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Baseline susceptibility of the diamondback moth, *Plutella xylostella* (Linnaeus) to *Bacillus thuringiensis* Cry1A toxins in India

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Abstract

Toxicity of *Bacillus thuringiensis* Cry1A δ-endotoxins to the 6-day old larvae of 14 different field populations of the diamondback moth, *Plutella xylostella* (Linnaeus) collected from seven states in the country was determined by leaf dip bioassay method. Cry1Ab was two times more toxic than Cry1Ac. The median lethal concentrations, LC_{50} 72 h, varied from 0.002 to 0.386 μg/ml for Cry1Ab and from 0.011 to 0.324 μg/ml for Cry1Ac. The winter population of *P. xylostella* showed significantly lower (4-fold) susceptibility to Cry1Ab than the summer population at Hisar. The discriminating concentration of 15.8 μg/ml for Cry1Ab over a 72 h assay based upon LC_{99} for the most resistant strain is proposed for the routine monitoring of resistance.

Keywords: Diamondback moth; *Plutella xylostella* (Linnaeus); *Bacillus thuringiensis*; Cry toxins; Baseline susceptibility

1. Introduction

Bacillus thuringiensis is a gram positive and soil inhabiting bacterium which is environmentally safe and effective for the control of insects. *B. thuringiensis* owes its insecticidal activity to the presence of parasporal crystalline proteinaceous δ-endotoxins. These insecticidal crystal proteins are divided into five major classes with specific insecticidal activity, namely Cry1 (Lepidoptera), Cry2 (Lepidoptera and Diptera), Cry3 (Coleoptera), Cry4 (Diptera) and Cry5 (Lepidoptera and Coleoptera) (Cannon, 1996; Crickmore et al., 1998).

B. thuringiensis has been in use as conventional insecticide for more than 60 years, but recent biotechnological advances have led to development of *B. thuringiensis* transgenic crops; which express Cry toxins constitutively. As a result, about 12.2 million hectares of *B. thuringiensis* transgenic crops like cotton, corn, potato were cultivated in 2003 (http://www.isaaa.org/kc/CBTNews/press_release/briefs30/es_b30.pdf). Similar efforts are being made to develop *B. thuringiensis* transgenic cole crops to protect from the attack of the diamondback moth, *Plutella xylostella* (Plutellidae: Lepidoptera) (Cao et al., 1999; Sharma et al., 2003). *P. xylostella* is the most destructive pest of cruciferous plants throughout the world, whose control costs about $1 billion annually (Talekar, 1992). In India, the damage to cole crops cultivated over about 501,700 hectares due to *P. xylostella* is estimated at $ 16 millions annually (Mohan and Gujar, 2003).

P. xylostella has developed resistance to as many as 73 insecticides including *B. thuringiensis* strains and its toxins (http://www.pesticideresistance.org/DB/speciesprofile.php?arthropodid=395). It has also developed resistance to the foliar sprays of *B. thuringiensis* formulations under field conditions (Tabashnik, 1994; Wright et al., 1997; Ferré and van Rie, 2002, Gujar and Mohan, 2002). The successful *B. thuringiensis* resistance management is based up on the high toxin expression and maintenance of refugia for transgenic cotton and maize crops. It requires a good database on the susceptibility status of pest species for high toxin expression in plant and routine resistance monitoring.

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This communication reports extensively susceptibility pattern of *P. xylostella* to two different *B. thuringiensis* Cry toxins.

2. Materials and methods

2.1. Collection and maintenance of P. xylostella

The different field populations of *P.xylostella* were collected from different geographical regions of India viz., Iruttupallam (11°3′ 0N; 76°58′E), Coimbatore (11°0′ 0N; 76°58′ 0E), Guntur (16°18′ 0N; 80°27′ 0E), Mangalagiri (16°25′ 60N; 80°32′ 60E), Eluru (16°41′ 60N; 81°50′E), Hyderabad (17°22′ 60N; 78°28′ 0E), Getalsud (23°26′ 60N; 85°31′ 0E), Najafgarh (28°37′ 0N ; 76°58′ 0E), Gurgaon (28° 28′ 0N; 77°16′ 0E), New Delhi (28° 40′ 0 N; 77°13′ 0E), Panipat (29° 23′ 0N;76° 58′ 0E), Hisar (29°10′ 0 N; 75°43′ 0E), Palampur (32° 13′ 0N; 76°19′ 0E), Ludhiana (30°56′ 0N;75°54′ 0E).

The different populations were maintained separately in laboratory. The adult moths were released into insect cages (glass and wired cases) for mating. They were allowed to lay eggs on Parafilm M coated with cabbage leaf extract. Then, these papers were transferred to cabbage or cauliflower leaves for feeding. Adults were fed on 15% honey solution fortified with drop each of multivitamins (ABDEC® forte drops, Pfizer Ltd.) and vitamin E (Evion®, Merck) for better egg laying. Insects were maintained at 27±1 °C, 60–70% RH and a photoperiod of 14 h light and 10 h dark. Under these conditions, egg period: 3–4 days, larval period: 8–12 days, pupal period: 4–6 days which takes 15–22 days for completion of one generation from egg to adult. For temporal variation, we collected *P. xylostella* population at frequent intervals in the single crop season from Najafgarh whereas Hisar, Eluru and Ludhiana populations were collected in different crop seasons.

2.2. B. thuringiensis toxins and their purification

Cry1Ab and Cry1Ac toxins were prepared from *Escherichia coli* strains according to the method described by Lee et al. (1992). Protoxins were converted to toxins by treatment with trypsin kept at 37 °C for 5 h and quantified by eluting Coomassie brilliant blue R-250 dye from stained toxin bands on sodium dodecyl sulphate-polyacrylamide gel electropherogram as described by Ball (1986).

2.3. Toxicity bioassays

We used leaf dip method for conducting bioassays on *P. xylostella* (Mohan and Gujar, 2003). The cabbage (*Brassica oleracea* var. *capitata*; cv. Golden acre) leaves were first washed with distilled water containing 0.1% Triton X-100 and air dried for about 10 min. Leaf discs (4.5–5.0 cm dia) were cut from the centre of the middle leaves of cabbage plants using a plastic punch and dipped in different concentration of respective toxins for 10–15 s and then allowed to air-dry for 30 min to 1 h at room temperature. Control leaf discs were immersed in distilled water containing Triton X-100 (50 μg/ml). The leaf discs were placed in individual plastic Petri dishes (6 cm in dia) containing single, dry filter paper (4.5 cm dia). Ten, 6-day old (3rd instar) larvae were released on each leaf disc with 3–4 replicates per treatment. Five or six treatments were kept for each bioassay based on availability of larvae. Larvae were allowed to feed on treated disc for 72 h at 27±1 °C and 60–70% RH. Larval mortality was recorded at 24, 48, and 72 h from the date of experiment. Experiments with control mortality more than 20% were discarded and repeated.

2.4. Statistical analysis

The LC_{50} were estimated for 72 h mortality data by using Maximum Likelihood Programme (Ross, 1987). The significance of difference between two LC_{50} was determined on the basis of overlap of 95% fiducial limits at 1% level (Litchfield and Wilcoxin, 1949).

3. Results

The Delhi population (IARI fields) was most susceptible to both Cry1Ab and Cry1Ac (0.002 and 0.011 μg/ml, respectively) when compared with the other field populations (Tables 1 and 2). Hence, it was used for the calculation of resistance ratios with respect to other populations. There was a wide variation (resistance ratio of 193-fold) in susceptibility of *P. xylostella* to Cry1Ab (Table 1). The toxicity of Cry1Ab ranged from 0.002 to 0.386 μg/ml among different populations. The Eluru population, which showed highest resistance to Cry1Ab, and was at par with that from Mangalagiri, differed significantly in susceptibility with those from Iruttupallam, Hyderabad, Ludhiana, Guntur, Getalsud, Gurgaon, Panipat, Hisar, Najafgarh, Coimbatore and New Delhi populations.

Field populations of *P. xylostella* showed less variation in susceptibility to Cry1Ac (29-fold) with LC_{50} ranging from 0.011 to 0.324 μg/ml (Table 2). The Eluru population, which exhibited the highest tolerance to Cry1Ac, differed significantly in its susceptibility to Cry1Ac with those from Iruttupallam and New Delhi.

There was a variation in susceptibility of *P. xylostella* to both Cry1Ab and Cry1Ac temporally (Table 3). The winter population of *P. xylostella* was four-fold less susceptible than the summer population to Cry1Ab at Hisar. The populations of Eluru and Ludhiana showed little difference in susceptibility levels over a year for