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# Potential of Cry1Ac from *Bacillus thuringiensis* against the tomato pinworm, *Tuta absoluta* (Meyrick) (Gelechiidae: Lepidoptera)

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## Abstract

The tomato pinworm, *Tuta absoluta* (Meyrick) (Gelechiidae: Lepidoptera), is an introduced serious pest of tomato in India. Management of this insect pest mainly relies on insecticides because of its high infestation levels on all plant parts and life stages of tomato crop. This laboratory study investigated the efficacy of Cry1Ac protein of *Bacillus thuringiensis* against *T. absoluta*. The LC<sub>50</sub> and LC<sub>95</sub> values for 2nd, 3rd, and 4th larval instars were 0.12, 0.27, and 0.43 µg/ml and 0.63, 0.71, and 2.64 µg/ml, respectively. Experimental results showed that Cry1Ac is effective against different larval instars of tomato pinworm.

**Keywords:** *Bacillus thuringiensis*, *Tuta absoluta*, Bioassay, Cry1Ac toxin

## Background

The tomato pinworm (TPW), *Tuta absoluta* (Meyrick) (Gelechiidae: Lepidoptera), is an important pest of tomato in India. It is a micro-lepidopteran and oligophagous pest native of South America. It was described by E. Meyrick in Peru during 1917. Tomatoes are grown both under greenhouse and open field conditions. One of the major limiting factors in tomato production is *T. absoluta*, a global invasive pest. In India, this pest was initially observed in Pune, Maharashtra, in both polyhouse and open field tomatoes in October 2014. The maximum level of infestation causes 80–100% yield loss (Tropea Garzia et al. 2012; Shashank et al., 2015).

Among several management options, more reliance on insecticides may not be viable as they provide ephemeral benefits, often with adverse side effects. Evolution of pesticide-resistant strains has been reported in South

America and Europe due to repeated and heavy use of pesticides (Silva et al., 2011). One alternative to insecticides is the use of biological insecticides like *Bacillus thuringiensis* (*Bt*) that expresses insecticidal crystal (CRY) proteins during sporulation phase of its growth cycle. These crystal proteins, which are sequestered in bacteria as crystalline inclusions, mediate specific pathogenicity against insects. It has been found to be a very effective, environmentally safe, and insect-specific bio-pesticide. Toxicity of *Bt* spray-able formulations, which are considered highly effective, selective, safe, and compatible in integrated pest management, is largely due to Cry toxins (Sanyasi and Govind, 2011).

Alternatives like biological preparations from *Bt* reduced *T. absoluta* damage up to 90%, but showed poor field persistence and need for repeated applications (Gonzalez-Cabrera et al. 2011). *Bt* var. *kurstaki* still exhibits a satisfactory efficacy against *T. absoluta* larval infestations in Spanish outbreaks (Sanda et al., 2018). According to the different varieties, *Bt* is highly specific to some insect orders including Lepidoptera, Diptera, and Coleoptera. *Bt*

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formulates are very effective against *T. absoluta* under laboratory, greenhouse, and field conditions (Ghazwan et al., 2017). The present study was undertaken to evaluate the potential of *Bt* toxin Cry1Ac against larval instars of *T. absoluta* under laboratory conditions.

## Materials and methods

Purified Cry1Ac toxin was obtained from the Centre for Plant Molecular Biology and Biotechnology (CPMB& B), Tamil Nadu Agricultural University (TNAU), Coimbatore, India. Original JM (Jockey Mango) 103 *E. coli* strain expressing Cry1Ac gene was originally obtained from Dr. Neil Crickmore's Lab, University of Sussex, UK, and Cry1Ac was extracted as described by Sayyed et al. (2000). Cry1Ac toxin was stored at  $-20^{\circ}\text{C}$ .

All the experiments were conducted at Horticultural College & Research Institute, Periyakulam, TNAU. The culture of tomato pinworm was maintained on tomato plants (PKM1) for 2 generations prior to use in leaf dip bioassay studies under laboratory conditions. The toxin dilutions were freshly prepared for each assay (Dakshina and Gary, 2003). For  $\text{LC}_{50}$  estimation, bioassay studies were conducted by different concentrations. The concentrations of *Bt* protein were prepared separately for 2nd instar (0.05, 0.10, 0.15, 0.20, 0.25, and 0.30  $\mu\text{g/ml}$ ), 3rd instar (0.15, 0.20, 0.25, 0.30, 0.35, and 0.40  $\mu\text{g/ml}$ ), and 4th instar (0.10, 0.20, 0.30, 0.40, 0.50, and 0.60  $\mu\text{g/ml}$ ) in separate experiments. All concentrations were made up to a total volume of 25 ml in a glass beaker. To determine  $\text{LC}_{50}$  values for toxic concentrations in the exposure experiments, preliminary bioassays were conducted. Different known concentrations of Cry1Ac protein were used and prepared in 0.02% Tween 20, using double distilled water.

Fresh leaves were placed in a plastic container (2-cm diameter and 3-cm height) into which 2% melted agar (allowed to cool) was added and then leaf discs (1-cm diameter) were placed to maintain turgidity, and individual larvae per container were released. Pot-cultured 45-day-old plant tomato (PKM1) leaf discs were immersed in each suspension for 1 min, and then air dried. Groups of 10 *T. absoluta* larvae (2nd, 3rd, and 4th separately), pre-starved for 4 h, were allowed to feed on the treated leaves. For untreated check, the larvae were fed on untreated leaves (leaves dipped in 0.02% Tween 20 and air dried). A minimum of 30 insects per concentration were used; each concentration had 3 subsets as replicates. Each assay was performed 2–3 times. All bioassays were carried out under a controlled environment at  $25 \pm 5^{\circ}\text{C}$  and 80% RH with a 12:12 h (D:L).

## Data analysis

Mortality rates of larvae at 24-h intervals for 5 days after initiation of the experiment were recorded. Any larva

failed to move when touched repeatedly was considered dead. Corrected mortality percentages were worked out, using Abbott's formula (Abbott, 1925), and subjected to probit analysis (Finney, 1971) from EPA Probit Analysis Program (version 1.5).

## Results and discussion

The results of the probit regression analysis of concentration-response mortality data for the bioassays of Cry1Ac to *T. absoluta* were recorded. The slope values of different larval instars varied significantly, indicating variability in the susceptibility to Cry1Ac among the larval stages. *T. absoluta* showed variable responses to Cry1Ac as reflected in the  $\text{LC}_{50}$  values for 2nd, 3rd, and 4th instar larvae. Cry1Ac showed toxicity to all larval instars of pinworm. Based on the concentration mortality response to Cry1Ac, the  $\text{LC}_{50}$  and  $\text{LC}_{95}$  values for 2nd, 3rd, and 4th instars were 0.12, 0.27, and 0.43  $\mu\text{g/ml}$  and 0.63, 0.71, and 2.64  $\mu\text{g/ml}$ , respectively (Table 1). The susceptibilities of different larval instars of tomato pinworm to Cry1Ac protein produced by *Bt* var. *kurstaki* were presented in Fig. 1. At  $\text{LC}_{50}$ , 50% mortality was observed in the 3rd day of treatment, in all the instars tested. Based on the present study, it is evident that all the larval instars of the pest were susceptible to the Cry1Ac of *Bt*. The results indicated that susceptibility of larvae decreased with larval developmental stage.

Variations in susceptibility of tomato pinworm depend on the age of the insect, and susceptibility decreased with the age of the insect. Control measures are sometimes ineffective because larvae feed inside the galleries formed from mesophyll tissues (Desneux et al., 2011).

Obtained results are in accordance with Dakshina and Gary (2003) who reported that CRY I toxin was toxic to tomato pinworm, *Keiferia lycopersicella*, with  $\text{LC}_{50} = 17.68$  to 56.00  $\mu\text{g/ml}$  in Florida, with 3.17-fold difference (lab population; Homestead  $\text{LC}_{50}$  value 17.68 with resistant population Guasava 1 with  $\text{LC}_{50}$  value 56.00). Similarly, when *Helicoverpa armigera* (Hub.) was continuously exposed to Cry1Ac toxin under laboratory conditions, resistance has been developed (Kranthi et al., 2000) as well, against the beet armyworm, *Spodoptera exigua* (Hubner) (Muhammad et al., 2019). The present results are in accordance with Kannan and Uthamasamy (2006) who reported the  $\text{LC}_{50}$  values were 0.119  $\mu\text{g/ml}$  for *H. armigera*. Susceptibility to Cry1Ac for *H. armigera* geographically varied between India, America, and China.

However, a repeated and intensive use of *Bt* formulations may lead to resistance in the case of *Plutella xylostella* (Tabashnik and Carriere, 2017). The use of biopesticides is one effective way of coping with insect pests. Of the total production of biopesticides, entomopathogenic bacteria (mostly *Bt*) amount to 90%. *Bt* has

**Table 1** Toxicity of Cry1Ac protein from *Bacillus thuringiensis* to 2nd, 3rd, and 4th larval instars of tomato pinworm, *Tuta absoluta*

Larval stage	Slope	SE <sup>a</sup>	$\chi^{2b}$	LC <sub>50</sub> ( $\mu$ g/ml)	Confidence limits (95%)		LC <sub>95</sub> ( $\mu$ g/ml)	Confidence limits (95%)	
					Lower limit	Upper limit		Lower limit	Upper limit
<b>Second instar</b>	2.13	0.56	0.10	0.12	0.07	0.16	0.63	0.40	1.72
<b>Third instar</b>	3.96	1.12	0.34	0.27	0.22	0.33	0.71	0.49	2.18
<b>Fourth instar</b>	2.10	0.63	0.17	0.43	0.30	0.65	2.64	1.29	37.4

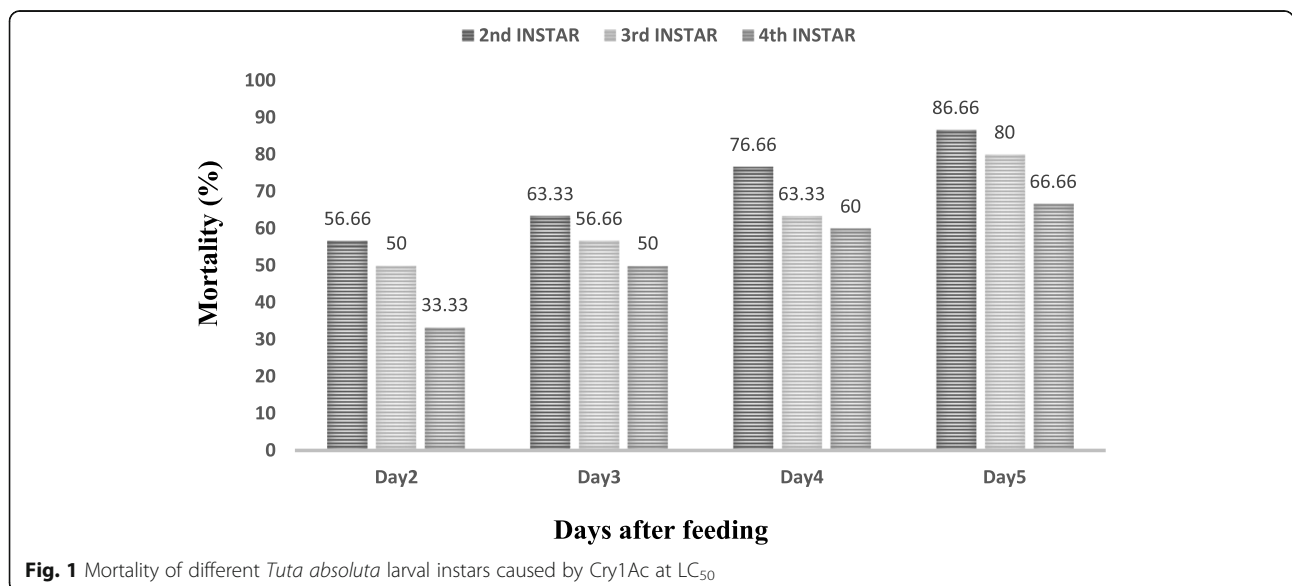
<sup>a</sup>Standard error, <sup>b</sup>chi-square test

been commercially used in the biological control of insect pests for the last 4 decades. *Bt* strains can produce toxic compounds of numerous chemical structures and properties. Selectivity of *Bt*  $\delta$ -endotoxins against the larvae of target insects was documented earlier (Stepanova et al., 1996). As Cry1Ac was toxic to all the instars of TPW, yield loss due to this pest can be efficiently reduced by *Bt*-based formulations. From the bioassay LC<sub>50</sub> values obtained for all instars, it is evident that the populations of Tamil Nadu were enough susceptible for effective control. Further, if we can establish the quantity of *Bt* toxin required, the information will be useful, while developing transgenic tomato crops, it will give an idea about the required level of *Bt* toxin expression.

*Bt* products/ commercial formulations are environmentally safe and provide good yield without any chemical residues and exhibited satisfactory efficacy against *T. absoluta*. Srinivasan and Dilipsundar (2019) reported that Dipel (*Bt* var. *kurstaki*) was very effective against tomato pinworm with 78.67% reduction in larval population by field evaluation. Youssef and Hassan (2013) reported that the commercial formulation “Protecto” was highly effective against tomato pinworm with 96.7% mortality and other *Bt* isolates with 93.3, 90, 86.7, and 80% mortalities. Gowtham et al. (2018) stated that

standard *Bt* strain (HD1) and *Bt* isolate KGS2 showed 95 and 100% mortality against tomato pinworm. Hatice et al. (2017) transferred a modified *Bt* Cry1Ac gene to tomato plants through *Agrobacterium tumefaciens*-mediated transformation. Cry1Ac-expressed tomato plants resulted in mortality rates at 38–100% depending on the transgenic line. In infested leaves, gallery formation was reduced to 57–100% of transgenic plants. This is the first report on the development of transgenic tomato plants resistant to *T. absoluta*.

Bioassays of the viable biocide in the laboratory showed high efficacy in dropping the damage caused by various larval instars of *T. absoluta* at different concentrations compared to untreated control. First and 2nd instars recorded the highest mortality rates, while it was less in 3rd and 4th larval instars. Quite a few pest instars were found to be susceptible to *Bt* to a different extent (Giustolin et al., 2001). Also, in the later instars, the lowest mortality rate was probably due to increased maturation immunity of the larvae. On the other hand, the early instars suffered from higher mortality compared to the late instars. The capacity of *Bt* subsp. *kurstaki* as commercial biocide in reducing pests of economic importance is well known as a key part of IPM programs (Roh et al., 2007).



**Fig. 1** Mortality of different *Tuta absoluta* larval instars caused by Cry1Ac at LC<sub>50</sub>

## Conclusion

Different instars of *T. absoluta* to Cry1Ac protein produced by *Bt* var. *kurstaki* showed susceptible reactions. LC<sub>50</sub> values for different instars varied significantly, indicating variability in the susceptibility among the instars to the Cry1Ac. This information may establish a base for selecting *Bt* Cry1Ac to be used for the control of tomato pinworm.

## Abbreviations

TPW: Tomato pinworm; *T. absoluta*: *Tuta absoluta*; *Bt*: *Bacillus thuringiensis*

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## Authors' contributions

BV performed the idea of this article, and SKJ and BV wrote the manuscript. JJ and MS participated in writing the manuscript and statistical analysis. MT, SI, and SP contributed the material and helped in the maintenance of *Tuta absoluta*, while all authors equally did the bioassay experiments. The authors read and approved the final manuscript.

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## Consent for publication

I agree to publish this paper in the *EJBP*.

## Competing interests

The authors declare that they have no competing interests.

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