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TOXICITY OF *BACILLUS THURINGIENSIS* STRAINS TO THE COTTON APHID, *APHIS GOSSYPII* (HOMOPTERA : APHIDIDAE)

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Abstract: Mortality bioassays were used to investigate the toxicity of *Bacillus thuringiensis* strains against cotton aphids, *Aphis gossypii*. These strains were isolated from samples collected from different localities of Pakistan. Of the total isolates screened, nine strains showed activity against cotton aphids. Two isolates viz., Fic 16.15 and Ins 2.13 gave the lowest LC_{50} values.

Key words: *Aphis gossypii, Bacillus thuringiensis*, bio-insecticide, toxicity.

INTRODUCTION

The cotton aphid, *Aphis gossypii* Glov. is a significant pest of cotton in Pakistan (Mohyuddin, 1985). Both adults and nymph imbibe liquid from host plant tissues by probing inter- and intracellularly with their maxillary and mandibular stylets (Pollard, 1973). These results in stunted growth, curled leaves and death of small plants. Heavy infestation on cotton later in the season caused shedding of curled leaves and premature opening of the bolls, thus reducing the yield and grade of lint. Black sooty mould develops on honey dew excreted by both adults and nymphs which makes cotton sticky and stained (Roy and Behvra, 1983).

The only control method available for the aphids is the foliar spray of insecticides. However, the development of resistance to chemical insecticides by many pest insects and general concern for environmental damage have shifted the trend towards biological control agents.

Subspecies of *Bacillus thuringiensis* produce insecticidal, proteinaceous and parasporal protoxins that have specific activity spectrum (Feitelson *et al.*, 1992). The inactive protoxins in the larval midgut are activated to the insecticidal toxins by solubilization in the high pH (ca 10.5) and cleavage by the specific proteases. Since *B. thuringiensis* is insecticidal, there has been great interest in its use for the control of agricultural pests and human disease vectors (Dulmage, 1981). The main objective of the present study was to evaluate the toxicity of the *B. thuringiensis* strains on the cotton aphids and to determine survival of the insects eater intoxication.

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MATERIALS AND METHODS

Bacillus thuringiensis subspecies were obtained from CAMB culture collection and grown in G. medium (pH 7.0), containing 0.2 g $MgSO_4$, 0.6 g K_2HPO_4, 0.4 g KH_2PO_4, 2 g $(NH_4)_2SO_4$, 0.08 g $CaCl_2$, 2 g yeast extract, 1 g glucose, 0.4 g caseamino acid, 10 ml Tris HCl (1M, pH 7.6) and 2.5 ml each of 1% $ZnSO_4$, 1% $CuSo_4$, 10% $MnSO_4$ and 0.04% $FeSO_4$ per litre.

All cultures were grown at 30°C with shaking (150 rpm) for three days. After three days, the bacterial culture consisting of vegetative cells, sporangia, spores and crystals were harvested by centrifugation (7,000 K, 10 min, 4°C). The pellets were washed with 0.5M NaCl, 10mM EDTA and twice with distilled H_2O. Pellets were resuspended in solubilizing buffer (50mM sodium carbonate and 10mM Dithiothretol, pH 10) and placed at 37°C for 24 h. The suspension was centrifuged (7,000 K, 10 min, 4°C) and supernatant was assayed for total protein using Bio-Rad protein assay method with bovine serum albumin as the standard. The protoxin (20 μg) was digested with 1 μg trypsin at pH 7.0, 30°C for three hours. The toxins were dialysed against distilled water (pH 7.0), using filter paper of 0.025 μm.

Bioassays were carried out on adult aphids by feeding them different dilutions of activated toxins in 18% sucrose solution of pH 7.5-8.0 (Auclair, 1965; Walters *et al.*, 1970). The diet was presented to aphids in a stretched parafilm sachets. The sachet was made on the upper rim of small plastic tube. The base of the tube had a hole covered with paper for aeration. Bioassays were performed in triplicate samples of 45 aphids per dose. The concentrations of toxin proteins were 32, 62, 125 and 250 μg/ml. The tests were repeated many times for each toxic strain. Survivorship was monitored daily by viewing through the sides of tube. The median lethal dose (LD_{50}) for each test strain was calculated after 48 hrs of toxin ingestion by probit analysis (Raymond, 1985).

RESULTS

A total of 100 *B. thuringiensis* strains, isolated from different sources and areas of Pakistan were assayed. The majority (91%) of the isolates were non-toxic to aphids. The remainder showed varying level of toxicity in the screening test. The strains of *B. thuringiensis* which caused 50% mortality in aphid population at concentration less than 300 μg toxin protein/ml were considered toxic. In total, nine strains of *B. thuringiensis*, isolated from soil, animal and bird droppings, wheat dust, pulse dust and dead insects were sufficiently toxic (Table I).

Among the active strains, Fic 16.15 showed the lowest and SHD 36.2 showed the highest LD_{50} values. The rank order of toxicity for the different strains is given in Table I. Among the toxic strains Fic 16.15 and INS 2.13 displayed the steepest slope.

All active strains, studied during the present experiments, showed a significant increase in mortality rate with the increase of dosage and exposure time of aphid population against toxin proteins. The mortality of non-toxic isolates was not significantly different from the mortality of the control aphids.

DISCUSSION

The discovery of *B. thuringiensis* isolates with toxic activity against different insect and pests (Dulmage, 1981) has initiated an interest for the search of strains which show susceptibility for cotton aphids (*Aphis gossypii*). The results of this study showed that nine *B. thuringiensis* isolates had variable level of toxicity for cotton aphids. In nature, these strains were not available to aphids that's why they did not take active part in their population control. In laboratory their toxic gene will be isolated and cloned in bacteria or plant or formulation will be prepared for spray. The strains Fic 16.15, Ins 2.13 and Fic 5.2A based on low LD_{50} values, were most active against aphids.

Table I: Median lethal concentration (LC_{50}, μg protein/ml) of *Bacillus thuringiensis* strains against cotton aphids, *Aphis gossypii*, exposed for three days.

STRAINS	SOURCE OF MATERIAL	LC_{50}	SLOPE	RELATIVE SENSITIVITY
Fic 16.15	Bird dropping	46	1.57±0.90	1.0
Ins 2.13	Dead insect	61	1.67±0.81	1.3
Fic 5.2A	Animal dropping	101	0.30±1.02	2.2
Gu 9.1	Pulse dust	113	0.94±0.86	2.5
Fic 11.2	Soil	139	1.48±0.71	3.0
CHT 17.6	Soil	140	0.77±1.17	3.0
Fic 3.16	Bird dropping	203	0.96±0.76	4.4
Hfz 24.8	Wheat dust	210	2.46±0.47	4.6
Shd 36.2	Bird dropping	296	1.87±0.55	6.4

Note: Mortality counted after 48 h.

a: LC_{50}/LC_{50} of Fic 16.5

The aphids feed on artificial diet at an estimated rate of 20 nl/h (Wright *et al.*, 1985). Assuming constant feeding an aphid took up approximately 1 μl of toxin solution in the present study. The toxin proteins required for 50% mortality of population ranged from 21 to 135 ng. These doses are higher than the activated toxin required by Lepidoptera and Coleoptera for the 50% mortality of population. It may be due to difference in food conservation time in gut of these insects. Since aphid rapidly excrete large volume of their liquid diet after ingestion, therefore midgut residence time of imbibed toxin may be low (Walters and English, 1995). At present, the activation process within the aphid gut is unknown. Aphid may have rapid brush border membrane repair system to avoid destruction induced by delta endotoxin on *B. thuringiensis* (Percy and Fast, 1983; Bauer and Pankratz, 1992).

This further suggest that these strains, mentioned in Table I, are more toxic than other strains and their effect on membrane of gut at the LD_{50} concentration is irreversible.

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