PATHOGENICITY OF THE FUNGUS VERTICILLIUM LECANII TO THE WHITEFLY BEMISIA TABACI(ALEYRODIDAE: HOMOPTERA).

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Abstract: Pathogenicity tests utilizing the Fungus *Verticillium Lecanii* and the whitefly, *Bemisia Tabaci* were done in the laboratory. These tests were conducted on 1- day – old and 2 - day – old of eggs in addition to the 3rd instar nymphs. Eggs were found to be immune to infection but mortality of hatching nymphs reached 80 – 90%. The rate of coming nymphs infection depended on the age in which the eggs were treated. Mortality as LC_{50} was 0.22×10^{7} and recorded for nymphs on the third days after treatment. Analysis of treated insects revealed a reduction in total protein, fat and amylaze enzyme.

Introduction

Whitefly, *Bemesia tabaci (Aleyrodidae :Homoptera)* is one of the most important arthropods pests of greenhouse and field crops, *(Obsorne & Landa, 1992)*. Direct damage occurs due to sucking plant sap from the pheloem and in very heavy infestations leaves drop, maturing of fruits is prohibited and the plant dies. Also the excretion of honeydew cause the growth of mould, inhibiting photosynthesis, changing the crops like cotton which become difficult to process. The insects transmit about 20 viruses *(Wisler et al. 1998)*, and loss in crops, cotton, tobacco, vegetables and ornamentals. The majority of diseases associated with whiteflies are caused by *Bemisia tabaci* and the sweat potato whitefly *(Duffus, 1987)*. Pathogens of Aleyrodidae are restricted to fungi, because they are the only group of organisms that can penetrate the cuticle and so infect these plant – sucking insets *(Fransen, 1990)*.

Mycotal, *verticillium lecanii* strains were first introduced commercially in the U.K. for whitefly on vegetable crops, it can be used in protected crops such as cucumbers, tomatoes, sweet peppers, beans, aubergine, lettuce, ornamentals and cut flowers. V.*lecanii* is a well documented entomopathogen of insect order Homoptera, most commonly aphids, scale insects and whiteflies. In general, *V.lecanii* was the most pathogenic species. Immature whiteflies appeared to be more susceptible to fungal infection than

adult houseflies (*Steenberg & Humber, 1999*). Mycotal dose not kill the eggs of whitefly population, thus the pathogenicity was determined by calculating the percentage of infected nymphs among the total number of hatched nymphs from the treated eggs. (*Gindin, et. al., 2000*). The present study provides information on the pathogenicity of *V lecanii* against *B tabaci*, and some changes in certain biochemical components of infested insects

Materials And Methods

Test Insects:

Bemisia tabaci was reared on tomato plants under controlled conditions in glasshouse at 25±3C° ,50±20% RH and a photoperiod of 16 8 (light : dark). In order to obtain *B.tabaci* of uniform age, 50-100 adults were placed on small tomato plants for 24-36 h. Theu all adults were removed and plants with eggs were transferred to environmental growth chambers for further development of homogeneous populations

The fungus:

The product Mycotal is based on the entomogenous fungns *verticillium lecanii*. It showed very promising results for enhancing the efficacy of the fungus for controling the whiteflies and thrips(*Van Der Pas, et. al.,1998*).We prepared the stok solntion by adding lgr. powder to 1 L of water, and then we prepared 4 concentrations of suspension; (2.3×10^7, 1.15×10^7, 0.575×10^7 and 0.2875×10^7 spores /ml.) in addition to the control.

Bioassay procedure for nymphs and eggs of B.tabaci:

Third instar *B. tabaci* nymphs were used. Individual tomato leaves with uniformly insects were selected for the treatments. Leaf sectors with approximately 50 to 100 insects were used. These leaf pieces bearing 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 leaf sectors were then placed in petri dishes and incubated in growth chambers at alternating temperatures of 25C° (14 h in light) and 20 C° (10 h in the dark). Relative humidity close to 100% was reached by placing the treated leaf sectors on a moist filter paper in each petri dish. For aeriation purposes, each petri dish was opened daily for 25-30 min. This procedure was necessary to a void development of saprophytic fungi on whitefly honeydew. Larval mortality was determined daily by counting the number of infected and non-infected individuals per leaf. The test was repeated twice using 4 replicates Eggs of uniform age (one – day old age, 4- day old age) were obtained as described earlier. Pathogenicity was determined by Calculating the

percentage of infected nymphs among the total number of emerged nymphs

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 nymphs that were weighed.

Determination of total protein:

The nymphs were immersed in 96% ethyl alcohol and left 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 frigdaire until analysis. Total protein content was determined by the method of (Lowry et al. 1951).

Determination of fat content of treated nymphs :

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 of the chloroform layer which contained the lipids. This layer was taken in clean dry 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 the amylaze Enzyme in the treated nymphs :

The enzyme activity was assayed according to Rick and Stegbauer (1974)

Results And Discussion

Pathogenicity of *V . lecanii* on nymphs:

Present data indicate that the nymphs of *B.tabaci* are susceptible to the fungus *V lecanii*. The successful infection by *V lecanii* was also reported for some other homopterous insects such as *Bemisia argentifollii* (Gindin et.al., 2000) 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 0.22×10^{7} and LC_{90} was 0.75×10^{7} spors/ml (Fig.1). These date are in agreement with Cheol-sik Yoon, et. al (1996), who recorded that the LC_{50} of *V lecanii* against the whitfly on tomato plants in the greenhouse was 2.3×10^{6} conidia/ml The fungi appeared clearly on the treatment dead nymphs after putting in 100% moisture at 25C° as recommended by Butt & Goettel (2000), (see picture,(1))

Pathogenicity of *V.Lecanii* on eggs and hatched nymphs:

Eggs of *B.tabaci* are immune to infection by *V.lecanii* 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 after hatching. One- and 4-day-old eggs were treated with suspension of *V .lecanii* 10^7 spors/ ml 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 of the treated eggs. The first infection of the emerging nymphs from the treated one- day old eggs were observed after 8 and 10 days after tratment The mortality mean for 3 replieats was 9,33%. and 18,27% after 8 and 10 days, respectively . A significant increase in nymphs mortality was obtained when 4-day – old eggs were treated. Mortality mean was 8.33% at 6 day, 35% at 8 day and 30% at 10 days after treatment of 4- day- old eggs These date indicate that the egg tretment with *V.lecanii* has no effect on egg hateling rate, but did affect the

Table(1): Susceptibility of 3th instar nymphs of *B.tabaci* ro the entomopathogenic Fungus *V.lecanii* 3 days after treatment.

Concentrations	No of treated Larvae (mean 3 Rep.)	Mort (Mean) (%)	Correct Mort (%)
2.3×10^7 spores/ml	80	83.33	81.66
1.25×10^7 spores/ml	100	82.33	80.68
0.575×10^7 spores/ml	120	67.33	65.98
0.288×10^7 spores/ml	50	57.33	56.18
Control	100	2	0.0

Mortality of hatching nymphs. The rate 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

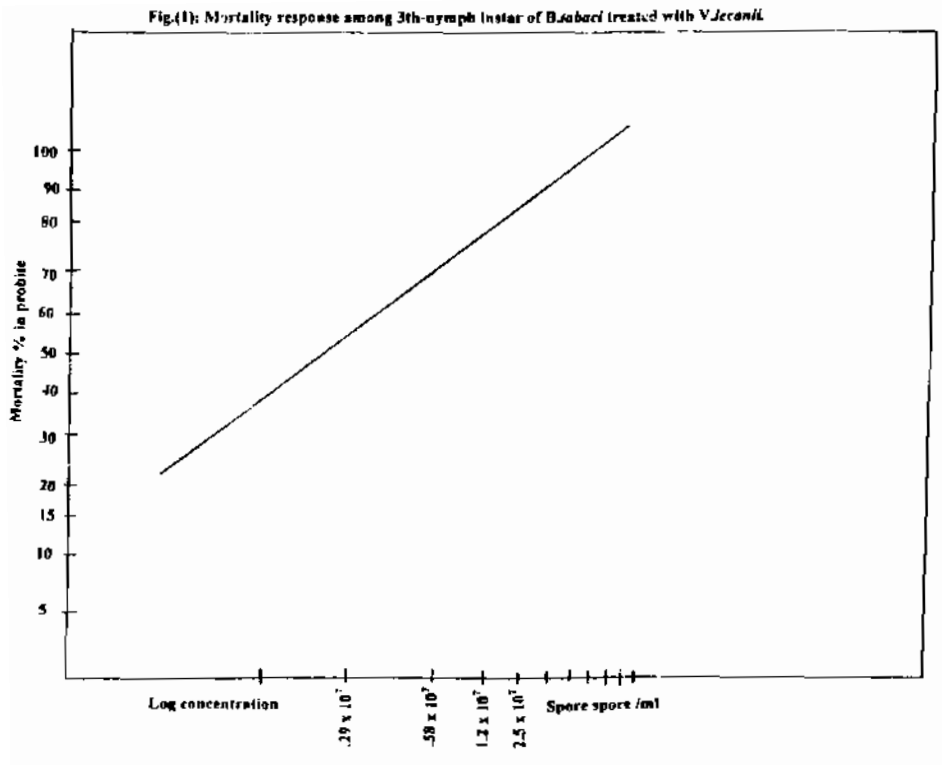
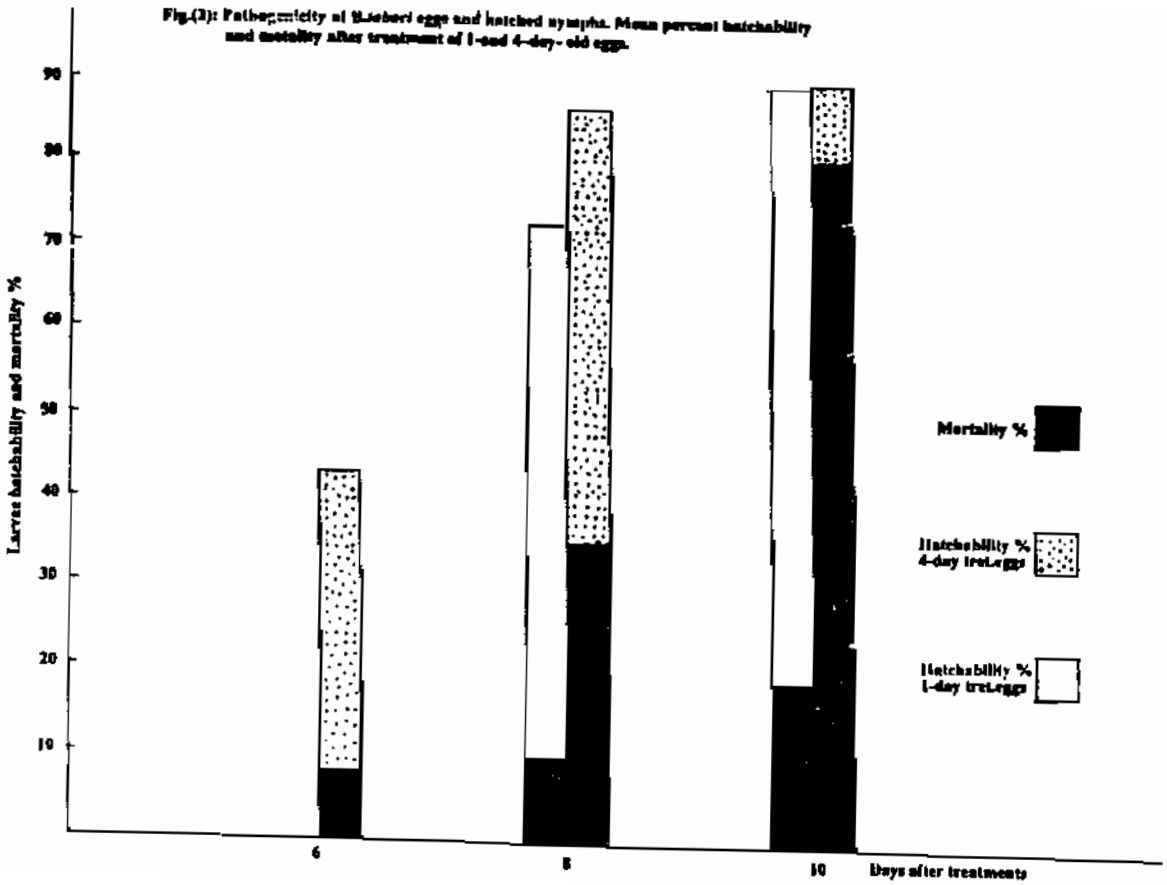


Fig (1): Mortality response among 3th-nymph instar of *B.tabaci* treated with *V.lecanii*

Fig.(3): Pathogenicity of H.zeheri eggs and hatched nymphs. Mean percent hatchability and mortality after treatment of 1-and 4-day- old eggs.



sam 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 *Bemisia argentifolii* and proved that the rate of infection depends on the time required for nymph emergence after treatment.

*** Determination of total Protein:**

Present data in table (2) indicate the effect of *V.lecanii* on the total soluble protein of *B.tabaci* the data revealed that the fungus reduced the amount of soluble protein in the treated nymphs than the control. The mean of the total protein in the treated nymphs was 1.214 Protein per weight (gr.) and control was 2.6858 per gr. The percentage of the decrease than control was 54,799% 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 & 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 haemolymph 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 haemalymph protein and amino acids in Aphis due to the 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 Also These data are in agreement 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 nymphs:

Present data in table (3) indicate the effect of *V.lecanii* on the lipid contents of *B.tabaci* nymphs. The data revealed that the fungus reduced the lipid content in the treated nymphs than the control. The Percentage of the lipids content from 4 replicates of sample treated with fungus was 13,4715% but in control was 22,8570%. These data indicated that the fungus infected the nymphs and decreased the lipid contents by affecting the metabolism of the

treated nymphs. These data are 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 conidia of fungi, *B.bassiana*, and this fung can colonize the haemolymph of clorado beetle larvae, starting in the degradation process, (Cermakova & 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 ststed that the fungi spreads through the blood system, faty bodies, glandular tissues, digestive trac and nervous system of the host.

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

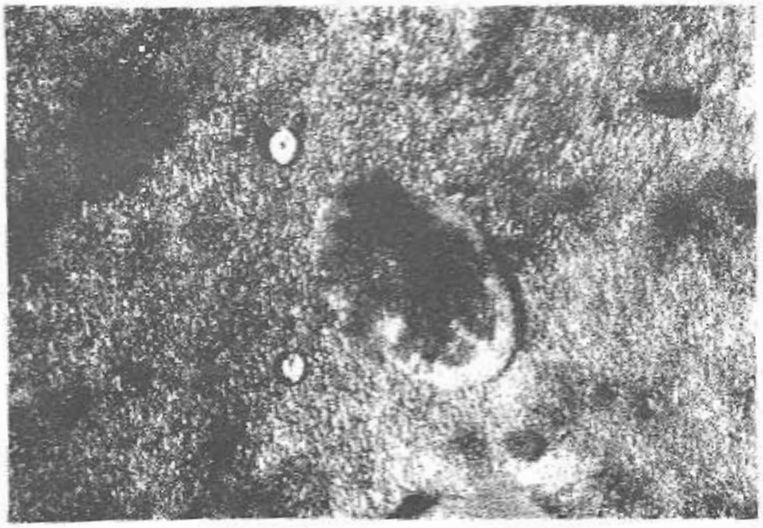
Replicates	Amounts of total soluble protein me per gm	Control	Decrease than control %
1	1,527	3,143	-
2	1,053	2,540	-
3	1,063	2,180	-
4	1,2133	2,880	-
Mean	1,214	2,6858	54,799

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

Replicates		Sample weight (gm)	Lipid content	Lipid content %
Control	1	0,500	0,0942	18,840
	2	0,7663	0,1124	14,668
	3	0,500	0,053	10,6
	4	0,450	0,044	9,778
	Mean	0,5541	0,06398	13,4715
treatment	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

Table (4):Effect of fungus infection on the – amylaze of *B. tabaci*, nymphs

Replicates	Amounts of x-amylaze per gm	Control
1	0.150	1.230
2	0.130	1.243
3	0.100	1.01
4	0.145	1.245
Mean	0.13125	1.182



Picture (1): nymph instar B. tabaci infested *V. lecanii*.

Determination of – amylaze enzyme of treated nymphs:

Data in table (4) indicate the effect of *V.lecanii* on the –amylaze enzyme in the treated nymphs. The date showed a reduction in the amount of –amylaze of *B.tabaci* nymphs treated with the fungus *V .lecanii* than control. These results demonstrate that the fungal toxin is an inhibitor of insect digestive enzymes and act as a 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 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 & Grula, (1980) who recorded a decreasc in certain haemolymph proteins, aminoacids and carbohydrate in insects infected by the fungi. Data were also in agreement with smith and Grula, (1982). they 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.

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اختبارات ممرضة للفطر فيرتيكيلم ليكانى على الذبابة البيضاء

صفاء حسنين على

مركز البحوث الزراعية – معهد وقاية النبات – قسم اختبارات المبيدات على آفات القطن – مصر

اظهرت الدراسة الحالية التاثير الممرض لفطر فيرتيكيلم ليكانى على حشرات الذباب الابيض تحت الاختبارات الممرضة على حوريات العمر الثالث وأيضا على أعمار مختلفة من البيض (عمر يوم ... حتى عمر اربعة أيام).واظهرت النتائج انخفاض فى البروتين الكلى والدهون وانزيم الاميليز فى الحشرات المصابة كان البيض مقاوما للإصابة لكن موت الحوريات الناتجة وصل إلى ٨٠ – ٩٠% وتعتمد نسبة إصابة الحوريات على أعمار البيض وقت المعاملة . سجل الموت فى الحوريات بعد ثلاثة أيام من المعاملة فكانت الـــ LCSO = ٠٢٢, × ١٠^٢ و الـــ LCGO = ٠٧٥, × ١٠^٢.