ESTIMATION OF INSECTICIDAL CAPABILITY OF BACILLUS THURINGIENSIS (SOTTO) AGAINST PINK BOLLWORM PECTINOSPHORA GOSSYPIELLA (SAUNDERS)

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SUMMARY: The bioassay on pink bollworm Pectinophora gassypiella (Saunders) was carried out using δ - endotoxin or purified crystals of an indigenous isolate of bacillus thuringiensis sotto. The crystals were obtained by two phase liq/liq separation technique. LD 50 of the target insect was determined to be 30 µg/ml of purified δ - endotoxin per milliliter of the diet.

Key Words: Insecticidal activity of indinous isolate.

INTRODUCTION

The indiscriminate use of chemical insecticides has created a serious problem. Pests develop resistance to the chemicals and at times their accumulation in food substance causes food poisoning Steinhaus 1949. Proposed that microorganisms can be used to control insects. Hannay (4) discovered extra sporal inclusion body; (δ - endotoxin) attention was paid to its nature and significance (3-8). since then δ - endotoxin is used as a potent microbial insecticide.

The objective of the present work is to determine the insecticidal activity of δ - endotoxin produced by the local/indigenous isolate of *bacillus thuringiensis* Sotto against the neonatal larvae of pink bollworm *Pectinosphora gossypiella*.

MATERIALS AND METHODS

Insecticidal capability of locally isolated strain of *Bacillus thuringiensis* sotto was carried out the purified crystals were isolated by the method of Sheikh *et al.* (10). Serological typing was done at Institue Pasteur Paris by Dr. H de berjae. *Pectinophora gossypiella* (Saunders) was used as a test insect.

Preparation of toxin doses

The purified crystals of B. t. sotto were obtained by 2 phase liquid/liquid separation technique. Sheikh *et al.* 1978, the resulting material which was in powdered form was stored in sterile vials at 4°C. The dilutions of δ - endotoxin were made in sterile distilled water and mixed with the artificial diet.

Infestation

The newly hatched larvae of *P. gossypiella* with the help of a fine camel hair brudh were transferred to each vial containing a mixture of diet and purified crystals and incubated for 48 days, observations were carried out when the larvae in control start pupting. Then the percentage of live and dead were calculated (Table 1). For 4 to 5 days the vials were incubated in total darkness because neonatal larvae are photosensitive.

Rearing of the test insect

Different larval stages of pink bollworm *Pectinophora gossypiellla* (Saunders) were collected from different agricultural areas of sind. The insects were reared on a mass scale. The diet formula of Vanderrzant Adkision wheat germ diet has been modified by replacing wheat germ with Lobia bean along with an addition of vitamin E powder, Sheikh *et al.* (10).

Controls

Controls using sterile distilled water as the only additive to the artificial diet were run parallel to every set of experiment.

Filling of Vials

Small homeopathic vials were used for conducting bioassay on *Pectinophora gossypiella*. A batch of 100 vials was used for each test and control experiment.

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Table 1

Conc. of toxin (δ-endotoxin)	Average mortality in percentage	Mortality in control	Net. mortality percentage after correction
10 μg/ml	76.9 %	45.4 %	31.6 %
20 μg/ml	88.1 %	45.4 %	42.8 %
30 μg/ml	78.0 %	24.6 %	53.4 %
40 μg/ml	87.2 %	24.6 %	62.6 %
50 μg/ml	84.6 %	13.8 %	70.8 %
60 μg/ml	88.6 %	13.8 %	74.6 %

RESULTS AND DISCUSSIONS

The bioassay on first instar larvae of pink bollworm *Pectinosphora gossypiella* (Saunders) using purified δ -endotoxin of B. t. sotto was carried out in concentrations range from 10 μg/ml to 60 μg/ml diet for 48 days until 10% pupation was achieved in control batches. Since each set of experiment (100 vials) was repeated three time. Therefore average mortality percentage for each concentration of toxin was calculated from the total number of dead larvae. The average mortality rate of each concentration of toxin was compared with the average mortality percentage of its control batch. After subtraction this gave the mortality percentage of different concentration of toxins (Table 1, Figure 1).

The data presented in the Table 1 indicated that every 10 μg/ml increase in the concentration of toxin from 10 μg/ml to 50 μg/ml resulted in 10% gradual increase in mortality rate of larvae. From 50-60 μg/ml concentrations of toxin only 4% increase in mortality rate was discovered. These observations lead to the conclusion that the saturation point of the toxin may have been attained and further increase in the toxin concentration may not contribute to still elevated rate of mortality, 50% mortality was observed at 30 μg/ml of the toxin. Thus the LD 50 for *Pectinophora gossypiella* utilizing purified δ -endotoxin (crystals) of *bacillus thrungiensis* Sotto was determined as 30 μg/ml of diet.

The present results confirm the studies of Dulmage (12) that as the concentration of the toxin in the diet were increased, it sharply affected the growth and development of larvae. Hence the retardation on development and mortality are directly related to the amount of toxin present. Ignoffo and Graham (13) also observed marked reduction in the population of the pink bollworm *Pectinophora gossypiella* with high dosage of *B. thuringiensis*. Similarly Bullock and Dulmage (14) achieved good control of *P. gossypiella* by the application of *B. thuringiensis*.

The graph plotted with mortality rate against toxin concentration clearly shows a rise in mortality with the increase in toxin concentration (Plate 1).

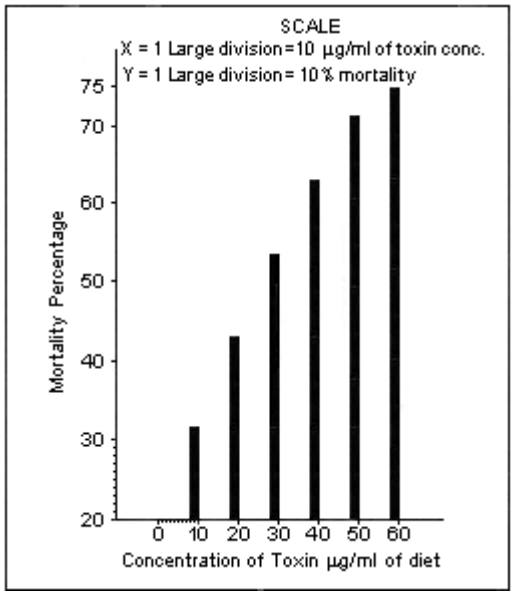

Plate 1: Determination of lethal activity of the crystal (endotoxin) with *Pectinophora gossypiella*.

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