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RESEARCH ARTICLE

Relative toxicity of subspecies of *Bacillus thuringiensis kurstaki* HD-1 and HD-73 against the larvae of legume pod borer, *Maruca vitrata*, F. (Lepidoptera: Crambidae)

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Abstract

The legume pod borer, *Maruca vitrata* F. (Lepidoptera: Crambidae), is a major insect pest of many edible legumes in various regions of America, Asia and Africa. The larvae cause serious damage to the reproductive parts of cowpea, pigeon pea and beans in India. Promotion and use of biopesticide containing *Bacillus thuringiensis* (*Bt*) is viewed as a viable alternative to synthetic pesticides. In this study, field populations of *M. vitrata* were collected from intensive legume-growing regions of India during years 2023 and 2024. A commercial formulation, *Bt* sub.sp. *kurstaki* HD-1 (Delfin®), along with reference strains of *Bt* sub.sp. *kurstaki* HD-1 and HD-73 were tested on the larvae of different *M. vitrata* populations collected across India to evaluate their relative effectiveness. The LC_{50} values estimated for *Bt kurstaki* HD-1 and *Bt kurstaki* HD-73 strains and a commercial formulation of *Bt kurstaki* HD-1 (Delfin®) against different field-collected populations of *M. vitrata* ranged from 1.097 to 1.829 ppm, 6.228 to 7.236 ppm and 2.894 to 4.930 ppm, respectively. The *Bt kurstaki* HD-1 strain harbouring multiple crystal proteins (Cry1Aa, Cry1Ab, Cry1Ac, Cry2A, Cry2B) were relatively more toxic to the larvae of *M. vitrata* than *Bt kurstaki* HD-73 which harbours a single Cry toxin i.e., Cry1Ac.

Keywords

Bacillus thuringiensis; biopesticides; cry toxins; *Maruca vitrata*; toxicity

Introduction

Maruca vitrata F. (Lepidoptera: Crambidae), also known as bean or legume pod borer, spread across tropical and sub-tropical regions of the world is a serious threat to realizing the potential yield of many food legumes. The pest can damage the reproductive parts such as flowers and pods of cowpea, beans and pigeon peas (1, 2), which affects the quantity and quality of marketable vegetables and grains. *M. vitrata* is responsible for 79.05 % of avoidable losses in pigeon pea production in India (3). The frequent use of these synthetic insecticides has raised several concerns, including the development of insecticide resistance, environmental contamination and adverse effects on non-target organisms and beneficial insects such as ecosystem disruption (4). Hence, development of sustainable management strategies is very important to reduce the usage of synthetic insecticides (5). One such promising alternative to chemical insecticides is the use of *Bacillus thuringiensis* (*Bt*) based biopesticides. *Bt* is a soil bacterium that produces crystal (Cry) proteins and vegetative insecticidal proteins (Vip), which are toxic to a wide range of lepidopteran pests (6). *Bt*-based biopesticides have gained popularity due

to their highest sensitivity to target pests and their relative safety for non-target organisms and the environment (7, 8). The Cry proteins bind to specific receptors in the insect midgut, leading to pore formation, cell lysis and eventual insect death (9). However, the efficacy of *Bt* proteins against *M. vitrata* can vary among different strains and formulations and can be influenced by the geographical distribution and genetic diversity of pest populations (10). Therefore, to validate the efficacy of *Bt* strains and provide a consistent recommendation for its management, testing of many field-collected populations of *M. vitrata* was conducted.

Materials and Methods

Insect collection and maintenance

To screen the toxicity of *Bt* strains, field populations of *M. vitrata* were collected during year 2024 from the major leguminous crop-growing regions across India. The selected places are known for intensive cultivation of pulses: Hyderabad in Telangana (17.5111° N, 78.2752° E), Dharmapuri in Tamil Nadu (12.229615° N 78.1543° E), Guntur in Andhra Pradesh (16.2874° N, 80.3707° E), Kolar in Karnataka (13.543357°N 78.333298° E) and Vellayanikara in Kerala (8.4316°N, 76.9860° E). Larvae were maintained at 25 ± 1°C, 70 ± 5 % humidity and a 12:12 light-dark cycle on a semi-synthetic diet, changed every three days. Paper folds were provided for pupation. Adults were transferred to rearing cages with 10% honey solution, Vitamin E and pigeon pea twigs for oviposition. Eggs laid on pigeon pea plants were collected and neonate larvae were moved to a semi-synthetic diet for development under controlled conditions.

An insecticide-susceptible iso-female colony of *M. vitrata* (National Accession Number NBAIR-IS-CRA-02) was also included in the toxicity assays. This colony was originally collected from a field bean crop near Bengaluru (12.9716°N, 77.5946°E) and maintained at the Insect Genomic Resources Laboratory of the Indian Council of Agricultural Research - National Bureau of Agricultural Insect Resources (ICAR-NBAIR), Bengaluru for more than 70 generations. All the populations were maintained on the modified semi-synthetic artificial diet using chickpea flour as the protein base.

Bacillus thuringiensis strains

Bt sub.sp. *kurstaki* HD-1 and HD-73 strains were procured from *Bacillus* Genetic Stock Centre, Colombus, USA. Acetone-lactose co-precipitation method (11) was employed to prepare the spore-crystal formulation of these strains. The acetone-lactose co-precipitation method involves harvesting spores and crystals, washing with acetone to remove debris, precipitating with lactose and centrifuging the mixture to obtain a purified spore-crystal powder. The commercial formulation of *Bt kurstaki* HD-1 strain (Delfin®), a product of MARGO Biocontrols Pvt. Limited, Bangalore, was used in this study. The Cry toxin content in these formulations was determined as per standard protocol (12). The specifications regarding these strains are supplied in the Table 1.

Bioassay protocol

Dose-response bioassays were conducted against pre-starved third-instar larvae of *M. vitrata*. Five to seven appropriate concentrations of each formulation were used in such a way that would cause 5 to 95 % larval mortality with triplicates. One millilitre of the diet containing different concentrations of the *Bt* toxin was added to each well of the bioassay tray. Then, one 3rd instar larva was placed in each well and sealed with a self-adhesive label. The bioassay trays were incubated in a BOD incubator set at 26 ± 2 °C, 70 ± 5 % relative humidity and a 12:12 light-dark cycle. The larval mortality was determined after four days of continuous feeding on the treated diets. Control larvae were maintained on the plain diets without any toxins. Moribund larvae which did not respond to probing were considered as dead.

Statistical Analysis

The data was subjected to Probit analysis according to Finney (13) using Polo-PC® LeOra software Petulama, California, USA (14) and assessed LC_{50} (lethal concentrations causing 50 per cent mortality), 95 per cent fiducial limits and slope value of probit line. Abbott's formula (15) was used to determine the adjusted larval mortality. The estimated parameters included the median lethal concentration (LC_{50}), slope and intercept of the model. GraphPad Prism software (16) was used to visualize the results and goodness of fit analysis was performed for everyone.

Results

Relative toxicity of B. thuringiensis strains against M. vitrata populations

The representative population of *M. vitrata*, collected from different locations indicated a similar level of susceptibility to all the three *Bt* strains tested. The toxicity findings against *M. vitrata* third instar larvae are presented in Tables 2, 3 and 4. The commercial strain of *Bt kurstaki* HD-1 as well as the *Bt kurstaki* HD-1 strain obtained from BGSC, Columbus exhibited significantly higher toxicity compared with *Bt kurstaki* HD-73 strain. The LC_{50} values estimated for the laboratory colony of *M. vitrata* were 1.097 ppm for HD-1, 6.223 ppm for HD-73 and 2.894 ppm for Delfin.

The bioassay results revealed the levels of sensitivity among *M. vitrata* populations to different strains of *Bt kurstaki*. The LC_{50} values for *Bt kurstaki* HD-1 ranged from 1.097 to 1.829 ppm, indicating a high sensitivity to this strain. In contrast, the LC_{50} values for *Bt kurstaki* HD-73 ranged from 6.228 to 7.934 ppm, showing moderate sensitivity across the tested populations. Additionally, the commercial formulation of *Bt kurstaki* HD-1 (Delfin®) had LC_{50} values between 2.894 and 4.930 ppm, reflecting higher sensitivity, though slightly less potent compared to the HD-1 strain. Overall, *M. vitrata* populations showed the greatest susceptibility to the HD-1 strain.

Table 1. Details of toxin tested against *M. vitrata* larvae

*Bt** strains	Subspecies	Source	Crystal toxins identified
Bt kurstaki HD-1	*kurstaki*	BGSC,Columbus,USA	Cry1Aa, Cry1Ab, Cry1Ac, Cry2A, Cry2B
Bt kurstaki HD-73	*kurstaki*	BGSC,Columbus,USA	Cry1Ac
Bt kurstaki HD-1 commercial formulation (Delfin®)	*kurstaki*	Margo Biocontrols Pvt. Ltd.	Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ia, Cry2Aa, Cry2Ab, Vip 3Aa10

* *Bt- Bacillus thuringiensis*

Table 2. Relative toxicity of *Bt kurstaki* HD-1 strain against various populations of *M. vitrata*

Bt Strain	*M. vitrata* population source	Slope ± SE	X^2 (df)	LC_{50} (ppm)	95 % fiducial limits	
					Lower limit	Upper limit
***Bt kurstaki* HD-1**	Susceptible (NBAIR-IS-CRA-02)	1.897 ± 0.348	1.688 (4)	1.097	0.678	1.577
	Vellayanikara	1.710± 0.328	3.222 (4)	1.151	0.691	1.698
	Guntur	2.025 ±0.360	3.720 (4)	1.461	0.976	2.053
	Hyderabad	2.320 ± 0.405	2.759 (4)	1.656	1.157	2.251
	Kolar	2.454 ± 0.424	3.221 (4)	1.046	1.156	2.835
	Dharmapuri	2.558 ± 0.438	3.459 (4)	1.829	1.004	2.943

* SE-Standard error; X^2- chi square value; df- degrees of freedom; ppm-parts per million; LC_{50}- lethal concentration 50

Table 3. Relative toxicity of *Bt kurstaki* HD-73 strain against various populations of *M. vitrata*

Bt Strain	*M. vitrata* population source	Slope ± SE	X^2 (df)	LC_{50} (ppm)	95 % fiducial limits	
					Lower limit	Upper limit
***Bt kurstaki* HD-73**	Susceptible (NBAIR-IS-CRA-02)	3.135 ± 0.568	0.435 (4)	6.228	4.686	8.240
	Vellayanikara	3.632 ± 0.657	3.293 (4)	6.991	5.412	9.123
	Guntur	3.587 ± 0.643	5.932 (4)	7.563	5.308	11.116
	Hyderabad	3.605 ± 0.649	4.433 (4)	7.271	5.395	9.977
	Kollar	3.370 ± 0.600	4.331 (4)	7.934	5.218	12.557
	Dharmapuri	2.811 ± 0.500	5.722 (4)	7.236	4.059	13.748

* SE-Standard error; X^2- chi square value; df- degrees of freedom; ppm-parts per million; LC_{50}- lethal concentration 50

Table 4. Relative toxicity of commercial formulation of *Bt kurstaki* HD-1 (Delfin®) against various populations of *M. vitrata*

Bt strain	Location	Slope ± SE	X^2 (df)	LC_{50} (ppm)	95 % fiducial limits	
					Lower limit	Upper limit
***Bt kurstaki* HD-1 (Delfin®)**	Susceptible (NBAIR-IS-CRA-02)	2.094 ± 0.369	1.017 (4)	2.894	1.891	4.135
	Vellayanikara	2.566 ± 0.472	0.863 (4)	3.401	2.347	4.644
	Guntur	2.445 ± 0.444	2.528 (4)	4.044	2.821	5.565
	Hyderabad	2.767 ± 0.511	1.474 (4)	3.935	2.807	5.299
	Kollar	2.442 ± 0.439	2.051 (4)	4.667	3.291	6.426
	Dharmapuri	2.615 ± 0.472	1.771 (4)	4.930	3.538	6.702

*SE-Standard error; χ2- chi square value; df- degrees of freedom

The dose-response curves (Slope ± SE) suggest a relatively uniform sensitivity across the populations. The populations' dose-response curves had a generally similar slope, with values for *Bt* subspecies HD-1 at 1.710 ± 0.328 to 2.558 ± 0.438, *Bt* subspecies HD-73 at 2.811 ± 0.500 to 3.632 ± 0.657 and *Bt kurstaki* HD-1 (Delfin®) at 2.094 ± 0.369 to 2.615 ± 0.472. Greater variability is indicated by lower slopes, while less variance in sensitivity within the population is suggested by higher slopes. The dose-response curves (Slope ± SE) suggest a relatively uniform sensitivity across the populations. Additionally, chi-square (x^2) values for all the toxins demonstrate a generally good fit for the model used in the analysis. The accuracy, precision and sensitivity of different *M. vitrata*

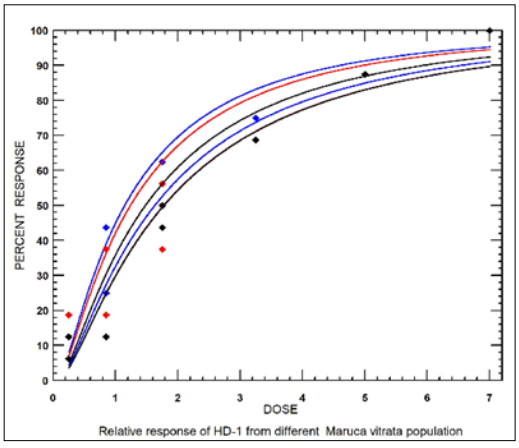

Fig. 1a: Dose-response curve of *Bt kurstaki* HD-1 for different *M. vitrata* populations.

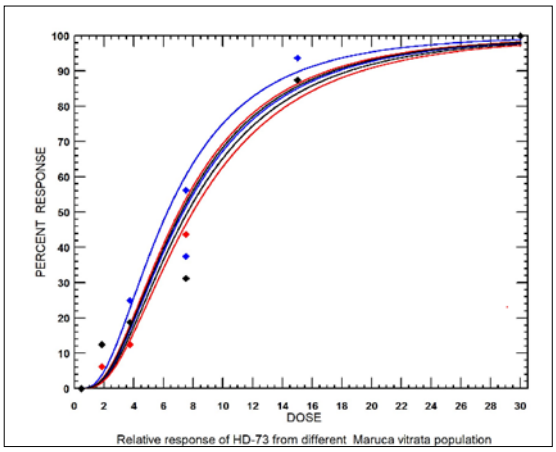

Fig. 1b: Dose-response curve of *Bt kurstaki* HD-73 for different *M. vitrata* populations.

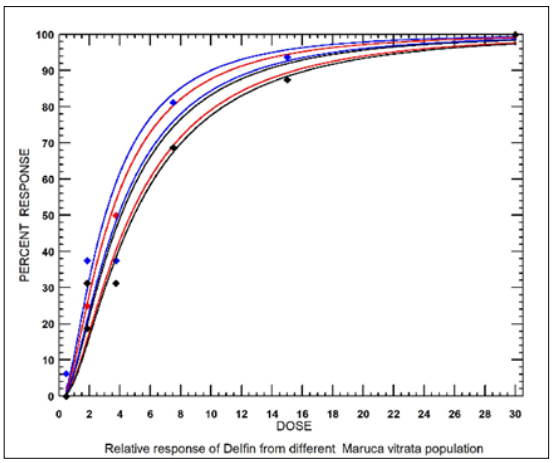

Fig. 1c: Dose-response curve of *Bt kurstaki* HD-1 (Delfin®) for different *M.vitrata* populations.

Fig. 1. Dose-response curve of different toxins for *M. vitrata* populations.

populations towards different toxins was depicted through the probit response curve (Fig.1a,1b,1c).

Pairwise correlation coefficient of B. thuringiensis insecticidal proteins for M. vitrata

According to Pearson's correlation analysis, the two toxins that have the largest positive correlation (0.790) are Delfin-5 % and HD-73. This indicates a strong association where both toxins tend to increase concurrently and the low p-value suggests that the correlation is likely significant. The slightly positive correlation (0.509) between Delfin-5 % and HD-1 points to a potentially significant and relatively strong relationship. Like the Delfin-5 % and HD-73 pair, HD-1 and HD-73 show a moderate association (0.386) but not statistically significant. By comparison, the correlation between HD-1 and Delfin-5 % is poor (0.151), suggesting a slight relationship that might not be statistically significant. Ultimately, there appears to be no significant correlation between HD-73 and Delfin-5 %, as evidenced by their nearly insignificant correlation (0.031) (Fig. 2).

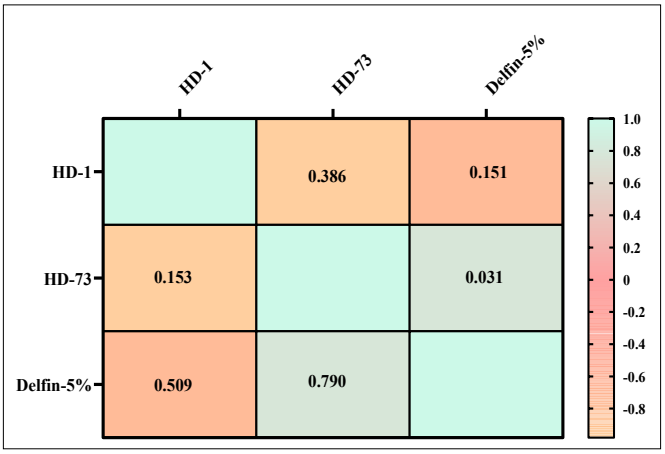

Fig. 2. Pairwise correlation analysis of LC_{50} values of three toxins in field populations of *M. vitrata*. *Values below the unfilled diagonal squares indicate the correlation coefficient r and above the unfilled diagonal squares the corresponding P-values. The scale colours of the filled boxes indicate the magnitude of the correlation.

Discussion

The relative toxicities of strains of *Bt kurstaki* HD-1 and HD-73 were assessed against larvae of multiple field-collected populations of *M. vitrata*. The results revealed that the *Bt kurstaki* HD-1 strains which reportedly harbour Cry1Aa, Cry1Ab, Cry1Ac, Cry2A, Cry2B toxins exhibited high levels of mortality against all the populations of *M. vitrata* as compared to *Bt kurstaki* HD-73 which produced only Cry1Ac toxin. The extreme toxicity of *Bt kurstaki* HD-1 against diamondback moth was reported (17). The toxin Cry1Ac present in HD-73 strain was least effective against *M. vitrata* (18). Similarly, Cry1Ab was more toxic than Cry1Ac against *Chilo suppressalis* (19). The presence of cry genes and vip genes in the midgut of insects may result in increased toxicity as well as assist prevent or delay the development of resistance in insect populations (20). These genes create numerous insecticidal toxins with varied binding affinities. The high susceptibility of *M. vitrata* larvae to *Bacillus thuringiensis kurstaki* formulations, resulting in significant larval mortality and reduced crop damage in field conditions (21). A recent study found that toxicity to *Bt* toxins in *Spodoptera frugiperda* varied substantially across different regions, with LC_{50} values ranging widely due to differences in exposure history, local agricultural practices and ecological conditions (22).

The minimal variation in resistance levels detected is in on par with findings from bioassays conducted on different insect species that are not frequently exposed to *Bacillus thuringiensis* (*Bt*) formulations. The toxicity rations below 10-fold is considered as natural variations among the populations and should not be considered as a sign of resistance development (23). Likewise, some other studies have revealed the importance of incorporating biopesticides such as *Bt* based formulations in rotation or combination with other pest management strategies for sustainable management of the insect pests over time (24). The information generated in the present study serves as a baseline data for monitoring the development of resistance against *Bt* in *M. vitrata* populations in future in the event of increased use of *Bt* based formulations for its management.

Conclusion

Due to the development of resistance to synthetic insecticides, Bt kurstaki HD-1-based formulations are considered a viable alternative for managing *M. vitrata*. Rotating Bt products with these toxins can effectively help prevent resistance development in *M. vitrata*. These Bt-based products can be successfully integrated into Integrated Pest Management (IPM) programs for managing legume pod borer infestations.

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Authors' contributions

NV took the lead in writing the original draft and contributed to visualization, validation, methodology, investigation, and formal analysis. MS focused on reviewing and editing the manuscript, visualization, software development, and data curation. NA provided overall supervision for the project. MM contributed through supervision and provision of resources. SP also supported the work with supervision and resources. SV was responsible for formal analysis and data curation. MJ offered supervision and resources, while RG contributed to visualization, software development, and data curation. MM played a pivotal role in conceptualization, methodology, reviewing and editing the manuscript, supervision, resource allocation, and project administration.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships

that could have appeared to influence the work reported in this paper.

Ethical issues: None

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