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PATHOGENICITY OF *VERTICILLIUM LECANII* TO *APHIS CRACCIVORA* (Homoptera: Aphididae)

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ABSTRACT : Research studies were undertaken to investigate the possibility of using *Verticillium Lecanii* as a biological control agent for the control of *Aphis craccivora*. The Present work reported successful infection by *V.Lecanii* to the aphid *Aphis craccivora*. Pathogenicity test as well as the effect on some changes in certain biochemical components of treated nymphs were done. Results indicated that LC_{50} and LC_{90} were 0.85 x 10^7 and 2.7 x 10^7 spores/ml., respectively The tests proved reductions in total protein, fat, and- amylase enzyme.

INTRODUCTION

Aphis craccivora is an important pest causing a great damage to many agricultural crops in the field as well as in glasshouses, such as lentils, alfalfa, clover, beans cowpeas, bandelions, lambsquarters, mustard, and peas. Young adult feed by sucking plant juices. Eggs develop within the mother and nymphs are born live. Within a few days, nymphs mature into reproductive adults and population density can increase very rapidly, (Schreiner, 2000) without mating and colonies consist females. Adult aphids have no wings, but as they become crowded winged forms appear. They are found on growing points as tips, flowers and developing been pods. Cowpea aphids are known to transmit a number of plant virues. Many species of entomopathogens are employed as biological control agents of insect pests in row and glasshouse crops, orchards, ornamentals, range, turf and lawn, stored products and forestry and for abatement of pest and vector insects of veterinary

and medical importance. Using of these microbial agents include safety for humans and other nontarget organisms, reduction of pesticide residues in food, preservation of other natural enemies and increased biodiversity in managed ecosystems, (Lacey, et.al. 2001).

Verticillium lecanii is a well documented entomopathogen of insect order Homoptera, most commonly aphids, scale insects and whiteflies in tropical and subtropical regions. It has been commercialized and applied successfully for years in Europe to control aphids or whiteflies in greenhouse (Obsorne & Landa, 1992). The entomopathogenic fungus, *Verticillium lecanii* was used to control the major aphid pests on chrysanthemums (Hall, 1980) and cotton (Hall, 1982). *V.lecanii* is an microbial insecticide against aphids, scales and thrips insects (Hall, 1981). The pathogenicity and potential of *V.lecanii* as a microbial agent for biocontrol was demonstrated for some whiteflies, aphids and thrips, (Chandler et.al. (1993), , Helyer, (1993) Hsiao et al. (1992). The present study provides information on the pathogenicity of *V.lecanii* against *A.craccivora* and some changes in certain biochemical components of infested insects.

MATERIALS AND METHODS

Test Insects:

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

The fungus:

The product Mycotal is produced from the entomogenous fungus *Verticillium lecanii.* It showed very promising results for enhancing the efficacy of the fungus for control of Aphids, (Humber & Hansen, 2001). The stok solution was prepared by adding 1 gram of the myctoal powder to 1 L. of water, and 4 concentrations of suspension; (2.3×10^7, 1.15×10^7, $.575 \times 10^7$, $.2875 \times 10^7$ spores/ml.) were prepared.

Bioassay procedure for nymphs of *A.craccivora:*

Third instar *A.craccivora* nymphs were used. Individual bean plant with uniformly insects were treated with the 4 concentrations of spore

suspensions and the untreated control. The samples were collected from each treatment and control. 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 fungal 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 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 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 was 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 aclean dry beaker (weighted 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 *V.lecanii* on nymphs:**

Present data indicate that the nymphs of *A.craccivora* are susceptible to the fungus *V.lecanii*. the successful infection by *V.lacanii* was also reported for some other aphids, such as *Aphis gossypii* (Hall, 1982) and *Myzus persicae* (Erkilic, 1992). Also *V.lecanii* provide good biological control against aphids in greenhouse (Greer, 2000). 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 0.85 x 10^7 and LC_{90} was 2.5 x 10^7 spores/ml. (Fig (1)). These data werer in agreement with Hall, (1981) who used concentration of 5 x 10^7 spores/ml of *V.lecanii* to control aphids and scale insects and found that concentration of 10^7-10^8 was 2-3 times faster than the lower concentration of 10^5 spores/ml. The fungi appeared clearly on the dead nymphs after putting under 100% moisture at 20°C as recommended by Butt & Goettel (2000).

Fig.(1) Mortality response of the 3rd instar nymphs of *A.craccivora* to *V.lecanii*

*** Determination of total protein:**

Present date in table (2) indicate the effect of *V.lecanii* 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 1.0798 mg protein per weight (gm.) and control was 2.5493 mg per gm. The percentage of the decrease than control was 57.6433%. These data were in agreement with Eman & Sewify, (1991), they recorded a decrease in the concentration of 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, concluded 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. Jackson et.al. (1985) indicated highly significant quantitative differences in haemalymph protein and amino acids in aphids 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 also 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 insects:**

Present data in table (3) indicate the effect of *V.lecanii* on the lipid contents of *A.craccivora* insects. The data revealed that the fungus reduced the lipid content in the treated insects more than the

control. The percentage of the lipids content from 4 replicates of sample treated with the fungus was 5.0702% but in the control it was 9.5798%. These data indicated that the fungus infected nymph decrease the lipid contents as on effect on the metabolism in the treated insects. These data were in greement with Smith and Grula, (1982), they 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 sources for germination of the conidia of *B.bassiana,* and this fungus can colonise the haemolymph of clorado beetle larvae, starting in the degradation process, Cermakova & Samsinakova, (1960). Also,Jackson et.al (1985) 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. Furthermore,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 *V.lecanii* 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 *V.lecanii* than that of the control. These results demonstrate that the fungal toxin is an inhibitor of insect digestive enzymes and act as 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) who 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 condia of the fungi, *B.bassiana.* Zacharuk, (1981) stated that the degradative changes in insect tissues and organs occure before the fungus hyphal invasion due to certain metabolites of fungal origin that are mainly toxic substances.

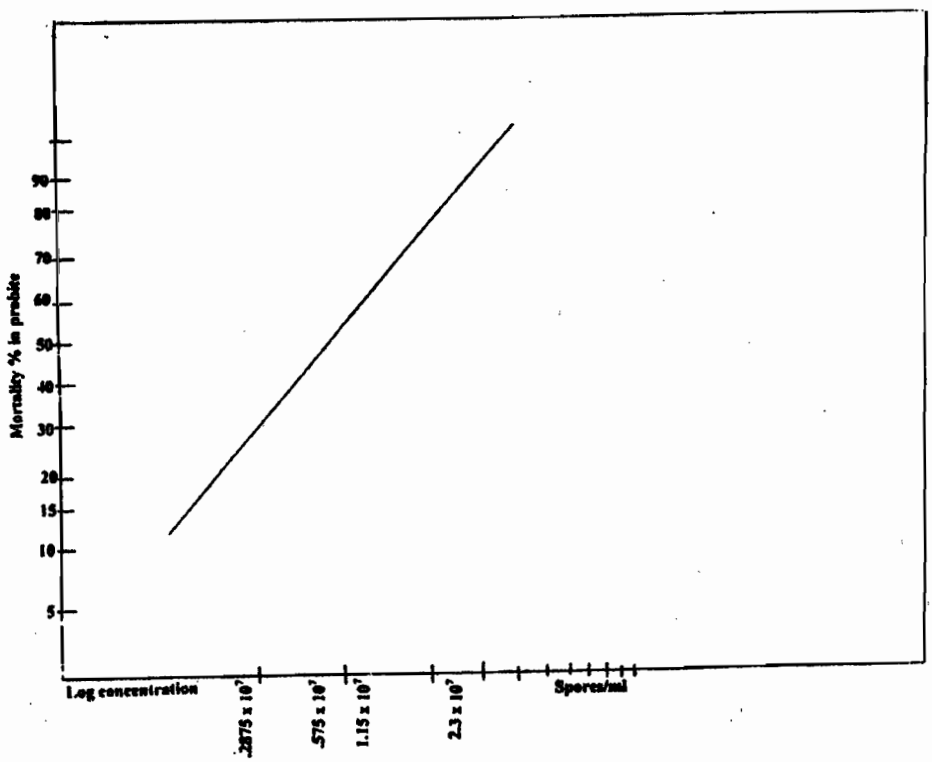

Fig. (1) Mortality response of the 3rd instar nymphs of *A. cracclvora to V. lecanii*

Table. (1) Suseptibility of 3rd instar larvae of A.craccivora to the entomopathogenic fungus *V.lecanii* 3 days after treatment.

Concentrations	No. of treated nymph (mean 3 Reps.)	Mort (Mean) (%)	Correct Mort. (%)
2.3 x 10^7 spores/ml	85	86	84.796
1.15 x 10^7 spores/ml	70	44.3	43.68
.575 x 10^7 spores/ml	68	32.5	32.45
.2875 x 10^7 spores/ml	50	17	16.762
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 per gm	Control	Decrease than control %
1	0.818	2.86	-
2	1.253	2.48	-
3	1.287	2.077	-
4	0.961	2.78	-
Mean	1.0798	2.5493	57.6433

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

Replicates		Sample weight (gm)	Lipid content	Lipids content %
Treatment	1	0.5972	0.036	6.0281
	2	0.50	0.031	6.2
	3	0.556	0.024	4.3166
	4	0.455	0.017	3.736
	Mean	0.5271	0.027	5.0702
Control	1	0.7012	0.0584	8.329
	2	0.573	0.063	10.995
	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	0.12	0.403
2	0.24	0.51
3	0.243	0.407
4	0.257	0.477
Mean	0.2150	0.4493

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التأثير المرضي للفطر *Verticillium Lecanii* على *Aphis craccivora*

صفاء حسنين علي

قسم إختبارات مبيدات آفات القطن – معهد بحوث وقاية النبات
مركز البحوث الزراعية

هـدف هذه الدراسة هو تأكيد أنه من الممكن استخدام الفطر *V.lecanii* في المكافحة البيولوجية لحشرة *Aphis craccivora* بنجاح.

أجريت تجارب باستخدام الفطر *V.lecanii* على حشرة *A.craccivora* وقـد أثبتـت التجارب أن الفطر قد نجح في إصابة طور الحوريات وكانت قيمة التركيز النصفى القاتل (٠,٨٥ ×١٠^{٧}) والتركيز القاتل بنسبة ٩٠% هو ٢,٧ × ١٠^{٧} كونيديا/مل. وقد أثبتت التجارب انخفاض في نسبة البروتين والدهون وأنزيم الأميليز في الحشرات المعاملة بالفطر عن الحشرات الغير معاملة بالفطر.