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Efficacy of *Bacillus thuringiensis* Berliner var. *kurstaki* and var. *morrisoni* against *Helicoverpa armigera* (Hübner)

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ABSTRACT: **Comparative studies on the efficacy of *Bacillus thuringiensis* Berliner var. *kurstaki* (HD-1) and *morrisoni* (HD-12), when used alone against second instar larvae of *Helicoverpa armigera* (Hübner) revealed that the strain *kurstaki* (HD-1) was more toxic than *morrisoni* (HD-12). Strain *kurstaki* (HD-1) could cause 79.38 per cent mortality of second instar larvae of *H. armigera at* 0.00963 per cent concentration, while *morrisoni* (HD-12) could cause 89.66 per cent mortality at 0.0576 per cent concentration. The LC_{50} value of HD-1 was 0.0055, whereas that of HD-12 was 0.02%.**

KEY WORDS: *Bacillus thuringiensis* var. *kurstaki, morrisoni, Helicoverpa armigera*

INTRODUCTION

Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) a polyphagous pest has attained the status of International pest in the recent years due to severe damage caused to several crops. The management of this pest has become difficult as it has developed resistance to different insecticides (Mehrotra and Phokela, 1992; Lande and Sarode, 1995). In view of this, efforts are being made to evolve alternate strategies for the management of this pest. Investigations around the world have indicated the usefulness of *Bacillus thuringiensis* to combat the menace of insect pests. *Bacillus thuringiensis kurstaki* has been found to be the most effective against early second instar larvae of *Helicoverpa armigera* (Brownbridge and Onyango, 1992). However, input cost is a limiting factor in the use of this bacterium. Therefore, in order to evolve economically viable treatment, the investigations were undertaken to evaluate the efficacy of *Bacillus thuringiensis* Berliner var. *kurstaki* and var. *morrisoni* individually against *H. armigera.*

MATERIALS AND METHODS

The present experiment was conducted to study the efficacy of different concentrations of *Bacillus thuringiensis* var. *kurstaki* and var. *morrisoni* against *H. armigera* during the year 1997-98. *B. thuringiensis* var. *kurstaki* (HD-1) was obtained from the Biotechnology Centre of Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, while *B. thuringiensis* var. *morrisoni* (HD-12) was procured from the National Research Centre on Plant Biotechnology, IARI, New Delhi. *Bt* strains were cultured and multiplied in Luria agar media.

Collection of *Helicoverpa armigera*

Five to six day old *H. armigera* larvae were collected from the cotton and pigeonpea crops and were brought to the laboratory for multiplication.

Each larva was placed separately in each of the plastic containers to avoid cannibalism. The culture of *H. armigera* was maintained on a chickpea based semisynthetic diet as per procedure suggested by Armes *et al.* (1992) in BOD incubator set at 27 ±2°C and a photoperiod of approximately 13L:11D. Five to six day old larvae of subsequent generations were used for bioassay.

Protein Estimation

By multiplying the *Bt* strains in Luria agar media, protein from *Bt* was isolated by acetone precipitation method. The spore crystal protein complex was extracted as per the procedure of Dulmage *et al.* (1970). The comparison between *Bt* strains var. *kurstaki* and *morrisoni* was done by working out LC_{50} of each strain by Probit analysis (Finney, 1954). The soluble protein toxin content was estimated by Lowry's method (Lowry *et al.*, 1951).

Bioassay studies

The bioassay studies were conduct by using diet incorporation method. Initially the protein was dissolved in an alkaline carbonate buffer pH 9.5. Stock solution (0.1M) of *Bt* toxin was prepared based on the protein content and serial dilutions were made incorporating into the artifical diet. On the basis of log concentration, six concentrations of protein from each strain were prepared and were fed to six day old larvae of *H. armigera* using 30 larve per treatment along with a control, having 3 replications, as suggested by Stone *et al.* (1989). On the basis of toxicity studies of Biobits, a commercial formulation of *B. thuringiensis* var. *kurstaki*, the concentrations tested were 0.00160, 0.00321, 0.00481, 0.00642 and 0.00963 per cent where as for var. *morrisoni* these were 0.0144, 0.0216, 0.0288, 0.0360, 0.0432 and 0.0576 per cent. These were tested against six-day old larvae (second instar) of *H. armigera* in the laboratory. This instar was chosen based on the studies of Douressamy and Regupathy (1993) who had found that first two instars were more susceptible than the later instars. The mortality counts of *H. armigera* larvae were taken after 24 hours up to seven days. The moribund larvae were also considered dead. The per cent mortality of treated larvae in each treatment was worked out. The corrected mortality was calculated using Abbott's formula (Abbott, 1925). Further, data were subjected to Probit analysis and median lethal concentration (LC_{50}) values were calculated from the reggression equation.

RESULTS AND DISSCUSSIONS

Toxicity of *B. thuringiensis* var. *kurstaki* (HD-1) against *H. armigera*

Per cent mortality in treatments with *B. thuringiensis* var. *Kurstaki* ranged from 20 to 80 as against 10 in the control. Corrected mortality varied from 17.52 per cent in the lowest to 79.38 per cent in highest concentrations (Table 1). The data on the doses kill and log concentrations were plotted and the straight eye fitting regression line was drawn. The heterogeneity was observed to be $\chi^2(5) = 2.61$. The equation was worked out as $Y = 1.36 + 2.09x$ and further LC_{50} was calculated, which was found to be 0.0055. The fiducial limits were observed to be 0.0044 and 0.0068 (Table 3).

Toxicity of *B. thuringiensis* var. *morrisoni* (HD-12) against *H. armigera*

The dose mortality data (Table 2) reveal that mortality in different concentrations varied from 20 to 80 per cent in the treatments and 10 per cent in the control. The corrected mortality varied from 17.52 per cent at the lowest concentration to 89.69 per cent at the highest concentration. The data on doses mortality thus obtained were further analysed. The Probit kill and log concentrations were drawn. The heterogeneity was observed to be $\chi^2(5) = 0.96$. The regression equation was worked out as $Y=3.8x - 0.59$ and further LC_{50} was calculated which was found to be 0.029. The fiducial limits were 0.026 and 0.033, (Table 3).

Relative toxicity of different *Bt* strains of *H. armigera*

The LC_{50} values of *Bt* var. *kurstaki* and *Bt* var. *morrisoni* against *H. armigera* larvae were

0.0055 and 0.029, respectively. The LC_{50} value of *kurstaki* was used as unity *i.e.* one, because it is widely used for calculating relative toxicity of these *Bt* strains *i.e.* var. *kurstaki* and var. *morrisoni.* Hence, it is concluded that toxicity of var. *kurstaki* is 5.27 times more than that of var. *morrisoni.* These results agree with those of Bindu (1997). Studies on the efficacy of two *Bt* strains var. *kurstaki* and *morrisoni* against the larvae of *H. armigera* revealed that the *Bt* var. *kurstaki* (HD-1) is more toxic than *Bt* var. *morrisoni* (HD-12), since their LC_{50} values were 0.005 and 0.029 IU/ml, respectively. These observations are in conformity with those of Salama *et al.* (1981) and Ali and Young (1992). Similarly, Bindu (1997) also noticed that out of the four strains of *Bt, Bt kurstaki* was found to be more effective against *H. armigera* as compared to *Bt morrisoni.* The results of present study are also comparable to those of Natarajan and Martouret (1994) and Salama *et al.* (1983) who reported that HD-1 was effective as compared to other *Bt* strains, var. HD-73 *aizawai* and HD- 263 *i.e. sotto.*

Table 1. Toxicity of *Bt kurstaki* strain against *H. armigera* after seven day

Bt concentration (%)	Log conc. (X) MF=10^4	No. of tested larvae	Actual mortality	Average (%mortality)	Corrected (% mortality)
0.00160	1.204	30	6	20	17.52
0.00321	1.506	30	9	30	27.83
0.00481	1.682	30	12	40	38.14
0.00642	1.807	30	15	50	48.45
0.00803	1.904	30	18	60	58.76
0.00963	1.983	30	24	80	79.38
Control	0.0	30	03	10	Nil

Table 2. Toxicity of *Bt morrisoni* strain against *H. armigera* after seven days

Bt concentration (%)	Log conc. (X) MF=10^3	No. of tested larvae	Actual mortality	Average (%mortality)	Corrected (% mortality)
0.0144	1.15	30	6	20	17.52
0.0216	1.33	30	9	30	27.83
0.0288	1.45	30	12	40	38.14
0.0360	1.55	30	15	50	48.45
0.0432	1.63	30	18	60	79.38
0.0576	1.76	30	24	80	89.69
Control	0.0	30	03	10	Nil

Table 3. Relative toxicity of different *Bt* strains against *H. armigera*

Sl. No.	Strain	Heterogeneity	Regression	LC_{50}	Fiducial limits	Relative toxicity
1.	Bt *kurstaki*	χ^2 (5)=2.61	Y=2.09 x +1.36	0.0055	0.0044 0.0068	1
2.	Bt *morrisoni*	χ^2 (5)=0.96	Y=3.8 x -0.59	0.029	0.026 0.033	5.27

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