Bioefficacy of *Bacillus thuringiensis* isolates crude protein against *Plutella xylostella* L.

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ABSTRACT

A laboratory experiment was carried out to isolate and study the effect of different concentrations of *Bacillus thuringiensis* (*Bt*) crude protein on Diamond back moth (*Plutella xylostella*). Crude protein from thirteen *Bt* isolates was extracted and used at different concentrations *viz.*, 100, 250, 500, 750, 1000 and 2000 ppm for bioassay against third instar larvae of *P. xylostella*. The per cent mortality was recorded after 24, 48 and 72 hrs of treatment. A cumulative mortality of 100 per cent was recorded by three isolates *viz.*, UK-13C, UK-762D, UK-25A as well as by the reference strain HD1, followed by UK-52A, UDP-420B, DK-45B and DK-6B (93.00, 86.67, 86.67 and 83.33 respectively) at 2000 ppm after 72 hrs. The crude protein of other isolates caused larval mortality in the range of 66.67 to 80.00 per cent at 2000 ppm after 72 hrs. No larval death was observed in the control. Irrespective of the crude protein concentration, the larval mortality was highest on third day, followed by second day.

Key words: *Bacillus thuringiensis*, bioassay, crude protein, diamond back moth

INTRODUCTION

It has been estimated that 9000 species of insect pests affect commercial crops in the world. Among the lepidopteran insect pests of cultivated plants, diamond back moth (*Plutella xylostella*) is a widely distributed, serious pest of cruciferous crops (Salinas, 1977). The pest is distributed all over India and direct crop losses due to its damage are worth several crores of rupees. Satpathy *et al.* (2005) reported 50-80 per cent loss in marketable yield of cabbage due to attack of *P. xylostella*. Caterpillars of the DBM are the dominant and most damaging to cabbage and cauliflower crops. In India, the losses of cabbage and cauliflower due to DBM is about 35 per cent with chemical control but can go upto 90 per cent in the absence of chemical control (Mohan and Gujar, 2003).

Under this situation, the usage of pesticides has become inevitable. Inadvertent use of chemicals for pest control has posed serious threat to our environment and effective alternatives for chemical insecticides are being screened. One of the solutions for pest insurgence problem is the use of biological agents. Biological control of insect pests has become popular and provides a safer means to reduce insect damage (Dhaliwal and Arora, 1998). Among various options available, the use of soil bacterium *B. thuringiensis* has immense potential for use as biopesticide.

MATERIALS AND METHODS

Soil and leaf samples were collected from Western Ghat regions of Uttara Kannada, Dakshina Kannada and Udupi districts of Karnataka and used for the isolation of *B. thuringiensis*. The isolation of *Bt* was carried out by following sodium acetate selection method (Travers *et al*., 1987). The isolates were purified and subjected for crystal staining as per the procedures outlined by Sharif and Alaeddinoglu (1988).

The isolates obtained were tested for their efficacy against diamond back moth (*Plutella xylostella*). As cabbage is the most preferred host plant for *P. xylostella.* Cabbage seedlings were raised in pots one month prior to rearing of the insect larvae. Diamondback moth was mass cultured in the laboratory as per the method described by Liu and Sun (1984) with little modification. Extraction of crude protein from *B. thuringiensis* was carried out by following the method of Dulmage (1970). The bioefficacy of crude protein extracts of *B. thuringiensis* isolates were tested at different concentrations against third instar larvae of *P. xylostella* in comparison with the crude protein extract of the reference *Bt* strain HD1 (Howard Dulmage) which was obtained from Bacillus Genetics stock center (BGSC), Department of Biochemistry, Ohio State University, Columbia.

Six different concentrations of crude protein (100, 250, 500, 750, 1000 and 2000 ppm) of each isolate including reference strain HD1 were prepared by dissolving the calculated amount of crude protein in sterile distilled water. Fresh cabbage leaf discs of 7.5cm diameter were dipped separately in solutions of different concentrations of crystal protein and air dried. The air dried leaf discs were fed to the third instar larvae of *P. xylostella,* which were subjected to starvation for 6 h prior to treatment. The larvae fed with leaf discs dipped in sterile distilled water served as control. For each isolate, separate plastic containers were used and ten larvae were released per container. Three replications were maintained for each isolate. The observations on larval mortality were recorded at an interval of 24 hrs for three days. Results of the dose mortality response (LC_{50} and LC_{99}) were analyzed by using the method proposed by Finney (1952) with the help of MLP package.

RESULTS AND DISCUSSION

Thirteen *Bt* isolates were obtained from Western Ghats of Karnataka, of which five isolates were obtained from Uttara Kannada district and four each from Dakshina Kannada and Udupi districts of Karnataka. These isolates showed different crystal morphologies under phase contrast microscope (Table 1). Among 13 isolates, 8 had spherical shaped crystals, 3 with irregular type and two showed bipyramidal shaped crystals indicating predominance of spherical crystals in the isolates. The dominance of spherical crystals has been reported by Arrieta *et al.* (2004) in the isolates of coffee plantations whereas only bipyramidal inclusions were reported by Wangondu *et al.* (2003).

The crude protein extracted from the 13 *B. thuringiensis* isolates was tested at six different concentrations (100, 250, 500, 750, 1000 and 2000 ppm) against third instar larvae of *P. xylostella* and the results are presented in Table 2. In general, the per cent mortality was found to increase with increase in concentration of crude protein of all the isolates. A cumulative mortality of 100 per cent was recorded by three isolates *viz*., UK-13C, UK-762D, UK-25A as well as by the reference strain HD1, followed by UK-52A, UDP-420B, DK-45B and DK-6B (93.00, 86.67, 86.67 and 83.33 respectively) at 2000 ppm after 72 hrs of treatment. The crude protein of other isolates caused larval mortality in the range of 66.67 to 80.00 per cent at 2000 ppm after 72 hrs. No larval death was observed in the control. Irrespective of the crude protein concentration, the larval mortality was highest on third day followed by second day.

Table 1. Location, source and crystal morphology of *B. thuringiensis* isolates

Isolate No.	Source	Location	Crystal morphology
UK-13C	Soil	Uttara kannada district of Karnataka	Bipyramidal
UK-25A	Leaf	-do-	Spherical
UK-46B	Soil	-do-	Spherical
UK-52A	Soil	-do-	Irregular
UK-762D	Compost	-do-	Bipyramidal
DK-6B	Soil	Dakshina kannada district of Karnataka	Spherical
DK-45B	Soil	-do-	Spherical
DK-140D	Soil	-do-	Spherical
DK-189B	Soil	-do-	Spherical
UDP-346B	Soil	Udupi district of Karnataka	Spherical
UDP-358A	Soil	-do-	Irregular
UDP-416D	Soil	-do-	Irregular
UDP-420B	Soil	-do-	Spherical

Among the isolates which brought one hundred per cent mortality, UK-13C and UK-762D possessed bipyramidal crystals whereas UK-25A formed spherical crystals. Generally the lepidopteran active isolates of *Bt* have been known to produce bipyramidal crystals (Opondo *et al.*, 2010) with some exceptions of spherical crystals (Wasano *et al.*, 2000). Although the relationship between the type of crystal morphology and the level of insecticidal activity is not clear, it has been reported that the strains with bipyramidal crystals

Table 2. Bioefficacy of crude protein of selected *Bt* isolates of Western Ghats on third instar larvae of *P. xylostella*

Isolates	Concentration (ppm)	Per cent mortality at 72 hrs	LC_{50} (ppm)	Fiducial limit		LC_{99} (ppm)	Fiducial limit	
				Lower	Upper		Lower	Upper
UK-13C	100 250 500 750 1000 2000	13.33 26.67 53.33 80.00 93.33 100.00	434.82	324.59	522.55	1984.15	1368.43	4423.45
UK-25A	100 250 500 750 1000 2000	10.00 13.33 30.00 50.00 86.67 100.00	688.64	558.77	783.64	1869.83	1366.82	4645.31
UK-46B	100 250 500 750 1000 2000	10.00 16.67 30.00 50.00 66.67 80.00	823.26	640.06	1072.99	8566.41	4274.52	39871.99
UK-52A	100 250 500 750 1000 2000	10.00 20.00 40.00 60.00 80.00 93.00	604.98	473.33	737.40	3928.68	2436.58	10334.15
UK-762D	100 250 500 750 1000 2000	10.00 16.67 33.33 60.00 90.00 100.00	633.22	501.84	723.13	1762.26	1298.09	4152.65
DK-6B	100 250 500 750 1000 2000	6.67 13.34 26.67 46.67 63.33 83.33	869.14	698.58	1093.20	6162.55	3500.46	20292.83
DK-45B	100 250 500 750 1000 2000	10.00 16.67 30.00 50.00 70.00 86.67	767.15	605.45	957.49	5759.78	3242.95	20092.82
DK-140D	100 250 500 750 1000 2000	6.67 13.34 23.33 43.33 60.00 76.67	977.25	776.07	1286.47	8429.60	4329.49	36872.68
DK-189B	100 250 500 750	6.67 13.34 20.00 46.67	1006.01	794.57	1346.97	9380.64	4653.59	45004.55

	1000 2000	53.33 73.33						
UDP-346B	100 250 500 750 1000 2000	0 6.67 20.00 40.00 56.67 70.00	1119.40	899.97	1485.56	8455.53	4557.15	30008.61
UDP-358A	100 250 500 750 1000 2000	0 6.67 13.34 26.67 53.33 66.67	1302.12	1048.80	1779.94	8484.62	4519.83	34640.61
UDP-416D	100 250 500 750 1000 2000	6.67 13.34 20.00 40.00 56.67 73.33	1073.09	847.52	1462.22	9753.21	4744.18	52065.59
UDP-420B	100 250 500 750 1000 2000	10.00 16.67 33.33 53.33 70.00 86.67	736.10	577.79	920.93	5894.93	3297.06	20356.20
HD1 (Ref)	100 250 500 750 1000 2000	13.33 30.00 60.00 83.33 100.00 100.00	390.98	288.97	468.87	1586.83	1129.03	3340.52

are more toxic to lepidopteran larvae (Obeidal *et al.,* 2004; JianHong *et al.,* 2000; Asokan and Puttaswamy, 2007 and Monnerat *et al.,* 2007).

The dose mortality response (LC_{50}) of *P. xylostella* to the crude protein of selected *B. thuringiensis* isolates indicated differences in their ability to kill the larvae. The dose mortality response of third instar larvae of *P. xylostella* to crude protein of native *B. thuringiensis* isolates revealed that the isolate UK-13C showed the least LC_{50} value of 434.82 ppm with fiducial limits ranging from 324.59 to 522.55 ppm followed by the isolates UK-52A, UK-762D and UK-25A with LC_{50} values of 604.98, 633.22, 688.64 and 688.64 ppm respectively (Table 2). The isolate UDP-358A exhibited the maximum LC_{50} value of 1302.12 ppm with fiducial limits ranging from 1048.80 to 1779.94 ppm. The reference strain HD1 however showed the LC_{50} value of 390.98 ppm with fiducial limits ranging from 288.97 to 468.87 ppm.

Dosage mortality response (LC_{99}) of third instar larvae of *P. xylostella* to crude protein of native *B. thuringiensis* isolates revealed that the isolate UK-762D showed the least LC_{99} value of 1762.26 ppm with fiducial limits ranging from 1298.09 to 4152.65 ppm. This was followed by the isolates UK-25A, UK-13C and UK-52A with LC_{99} value of 1869.83, 1984.15 and 3928.68 ppm respectively (Table 2). The isolate UDP-416D recorded the maximum LC_{99} value of 9753.21 ppm with fiducial limits ranging from 4744.18 to 52065.59 ppm. The reference strain HD1 showed LC_{99} value of 1586.83 ppm with fiducial limits ranging from 1129.03 to 3340.52 ppm.

A very wide variation exists for the effectiveness of *B. thuringiensis* isolates against target insects (Yaradoni, 1999). Degree of pathogenicity varied with concentration of bacterial isolate as well as the period of exposure (Savitri and Muralimohan 2003). Similar differences in effectiveness among the strains and subspecies of *B. thuringiensis* against *P. xylostella* and other insects have been reported earlier (Arora *et al.*, 2006; Kaur *et al.*, 2006; Monnerat *et al*., 2007; Netravathi, 2010; Patel and Ingle, 2012; Geeta *et al.*, 2012). The differences in the efficacy of different isolates of *B. thuringiensis* has been suggested to be due to the difference in the carbohydrate affinity of the domain II which results in variable binding specificity with the receptors at the brush border membrane of the insect larvae, causing difference in toxicity of the cry protein (Burton *et al*., 1999).

The present study thus reports the isolation of insecticidal *Bt* strains which caused mortality of *P. xylostella*. Hence, *Bt* can be incorporated as one of the effective component in the integrated pest management of *P. xylostella* on cabbage.

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