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COMPARISON OF INSECTICIDAL CAPABILITY OF LOCALLY ISOLATED *B. THURINGIENSIS* (KURSTAKI) WITH STANDARD AMERICAN STRAINS AGAINST CORN EARWORM *HELIOTHIS ARMIGERA* (HUBN)

M. Rafi Shaikh, Dilnawaz Shaikh,* Baqar Naqvi

Department of Microbiology, University of Karachi, Karachi-32

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The insecticidal capability of locally isolated *B. thuringiensis* (Kurstaki) 448 was compared with USDA strains HD 241, HD 244 using newly hatched larvae of (Corn Earworm) Heliothis armigera (Hubn); in local isolate 50% mortality was achieved at a concentration of 11.25 μg/ml, while in case of the American strains it was between 5.6 to 0.75 μg/ml. With the standard HD-1-S 1980 it was noted to be 41.5% to 56.0%. The test material consisted of 60 μg/ml to 3.75 μg/ml of live spores and δ-endotoxin crystals.

Key words: Insecticidal activity of *Bacillus thuringiensis.*

INTRODUCTION

Bacillus thuringiensis is well known for its insecticidal capabilities [1-14]. Commercial preparations based on spores and/or crystals of this bacterium are used to control insects in many countries [15]. Search for new promising strains is in progress in many countries; Shaikh *et al.* [16] isolated a new Pakistani variety belonging to serotype XIII. Some of the isolates belonging to other groups demonstrated marked insecticidal activity. The present study describes results of and comparison with standard USDA strains against the target insect.

MATERIAL AND METHODS

Culture strains. Local isolate: *B. thuringiensis* 448 (Kurstaki) serotype (3 ab) was isolated by the method of Shaikh *et al.* [16]. Serological typing was done at the Institute Pasteur Paris by Dr. H. de Barjac.

USDA Strains. The American strains were supplied by the United States Department of Agriculture, Cotton Insects Research Station, Brownsville, Taxas. All cultures were maintained on nutrient agar (E. Merck) slopes.

Preparation of toxic mixture. An overnight nutrient broth (E. Merck) culture of each strain was inoculated (4 ml quantities) into sterile Roux bottles, the two sides of which were previously coated with a layer of nutrient agar medium. Inoculated bottles were incubated at 30° for 48 hours. Harvesting of bacterial growth was done in sterile distilled water. Harvested material was washed twice at 3000g. with distilled water; the sediment was suspended in 50 ml of xylene (E. Merck). The volatile material was evaporated in an incubator at 37°. Dry powder was scraped off and stored in stoppered bottles in a cool dry place.

Rearing of test insect. Different larval stages of *Heliothis armigera* (Hubn) were collected in agricultural areas of Sind. The adults were identified by USDA labs. at Brownsville and Maryland USA. The insect was reared on mass scale over a diet developed in our labs. The ingredients and quantities used in the preparation of diet are tap water (2000 ml), powdered agar (50 g) bean powder (*Vigna unguiculata* 400 g), ascorbic acid (11 g), sorbic acid (3 g), dried active yeast (baking granules 20 g), methyl-para hydroxy-benezoate (7 g), formal-dehyde 10% (6 ml) and vitamin mixture (4 ml).

Buffer solution. 26 g of sodium dihydrogen phosphate were dissolved in 1 litre of distilled water (Sol. A), and 18 grams disodium hydrogen phosphate were dissolved in 1 litre of distilled water (Sol. B). 35 ml. of Sol. A was mixed with 65 ml of solution B to give a pH of 7.00. This buffer solution having a molarity of 0.17 was used throughout in making dilutions of the toxin.

60 mg of dry toxic mixture was suspended in 100 ml of buffer solution (pH 7.00) and subsequent dilutions (60 μg to 3.75 μg/ml) were prepared.

Infestation of Larvae. With the help of a fine camel hair brush a single newly hatched (neonatal) larva of *Heliothis armigera* (Hubn) was transfered into each vial. The vials were then tightly plugged with cotton wool and incubated for seven days. After seven days of incubation when the larvae in control started pupating, the percentage of live and dead larvae were calculated.

* Department of Pharmaceutics, Faculty of Pharmacy, University of Karachi.

RESULTS AND DISCUSSION

Bioassays using spore-crystal (δ-endotoxin) complex of different strains in concentration ranging from 60 μg/ml to 3.75 μg/ml diet were carried out for seven days on 25 first stage larvae of *Heliothis armigera* (Hubn). The experiment was performed in triplicate using HD-1-S-1980 as standard strain. At the end of incubation time, average and net mortality was recorded (Table 1, 2, 3) and Fig 1, 2, & 3.

Data presented in Tables 1,2,3 indicated that at the dilution (7.5 μg/ml) net mortality caused by strain HD-448 HD-241 & HD-244 were 34.8%, 60% and 66.8% respectively while the net mortality rate in case of HD-1-S-1980 varied from 41.6% to 56.0%. At the highest concentration (60 μg/ml) HD-241 showed 96% mortality, HD-244 indi-

Table 1. Mortality rate of *Heliothis armigera* (Hubn) of three replicate experiment using nine serial dilution of spore-endotoxin complex of HD-244 with standard HD-1-S-1980* (USDA Method).

Conc. of toxin μg/ml	Average mortality (%)			Net mortality**(%)	
	HD-244	HD-1-S-1980	Control	HD-244	HD-1-S-1980
3.75	50.4	40.0	2.4	48.0	37.6
5.625	50.4	36.0	2.4	48.0	33.6
7.5	69.2	44.0	2.4	66.8	41.6
11.25	70.8	61.2	2.4	68.4	58.8
15.0	77.2	57.2	2.4	74.8	54.8
22.5	78.8	81.2	2.4	76.4	78.8
30.0	84.0	76.0	2.4	81.6	73.6
45.0	92.0	89.2	2.4	89.6	86.8
60.0	97.2	92.0	2.4	94.8	89.6

* Results were compiled from three replicates.
** Net mortality percentage was calculated after correcting the values, viz a viz deaths in control.

Table 2. Mortality rate of *Heliothis armigera* (Hubn) of three replicate experiments using the nine serial dilutions of spore-endotoxin complex of indigenous strain Bt-448 with standard HD-1-S-1980*.

Conc. of toxin μg/ml.	Average mortality (%)			Net mortality** (%)	
	Bt-448	HD-1-S-1980	Control	Bt-448	HD-1-S-1980
3.75	25.2	26.4	1.2	24.0	25.2
5.625	33.2	40.0	1.2	32.0	38.8
7.5	36.0	57.2	1.2	34.8	56.0
11.25	53.2	58.4	1.2	52.0	57.2
15.0	64.0	62.4	1.2	62.8	61.2
22.5	72.0	73.2	1.2	70.8	72.0
30.0	77.2	77.2	1.2	76.2	76.0
45.0	93.2	89.2	1.2	92.0	88.0
60.0	97.2	92.0	1.2	96.0	90.8

* Results were compiled from three replicates.
** Net mortality percentages was calculated after correcting the values viz a viz deaths in control.

Table 3. Mortality rate of *H. armigera* (Hubn) of three replicate experiments using seven serial dilution of spore-δ-endotoxin compled of HD-241 with standard HD-1-S-1980* (USDA method).

Conc. of toxin μg/ml.	Average mortality (%)			Net mortality**(%)	
	HD-241	HD-1-S-1980	Control	HD-241	HD-1-S-1980
7.5	62.5	53.2	2.4	60.0	50.8
11.25	72.0	57.2	2.4	69.6	54.8
15.0	72.0	69.2	2.4	69.6	66.8
22.5	78.4	72.0	2.4	76.0	69.6
30.0	94.4	82.4	2.4	92.0	80.0
45	97.2	84.0	2.4	94.8	81.6
60	98.4	94.4	2.4	96.0	92.0

* Result were compiled from three replicates.

** Net mortality percentage was calculated after correcting the values viz a viz deaths in control.

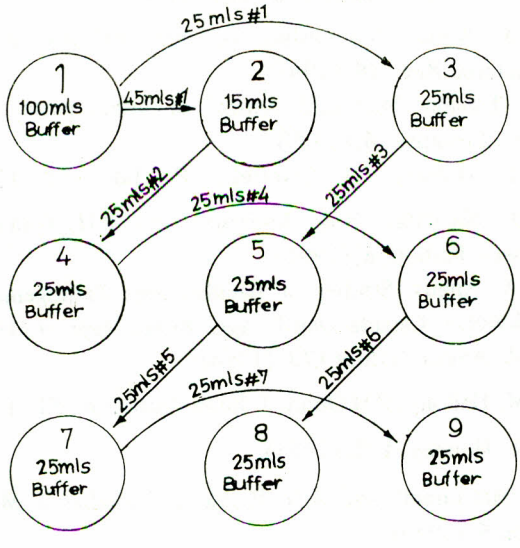

Fig. 1. Diagrammatic representation of the preparation of nine serial dilution of spore-crystal complex.

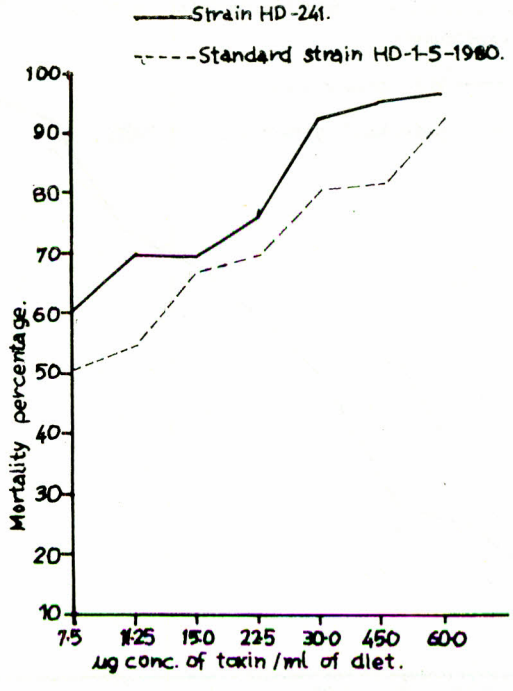

Fig. 2: Lethal activity of *Bacillus thuringiensis*-δ-endotoxin spore complex of USDA strain HD-241 and U.S. standard strain HD-1-S-1980 against *Heliothis armigera* (Hubn). Mortalities at various conc. of toxins were based on 7 days bioassays, compiled 3 replicated experiments.

cated 94.8% of insecticidal activity, Bt. 448 exhibited 96% mortality, while in case of standard strain HD-1-S-1980 it varied from 89.6% to 92.0%. It can be concluded from the results that the strain HD-241 was highly effective against the test strain in high dose of 60 μg/ml concentration and was the most potent one. Strain HD-244 was also found to be effective but less than HD-241 whereas the indigenous strain Bt-448 was found to be highly effective at a high concentration but not at a low concentration. The LD_{50} observed in case of Bt-448 was close to 11.25 μg/ml, whereas in case of HD-244 and HD-241, it was between 5.6 to 7.5 μm/l.

These results are in conformity with H.T. Dulmage [17], as the concentration of toxin in the diet was increased to more toxic levels it sharply effected the rate of mortality. Present results also confirm the studies of Beegle

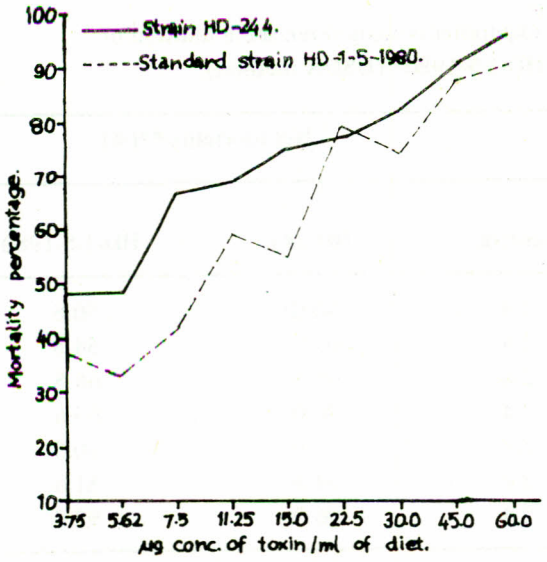

Fig. 3. Determination of lethal activity of spore-δ-endotoxin complex of USDA strain HD-244 and standard strain HD-1-S-1980 against *Heliothis armigera* (Hubn). Mortalities at various conc. of toxin were based on 7 days bioassays and on average of three replicated experiments.

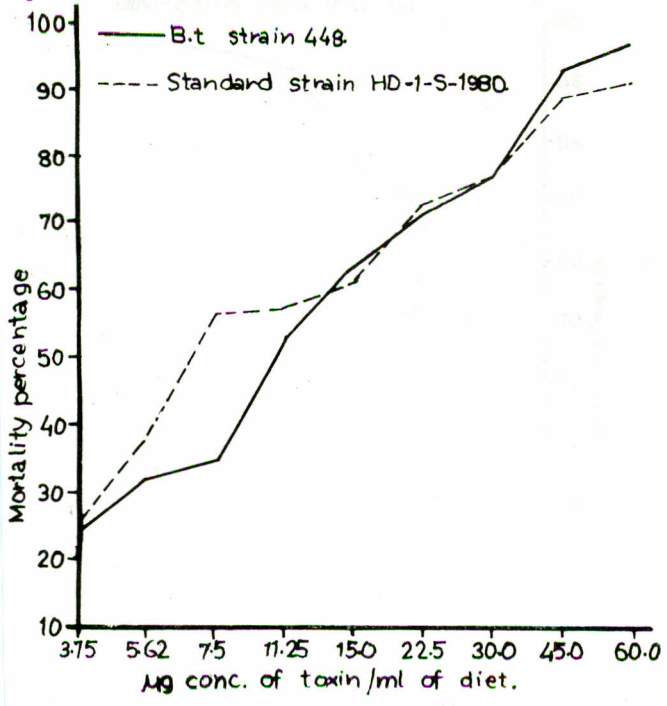

Fig. 4: Toxicity of *Bacillus thuringiensis*-δ-endotoxin spore complex of indigenous strain B. t-448 and U.S. Standard Hd-1-S-1980 against *Heliothis armigera* (Hubn). Mortality results at different conc. levels of toxins were based on 7 days bioassays of three replicated experiments.

carried out at Cotton Insect Research Unit, Brownsville Texas, USA (Personal Communication).

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