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PATHOGENICITY OF *Beauveria bassiana* TO *Bemisia tabac*i (Gnnandius) (HOMOPTERA: ALEYRODIDAE).

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ABSTRACT

Present test reported successful infection by *Beauveria. Bassiana* against the whitefly, *Bemisia. Tabaci*. The fungus tested for its pathogenicity to the third – instar nymphs, and tested also against one-and 4-day-old eggs. Also the tests proved a reduction in total protein, fat, and ∝ -amylase enzyme in the treated insects. Eggs were found to be immune to infection, but mortality of hatching nymphs reached 80-90%. The rate of hatching nymphs infection depended on the age of which the eggs were treated. Mortality of nymphs recorded on the third day after treatment and LCso was 1.12×10^3 and LC_{90} was $3\,8 \times 10^3$ spores/ml.

INTRODUCTION

Bemesia tabaci, is a major pest of a wide range of wild and cultivated plants in warm climates worlwide (Osborne, and Landa, 1992). It affects crops grown in greenhouse and field, all year round with short development cycles and high fecundity . It is known as a principal pest of some crops such as vegetables, caused direct damage sucking plants sap and indirect damage by transference the virues. Although mainly chemical control has been used against *B.tabaci,* it is not always successful, possibly due to the waxy nature of the cuticle (Byrne and Bellows, 1991). And rapid development of resistance (Smith, 1993). The reduced efficiency of insecticidal control and ecological awareness revived the interest in biological control. In this regard, *B. tabaci* is attacked by parasitoids, predators and entomopathogenic fungi. This fungi is among natural occuring antagonists to be considered in aspects of biological control. *Beauveria bassiana* provide good control of aphids and whiteflies (James and Elzen, 2000). The fungus work by attaching to thehost surface, then penetrating into the body and killing it. The fungus is available commercially for greenhouse ornamentals and vegetables (Greer, 2000) The potential use of a fungus as a biological control agent is greatly influenced by the susceptibility of the various development stages of the insect. To the pathogene as well as its ability to initiate infection in early stages of the whitefly's development. *Beauveria bassiana* is an insect pathogen for good biological control of whitefly, aphids, thrips, psyllids, weevils and mealybugs in ornamental and vegetables in greenhouses and fields. Bastiaan (1997) used the *B bassiana* against sweetpotato, silverleaf whitefly in the greenhouse. The present study provides information on the pathogenicity of *B.bassiana* against *B.tabaci.*

MATERIALS AND METHODS

Tested Insects:

Bemisia tabaci was reared on tomato plants under controlled conditions in glasshouse at 25 ± 3 °C, 50 ± 20% RH and a photoperiod of 16:8 (light: dark). In order to obtain *B.tabaci* individuals of uniform age, 50-

100 adults were placed on small tomato plants for 24-36 h. Then all adults were removed and plants with eggs were transferred to environmental growth chambers for further development of homogeneous populations.

Tested fungicide:

The product Bio-fly (30 x 10^6) cells / ml L is based on the entomogenous fungus *Beauveria bassiana*.

Was prepared by addide 1cm to 1L. of water, and tnen we prepared 4 concentrations of suspension; (7.5 x 10^3, 3.75 x 10^3 , 1.875 x 10^3, 0.937 x 10^3 spores /ml.) and control.

Bioassay procedure for nymphs and eggs of *B.tabaci*:

Individual tomato leaves with uniformly insects were selected to treatments. Leaf sectors with approximately 50 to 100 insects were used. These leaf pieces bearing 3^{ed} inster *B.tabact* nymphs were immersed in a spore suspension and control for 10 sec. To prevent development of saprophytic fungi, treated leaves were placed for 20-30 min on filter paper to remove excess moisture. The leaves were then placed in petri dishes which were incubated in growth chamber at alternating temperatures of 25 °C (14 h in light and 10 h in the dark). Relative humidity close to 100% was reached by placing a moist filter paper in each petri dish. For aeration purposes, each petri dish was opened daily for 25-30 min. This procedure was necessary to avoid development of saprophytic fungi on whitefly honeydew. Larval mortality was determined daily by counting the number of infected or non-infected individuals per leaf. The test was repeated twice using 4 replicates. Eggs of uniform age (0-1 –day old age,and 4-day old age) were obtained as described earlier. Pathogenicity was determined by calculating the percentage of infected larvae among the total number of emerged larvae.

Biochemical analysis:-

Sampling of Individuals started 72hr. after they were immersed in the suspensions. Subsequently, samples were collected at random from each treatment as well as from control. Each sample consisted of about 150-200 alive insect that were weighed.

Determination of total protein:-

The insect was immersed in 96% ethyl alcohol and lift 24hr. 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 frigidaire 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 as weighed and homogenized with a mixture of chloroform and methanol to produce a diphasic system, the chloroform layer contained the lipids. This layer was taken in clean dray beaker (weight 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 – Emylaze 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 *B.tabaci* are suceptible to fungus *B.bassiana*. The successful infection by *B.bassiana* was also reported by Bastiaan, (1997) who reported that *B.bassiana* is a fungal pathogen of whiteflies and used it in the management of whiteflies in the greenhouse. Data in table (1) show that the nymphs of *B.tabaci* are susceptible to fungus and the high hazard appeared at the higher concentration than those at the lower concentration. The LC_{50} for the third-instar nymphs was 1.12×10^3 and LC_{90} was 3.8×10^3 spors/ml. [Fig (1)].

Table(1): Suseptibility of 3th instar larvae of *B.tabaci* to the entomopathogenic Fungus *B.bassiana* three days after treatment.

Concentrations	No.of treated Larvae (mean 3 Rep.)	Mort (Mean) (%)	Correct Mort. (%)
7.5×10^3 spores/ml	100	98	96.04
3.75×10^3 spores/ml	70	89	87.22
1.875×10^3 spores/ml	100	72	70.56
0.937×10^3 spores/ml	90	40	39.2
Control	100	2	0.0

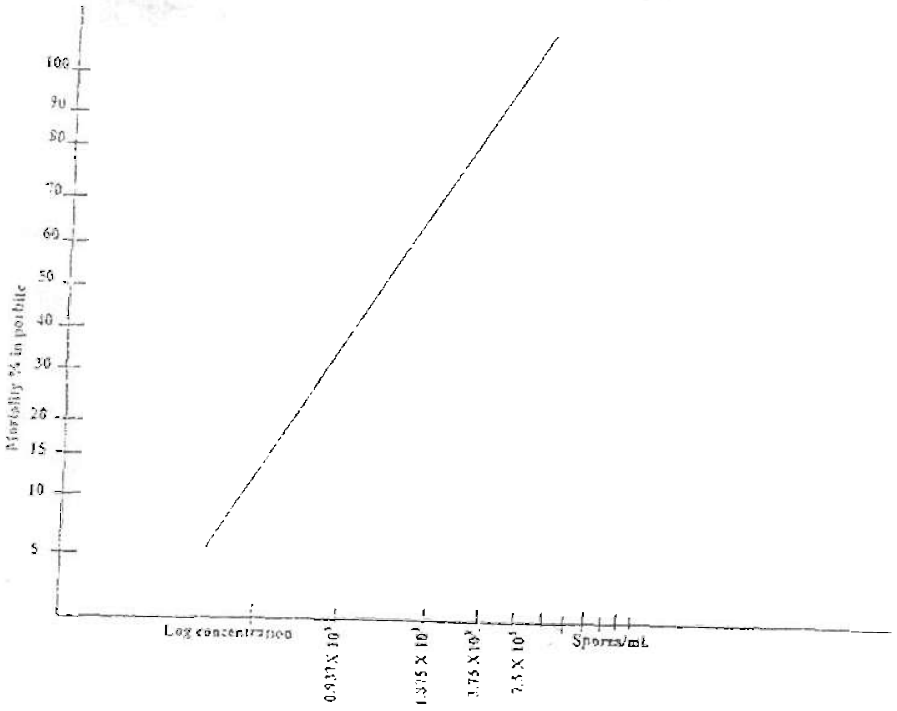
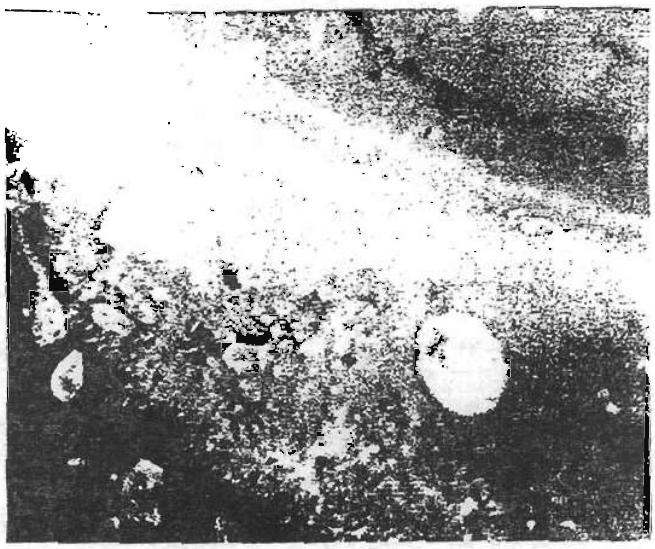


Fig (1): Mortality response among 3rd-nymph instar of *B.tabaci* treated with *B.bassiana*

These data are in agreement with wraight, *et. al.* (1998), who recorded the pathogenicity of *Beauveria bassiana* against the silverleaf whitefly, *Bemisia argentifolii.* The fungi appeared clearly on the treatment dead nymphs after putting in 100% moisture at 25 C° as recommended by Butt & Goettel (2000), [see picture (1)].

Picture (1): nymph instar of B.tabaci infested by *B.bassiana*



Pathogenicity of *B.bassiana* on eggs and hatched nymphs:

Eggs of *B.tabaci* are immune to infection by *B. bassiana* however, in preliminary studies we have noticed that when there is a population consiting of different stages, and eggs are found in the vicinity of infected nymphs or adults, the eggs may become covered with fungal hyphae. Although the chorion of these eggs was not invaded by any of the fungi, the eggs covered with hyphae either did not hatch or hatched with a delay of 3-4 days. The hyphae present on the eggs were found to infect the nymphs immediately upon hatching. 0-1 and 4-day-old eggs were treated with suspension of *B.bassiana* 10^7 spors/ml (Gindin *et al.*,2000)and the mortality of hatching nymphs was recorded (Fig (2)).

Always, first-instar emergence began in 7-8 day old eggs, regardless of the time of treatment, and reached approximately 80-90%. The rate of infection of hatching nymphs was found to depend on the age at which the eggs were treated. The firstinfection of nymphs emerging from treated one-day old eggs were observed after 8 days, and the mortality mean for 3 Replication was 12.33%. And the mortality mean was 21.27% after 10 days of treatment. A significant increase in nymphs mortality was obtained when 4-

day-old eggs were treated. Mortality mean was 9.83% at 6 day, 36.5% at 8 day and 81.5% at 10 days after treatment of 4-day-old eggs. These date indicate that the egg treatment with *B.bassiana* has no effect on egg hatch rate, but does affect the mortality of hatching nymphs.

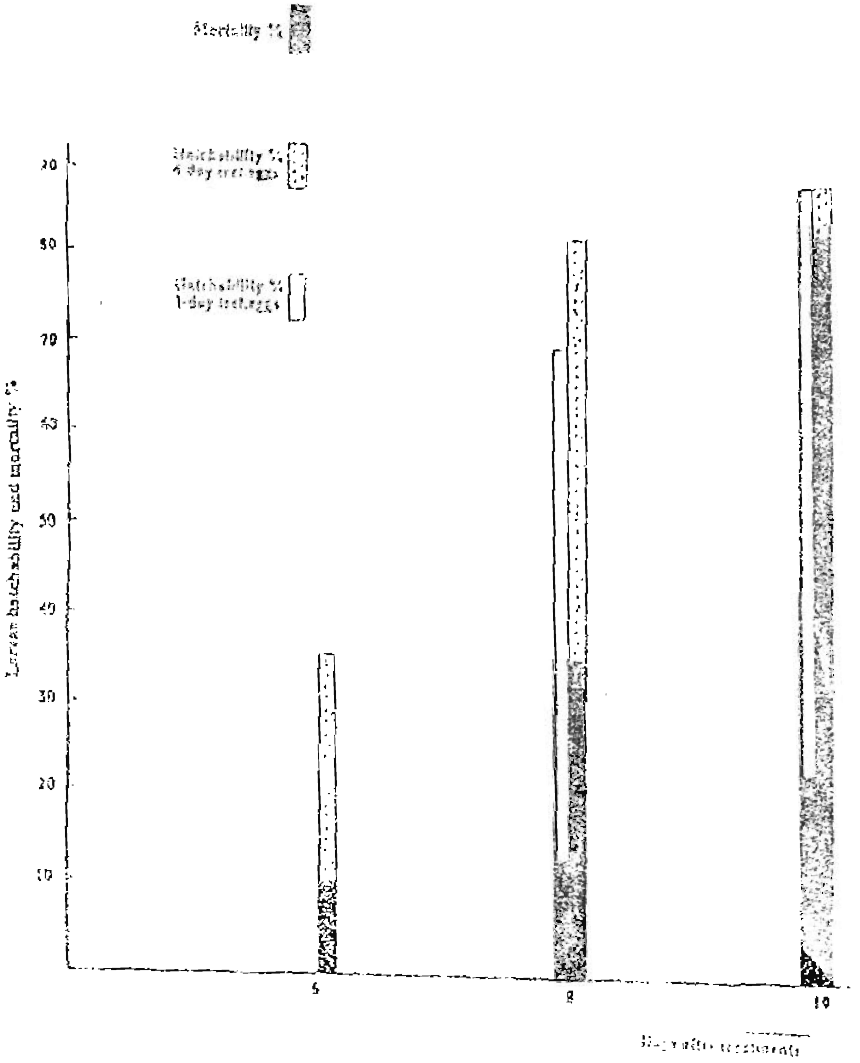


Fig.(2):Pathogenicity of *B. tabaci* eggs and hatched nymphs. Mean percent hatchability and mortality after treatment of 1-and 4-day-old eggs

The rat of infection depends on the time required for nymph emergence after treatment. The treatment of one- 4-day-old eggs caused lower infection of hatching nymphs at the same time after nymph emergence. This fact reflects a decline in efficiency of inoculation with time on the one hand, but on the other, indicates the survival of the inoculum on leaves for at least 8 days. These data was in agreement with Gindin, *et al.*(2000) who studed the pathogenicity of V.*lecanii* to different stages of *B. argentifolii* and proved that the rate of infection depends on the time required for nymph emergence after treatment.

Determination of total protein:

Present date in table (2) indicate the effect of *B.bassiana* on the total soluble protein of B.*tabaci*. 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 1.356 protein per weight (gr.) and control was 2.6858 per gr. The percentage of the decrease than control was 49.512. These data are in agreement with Eman & Sewify, (1991), who recorded a decrease in concentration of the total protein in Aphis insects treated with V.*lecanii*. These data also are in agreement with Gardner *et al.*(1979) and Cheung and Grula (1980), who 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. Also are in agreement with Gabriel, 1968, Kucera, 1980, Ignofoo, 1981 and Brey and Latge 1986, who stated that the ability of fungi to produce extracellular enzymes lead to changes in hacmolymph proteins and amino acids by breaking down proteins bound to chitin and to deterioration of the attached organs. Also this data are in agreement with Jackson *et al.* (1985) who stated that the highly significant quantitative differences in hacmalymph protein and amino acids in Aphis due to 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 are in agreement also with Leger *et al.*(1986) who cleared the potentiality of fungal enzymes to degrade the protein and chitin in locust cuticle.

Table (2): Effect of fungus infection on the total soluble protein of *B.tabaci*.

Replicates	Amounts of total soluble protein mg per gm	Control	Decrease than control %
1	1.465	3.143	-
2	1.39	2.540	-
3	1.27	2.180	-
4	1.30	2.880	-
Mean	1.356	2.6858	49.512

Determination of fat content of treated Insects:

Present data in table (3) indicate the effect of *B.bassiana* on the lipid contents of B.*tabaci* 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 13.353% but in control was 22,8570%. These data indicated that the fungus infected larvae decrease the lipid contents as effect on the metabolism in the treated insects. These data are ingreement 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 conidia of fungi, B.*bassiana*, and this fungus can colonize the haemolymph of clorado beetle larvae, starting in the degradation process, Cermakova and Samsinakova (1960). These results also are in agreement with Jackson et.al,(1985) who referred to the ability of all isolates of V.*lacanii* to degrade lipid and protein by extracellular enzymes in the host. Also data are in agreement with Jagatap, (1973), who stated that the fungus spreads through the blood system, fat bodies, glandular tissues, digestive tract and nervous tissues of the host.

Table (3):Effect of fungus infection on the lipid contents of B.*tabaci*.

Replicates		Sample weight (gm)	Lipid content	Lipids content %
Treatment	1	0.500	0.0245	4.90
	2	0.5276	0.0772	14.632
	3	0.7144	0.1056	14.782
	4	0.530	0.1060	20
	Mean	0.568	0.0783	13.3535
Control	1	0.565	0.110	19.4690
	2	0.5578	0.174	31.194
	3	0.493	0.1004	20.3651
	4	0.500	0.102	20.400
	Mean	0.52895	0.1216	22.8570

Determination of ∝ - amylase enzyme of treated insect:

Data in table (4) indicate the effect of *B.bassiana* on the -amylaze enzyme in the treated insect. The data showed a reduction in the amount of -amylaze of B.*tabaci* insects treated with the fungus *B.bassiana 0.11* than control w 82 1 gm . These results demonstrate that the fungi toxin is an inhibitors of insect digestive enzymes act as growth inhibitors 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 are in agreement with Samsinakova and Misikova, (1973) who examined the degradative enzymes as chitinase, protease, and lipase by fungal strains of diverse origins, in relation to their virulence against greater wax moth. Also these data are in agreement with Gardner *et al.*,(1979) and Cheung and Grula, (1980) who recorded a decrease in certain haemolymph

proteins, aminoacids and carbohydrate in insects infected by the fungi. Data are in agreement also with smith and Grula, (1982) who stated that a wide variety of natural compounds such as glucose, several aminoacids, chitin, starch and falfy acids can be used as carbon and energy source for germination of condia of fungi, B.*bassiana*. Also the data are in agreement with Zacharuk, (1981) who 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.

Table (4):Effect of fungus infection on the ∝- amylase of B.*tabaci*.

Replicates	Amounts of ∝-amylase per gm	Control
1	0.09	1.230
2	0.183	1.243
3	0.067	1.01
4	0.10	1.245
Mean	0.11	1.182

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التأثير الممرض للفطر ***Beauveria bassiana*** على الذبابة البيضاء ***Bemisia tabaci***

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هذه الدراسات أثبتت نجاح الفطر *B.bassiana* في إصابة الذبابة البيضاء *B.tabaci* تم دراسة تأثير الفطر على حوريات عمر ثالث وعلى بيض عمر يوم واحد و أربعة ايام من الوضع. أثبتت الدراسات انخفاض نسبة البروتين والدهون وأيضا أنزيم الأميليز داخل الحشرات المعاملة. وقد وجد مناعة البيض للإصابة بالفطر *B.bassiana* ولكن نسبة موت الحوريات الفاقسة وصل ٨٠-٩٠%. وقد وجد أن نسبة إصابة الحوريات الفاقسة تعتمد على عمر البيض المعامل. وقد سجل للحوريات عمر ثالث LC_{50} ١,١٢×١٠^{٣}، LC_{90} ٣,٨ ×١٠^{٣} كونيديا/ملل. أظهرت النتائج أنه من الممكن استخدام الفطر *B.bassiana* في المكافحة البيولوجية لحشرة *B.tabaci* .