Bioefficacy of native Sikkim *Bacillus thuringiensis* (Berliner) isolates against lepidopteran insects.

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ABSTRACT

Investigations were carried out to assess the efficacy of Sikkim *Bacillus thuringiensis* isolates against lepidopteran insects *viz.*, Cabbage leaf webber, Diamond Back Moth and bioassay were also done to assess the safety nature of *Bt* to silkworm. *Bacillus thuringiensis* isolates of Sikkim (Thirty five isolates) were assessed. Among these isolates mortality of each isolate was varied from insect to insect. The mortality of 86.67% was registered by the isolates 1554/b, 1526B/b and 1598d against Cabbage leaf webber. The isolates 6a4, 1598a, 1526B/b, 1622A/a and 1642A/a recorded maximum (93.33%) mortality against Diamond Back Moth. Whereas, 93.33 and 90.00 per cent mortality was recorded in 1620b and 1598d, respectively, against silkworm.

Key words: *Bacillus thuringiensis, Pluttella xylostella, Crocidolomia binotalis*

INTRODUCTION

The insecticidal properties of *Bacillus thuringiensis* (Berliner) (Bt) have been known for over a century and commercial products based on this organism have been available for 70 years, occupying >90% of the bio-pesticide market (Glare *et al.*, 2000). *B. thuringiensis* is reported to be the most successful commercial biocontrol agent against insect pests which is a rod shaped gram positive entomopathogenic bacterium abundant in soil. It is aerobic spore former well known for its ability to produce a proteinacious crystal during sporulation (Krieg, 1961; Heimpel, 1963). The crystal protein designated as delta endotoxin is toxic by ingestion for many insect larvae (Heimpel, 1963; Yadugiri, 2010). Steven and Naranjo highlighted the impact of *Bt* on various organisms in an elaborate manner. However, in the review nothing has been mentioned about the impact of Bt on *Bombyx mori*, *Crocidolomia binotalis* and *Pluttella xylostella* (L.). Impact of *Bt* on silkworm, *Antheraea pernyi* has reported earlier by Li *et al.* (2005). In the present study impact of some native isolates of Bt (2a4, 3a4, 4a4, 6a4, 7a4, 9a6, 1554b, 1598a, 1532/2, 1526Bb, 1544/3, 1706/4, 1711/1(a), 1622A/a, 1611C, 1642a, 1614C/b, 1742/1, 1710/1, 1567/4, 1650b, 1716/1, 1533b, 1707B/4, 1711/1, 1587a, 1642A/a, 1598d, 1640B/a, 1528a, 1621a, 1610c, 1602a, 1640A/a and 1620b) on *Bombyx mori*, *Crocidolomia binotalis* (Z.) and *Pluttella xylostella* are studied under laboratory conditions.

MATRIALS AND METHODS

Mass multiplication of test insects

Silkworm eggs were brought from grainages of Karnataka state Department of Sericulture, Dharwad. Egg cards were placed in plastic tubs. The egg cards were covered with black paper sheet to enhance the maximum emergence and were surrounded by foam to maintain humidity for egg hatching. After hatching of eggs, neonates were transferred on to mulberry leaves using camel hair brush. Neonate larvae scraped the green matter of leaves. Twice in a day (morning and evening) leaves were fed to larvae and humidity also maintained. Five day old larvae were used for bioassay tests.

Diamond back moth

The larvae collected from the fields were reared separately on cabbage leaves raised in green house under insecticide free condition. Pupae thus obtained were kept in a petriplate and placed in a cage of 25cm^3 for adult emergence. When moths started emerging mustard seedlings were provided for oviposition (Liu and Sun, 1984). Plastic cups of 6 cm height and 4.5 cm diameter were filled with sterilized vermi compost and Bavistin (2g/kg) treated presoaked mustard seeds were sown and allowed to germinate under normal conditions. Within 4-5 days after germination, they were placed in the oviposition cage and replenished at 24 h interval. Moths laid eggs on both the sides of cotyledons. The cups with eggs were transferred to plastic tubs of size 45 cm X 30 cm X 15 cm for mass rearing. Ten per cent honey solution containing multivitamin powder was provided for

the adults as food through cotton swab kept in a sterilized Petri plate. Eggs hatched in 2-3 days and neonates mined the mustard cotyledons and continuously fed on them. When the seedlings are completely consu med, larvae were transferred to fully expanded cabbage leaves with and its petiole covered in wet cotton swab to maintain leaf turgidity. Five day old F_1 generation larvae were used for the bioassay.

Leaf Webber

Cabbage leaf Webber was mass reared in the insectary. The 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 tubs of size 45 cm X 30 cm 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 generation larvae were used for bioassay.

Bioassay

Sikkim *B. thuringiensis* isolates were subjected for bioassay to ascertain their insecticidal activity against test insect. To multiply the isolates population they were streaked on plain Luria Agar (LA) plates and kept in incubation for 24 h and was inoculated in Luria broth (LB) of 1ml in eppedorf tube and was kept for growth under shaking condition at 28^{0}C and incubated for 24 h. Then the culture was reinoculated in Modified Glucose Media (MGM) (Aronson *et al*., 1971) and kept for 72 h at 30^{0}C on a shaker at 200 rpm. The serial dilution of culture from 10^0 to 10^7 was done at 9:1 ratio and 1ml of serial diluted culture from 10^{-6} and 10^{-7} were spread separately on LA plates and incubated for 24 h at 37^{0}C. Colony count were taken after 24 h and calculations were done using standard formula ($1.2X10^{-6}$ cfu/ml) (Shilpa, 2005) to fix the concentration of *B. thuringiensis.*

Insecticidal activity

Leaf dip bioassay described by Tabashnik and Cushing (1987) was adopted. Leaf disc of 6cm diameter were cut covering either side of midrib from untreated mulberry leaves to *B. mori* and cabbage leaves to *C. binotalis* and *P. xylostella*. These discs were dipped in aqueous solution of the test isolates for about 30 seconds. Excess fluid was drained off and discs were dried under shade for 10 min 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. HD1 served as standard check, Dipel 8L served as standard commercial *B. thuringiensis* formulation and leaf disc dipped in distilled water alone served as control. Later mortality was observed at 24 h, 48 h, 72 h and 96 h after treatment and data were subjected to analysis of variance after suitable transformation (arcsine)

Table 1. List of thirty five native isolates and their crystal morphology

Isolates	Location	Morphology
2a4	Sikkim	Spherical
3a4	Sikkim	Spherical
4a4	Sikkim	Spherical
6a4	Sikkim	Spherical
7a4	Sikkim	Spherical
9a6	Sikkim	Spherical
1554b	Sikkim	Bipyramidal
1598a	Sikkim	Irregular
1532/2	Sikkim	Rhomboidal
1526Bb	Sikkim	Triangular
1544/3	Sikkim	Spherical
1706/4	Sikkim	Spherical
1711/1(a)	Sikkim	Spherical
1622A/a	Sikkim	Spherical
1611C	Sikkim	Spherical
1642a	Sikkim	Triangular
1614C/b	Sikkim	Spherical
1742/1	Sikkim	Spherical
1710/1	Sikkim	Spherical
1567/4	Sikkim	Spherical
1650b	Sikkim	Irregular
1716/1	Sikkim	Spherical
1533b	Sikkim	Bipyramidal
1707B/4	Sikkim	Spherical
1711/1	Sikkim	Spherical
1587a	Sikkim	Spherical
1642A/a	Sikkim	Irregular
1598d	Sikkim	Irregular
1640B/a	Sikkim	Irregular
1528a	Sikkim	Spherical
1621a	Sikkim	Spherical
1610c	Sikkim	Spherical
1602a	Sikkim	Rhomboidal
1640A/a	Sikkim	Irregular
1620b	Sikkim	Spherical

Table 2. Efficacy of Sikkim *Bacillus thuringiensis* isolates against test insects

Isolates	Mean per cent mortality at 96 h after treatment		
	Bombyx mori	*Crocidolomia binotalis*	*Plutella xylostella*
2a4	83.33(66.18)def	73.33(59.03)cdef	66.67(54.81)cde
3a4	70.00(56.82)fgh	76.67(61.25)bcde	53.33(46.95)e
4a4	80.00(63.47)defg	83.33(66.18)abc	86.67(68.89)ab
6a4	76.67(61.25)defgh	83.33(66.18)abc	93.33(77.75)a
7a4	73.33(59.03)efgh	70.00(56.82)defg	80.00(63.47)bc
9a6	73.33(59.03)efgh	73.33(59.03)cdef	73.33(59.03)bcd
1598a	86.67(68.89)cde	80.00(63.47)bcd	86.67(68.89)ab
1554b	80.00(63.47)defg	86.67(68.89)ab	73.33(59.03)bcd
1532/2	80.00(63.47)defg	63.33(52.80)fghi	86.67(68.89)ab
1526Bb	73.33(59.03)efgh	86.67(68.89)ab	93.33(77.75)a
1544/3	80.00(63.47)defg	76.67(61.25)bcde	80.00(63.47)bc
1706/4	70.00(56.82)fgh	63.33(52.80)fghi	60.00(50.79)de
1711/1(a)	73.33(59.03)efgh	70.00(56.81)defg	60.00(50.79)de
1622 A/a	66.67(54.81)gh	73.33(59.03)cdef	93.33(77.75)a
1611C	80.00(63.47)defg	56.67(48.87)ghi	66.67(54.81)cde
1642 a	86.67(68.89)cde	53.33(46.89)hi	80.00(63.47)bc
1614 C/b	86.67(68.89)cde	73.33(59.03)cdef	73.33(59.03)bcde
1742/1	66.67(54.81)gh	50.00(45.02)i	46.67(43.10)h
1710/1	73.33(59.03)efgh	56.67(48.87)ghi	53.33(46.95)fgh
1567/4	76.67(61.25)defgh	80.00(63.47)bcd	76.67(61.25)bcd
1650b	76.67(61.25)defgh	63.33(52.80)fghi	60.00(50.79)efgh
1716/1	86.67(68.89)cde	56.67(48.87)ghi	53.33(46.95)fgh
553b	76.67(61.25)defgh	70.00(56.81)defg	73.33(59.03)bcd
1707 B/4	83.33(66.18)def	80.00(63.47)bcd	86.67(68.89)ab
1711/1	83.33(66.18)def	70.00(56.81)defg	66.67(54.81)cde
1587a	86.67(68.89)cde	76.67(61.25)bcde	80.00(63.47)bc
1642A/a	83.33(66.18)def	66.67(54.81)efgh	93.33(77.75)a
1598d	90.00(71.60)cd	86.67(68.89)ab	86.67(68.89)ab
1640A/a	73.33(59.03)efgh	63.33(52.80)fghi	60.00(50.79)de
1620b	93.33(77.75)bc	70.00(56.81)defg	66.67(54.81)cde
1640 B/a	86.67(68.89)cde	73.33(59.03)cdef	80.00(63.47)bc
1528a	76.67(61.25)defgh	63.33(52.80)fghi	60.00(50.79)de
1621a	83.33(66.18)def	60.00(50.79)ghi	66.67(54.81)cde
1610c	80.00(63.47)defg	70.00(56.81)defg	73.33(59.03)bcd
1602a	60.00(50.79)h	63.33(52.80)fghi	66.67(54.81)cde
Control	0.25(2.87)i	0.25(2.87)j	0.25(2.87)f
HD1	96.67(83.90)ab	80.00(63.47)bc	86.67(68.89)ab
Dipel	99.97(90.05)a	99.97(90.05)a	93.33(77.75)a
CV (%)	2.21	1.79	2.52
SEm±	3.39	2.50	3.74
CD at 1%	8.96	6.61	9.89

and the means were separated by Duncan's Multiple Range Test (DMRT) (Duncan, 1955).

RESULTS AND DISCUSSION

The three isolates 1554b, 1526B/b and 1598d recorded 86.67 per cent mortality, in 4a4 and 6a4 isolates treatment mortality of 83.33 per cent was noticed when were assayed against *C. binotalis* and were comparable to HD1 (80.00%). Similarly, Yadav (2007) reported 90 per cent mortality in native Sikkim isolates like 1707B/4, 1553/b, 1634/33/C and 1559/b to *C. binotalis* further he also noticed that the native Sikkim isolates were more toxic than the reference strain (HD1) against *C. binotalis*.

Isolates 6a4, 1526B/b, 1632A/a and 1642A/a caused 93.33 per cent mortality to *P. xylostella* and in 4a4 and 1598d (86.67%) morality was observed. Native isolates were comparable with HD1 (86.67%) strain Similar level of mortality (86.60%) to *P. xylostella* was recorded by Yadav (2007) in native isolate 1707B/4. An analogous result was reported by Shilpa (2005) and Marutesh (2007) and in their experiments also native isolates were found more toxic than the reference strain (HD1) against *P. xylostella.* But in case of *B. mori* isolates, 4a4, 6a4, 1554b, 1526B/b, 1642/a, 1642A/a and 1598d showed 80.00, 76.67, 80.00, 73.33, 66.67, 83.33 and 90 per cent mortality, respectively and HD1 showed 96.67% mortality. This finding is in line with Srinivas *et al*., (2002) who reported cent per cent mortality of third, fourth and fifth instars larvae of silkworm, using *B. thuringiensis* commercial products like BTK-I, BTK-II, BARC, Delfin and Dipel.

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 and the stage of metamorphosis of the silkworm (Savitri and Muralimohan 2003). Isolates which were efficient to kill larvae possessed different shaped crystals *viz.,* 4a4, 6a4, 1642/a possess spherical shaped crystals, 1526B/b irregular shaped crystals, 1554b bipyramidal shape and isolates 1642A/a, and 1598d possess irregular shaped crystals.

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