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Research Note

Bioefficacy of Chikkamagalur native *Bacillus thuringiensis* isolates against lepidopteran insects

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ABSTRACT: Laboratory bioassays were carried out to assess the efficacy of *Bacillus thuringiensis* isolates against lepidopteran insects, *viz.,* cabbage leaf webber (*Crocidolomia binotalis* (Zeller)) and diamondback moth, [*Plutella xylostella* (L.)] and for their safety to mulberry silkworm, *Bombyx mori* (L). The commercial products Dipel and HD1 gave significantly higher mortality, but both were on par with isolates 2422c and 2459c in their efficacy against *C. binotalis* and *P. xylostella*, respectively. Of the five tested isolates against lepidopteran insects, the efficacy of each isolate varied with the insect. The isolate 2422c recorded the maximum mortality (80%) against cabbage leaf webber, whereas the isolate 2459c reduced *P. xylostella* by 80.0%. Both commercial products gave 93.3-96.7% mortality, whereas the isolates 2422c and 2459c gave 83.33 and 80% mortality, respectively, against silkworm.

KEY WORDS: *Bacillus thuringiensis*, toxicity, *Crocidolomia binotalis*, *Plutella xylostella*, *Bombyx mori*

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INTRODUCTION

Chemicals are the frontline defense for insect pest suppression. Sole reliance on pesticides is, however, not without problems such as the resurgence of sucking pests, replacement of beneficial fauna, development of resistance and residue of toxic chemicals in food stuffs (Rao *et al*., 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 an aerobic spore former well known for its ability to produce a proteinacious crystal during sporulation (Krieg, 1961; Heimpel, 1963; Heimpel *et al*., 1959). The crystal protein designated as delta-endotoxin is toxic on ingestion for many insect larvae (Heimpel, 1963). Bt formulations do have certain drawbacks like lower environmental stability, reduced *in situ* multiplication of the bacterium, early removal from plant surfaces and non-systemic nature of the toxin that necessitate repeated application. However, *B. thuringiensis* based biopesticide sprays are being used worldwide for insect pest control. In India, *B. thuringiensis* based biopesticide formulations are being used on various crops for the management of lepidopterans (Dhaliwal and Arora, 1998). Hence, it is advised that evaluation of native isolates of *B. thuringiensis* would emerge as a potent tool in managing cabbage leaf webber, *Crocidolomia binotalis* (Zeller) and diamondback moth, *Plutella xylostella* (L.). The use of insect pathogens, particularly *B. thuringiensis* for the suppression of lepidopterous crop pests (Narayanan and Gopalakrishnan, 1988), is encouraging but weighed down with risk to silkworm. This consideration necessitated the investigation of the effect of *B. thuringiensis* isolates on mulberry silkworm, *Bombyx mori* (L.).

Mass multiplication of test insects: Diamondback moth, Plutella xylostella

The larvae collected from the fields were reared separately on cabbage leaves raised in a greenhouse under insecticide – free condition. The pupae thus obtained were kept in a Petri plate and placed in a cage of 25cm^3 for adult emergence. When moths started emerging, mustard seedlings were provided for oviposition. Plastic cups of 6 cm height and 4.5 cm diameter were filled with sterilized vermicompost and mustard seeds presoaked for 24 hrs and treated with Bavistin (2g kg^{-1}) were sown in cups and allowed to germinate under natural conditions. Within 4-5 days after germination, they were placed in the oviposition cage and replenished at 24 hrs interval. The cups with eggs on both the sides of cotyledons were transferred to plastic tubs (45 x 30 x 15 cm) for mass rearing. Honey solution (10%) containing multivitamin powder was provided for the adults as food through cotton swab kept in a sterilized Petri plate. The eggs hatched in 2-3 days and neonates mined the mustard cotyledons and fed on them. When the seedlings were completely

consumed, the larvae were transferred to fully expanded cabbage leaves with petiole covered in wet cotton swab to maintain leaf turgidity. Five-day-old F_1 generation larvae were used for the bioassay.

Leaf webber, Crocidolomia binotalis

The cabbage leaf webber was mass reared in the insectary, Department of Entomology, UAS, Dharwad. The larvae collected from the infested fields of cabbage were reared separately on cabbage leaves raised in a greenhouse under insecticide-free condition. The pupae thus obtained were kept in a sterilized Petri plate inside a cage of $25cm^3$ for adult emergence. When the moths started emerging, 25–30 days old small cabbage heads were provided for oviposition. The egg laid on ventral and dorsal surface of leaves were transferred to plastic tubs (45 x 30 x 15 cm) for mass rearing. Honey solution (10%) containing multivitamin powder was provided for the adults as food through cotton swab kept in a sterilized Petri plate. Five-day-old F_1 generations were used for bioassay.

Silkworm, Bombyx mori

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

Bioassay

Native *B. thuringiensis* isolates collected from Chikkamangalur, Karnataka – isolates 2422c, 2364, 2459c, 1201c and 1228a and preserved in the Department of Biotechnology, UAS, Dharwad, were used for bioassay to ascertain their insecticidal activity against test insects.

Preparation of B. thuringiensis culture for bioassay

To multiply the isolates, they were streaked on plain Luria agar (LA) plates, kept in incubation for 24 h and inoculated in Luria broth (LB) of 1ml in eppendorf tube and kept for growth in a shaker at 28°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°C on a shaker at 200 rpm. Serial dilution of the culture from 10° 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°C. Colony counts were taken after 24h and calculations were done using the standard formula ($1.2x10^{-6}$ CFU ml^{-1}) (Shilpa, 2005) to fix the concentration of *B. thuringiensis.*

Insecticidal activity of native B. thuringiensis isolates against test insects

Leaf dip bioassay described by Tabashnik and Cushing (1987) was adopted. Leaf discs of 6 cm diameter were cut covering either side of the midrib from untreated mulberry leaves for *B. mori* and cabbage leaves for *C. binotalis* and *P. xylostella*. These discs were dipped in aqueous solution of the test isolates for about 30 seconds. Excess fluid was drained and the discs were dried under shade for 10 min before transferring to plastic containers (10 cm height and 6 cm diameter) over a moistened filter

Table 1. Efficacy of Chikkamagalur *B. thuringiensis* isolates against test insects

Isolates	Mean per cent mortality at 96h after treatment		
	Bombyx mori	*Crocidolomia binotalis*	*Plutella xylostella*
2422c	83.33 (66.18)^{bc}	80.00 (63.47)^{b}	73.33 (59.03)^{c}
2364	63.33 (52.80)^{c}	66.67 (54.81)^{c}	73.33 (59.03)^{c}
2459c	80.00 (63.47)^{bc}	76.67 (61.25)^{b}	80.00 (63.47)^{bc}
1201c	76.67 (61.25)^{c}	73.33 (59.03)^{bc}	73.33 (59.03)^{c}
1228a	70.00 (56.82)^{c}	53.33 (46.95)^{d}	53.33 (46.95)^{d}
Control	0.25 (2.87)^{d}	0.25 (2.87)^{e}	0.25 (2.87)^{e}
HD1	93.33 (77.75)^{ab}	80.00 (63.47)^{b}	86.67 (68.89)^{b}
Dipel	96.67 (83.90)^{a}	90.00 (71.60)^{a}	99.97 (90.05)^{a}
CV (%)	3.37	1.62	1.85
SEM$\pm$	4.79	2.09	2.53
CD at 1%	14.00	6.12	7.42

*Values within parentheses indicate arcsine transformed values; values superscripted by the same alphabet(s) are statistically on par with each other by DMRT

paper. The leaf discs were placed slantingly so that the larvae can move and feed on either side. Bioassays were done with three replications per treatment and ten larvae of the test insects were released on each disc and the container was covered with a muslin cloth using a rubber band.

HD1 served as a standard check, Dipel 8L served as standard commercial *B. thuringiensis* formulation and leaf disc dipped in distilled water alone served as the control. Later mortality was observed at 24h, 48h, 72h and 96h after the treatments and the data were subjected to analysis of variance after suitable transformation (arcsine) and the means were separated by Duncan's Multiple Range Test (DMRT) (Duncan, 1955).

The bioassays of different isolates against *C. binotalis* and *P. xylostella* gave differential mortality. Commercial product Dipel gave 90% mortality of larvae after 96 h, followed by HD1 (80%) and native isolate 2422c (80%). The rest of the isolates, *viz.,* 2459c, 1201c, 2364 and 1228a registered 76.67, 73.33, 66.67 and 53.33% mortality, respectively, against *C. binotalis* (Table 1).

The highest mortality (99.97%) was obtained with Dipel against *P. xylostella* followed by the reference strain HD1 (86.67%), which was on par with the isolate 2459c (80%). The native isolates 1201a, 2364 and 2422c recorded 73.33% mortality, whereas the isolate 1228a registered only 53.33 per cent mortality against *P. xylostella*.

Dipel gave 96.67% mortality of *B. mori* after 96h and was on par with HD1 (93.33%). The isolates 2422c, 2459c, 2364, 1201c and 1228a caused 83.33, 80, 63.33, 76.67 and 70% mortality, respectively. This finding is in line with those of Marutesh (2007), who reported native isolates giving higher mortality of *P. xylostella*.

A very wide variation exists for the effectiveness of *B. thuringiensis* isolates against target insects (Yaradoni, 1999) and their infectivity against silkworm (Savitri and Muralimohan 2003; Chitra *et al.*, 1974). Knowles (1994) opined that the variations in efficacy against different lepidopterans may be due to varying number of *Cry* genes and the absence of specific binding sites.

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