was renewed each second day until the larvae reached the third larval instar. One hundred and twenty larvae, each five groups were treated with different concentration of *B. bassiana* suspension. The 6^{th} group was treated with distilled water containing 0.1 % tween 80 as a control. The larvae were individually treated by dipping in the fungus suspension for 3 sec.

Each 5 larvae were introduced with fine brush into a Petri dish having corn ear silk bundles. The larvae were examined daily, and its food renewed each second day. Number of dead larvae were recorded.

2.2.3 Larvae of *Spodoptera littoralis*:

A colony of cotton leaf worm was reared on Castor oil bean leaves as described by El-Defrawi et al., 1964[22]. One hundred and twenty larvae of early fourth instar were divided into six groups, each of 20 larvae. Larvae of 5 groups were individually treated with suspension of *B. bassiana* conidia containing 0.1 % Tween 80. Tested concentrations were, $1X10^7$, 2.5 X 10^7, $5X10^7$, 7.5 X 10^7, 1X 10^8 conidia / ml. After treatment, each group was introduced into Petri dish 15 cm diameter each of 5 larvae and fed on fresh castor- oil bean leaves. The sixth group treated with distilled water containing 0.1 % Tween 80 and used as a control and number of dead larvae were recorded. Mortality was corrected according to Abbot 1925 values of LC_{50}, LC_{90}, LT_{50} and LT_{90} were calculated using Litchfield and Willcoxon 1949[23].

2.2.4 Statistical Analysis:

Mortality rates of the tested insects were analyzed using ANOVA one-way test. Probity analysis was used to calculate values of the lethal concentration (LC50 and LC90) and the lethal time (LT50 and LT90) for each fungus[24].

3. Results

The cowpea seed beetle, *Collosobruchus maculates* and the clover seed beetle, *Bruchidus trifolii* :

Data presented in Table (1) indicated that *C. maculates* adults are susceptible to *B. bassiana* infection on the fourth day after treatment, all concentrations caused mortality in the insects, except for 1 $X10^7$ conidia/ ml. The highest number of dead adults for all concentrations was recorded between the 6^{th} and 8^{th}day after treatment. The moderate concentration ($5X10^7$ conidia / ml) yielded considerable mortality 90 %. Increasing the infection dose to 1 X 10^8 conidia / ml resulted in 100% mortality.

Table (1) Mortality, L_{C50}, L_{C90}, slop, and L_{T50} and L_{T90} of *Callosobruchus maculates* treated with the fungus *Beauveria bassiana* under laboratory conditions using dipping method.

Concentration Conidia / ml	Days after treatment						L_{C50} at 12 days	L_{C90} at 12 days	Slope value	L_{T50}	L_{T90}
	2	4	6	8	10	12					
1X 10^7	0	0	5	25	30	50	10.639000 conidia/ ml	57.973000 conidia / ml	0.741	12.1	22.2
$2.5X10^7$	0	5	20	40	60	70				9.1	17.1
$5X10^7$	0	10	30	60	75	90				7.2	12.7
7.5 $X10^7$	5	20	60	80	90	100				5.4	10.3
1X 10^8	10	25	70	95	100	100				4.6	8.5
Control	0	0	0	0	0	0	-	-	-	-	-

The calculated L_{c50} was 10.639 X 10^7 conidia / ml, while L_{c90} was 57.973 X 10^7 conidia / ml at 12 days of treatment.

Data presented in Table (2) revealed that this insect is susceptible to *B. bassiana* infection. Conidia concentration of 5 X 10^7 caused a mortality of 85%, increased to reach 100% by 7.5 X 10^7 and 1X 10^8 conidia / ml. For all concentrations, the highest number of dead adults occurred between the 8^{th} and 10^{th} days after treatment.

Table (2) Mortality, L_{c50}, L_{c90}, slop, L_{T50} and L_{T90} of clover seed beetle, *Bruchidus trifolii* treated with the fungus, *B. bassiana* under laboratory conditions by dipping method.

Conc. Conidia /ml	Days after treatment						Lc_{50} at 12 days	Lc_{90} at 12 days	Slope value	LT_{50}	LT_{90}
	2	4	6	8	10	12					
$1x10^7$	0	5	15	35	45	50	15.150.000	88.945.000	1.7	11.2	25.1
$2.5x10^7$	0	5	20	35	50	65				9.8	20
$5x 10^7$	5	15	30	50	85	100				7.1	15.3
$7.5x 10^7$	5	15	40	70	90	100				6.1	11.8
$1x10^8$	5	15	45	80	100	100				5.9	11.5
Control	0	0	0	0	0	0	0	0	0	0	0

The calculated Lc_{50} was 15.150.000 Conidia/ ml, while Lc_{90} was 88.945.000 Conidia/ml.

Larvae of *Sesamia cretica*:

Larvae of *S. cretica* seemed to be susceptible to *B. bassiana* infection. Table (3) illustrated percent mortality of *S. cretica* larvae treated with different concentrations; the first dead larvae were recorded on the 6^{th} day. The total larval mortality increased as the conidia concentration increased.

The calculated Lc_{50} was 8.537.000 Conidia/ ml. while, Lc_{90} was 15.070.000 Conidia / ml.

Table (3) Mortality, Lc_{50}, Lc_{90}, slop, Lt_{50} and Lt_{90} of *Sesamia cretica* 3^{rd} larval instar treated with *Beauveria bassiana* under laboratory conditions using dipping method.

Conc. Conidia/ ml.	Days of treatment							Lc_{50}at 14 days	Lc_{90}at 14 days	Slop value	Lt_{50}	Lt_{90}
	2	4	6	8	10	12	14					
$1x10^7$	0	0	0	5	10	20	30	8.537.000 Conidia/ ml.	15.070.000 Conidia/ ml.	0.517	17.4	39.6
$2.5x 10^7$	0	0	0	5	20	30	40				14.8	26.6
$5x 10^7$	0	0	5	20	40	55	70				11.0	18.00
$7.5x 10^7$	0	0	5	25	45	55	75				10.7	17.5
$1x10^8$	0	0	15	35	60	90	90				8.5	13.3
Control	0	0	0	0	0	0	0	0	0	0	0	0

Spodoptera littoralis larvae:

Table (4) Mortality, Lc_{50}, Lc_{90}, slop, L_{T50} and L_{T90} of *Spodoptera littoralis* larvae treated with the fungus, *Beauveria bassiana* under laboratory conditions using dipping method.

Conc. Conidia/ ml.	Days after treatment							Lc_{50}at 14days	Lc_{90}at 14days	Slop value	L_{T50}	L_{T90}
	2	4	6	8	10	12	14					
$1x10^7$	0	0	0	5	10	20	25	77.570.000 Conidia/ml.	101.230.000 Conidia/ml.	3.420	19.9	41.0
$2.5x 10^7$	0	0	0	5	10	30	45				14.6	32.6
$5x 10^7$	0	0	5	10	15	30	55				14.4	25.9
$7.5x 10^7$	0	0	10	15	20	55	60				12.6	23.3
$1x10^8$	0	5	20	30	50	60	65				10.5	17.5
Control	0	0	0	10	15	20	25	0	0	0	0	0

Table (4) shows mortality of *S. littoralis* the 4^{th} instar larvae with *B. bassiana*. However, all tested concentrations were not effective against the cotton leaf worm in the first six days after treatments. On the other hand, low concentrations; $1X10^7$ and $2.5X 10^7$ did not cause moderate mortality to the pest and that it could be concluded *S. littoralis* were not susceptible to *B. bassiana* infection. The infected larvae needed longer time to the killed by the fungus. The concluded L_{c50} was 77.573.000 conidia/ ml while L_{c90} was 101.230.000 conidia/ ml

In conclusion, the pathogenicity of the fungus *B.bassiana* against larvae of *S. cretica* and *S. littoralis* due to the fungus spores which germinate on the cuticle surface and produces an infection hypha, while penetrate the integument of the insect at any point except the head capsule. The penetration appears to be assisted by the production of enzymes that dissolve the chitin layer of the cuticle and by the mechanical pressure excerted by the fungus. After infection through the cuticle, the fat body is the first tissue to be attached. The infected larvae become sluggish and furls to respond to external stimuli. The pest remains soft until the mycelium has ramified and grown in the body tissues following this the body becomes rigid and mummified. No external signs of the fungus are evident as long as the pest is kept in a dry atmosphere. Soon after exposure to moist air, the white mycelium becomes apparent over the surface of the insect.

In general, the results illustrated in this study by using the fungus; *B. bassiana* are of great importance and encourage to use the fungus strain as a microbial insecticide against certain insect pests cause considerable damage to some important crops cultivated in regions which high relative humidity prevailing.

4. Discussion

The results of the current study showed that *C. maculates*, *B. trifolii* and *S. cretica* were sensitive to *B. bassiana* infection and different concentrations caused mortality in the insects. These results are consistent with those of Annop-Ongsakul and Surakrai- Permkam who reported that adults of *Callosobruchus chinesis* died in the laboratory one day after dipping them in *B. bassiana*[25].It proved to be highly virulent to many coleopterous insects such as *Alfalfa weevil*[26], *Hypera brunneipennis* [27], Cotton ball weevil, *Anthonomus grandis*[28], banana weevil, *Cosmpobtes sasdidus*[29].

Obtained results agree with those of Arshad and Hafez[30], who tested the effectiveness of *B. bassiana* against the adults of *Aeolesthes sarta* (Coleoptera: Cerambycidae). They found that the fungus induced 94.3% adult's mortality. Also, Frydocna *et al*.[31], investigated the efficiency of the fungus, *B. bassiana* on stored product past *Sitophlus granarium, Oryzaphilus surinamensis* and *Tribolium confusum* in laboratory and found that these insects were highly susceptible to the fungus.

These results were consistent with the results reported on different laboratory lepidopterous pests, *Zuzera pyrina* [32], *Pieris rapae* [8,33], *Zeuzera coffeae* [34,35].

El-Sufty et al. found that treating larvae with suspension of low and middle spore densities till 10^8 spores /ml yield not more than 16% mortality[8]. Fernandes *et al*. found that the fungus *B. bassiana*showed a higher ratio of halo / colony (H/C) index for amylase activity[36]. He added *B. bassiana* can use starch as a source of energy for its development and sporulation. Murad *et al* in their work was able to determine low concentrations of α-amylase produced by entomopathogenic fungus *M. anisopliae* growing on *Callosobruchus maculatus*shelves[37]. This result was expected by the authors because of the lack of starch in the composition of the shelves. The result also indicates that this enzyme is not very important for the infection process since it's not present in the cuticle of the insect [38-41].

In conclusion, the pathogenicity of the fungus *B.bassiana* against larvae of *S. cretica* and *S. littoralis* due to the fungus spores which germinate on the cuticle surface and produces an infection hypha, while penetrate the integument of the insect at any point except the head capsule. The penetration appears to be assisted by the production of enzymes that dissolve the chitin layer of the cuticle and by the mechanical pressure exerted by the fungus. After infection through the cuticle, the fat body is the first tissue to be attached. The infected larvae become sluggish and furls to respond to external stimuli. The pest remains soft until the mycelium has ramified and grown in the body tissues following this the body becomes rigid and mummified. No external signs of the fungus are evident as long as the pest is kept in a dry atmosphere. Soon after exposure to moist air, the white mycelium becomes apparent over the surface of the insect.

In general, the results illustrated in this study by using the fungus; *B. bassiana* are of great importance and encourage to use the fungus strain as a microbial insecticide against certain insect pests cause considerable damage to some important crops cultivated in regions which high relative humidity prevailing.

5. Significance Statement

The cowpea seed beetle, the clover seed beetle, larvae of *sesamia cretica* and the Egyptian cotton leaf worm are the four important pests in Egypt, though they decrease the economy.

In the present study a number of these insect pests were treated with the Egyptian strain of *B. bassiana* on artificial medium to evaluate their susceptibility to the fungus in five different spore concentrations under laboratory conditions.

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