International Journal of Plant Protection | **Volume 5 | Issue 1 | April, 2012 | 40-44**

IJPP

Research Article

Evaluation of spore crystal toxin complex of *Bacillus thuringiensis* isolates against *Helicoverpa armigera* larvae

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ARITCLE INFO

Article Chronicle :
Received : *04.10.2011*
Revised : *15.11.2011*
Accepted : *27.01.2012*

Key words :
Bacillus thuringiensis, H.armigera larvae, Spore crystal toxin complex

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ABSTRACT

In vitro toxicity testing was performed with six Bt. isolates *viz.,* 4D4, 4A3, 4J3, MTCC868, Bt1 and Bt9 procured from different sources and their spore crystal toxin (SCT) complex was produced which was evaluated by feeding to second instar larvae of *Helicoverpa armigera* at different concentrations (500µg/ml, 250 µg/ml and 125 µg/ml). Out of six Bt isolates tested, three isolates Bt1, MTCC868 and 4D4 proved very effective. Cent per cent mortality was obtained in Bt1 and MTCC868 which was at par with 4D4 (93.33%) at 500 µg/ml after 240 hours. The other isolates were less effective even at higher concentration but all the Bt isolates showed more than 50 per cent mortality at 500 µg/ml after 240 hours. The mortality at higher concentration (500µg/ml) was significantly better than middle concentration (250µg/ml) which in turn was significantly better than lower concentration (125 µg/ml) under laboratory condition.

How to view point the article : Joshi, Neelam, Jana, Sujata and Virk, J.S. (2012). Evaluation of spore crystal toxin complex of *Bacillus thuringiensis* isolates against *Helicoverpa armigera* larvae. *Internat. J. Plant Protec.*, **5**(1) : 40-44.

INTRODUCTION

Helicoverpa armigera is a serious pest of legumes, cotton and vegetables in South East Asia with an ability to develop a quick resistance to all kind of chemical insecticides. Keeping in view the side effects of insecticides, there is strong need for development and evaluation of eco-friendly strategies for the management of this pest. Among the several alternative methods for pest management, biological control by entomopathogens is promising one. Entomopathogens are the disease causing agents in the insect population, which may help in keeping the insect population below economic threshold level. It is a class of insecticides which is called as biopesticide. Biopesticides provide desired degree of plant protection which is totally eco-friendly (Cannon, 1990). Of all the biological agents that have been evaluated as insect control products, the most successful so far is *Bacillus thuringiensis* Berliner (Bt) which is being widely used as a biopesticidal formulation to control pest population among Lepidoptera, Diptera and Coleoptera (Beegle and Yamamoto, 1992, Schnepf *et al.,* 1998) because these are generally specific, economically viable, environmentally safe and compatible with the other methods of pest control (Jayaraj and Raghupathy, 1993).

Bt is extraordinary because of its unique capacity to synthesize crystal protein during sporulation. These crystal proteins have high insecticidal activity and quite selective in their toxicity to specific insect pests (Schnepf *et al.,* 1998, Whitlock *et al.,* 1991). Ingestion of delta endotoxin protein is a must for susceptible larvae; the crystal protein is solubilised in the alkaline midgut and then activated by digestive enzyme to yield a proteolyticaly stable toxin. The activated toxin passes through peritrophic membrane and binds to specific receptors in the brush border membrane vesicles. It opens up the K^+ channel in the membrane and eventually cell lysis causes death of the insects (Karim and Riazuddin, 1997). These naturally occurring insecticidal proteins have been commercially produced and used as insecticides for decades. Entomopathogens have shown varying degrees of virulence under different environmental conditions so, in present study different *Bacillus thuringiensis* isolates at different concentrations of spore crystal toxin were evaluated for their virulence against second instar larvae of *H.armigera*.

MATERIALS AND METHODS

In vitro toxicity test was performed with four Bt isolates

viz., 4D4, 4A3, 4J3, MTCC868, procured from different sources and two locally isolated Bt isolates Bt1 and Bt 9. The spore crystal toxin (SCT) complex was produced which was evaluated by feeding second instar larvae of *H. armigera* at different concentrations (500µg/ml, 250 µg/ml and 125 µg/ml). The SCT was produced as per the method of Salama *et al.* (1981) and Dulmage *et al.* (1970). The chickpea leaves were treated with uniform concentrations (500 µg/ml, 250µg/ml and 125µg/ml) of spore crystal toxin mixture of each strain by leaf dip method. Chickpea leaves were then air dried and fed as food to larvae. All this was done under aseptic conditions. There were three replications for each treatment and ten larvae per replication. The mortality of larvae was recorded daily and the data were analysed as per Completely Randomized Design.

Rearing of *Helicoverpa armigera* in laboratory

The larvae of the *H. armigera* were collected from chickpea fields of Entomological farm of PAU, Ludhiana. The larvae were reared on natural diet and reared for next generation. The second instar larvae from this reared generation was used for experimental purpose.

Production of spore crystal toxin (SCT) complex:

In vitro toxicity testing was performed with spore crystal toxin (SCT) mixture on the second instar larvae of *H. armigera*. The spore crystal toxin was produced as per the method of Salama *et al* (1981) and Dulmage *et al* (1970).The inoculums were prepared by transferring a loop full of cells from Nutrient agar slants into 50 ml of culture medium. After overnight incubation at 30±2°C at 200 rpm, the growth was employed to inoculate other culture flasks. Bt isolates were cultured in Glucose salt yeast (GSY) extract broth with following composition. Glucose (10g/l), Yeast extract (2g/l), Ammonium sulphate (0.8g/l) Dipot. hydrogen phosphate (0.8),Distilled water (1 l) at pH 6.5.

One ml of the overnight culture was used to inoculate each of 10 flasks containing 100 ml of GYE medium. The flasks were incubated at 30±2°C at 200 rpm. After three days, the bacterial spore crystal mixture was harvested in pellet by centrifugation at 12,000 rpm for 15 minutes.For each strain, the pellet obtained from one litre of culture was resuspended in 4-6 per cent lactose solution and the mixture was stirred. Then, the high purity acetone (4 volumes) was added with stirring to make a final volume of one litre. The mixture was filtered through Whatman Filter paper No. 1 to collect the precipitate containing spores and crystals complex Then the precipitate was allowed to dry and the dried powder was suspended in 100 ml of CMC-Triton X-100 (0.1% carboxy methyl cellulose in 0.01 per cent Triton X-100). The protein toxin content was estimated and a standard curve for protein was prepared under the same conditions using bovine serum albumin at concentration of 20-100 µg/ml (Fig 1).

Toxicity bioassays:

Toxicity of spore crystal toxin (SCT) complex was studied against second instar larvae of *H. armigera*. The chickpea leaves were treated with uniform concentrations (500 µg/ml, 250µg/ml and 125µg/ml) of spore crystal toxin mixture of each strain by leaf dip method. Chickpea leaves were then air dried and fed as food to larvae. All this was done under aseptic conditions. There were three replications for each treatment and ten larvae per replica . The mortality of larvae was recorded daily and the data was analysed as per Completely Randomized Design.

RESULTS AND DISCUSSION

The results obtained from the present investigation are presented below :

After 72 hours treatment :

All the treatments gave significantly higher mortality than control. The highest mean cumulative per cent mortality was recorded in Bt1(17.78) which was at par with MTCC868 (17.78%), 4A3 (14.44%) and Bt9 (12.22%). Lowest mean cumulative per cent mortality (10.00%) was recorded in isolate 4J3 (Table 1). The maximum mean mortality (20.95%) was recorded in 500 µg/ml concentration and it was significantly better than both the lower concentrations (250 µg/ml and 125 µg/ml). The interaction between treatments and concentrations was also significant. The maximum cumulative per cent mortality was found in isolate MTCC868 (33.33) at 500 µg/ml concentration and significantly better than all other combinations except Bt1 (30%), 4A3 (26.67%) and 4D4 (23.33%) at the same concentration.

After 168 hours treatment :

All the treatments were significantly better than control (1.11%) after 168 hours of treatment (Table 2). The highest mean cumulative per cent mortality was found in isolate MTCC868 (51.11) and it was significantly better than all the other treatments except Bt1 (50.00%), 4A3 (44.45%) and 4D4 (44.44%). The mean cumulative per cent mortality was lowest in 4J3 and Bt9 (35.56%). The mean mortality was maximum (53.81%) at 500 µg/ml concentration and it was significantly better than the other concentrations (Table 2). At 500 µg/ml concentration, the highest cumulative per cent mortality (80.00) was in Bt1 and it was significantly better than any other combination except 4D4 (73.33%), MTCC868 (66.67%) and 4A3 (66.66%).

After 240 hours treatment:

All the treatments were significantly better than control (3.33%) after 240 hours of exposure (Table 3). The mean cumulative per cent mortality was maximum in isolate Bt. 1

Table 1 : Efficacy of different concentrations of SCT complex of different Bt isolates for the control of *H. armigera* (72 hours after treatment)

Isolates	Cumulative per cent mortality (Different concentrations of SCT)			
	500 µg/ml	250 µg/ml	125 µg/ml	Mean
Bt 1	30 (33.31)	13.33 (21.57)	10.00 (18.90)	17.78 (24.59)
4A3	26.67 (31.11)	13.33 (21.57)	3.33 (9.00)	14.44 (20.56)
Bt9	16.67 (24.24)	10.00 (18.90)	10.00 (18.90)	12.22 (20.68)
MTCC868	33.33 (35.51)	16.67 (24.24)	3.33 (9.00)	17.78 (22.92)
4D4	23.33 (29.11)	6.67 (13.95)	3.33 (9.00)	11.11 (17.35)
4J3	16.67 (23.77)	6.67 (13.95)	6.67 (13.95)	10.00 (17.22)
Control	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)
Mean	20.95 (25.87)	9.52 (16.89)	5.24 (11.83)	

CD (P= 0.05) Treatment (T) = 5.56 Concentration(C) = 3.64
T x C= 9.62 Note: Values in parenthesis are arc sine transformations

Table 2 : Efficacy of different concentrations of SCT complex of different Bt isolates for the control of *H. armigera* (168 hours after treatment)

Isolates	Cumulative per cent mortality (Different concentrations of SCT)			
	500 µg/ml	250 µg/ml	125 µg/ml	Mean
Bt 1	80.00 (63.77)	43.33 (41.43)	26.67 (31.31)	50.00 (45.50)
4A3	66.66 (55.07)	46.67 (43.35)	20.00 (26.44)	44.45 (41.62)
Bt9	40.00 (39.43)	40.00 (39.43)	26.67 (31.31)	35.56 (36.72)
MTCC868	66.67 (55.37)	63.33 (53.05)	23.33 (28.44)	51.11 (45.62)
4D4	73.33 (60.35)	43.33 (41.43)	16.67 (24.24)	44.44 (42.01)
4J3	46.67 (43.35)	43.33 (41.35)	16.67 (24.24)	35.56 (36.31)
Control	3.33 (9.00)	0.00 (4.05)	0.00 (4.05)	1.11 (5.70)
Mean	53.81 (46.62)	40.00 (37.73)	18.57 (24.29)	

CD (P= 0.05) Treatment (T) = 5.74 Concentration(C) = 3.76
T x C= 9.95 Note: Values in parenthesis are arc sine transformations

Table 3 : Efficacy of different concentrations of SCT complex of different Bt isolates for the control of *H. armigera* (240 hours after treatment)

Isolates	Cumulative per cent mortality (Different concentrations of SCT)			
	500 µg/ml	250 µg/ml	125 µg/ml	Mean
Bt 1	100.00 (90.00)	73.33 (59.31)	56.67 (49.12)	76.67 (66.13)
4A3	80.00 (63.77)	50.00 (45.27)	33.33 (35.51)	54.44 (48.12)
Bt9	60.00 (51.13)	46.67 (43.35)	30.00 (33.31)	45.56 (42.60)
MTCC868	100.00 (90.00)	76.67 (62.06)	50.00 (45.27)	75.56 (65.77)
4D4	93.33 (89.96)	60.00 (51.13)	30.00 (33.51)	61.11 (58.20)
4J3	73.33 (59.31)	56.67 (49.21)	26.67 (31.31)	52.22 (46.61)
Control	6.67 (13.59)	3.33 (9.00)	0.00 (4.05)	3.33 (9.00)
Mean	73.33 (65.44)	52.38 (45.62)	32.38 (33.15)	

CD (P= 0.05) Treatment (T) = 4.59 Concentration(C) = 3.00
T x C= 7.95 Note: Values in parenthesis are arc sine transformations

(76.67) which was significantly better than all the other treatments except MTCC868 (75.56%). The maximum mortality (73.33%) was recorded in 500 µg/ml and it was significantly better than both the lower concentration (250 µg/ml and 125 µg/ml).The cent per cent mortality was found in Bt. 1 and MTCC868 at 500 µg/ml concentration and they were significantly better than all the other combinations except 4D4 (93.33%) .

It can be concluded that out of six Bt isolates tested, three isolates Bt1, MTCC868 and 4D4 proved very effective. Cent per cent mortality was obtained in Bt1 and MTCC868 which was at par with 4D4 (93.33%) at 500 µg/ml after 240

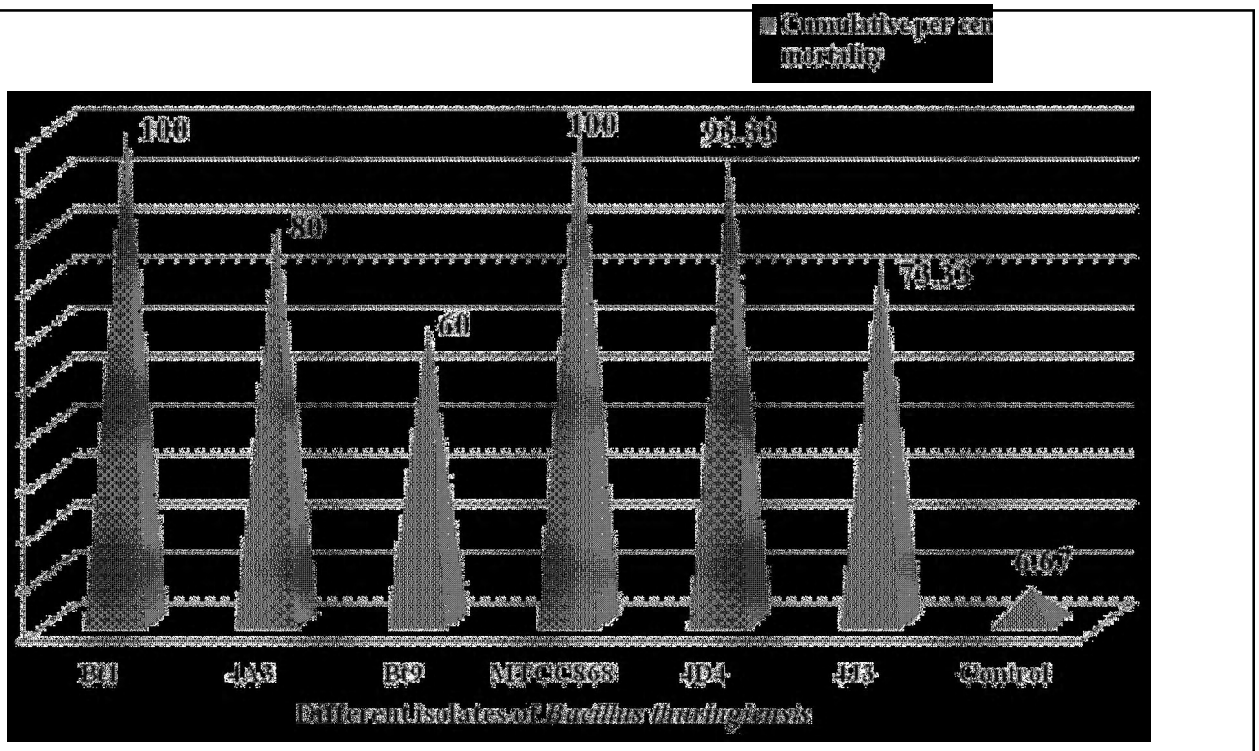

Fig. 1 : Efficacy of SCT complex (500 µg/ml) of different Bt isolates for the control of *H.armigera* (after 240 hours of treatment)

hours (Fig. 1). The other isolates were less effective even at higher concentrations but all the Bt isolates showed more than 50 per cent mortality at 500 µg/ml after 240 hours. The mortality at higher concentration (500µg/ml) was significantly better than middle concentration (250µg/ml) which in turn was significantly better than lower concentration (125 µg/ml), under laboratory condition.Therefore, different sub-species and isolates of *Bacillus thuringiensis* vary in their spectrum of activity against insect pests. The host-range of Bt isolates depends as the varying concentration of different toxic protein. The present results are in line with Tiwari (2000) who reported that toxicity of crystal protein is dose dependent. Many workers have used the culture with spores and crystals that contain multiple Bt proteins. The combined potencies of these Bt proteins account for the observed insecticidal spectra and efficacy of a strain (Krieg *et al.,* 1983, Jaquet *et al.,* 1987). However, Justin *et al.* (1989) reported that the primary cause of spectral differences lies in the spectrum of activity of the delta endotoxin. Mohan *et al.* (2009) also reported variation in toxicity of *B. thuringiensis* strains and their crystal toxins against high-altitude Himalayan populations of diamondback moth, *Plutella xylostella* L. Aramideh *et al.* (2010) also reported that out 48 *B. thuringiensis* strains isolated, 18.74 and 35.41 per cent of the isolates were toxic to larvae of *Culex pipiens* (L.) and *Pieris brassicae* (L.) respectively, causing more than 50 per cent mortality.

Acknowledgement:

The authors are thankful to Dr. Daniel R. Zeigler, *Bacillus* Genetic Stock Centre, Ohio State University, USA. and Institute of Microbial Technology, Chandigarh for providing us *Bacillus* isolates and Head, Department of Entomology for providing facilities.

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