

**EFFECT OF THE ENTOMOPATHOGENIC FUNGUS,  
*BEAUVERIA BASSIANA* ON *APHIS CRACCIVORA*  
(Homoptera : Aphididae)**

Safaa , H. Aly

Agricultural Research Center, Plant Protection Research Institute.  
Pesticides Tests of Cotton Pests Department, Egypt

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**ABSTRACT :** This study was undertaken to investigate possibility of using *B.bassiana* as a biological control agent against *Aphis craccivora*. Present test reported successful infection by *Beauveria bassiana* against the aphid, *Aphis craccivora*. The fungal pathogenicity to the nymphs, as well as the effect on some changes in certain biochemical components were investigated. Results showed that  $LC_{50}$  and  $LC_{90}$  of nymphs were  $1.69 \times 10^3$  and  $8.2 \times 10^3$  spores/ml, respectively. The tests proved a reduction in total protein, fat and - amylase enzyme.

## INTRODUCTION

*Aphis craccivora*, (Homoptera : Aphididae) is an important pest causing a great damage to many agricultural crops in the field and in glasshouses, such as lentils, alfalfa, clover, beans, cowpea, dandelions, lambsquarters, mustard, and peas. More than 50 genera of fungi contain species that are pathogenic to insects (McCoy, et.al. 1988). These species have extremely

heterogeneous life cycles, produce assorted pathologies in these infected host, and exhibit varying degrees of host specificity. Most fungal pathogens of insects are transmitted from infected hosts, and exhibit varying degrees of host specificity. Most fungal pathogens of insects are transmitted from infected hosts to susceptible hosts by environmentally resistant forms known as spores. In the typical fungal life cycle, when a spore encounters a susceptible host, it

adheres to the cuticle, germinates and penetrates the body wall of the host by means of its germ tube inside the host. The fungus grows vegetatively, as yeastlike hyphal bodies. The host usually dies, after which the fungus forms typical mycella that grow out through the cuticle and produce more resistant conidia. These conidia may then infect other hosts. The life cycles of many insect pathogenic fungi are more complicated than those of typical fungi and may involve alternate hosts, motile spores, and the production of toxins. Host specificity may be limited if any one of these events is circumvented. The details of fungal life cycles and pathogenesis are reviewed by, McCoy et al. (1988) Samson et.al. (1988). And Goettel et al. (1990)

*Beauveria bassiana* is a common but little understood fungal pathogen of many groups of insects. The fungus has been used for many years as a microbial insecticide in and some countries (Ferron, (1981), and McCoy, et.al. (1988). *B.bassiana* has been reported to infect more than 100 insect species in many different insects orders (McCoy et.al. 1988.).

*B.bassiana* and other fungal

pathogens infect the insect with contact and do not need to be consumed by their host to cause infection. Once the fungus has killed it's host, it grows back out through the softer portions of the cuticle, covering the insect with a layer of mold, and this mold produces millions of new infective spores. (Bacchus, 2000), recorded several varieties of target pests of *B.bassiana* such as, scarab beetles, leaf-feeding beetles, whitefly, aphids, thrips, psyllids, mealybugs, leafhoppers and plant hoppers, weevils, plant bugs, borers, leaf-feeding insects, grasshoppers, locusts and mormon crickets, stem-boring lepidoptera.

The present study provides information on the pathogenicity of *B.bassiana* against *A.craccivora*, and some changes in certain biochemical components of infested insects.

## MATERIALS AND METHODS

### Test Insect:

*Aphis craccivora* colony was reared under controlled conditions in a glasshouse on broad bean, *Vicia faba*, at 25°C, photoperiod of (15 L/9D) and 80-90% RH.

### **The Fungus:**

The product Bio-fly ( $30 \times 10^6$  spores/ml) is manufactured from the entomogenous fungus *Beauveria bassiana*. It showed very promising results for enhancing the efficacy of the fungus for control of aphids (Greer, 2000). The stock solution was prepared by adding 1gr. powder to 1 L. of water, and then 4 concentrations of suspension; ( $7.5 \times 10^3$ ,  $3.75 \times 10^3$ ,  $1.875 \times 10^3$ ,  $0.938 \times 10^3$  spores /ml.) and control, were prepared

### **Bioassay procedure for nymphs of *A.craccivora*:**

Third instar *A.craccivora* nymphs were used. Individual bean plants with uniformly insects were treated with the 4 concentration of spores suspensions and control. The samples were collected from each treatment as well as the untreated. Nymph mortality was determined daily by counting the number of infected versus non-infected individuals. The test was repeated twice using 4 replicates.

### **Biochemical analysis:-**

Sampling of individuals started 72hr. after treatment with the tested fungal suspensions. Subsequently, samples were collected at random from each

treatment as well as from control. Each sample consisted of about 150-200 alive insects that were weighed.

### **Determination of total protein:-**

The insect was immersed in 96% ethyl alcohol and left 24hrs. in alcohol then removed and the extract was taken for soluble protein analysis. The extract was concentrated to 2 ml, and then transferred to tightly closed bottle and kept in the refrigerator until analysis. Total protein content was determined by the method of (Lowry et.al. 1951).

### **Determination of fat content of treated insects:**

The rapid method of Bligh and Dyer (1959) was applied. Each sample was weighed and homogenized with a mixture of chloroform and methanol to produce a biphasic system, the chloroform layer contained the lipids. This layer was taken in a clean dry beaker (weighed before) and chloroform was evaporated by air current. Thenafter, the remained fat residues and beaker were re-weighed and the lipid content was calculated.

### **Determination of - amylase enzyme of treated insects:**

The enzyme activity was

assayed according to Rick and Stegbauer (1974).

## RESULTS AND DISCUSSION

### \* Pathogenicity of *B.bassiana* on nymphs:

Present data indicate that the nymphs of *A.craccivora* were susceptible to *B. bassiana*. The successful infection by *B.bassiana* was also reported for aphids, by Greer, (2000) who reported that the fungus works by attaching to the outside of the pest, then penetrating into the body and Killing it. *B.bassiana* was also used as a biological control agent against aphids in IMP program in USA, Cheryl (1999). Data in Table (1) show that the nymphs of *A.craccivora* are susceptible to the fungus and high hazard appeared at the higher concentration than those at the lower concentration. The  $LC_{50}$  for the third-instar nymphs was  $1.6 \times 10^3$  and the  $LC_{90}$  was  $8.2 \times 10^3$  spores/ml. (Fig 1). These data are in agreement with Knudsem et.al. (1990), Lecuona, et.al. (1997), Cheryl (1999) and Greer (2000) who recorded a good control of aphids by the fungus, *B.bassiana*. The fungus appeared clearly on the dead nymphs after putting in 100% moisture at 25°C as

recommended by Butt and Goettel (2000).

### \* Determination of total protein:

Present data in Table (2) indicate the effect of *B.bassiana* on the total soluble protein of *A.craccivora*. The data revealed that the fungus reduced the amount of soluble protein in the treated insects than the control. The mean of the total protein in the treated insects was 1.63275 mg / gm as compared with. 2.5493 mg / gm. for the untreated The percentage of the decrease than control was 35.953%. These data are in agreement with Eman & Sewify, (1991), who recorded a decrease in concentration of the total protein in *Aphis craccivora* insects treated with *V.lecanii*. Gardner et.al.(1979) and Cheung & Grula (1980), recorded a decrease in certain haemolymph proteins, amino acids and carbohydrates in insects infected by the fungi, and they mentioned that, this reduction is due to the pathological action of the fungi particular those of higher virulence. Gabriel, (1968), Kucera, (1980), Ignofoo, (1981) and Brey and Latge (1986), indicated that the ability of fungi to produce extracellular enzymes lead to changes in haemolymph proteins and amino acids by breaking down

proteins bound to chitin and to deterioration of the attached organs. The data were in agreement with Jackson et.al. (1985) who stated that the highly significant quantitative differences in haemolymph protein and amino acids in *Aphis* due to infection by fungus, *V.lecanii*, and they referred to the ability of all isolates of *V.lecanii* to degrade lipid and protein by extracellular enzymes in the host. These data were in agreement also with Leger et.al.(1986) who cleared the potentiality of fungal enzymes to degrade the protein and chitin in locust cuticle.

**\* Determination of fat content of treated insects:**

Present data in table (3) indicate the effect of *B.bassiana* on the lipid contents of *A.craccivora* insects. The data revealed that the fungus reduced the lipid content in the treated insects than the control. The percentage of the lipids content from 4 replicates of sample treated with fungus was 6.5048% but in control it was 9.5798%. These data indicated that the fungus infected nymph decrease the lipid contents as an effect on the metabolism in the treated insects. These data were in agreement with Smith and Grula,

(1982), who stated that a wide variety of natural compounds such as glucose, several amino acids, chitin, starch and fatty acids can be used as carbon and energy source for germination of *B.bassiana* conidia, and this fungus can colonise the haemolymph of Colorado beetle larvae, starting in the degradation process, (Cermakova & Samsinakova, 1960). These data were also in agreement with Jackson et.al.(1985), he referred to the ability of all isolates of *V.lacanii* to degrade lipid and protein by extracellular enzymes in the host. Jagatap, (1973), stated that the fungus spreads through the blood system, fat bodies, glandular tissues, digestive tract and nervous tissues of the host. Lecuona, et.al. (1997), recorded the effects of *B.bassiana* on insect lipids.

**\* Determination of -amylase enzyme of treated insect:**

Data in table (4) indicate the effect of *B.bassiana* on the -amylase enzyme in the treated insect. The data showed a reduction in the amount of -amylase of *A.craccivora* insects treated with the fungus *B.bassiana* than that of the control. These results demonstrate that the fungal toxin was an inhibitor of insect digestive enzymes and act as

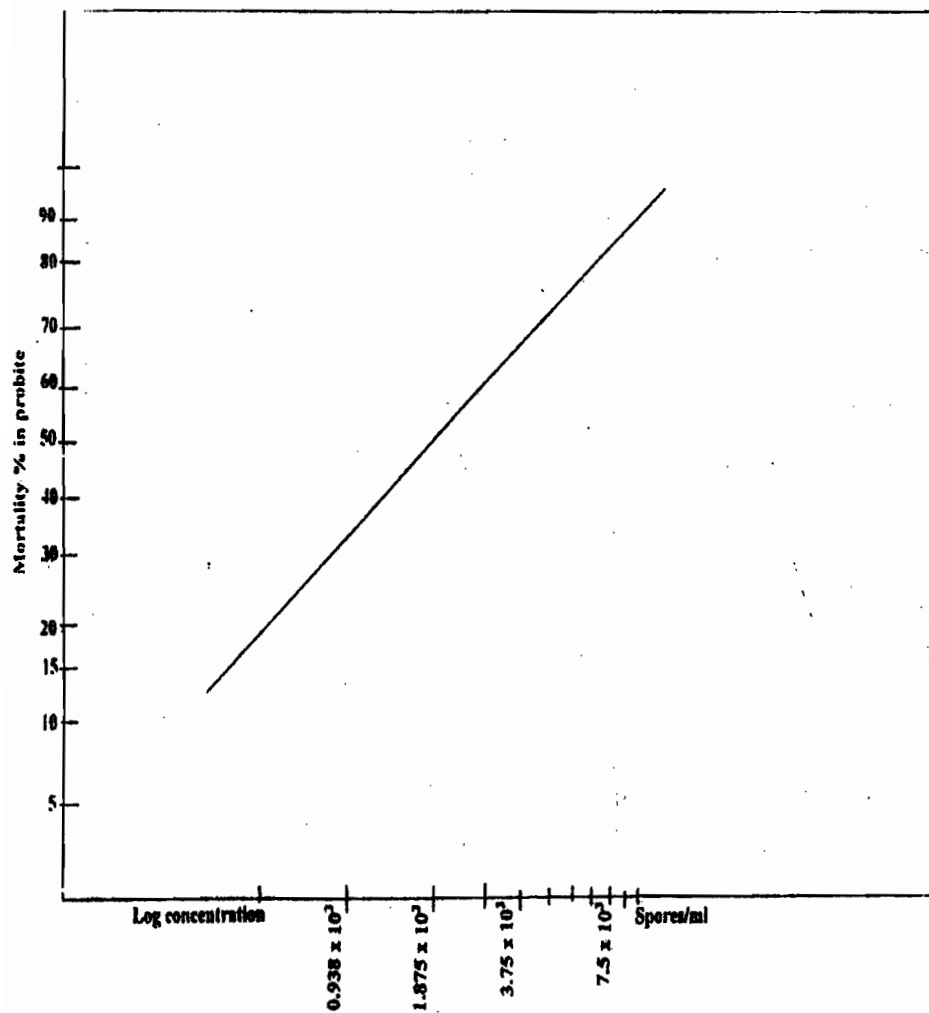


Fig. (1): Mortality response of the 3<sup>rd</sup>-instar nymphs of *A. craccivora* to *B. bassiana*

**Table. (1) Susceptibility of 3<sup>rd</sup> instar nymph of *A.craccivora* to the entomopathogenic fungus *B.bassiana* 3 days after treatment.**

Concentrations	No.of treated nymph (mean 3 Rep.)	Mort (Mean) ( % )	Correct Mort. (%)
7.5 x 10 <sup>7</sup> spores/ml	77	85	83.81
3.75 x 10 <sup>7</sup> spores /ml	90	96.4	68.428
1.875 x 10 <sup>7</sup> spores/ml	55	45	44.37
0.938 x 10 <sup>7</sup> spores/ml	80	32.7	32.242
Control	100	1.4	0.0

**Table. (2) Effect of the fungal infection on the total soluble protein of *A.craccivora***

Replicates	Amounts of total soluble protein mg/gm	Control	Decrease than control %
1	1.901	2.86	-
2	2.0	2.48	-
3	1.52	2.077	-
4	1.11	2.78	-
Mean	1.63275	2.5493	35.9530

Table. (3) Effect of the fungal infection on the lipid contents of *A.craccivora*.

	Replicates	Sample weight (gm)	Lipid content	Lipids content %
Treatment	1	0.480	0.0290	6.0417
	2	0.612	0.05	8.69
	3	0.4751	0.0298	6.2724
	4	0.560	0.031	5.536
	Mean	0.5318	0.03495	6.5048
Control	1	0.7012	0.0584	8.329
	2	0.573	0.063	120.995
	3	0.683	0.071	10.395
	4	0.50	0.043	8.60
	Mean	0.6143	0.0589	9.5798

Table. (4) Effect of the fungal infection on the - amylaze of *A.craccivora*

Replicates	Amounts of- amylaze per gm	Control
1	0.347	0.477
2	0.196	0.403
3	0.40	0.51
4	0.301	0.407
Mean	0.311	0.4493

growth inhibitor of insects. The pathological action of entomopathogenic fungi on various insect species has been studied in relation to the qualitative and quantitative modifications of the haemolymph components (Gardner et.al.,1979, Cheung and Grula, 1980). These data were in agreement with Gardner et.al.,(1979) and Cheung & Grula, (1980), they recorded a decrease in certain haemolymph proteins, aminoacids and carbohydrate in insects infected by the fungi. Smith and Grula, (1982) stated that a wide variety of natural compounds such as glucose, several aminoacids, chitin, starch and fatty acids can be used as carbon and energy sources for germination of conidia of *B.bassiana*. Zacharuk, (1981) stated that the degradative changes in insect tissues and organs occur before the fungus hyphal invasion due to certain metabolites of fungal origin that are mainly toxic substances.

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## تأثير الفطر *Beauveria bassiana* على حشرة *Aphis craccivora*

صفاء حسنين علي

قسم إختبارات مبيدات آفات القطن - معهد بحوث وقاية النبات

مركز البحوث الزراعية

الهدف من هذه الدراسة هو تأكيد أنه من الممكن استخدام الفطر *B.bassiana* في مكافحة البيولوجية للحشرة *A.Craccivora* بنجاح. أجريت تجارب باستخدام الفطر *B.bassiana* على حشرة *A.craccivora* وقد أثبتت التجارب أن الفطر قد نجح في إصابة طور الحوريات وسجل  $LC_{50} = 1,69 \times 10^3$  و  $LC_{90} = 8,2 \times 10^3$  كونيديا/مل. وقد أثبتت التجارب انخفاض في نسبة البروتين والدهون وانزيم الأميليز في الحشرات المعاملة بالفطر عن الحشرات الغير معاملة بالفطر.