Bioefficacy of crude protein of native *Bacillus thuringiensis* (Berliner) isolates against cabbage leaf webber, *Crocidolomia binotolis* Zel.

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ABSTRACT

Investigations were carried out to assess the efficacy of crude protein of some of *Bacillus thuringiensis* isolates against lepidopteran pest *viz.*, cabbage leaf webber (CLW) *Crocidolmia binotalis* Zel. mortality. The native isolate 1526B/b recorded highly virulent (LC_{50} = 682.420 ppm) where as M-126 recorded lowest virulent (LC_{50} = 6572.822 ppm). To identify the spectrum of insecticidal genes in the native *B. thuringiensis* isolates, the polymerase chain reaction (PCR) was carried out and the results show that none of the isolates amplified *cry*3, 4 and 18 genes.

Key words: *Bacillus thuringiensis*, *Crocidolmia binotalis*, crude protein, *cry*3, 4 and 18 genes.

INTRODUCTION

The human population is increasing in an alarming rate therefore there is a pressure on agriculture to produce huge amount of food grain to feed the increasing population. Due to increased growth of human population there is an ever increasing demand for 3F's *i.e.* food, fuel and fiber. The area under cultivation of crops could not be increased due to limited factor and there is a need to develop and adopt other technologies like high yielding crop varieties, intensive crop cultivation practices with plant protection strategies to meet increasing food demand (Reddy, 2000). Production in agriculture plagued mainly because of world wide insect pests. In India 5-50 per cent yield loss due to damage caused by insect pests was recorded (Durairaj, 1999).

Bacillus thuringiensis (Berliner) is reported to be the most successful commercial biocontrol agent against insect pests (Federici, 1999) which is a rod shaped gram positive entomopathogenic bacterium abundant in soil (Bora *et al*., 1993). It is aerobic spore former well known for its ability to produce a proteinacious crystal during sporulation (Krieg, 1961; Heimpel, 1963; Hempiel *et al*., 1963). The crystal protein designated as delta endotoxin is toxic by ingestion for many insect larvae (Heimpel, 1963). Umeh *et al.*, (2009) reported population dynamics of the Diamondback moth and control of lepidopteran insects on cabbage in the rainy season. Recently Sampurna Sattar *et al.* (2008) isolated and tested the native protein of *Bt* on hemipteran pests. Similarly Srinivasan and Hsu Yun-Che (2008) reported the use of *Bt* protein in vegetable management in Taiwan. The study was undertaken to evaluate the impact of native *Bt* protein on cabbage leaf webber.

MATRIAL AND METHODS

Mass multiplication of *Crucidolomia binotalis*

The CLW larvae collected from the infested fields of cabbage were reared separately on cabbage leaves raised in green house under insecticidal free condition. Pupae thus obtained were kept in a sterilized petriplate and placed in the cage of 25cm^3 for adult emergence. When the moth started emerging, 25–30 days old small cabbage heads were provided for oviposition. The moth laid eggs both on ventral and dorsal surface of leaves. Leaves with eggs were transferred to plastic tubes 45 x 30 x 15 cm for mass rearing. Ten percent honey solution was provided as food for adults in sterilized vial with cotton plug. Five day old F_1 generations were used for bioassay.

Extraction of crude protein from *B. thuringiensis*

The crude protein extraction was carried out by adopting the method suggested by Dulmage *et al*., (1970). Selected twelve *B. thuringiensis* isolates were inoculated to MGM broth in separate flasks and incubated for 72h. The pH of the culture broth of each isolate was brought down to 7 using 1N HCl and centrifuged at 8000 rpm for 20 min. The supernatant was discarded and the pellet was resuspended in six per cent lactose at 1/10 volume of the

Table 1. Larval mortality of Cabbage Leaf Webber, *C. binotalis* as influenced by different concentrations of crude protein of *B. thuringiensis* isolates

Isolates	Number of insects dead at different intervals				
	24h	48	72	96	% mortality
1642/a					
1000	1	8	13	15	50.00
2000	3	12	17	18	50.00
4000	8	16	20	21	70.00
6000	10	19	23	24	80.00
8000	13	22	25	27	90.00
10000	17	26	29	30	100.00
1642A/a					
1000	0	7	10	10	33.33
2000	2	8	12	13	43.33
4000	3	11	15	16	53.33
6000	6	13	15	16	53.33
8000	9	16	20	21	70.00
10000	13	20	23	24	80.00
1611/C					
1000	0	6	10	13	43.33
2000	1	8	11	15	50.00
4000	2	12	14	17	56.67
6000	5	15	17	21	70.00
8000	8	18	22	23	76.67
10000	10	22	26	26	86.67
1598d					
1000	2	9	14	16	53.33
2000	3	10	16	17	56.67
4000	6	13	19	20	66.67
6000	8	15	20	23	76.67
8000	10	17	22	25	83.33
10000	12	21	23	20	93.33
1526B/b					
1000	4	11	17	19	63.33
2000	6	13	19	21	70.00
4000	9	16	21	23	76.67
6000	10	19	24	25	83.33
8000	12	22	25	27	90.00
10000	15	23	27	29	76.67
M-86					
1000	0	5	8	10	33.33
2000	0	7	10	12	40.00
4000	3	9	13	16	53.33
6000	5	12	16	19	63.33
8000	7	14	18	22	73.33
10000	9	17	20	23	76.67
M-102					
1000	1	7	12	13	43.33
2000	3	11	14	15	50.00
4000	5	15	18	18	60.00
6000	6	15	20	21	70.00
8000	8	18	22	24	80.00
10000	11	22	26	27	90.00
M-126					
1000	0	2	5	6	20.00
2000	0	3	5	7	23.33
4000	0	5	8	11	36.67
6000	0	7	12	14	46.67
8000	1	10	15	17	56.67
10000	4	14	17	20	66.67
K-63					
1000	4	8	11	13	43.33
2000	5	10	12	14	46.67
4000	9	14	16	17	56.67
6000	12	16	19	20	66.67
8000	15	18	20	22	73.33
10000	18	21	23	25	83.33
Tx-353					
1000	0	8	11	11	36.67
2000	2	10	12	12	40.00
4000	5	10	14	14	46.67
6000	7	14	16	17	86.67
8000	9	17	19	19	63.33
10000	12	20	22	22	73.33
4BA1					
1000	0	7	12	13	43.33
2000	1	9	13	15	50.00
4000	3	10	16	18	60.00
6000	6	13	19	21	70.00
8000	8	16	22	23	76.67
10000	10	19	24	25	83.33
4G1					
1000	5	12	16	17	56.67
2000	7	15	19	20	66.67
4000	10	16	20	22	73.33
6000	13	18	23	25	83.33
8000	17	20	24	26	86.67
10000	20	22	25	27	90.00
HD1					
1000	3	16	20	20	66.67
2000	5	19	22	22	73.33
4000	8	20	25	25	83.33
6000	11	22	27	27	90.00
8000	13	25	28	28	93.33
10000	16	27	30	30	100.00
Halt					
1000	15	24	28	30	100.00
2000	23	27	30	30	100.00
4000	26	29	30	30	100.00
6000	27	30	30	30	100.00
8000	30	30	30	30	100.00
10000	30	30	30	30	100.00

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initial broth. The suspension was stirred for 30min over magnetic stirrer and four volume of acetone was added slowly followed by stirring for another 30 min. It was allowed to stand for 10min at room temperature and filtered through Whatsman No.1 filter paper. Again isolates were resuspended in 25ml acetone, stirred again for 30 min and filtered through Whatsman No.1 filter paper under suction. The acetone washing step was repeated thrice and finally the residue on the filter paper was allowed to dry overnight in a vaccum desicator at 25^{0}C. The white crystalline pellets obtained after drying was used to test against the insect pests.

Quantification of protein in the spore crystal complex

The protein content of the acetone powder was estimated following the method described by Lowry's (1951) as detailed below. A standard curve was prepared based on the UV absorption (280nm) of Bovine serum albumin at various known concentrations like 200ppm, 400ppm, 600ppm, 800ppm and 1000ppm. The concentrations were plotted along the X-axis and corresponding absorbance values were plotted along the Y-axis. Different concentrations of spore crystal complex from 500ppm to 5000ppm of the reference strain (HD_1) were prepared and the corresponding absorbance values were recorded from a UV-VIS spectrophotometer 117 (Systronyx). Out of this 5000ppm was found to be ideal and used for protein quantification. The spore crystal complex suspension of 5000ppm of the selected 12 isolates was prepared and UV absorbance was taken. The quantification of the crude protein was done by referring to the standard curve.

Bioefficacy of crude protein against test insects

The bioefficacy of *B. thuringiensis* isolates was tested at different concentrations against five day old larvae of *C. binotolis* in comparison with the crude protein isolated from reference strain HD1. Six different concentrations (1,000; 2,000; 4,000; 6,000; 8,000 and 10,000 ppm) of crude protein of each isolate including reference strain HD1 and standard check Halt (Commercial Bt formulation) were prepared by dissolving the calculated amount of spore crystal complex in sterile distilled water.

Bioassay

Leaf dip bioassay was adopted. Leaf disc of 6cm diameter were cut covering either side of midrib from untreated cabbage leaves *Crocidolmia binotalis*. These discs were dipped in aqueous solution of the test isolates for about 30seconds. Excess fluid was drained off and discs were dried under shade for 10min before transferring to plastic containers (10cm height and 6cm diameter) over a moistened filter paper. Leaf discs were placed slantingly so that larvae can move and feed on either side. Bioassays were done with three replications per treatment and ten larvae of test insects were released on each disc and the container was covered with muslin cloth using a rubber band.

RESULTS AND DISCUSSION

Commercial Bt formulation caused 100 per cent mortality to the five day old larvae at 24 h interval of treated period in 8000 and 10000 ppm concentration. The reference strain HD1 also efficient to cause 100 per cent mortality in 10,000 ppm concentration at 72 h of treatment. The mortality of test insect varies in different concentration, low mortality rate in low (1000 ppm) concentration and high in high (10000 ppm) concentration. The same trend was followed by native isolates also (Table 1 and 2). The native isolate 1642/a recorded highest (100%) mortality at 96 h interval in 10000 ppm concentration treatment, but isolate 1526B/b (96.67%) was efficient to eliminate more number of larvae in all concentrations than 1642/a and 1598d (93.33%) mortality in 10000 ppm concentration. LC_{-50} concentration of these isolates were 1453.150, 682.420 and 1274.680, respectively.

Table 2. Dosage mortality response of Cabbage leaf webber, *C. binotolis* to crude protein of *B. thuringiensis* isolates

Isolates	LC_{50} conc.	95% confidential limits	
		Lower limit	Upper limit
1642/a	1453.150	745.00	2105.273
1642A/a	3049.038	1649.971	4605.135
1611/C	2048.395	853.008	3170.406
1598d	1274.680	402.055	2088.478
1526B/b	682.420	99.278	1327.518
M-86	5080.595	1758.830	4285.205
M-102	1940.321	913.028	2897.384
M-126	6572.822	4435.832	11216.716
K-63	2187.627	867.237	3448.220
Tx-353	3704.00	1752.275	6658.175
4BA1	1949.148	752.541	3062.013
4G1	846.685	136.479	1588.679
HD1	779.328	257.677	1287.457
Halt	637.228	186.850	1083.204

Table 3. Cry profile in the native *B. thuringiensis* isolates

Isolates	*Cry* 1	*Cry* 2	*Cry* 3	*Cry* 4	*Cry* 11	*Cry* 18
1642/a	-	+	-	-	-	-
1642A/a	-	+	-	-	-	-
1611/C	-	+	-	-	-	-
1598d	-	+	-	-	-	-
1526B/b	-	-	-	-	-	-
M-86	-	+	-	-	-	-
M-120	-	+	-	-	-	-
M-126	-	+	-	-	-	-
K-63	-	-	-	-	+	-
Tx-353	+	+	-	-	+	-
4BA1	-	+	-	-	+	-
4G1	+	+	-	-	-	-

\+ Presence of crystal gene, - Absence of crystal gene

To identify the spectrum of insecticidal genes in the native *B. thuringiensis* isolates, the polymerase chain reaction (PCR), a widely used tool was employed, which identifies the presence of different *cry* gene through PCR amplification using the *cry* specific primers. A total of six sets of primers were used in PCR amplification. The profile of *cry* genes of the native *B. thuringiensis* isolates is tabulated in Table 3. Thirteen native isolates were diagnosed for presence of *cry* genes. Among 12 native *B. thur ingiensis* isolates *cry*1 amplified in two isolates (Tx-353 and 4G1), *cry*2 amplified in ten isolates except two isolates *viz.*, 1526B/b and K-63, *cry*11 amplified in three isolates *viz.*, M-126, K-63 and K-63 and none of the isolates amplified *cry*3, 4 and 18 genes. Among reference strains HD1 amplifies for *cry*1 and *cry*18, 4D4 amplified for *cry*2, *cry*3 amplified for 4AA1 and 4Q1 amplified for *cry*4 and 11. Among native isolates 1642/a (Sikkim isolate) did better than other isolates, followed by 1526 B/b. This isolate performed better against *C. binotolis* with least LC_{50} (682.420ppm) and comparable with reference strain HD_1 (779.328 ppm). Very wide variations exist for the effectiveness of *B. thuringiensis* isolates against target insects (Yaradoni, 1999). Knowles (1994) explored that the variations in efficacy against different lepidopteran may be due to varying number of *cry* genes and the absence of specific binding sites.

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