Egyptian Journal of Biological Pest Control

RESEARCH

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Response of pink bollworm *Pectinophora gossypiella* (Saunders) to Cry1Ac and Cry2Ab toxin

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Abstract

Background During field surveillance in Gujarat, India, in 2009, scientists found that pink bollworm was surviving in the first generation of Bollgard cotton, which contains a single protein. This survival was observed in four districts of Gujarat. From 2012 to 2014, surveys revealed that pink bollworm larvae had higher survival rates on Bollgard II cotton in Amreli and Bhavnagar districts. In 2016, a pink bollworm outbreak in Maharashtra caused significant losses in cotton production, leading to damage, yield losses, and increased management costs in several regions. The objective of the present study was to determine the reaction of *Pectinophora gossypiella* to Cry1Ac and Cry2Ab toxins.

Results Neonates of *P. gossypiella* collected from six distinct locations in Vidarbha were subjected to seven different concentrations of Cry1Ac. The highest LC_{50} value was recorded in the population collection of Yavatmal (1.362 µg/ml diet) but the lowest LC_{50} value of 0.600 µg/ml diet in Buldana population. The highest MIC_{50} value was recorded in the population collected from Yavatmal (0.743 µg/ml diet) and the lowest MIC_{50} value of 0.413 µg/ml diet in the Buldana population (0.431 µg/ml diet). The highest IC_{50} value was recorded in the population collected from Yavatmal (0.303 µg/ml diet) but the lowest IC_{50} value of 0.127 µg/ml diet in the Buldana population. Neonates of *P. gossypiella* collected from six different locations in Vidarbha were exposed to seven different concentrations (9.0, 3.0, 1.0, 0.333, 0.111, 0.037 and 0.012 for Cry1Ac and 27.0, 9.0, 3.0, 1.0, 0.333, 0.111 and 0.037 µg/ml for Cry2Ab) which revealed that the Yavatmal population had the highest LC_{50} value of 94.294 µg/ml diet and the lowest LC_{50} value was recorded in the Amravati population (42.144 µg/ml diet). The highest MIC_{50} value of 27.258 µg/ml diet was recorded in Buldana population, but the lowest MIC_{50} value was recorded in the Amravati population (12.881 µg/ml diet). The highest IC_{50} value of 3.209 µg/ml diet was observed in Yavatmal population, and the lowest one was recorded in the Amravati population (1.574 µg/ml diet).

Conclusions According to the LC_{50} values, all of the *P. gossypiella* populations in Vidarbha were extremely insensitive and had field-evolved resistance to the *Bt* proteins found in the transgenic cotton varieties of Bollgard II.

Keywords Cry1Ac, Cry2Ab, Bioassay, *Pectinophora gossypiella*, LC_{50}, MIC_{50}, IC_{50}

Background

Cotton (*Gossypium hirsutum* L.) is a vital commercial crop in India, known as the "king of fibers" or "white gold", mainly cultivated for its fiber. For many decades, cotton has been a significant contributor to the socio-economic status of millions of cotton farmers in the country. As of 2022, 9.9 million farmers depend on cotton cultivation in India. In 2002, genetically modified cotton (*Bt*) carrying genes encoding delta endotoxin proteins

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from the entomopathogenic soil bacterium *Bacillus thuringiensis* (*Bt*) was introduced in India. Currently, *Bt* cotton hybrids are cultivated in 12.5 million hectares with a production of 360 lakh bales, while non-*Bt* cotton is grown in an area of 8.50 lakh hectares (AICCIP 2022). Insect pests, specifically the pink bollworm (PBW), *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), are one of the primary factors contributing to the lowest yield of cotton in India. PBW originates in the Indo-Pak region and is distributed worldwide wherever cotton is grown. To manage the pink bollworm and other pests on cotton in India, synthetic pyrethroids were commonly used before the introduction of transgenic cotton and broad-spectrum insecticides. However, the extensive use of chemical insecticides led to ecological disruption, causing outbreaks of bollworm and secondary pests. To address this problem, genetically modified (*Bt*) cotton carrying genes from (*Bt*) was developed. Single-gene (Cry 1Ac) and dual-gene (Cry1Ac + Cry 2Ab) *Bt* cotton hybrids were launched, in India, in 2002 and 2006, respectively, targeting the pink bollworm, American bollworm, and spotted bollworm, as well as other minor lepidopteran insect pests (ICAC 2010). The resurgence of PBW on *Bt* cotton in India may be due to the development of resistance to the *Bt* toxin, along with other factors such as excessive use of chemical pesticides on non-*Bt* cotton and the presence of secondary pests. The exact reason is unclear, but the loss against this pest has caused significant damage to cotton growers. *Bt* cotton is the only approved genetically engineered technology in India and has shown excellent resistance against various pests, but there is concern about the potential for bollworms to develop resistance to the *Bt* toxin in the long run (Naik et al. 2017). Until 2006, Bollgard (BG-I) was the widely cultivated bollworm tolerant cotton variety, containing only one gene (CrylAc) belonging to the MON-531 event. *Bt* cotton was developed as an alternative for managing the American bollworm, which has developed high levels of resistance to numerous insecticides. However, PBW is difficult to manage as it is an internal feeder. In 2009, scientists in Gujarat detected PBW surviving on first-generation transgenic cotton expressing Cry1Ac, leading to confirmed resistance in four districts. Pest resistance poses a significant threat to the long-term efficacy of *Bt* toxins. The objective of the present study was to examine the reaction of the PBW to Cry1Ac and Cry2Ab toxins.

Methods

Maintenance of culture of PBW

Test insect

F_1 generation neonates of PBW from BG-II cotton fields were used for the experiment.

Larval rearing

Mature green bolls are collected at random from farmer cotton fields of BG-II in the Vidarbha districts of Akola, Buldhana, Amaravati, Wardha, Yavatmal, and Nagpur located in Maharashtra, India, in 2021. In 2016, PBW outbreak was discovered in the Vidarbha part of Maharashtra, causing losses of up to 80% in some areas and 20–30% in other areas. PBW infestations in many hotspots caused significant boll damage and yield losses in *Bt* cotton, as well as increased management costs in parts of Gujarat, Andhra Pradesh, Telangana, Maharashtra, and Karnataka (Naik et al. 2017). Twenty bolls are chosen at random from each field and collected from different locations to represent a district. The bolls are dissected and examined for the presence of PBW larvae, exit holes and frass. Healthy larvae of the second instar were scored as survivors and reared on an artificial diet to conduct bioassays on F-1 progeny (Muralimohan et al. 2009). Artificial diet was poured into bioassay trays (1-ml/well)/culture trays and allowed to cool for at least 30 min before transferring the neonates. Once the diet has cooled, active neonates are carefully transferred into each well of the bioassay trays using sterile camel hair brushes (0 or 1 No.) and covered with pull-and-peel tabs. Trays were labeled to indicate the date of transferring and the expected date of larvae those reach later instars; they were placed in an environmental chamber with a temperature of 25 ± 2 °C and relative humidity of 65 ± 5%. The trays containing the larvae were periodically checked for growth and development of larvae, as well as any changes in the diet, larval mortality, etc. Larvae were then reared until pupation on the artificial diet in 128-well bioassay trays and also in individual vials. Artificial diet (Table 1) was divided into three parts of which part A was weighed into one plastic beaker, part B into one Duran bottle and part C into a 50-ml centrifuge tube, MPHB into 50-ml centrifuge tube. Part A was blended with 200 ml of R.O. water for 3–5 min; retain 200 ml water. Agar was boiled (Part B) in 400 ml of water till the agar dissolves completely by intermittent shaking and cooling for 4–5 min. The molten agar was poured into blender containing part A, and the steam was allowed to escape for this 150 ml of water was added and blended for 5 min and allowed to cool down. The methyl parahydroxy benzoate was dissolved in 5 ml of 95% ethanol and added to the diet. Sorbic acid, Streptomycin, Bavistin, Wesson's salt and other micro-ingredients (Part C) were mixed completely by rinsing with 50 ml of water and blended for 2–3 min, and this diet was poured into a autoclaved Duran bottle maintained in a water bath at 58 °C. The bioassay trays (CD international TM)/culture vials/culture trays/bowls were irradiated for 20 min in a UV light chamber, and later the diet was dispensed hot diet in each bioassay tray

Table 1 Composition of artificial diet for mass rearing and bioassay for the pink bollworm, *Pectinophora gossypiella* (1000 ml)

Ingredients	*Pectinophora gossypiella*	
	Rearing	Bioassay
PART A		
Kabuli gram flour	30 g	–
Southland diet-Multi Sp	30 g	60 g
R.O. Water	400 ml	400 ml
PART B		
Agar Agar	14 g	14 g
R.O. water	400 ml	400 ml
PART C micro-ingredients and antimicrobials		
Wesson's salt	2.5 g	2.5 g
Casein	5 g	5 g
Cholesterol	0.5 g	0.5 g
Cysteine	0.1 g	0.1 g
Ascorbic acid	2 g	2 g
Multivitamin mix (Polybion SF)	6 ml	6 ml
Sorbic acid	0.5 g	0.5 g
Methyl parahydroxy benzoate (MPHP)	0.5 g	0.5 g
Streptomycin sulfate	0.5 g	0.5 g
Bavistin	2.5 g	2.5 g
Cifran (Ciproflaxacin injection)	3 ml	3 ml

cell/culture vial as required (hotness helps in equal distribution of diet). The trays are air-dried in laminar flow hood for 30 min and covered with aluminum foil and kept in refrigerated condition in inverted position which should be used within 2–3 days after preparation.

Pupal handling

Pupae that are one-day-old healthy and fully developed were collected from the bioassay trays and placed separately in plastic containers with mesh lids or perforated lids and placed in the environmental chamber to emerge.

Moth handling

Cotton squares and paper strips were kept in oviposition jars (transparent plastic container 28 cm height 24 cm diameter) for egg laying. For improving fecundity and egg fertility, adults were given a cotton swab soaked in a mixture of 10 percent honey solution fortified with 1–2 drops of multivitamin, ascorbic acid 1.2 g and methyl parahydroxybenzoate 0.2 g. Vigorous adults that emerge early (9–12 days) were released into the jars (25–30 pairs of moths/oviposition jar), which covered with a black cotton cloth. The neonate larvae of these field-collected populations (F_1) were used in bioassays after eggs were collected from mating that allowed to hatch.

Concentration–response bioassay protocol with insecticidal proteins (Cry1Ac and Cry2Ab) by diet incorporation

Bioassay was carried out at the Research laboratory, Mahyco-Jalna, Maharashtra, India, to compare the susceptibility of larvae (F_1) from six populations and exposing neonate larvae to Cry1Ac and Cry2Ab protein concentrations incorporated into an artificial diet to produce 0–100 percent mortality. To make a stock solution of 250 µg/ml of Cry1Ac, 12.69 mg of standard Cry1Ac formulation is suspended in 10 ml deionized water. The Cry1Ac protein used in the bioassays was derived from the commercial formulation MVP IIR (Mycogen Crop., USA), which contains delta endotoxin of *Bt* variety Kurstaki at 19.7 percent w/w of Cry1Ac encapsulated in killed *Pseudomonas fluorescens* (commercial product). From the primary stock, the top dose (9.0 µg/ml) of the assay was prepared by pipetting (concentrations were first calculated on paper and done with pipette) 1.404 ml protein plus 6.396 ml deionized water. In the case of Cry2Ab formulation (7.2 mg/g of corn leaf powder) (commercial product), 347.2 mg of standard Cry2Ab formulation was added into a centrifuge tube along with 9.653 ml deionized water to obtain 250 µg/ml mother stock and the top concentration of the assay is prepared by pipetting 27.0 µg/ml–4.212 ml protein plus 3.588 ml of deionized water. Seven concentrations were prepared by serially diluting the top concentration at the required concentrations 9.0, 3.0, 1.0, 0.333, 0.111, 0.037 and 0.012 µg/ml for Cry1Ac and 27.0, 9.0, 3.0, 1.0, 0.333, 0.111 and 0.037 µg/ml for Cry2Ab. Artificial diet was prepared and kept in hot water bath at 60 °C to prevent solidification. Following that various concentrations of Cry1Ac and Cry2Ab were mixed into the artificial diet (calculated amount of diet was poured in to each vial (50 ml centrifuge tubes) containing toxin and mixed thoroughly with the help of vortex mixer) and approximately 750 µl of Cry1Ac and Cry2Ab mixed diet (separately) was poured into each well of a 128-well bioassay tray (CD International trays, Massachusetts, USA). One neonate was placed in each well with a fine brush. Seven concentrations and one control (for control no toxin only artificial diet) were included for each population with 16 insects per treatment, and treatments were replicated three times resulting in at least 384 larval insects tested per location. Bioassay trays were placed in laminar air flow until solidified. Active, healthy neonate larvae were transferred using a fine hair brush (one larvae/well) and covered with self-adhesive pull-and-peel tabs (CD International pull-n-peel tabs). Infested trays were stored in an environmental chamber (25 ± 1 °C and RH 55–65%).

Observations recorded

- Mortality of instar (Determined from head capsule size) was recorded 21 days (artificial diet was not changed in between 21 days) after infestation (Tabashnik et al. 2000).
- Lethal concentrations (LC): Concentration that kills fifty percent of the test larval population
- Molt inhibitory concentration (MIC): Concentration of protein that severely limited larvae from reaching the second instar
- Inhibitory concentration (IC): Concentration of protein that prevented larvae from reaching the third instar
- Resistance ratios were calculated using the formula:

$$\text{Resistance ratio(RR) for } LC_{50} = \frac{LC_{50} \text{ of the test strain}}{LC_{50} \text{ of the susceptible (control) strain}}$$

$$\text{Resistance ratio (RR) for } MIC_{50} = \frac{MIC_{50} \text{ of the test strain}}{MIC_{50} \text{ of the susceptible (control) strain}}$$

$$\text{Resistance ratio (RR) for } IC_{50} = \frac{IC_{50} \text{ of the test strain}}{IC_{50} \text{ of the susceptible (control) strain}}$$

Data analysis

Probit analysis was carried out using POLO-PC, and concentration–response parameters were expressed in µg of Cry1Ac or Cry2Ab/ml of diet. Also, the LC/MIC/IC90 values were calculated.

Results

Median lethal concentration (LC_{50} & LC_{90}) of Cry1Ac protein to PBW neonates

LC_{50} values ranged from 0.600 to 1.362 µg/ml diet, with the highest LC_{50} value recorded in the Yavatmal population (1.362 µg/ml diet; 0.843–2.255) and the lowest LC_{50} value recorded in the Buldhana population 0.600 µg/ml diet (0.413–0.895). PBW populations collected in Akola, Amravati, Nagpur and Wardha had LC_{50} of 1.061 µg/ml (0.727–1.624), 0.681 µg/ml (0.462–1.038), 0.798 µg/ml, (0.554–1.187) and 1.096 µg/ml (0.0.621–2.211), respectively (Fig. 1). LC_{50} value for the susceptible population reared in the laboratory was 0.002 µg/ml (0.000–0.007) of diet. LC_{90} values ranged from 93.46 to 368.47 µg/ml diet with the highest LC_{90} value recorded in the Yavatmal population (368.47 µg/ml diet; 325.92–383.27) and the lowest LC_{90} value recorded in the Buldhana population 93.46 µg/ml (63.93–100.63) diet. PBW populations collected in Akola, Amravati, Nagpur, and Wardha had LC_{90} of 301.92 µg/ml (225.84–310.5), 100.62 µg/ml (83.25–123.93), 132.28 µg/ml (118.93–145.82) and 284.11 µg/ml

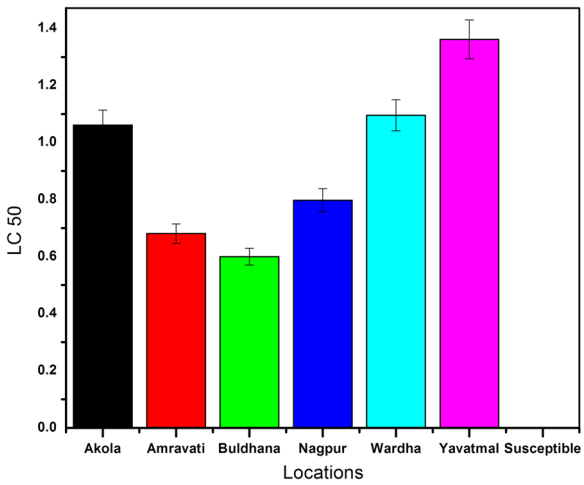

Fig. 1 Median lethal concentration (LC_{50}) of Cry1Ac protein to *Pectinophora gossypiella* neonates

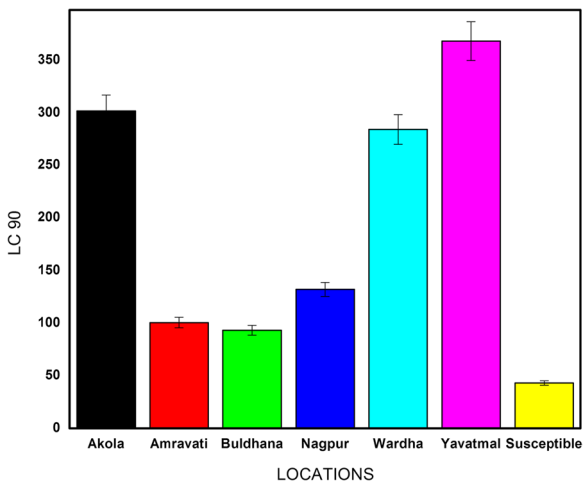

Fig. 2 Median lethal concentration (LC_{90}) of Cry1Ac protein to *Pectinophora gossypiella* neonates

(231.73–311.82), respectively. LC_{90} value for the susceptible population reared in the laboratory was 43.19 µg/ml (25.63–65.82) of diet. Resistance ratio was the highest in the Yavatmal population (681.00), followed by Wardha (548.00), Akola (530.50), Nagpur (399.0) and Amravati (340.50) (Fig. 2). The Buldhana population had the lowest resistance ratio (300.00).

Molt inhibitory concentration (MIC_{50} & MIC_{90}) of Cry1Ac protein to PBW neonates

The susceptibility of neonates to Cry1Ac in different populations was as follows: Akola (0.707 µg/ml diet; 0.485–1.602), Amravati (0.459 µg/ml diet; 0.275–0.792), Buldhana (0.431 µg/ml diet; 0.267–0.665), Nagpur (0.682 µg/ml diet; 0.128–01.254), Wardha (0.728 µg/ml diet; 0.408–1.423) and Yavatmal (0.743 µg/ml; 0.843–2.255) (Fig. 3). The highest MIC_{50} value was recorded in the Yavatmal population (0.743 µg/ml diet; 0.843–2.255), and the lowest MIC_{50} value was recorded in the Buldhana population (0.413 µg/ml diet; 0.267–0.665). MIC_{50} value for the susceptible population reared in a laboratory was (0.0013 µg/ml diet; 0.000–0.005). The highest MIC_{90} value was recorded in the Yavatmal population (401.57 µg/ml diet; 334.72–409.53), and the lowest MIC_{90} value was recorded in the Buldhana population (79.54 µg/ml diet; 67.45–87.943). MIC_{90} value for the susceptible population reared in a laboratory was (27.32 µg/ml diet; 18.92–29.635). The resistance ratio for Cry1Ac for molt inhibitory concentration was the highest in the Yavatmal population (571.54) followed by Wardha (560.00), Akola (543.85), Nagpur (524.62) and Amravati (353.08) (Fig. 4). The population of Buldhana had the lowest resistance ratio (331.54).

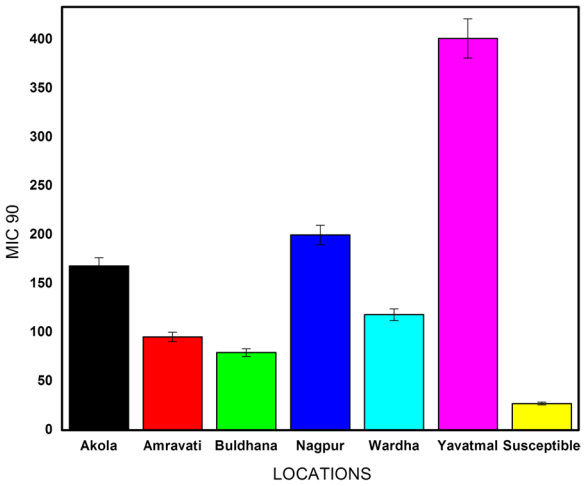

Fig. 4 Molt inhibitory concentration (MIC_{90}) of Cry1Ac protein to *Pectinophora gossypiella* neonates

Inhibitory concentration (IC_{50} & IC_{90}) of Cry1Ac protein PBW to neonates

IC_{50} values ranged from 0.127 to 0.303 µg/ml diet with the highest IC_{50} value recorded in the Yavatmal population (0.303 µg/ml diet; 0.171–0.490) and the lowest IC_{50} value recorded in the Buldhana population 0.127 µg/ml diet (0.084–0.182). PBW populations collected in

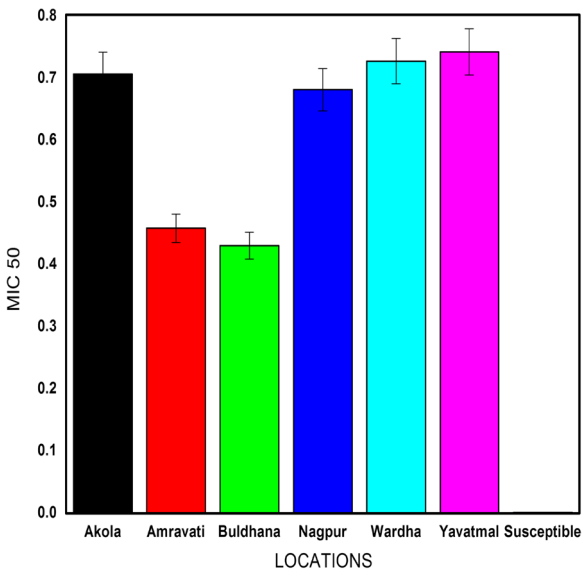

Fig. 3 Molt inhibitory concentration (MIC_{50}) of Cry1Ac protein to *Pectinophora gossypiella* neonates

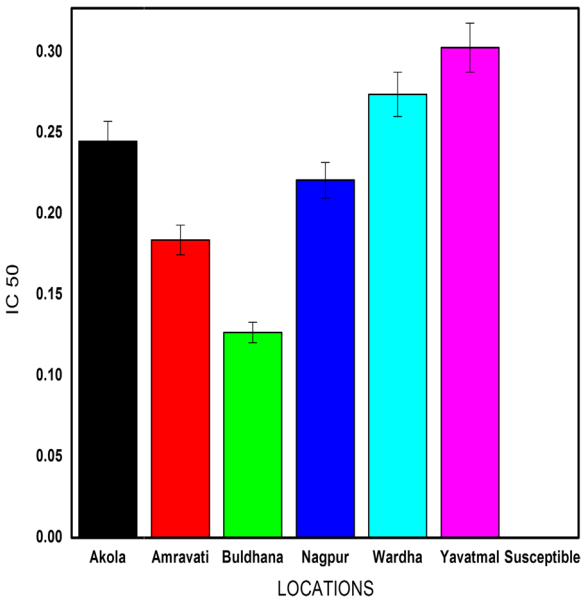

Fig. 5 Inhibitory concentration (IC_{50}) of Cry1Ac protein to *Pectinophora gossypiella* neonates

Akola, Amravati, Nagpur and Wardha had IC_{50} values of 0.245 µg/ml (0.175–0.342), 0.184 µg/ml (0.132–0.253), 0.221 µg/ml (0.143–0.319) and 0.274 µg/ml (0.166–0.447), respectively (Fig. 5). The IC_{50} value for the susceptible population raised in the lab was 0.0005 µg/ml (0.000 -0.003) of diet. The highest IC_{90} value was recorded in the Yavatmal population (74.308 µg/ml diet; 69.543–77.843) and the lowest IC_{90} value was recorded in the Buldhana population (27.543 µg/ml diet; 21.043–29.045) µg/ml diet. The IC_{90} value for the susceptible population reared in a lab was (27.04 µg/ml diet; 25.923–29.032). The resistance ratio for Cry1Ac for inhibitory concentration was the highest in the Yavatmal population (594.12), followed by Wardha (537.25), Akola (480.39), Nagpur (433.33) and Amravati (360.78) (Fig. 6). The population of Buldhana had the lowest resistance ratio (249.02).

Median lethal concentration (LC_{50} & LC_{90}) of Cry2Ab protein to PBW neonates

LC_{50} values ranged from 42.144 to 94.294 µg/ml of a diet with the highest LC_{50} value recorded in the Yavatmal population (94.294 µg/ml diet; 33.193–671.698) and the lowest LC_{50} value recorded in the Amravati population (42.144 µg/ml diet; 17.543–188.028). PBW populations collected in Akola, Buldana, Nagpur and Wardha had LC_{50} values of 90.557 µg/ml (30.156–719.020), 101.906 µg/ml (26.165–1858.338), 87.493 µg/ml (23.968–1272.505) and 66.187 µg/ml (20.475–669.526), respectively (Fig. 7). LC_{50} value was 0.050 µg/ml (0.032–0.073) of diet in the susceptible population raised in the laboratory. LC_{90} values ranged from 653.492 to 852.53 µg/ml of diet, with the highest LC_{90} value recorded in the Buldhana population (852.53 µg/ml diet; 826.28–871.93) and the lowest LC_{90} value recorded in the Amravati population (653.492 µg/ml; 635.74–673.26) of diet. PBW populations collected in Akola, Nagpur, Wardha and Yavatmal had LC_{90} of 837.721 µg/ml (813.83–843.83), 728.562 µg/ml (712.43–738.33), 770.386 µg/ml (743.85–784.37) and 833.735 µg/ml (819.94–852.48), respectively. LC_{90} value for a susceptible population reared in the laboratory was 31.54 µg/ml (19.38–32.05) of diet. The resistance ratio for Cry2Ab for median lethal concentration was the highest in the population of Buldhana (2038.12), followed by Yavatmal (1885.88), Akola (1811.14), Nagpur (1749.86)

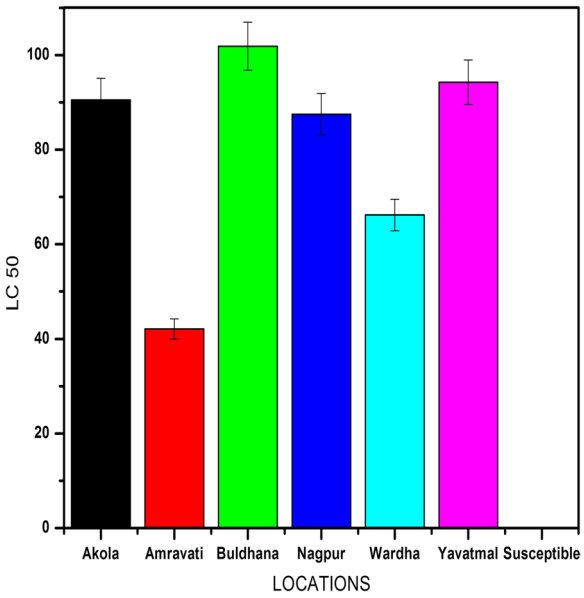

Fig. 7 Median lethal concentration (LC_{50}) of Cry2Ab protein to *Pectinophora gossypiella* neonates

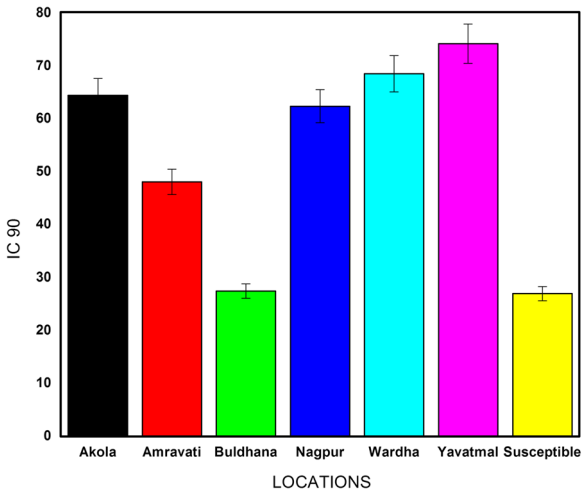

Fig. 6 Inhibitory concentration (IC_{90}) of Cry1Ac protein to *Pectinophora gossypiella* neonates

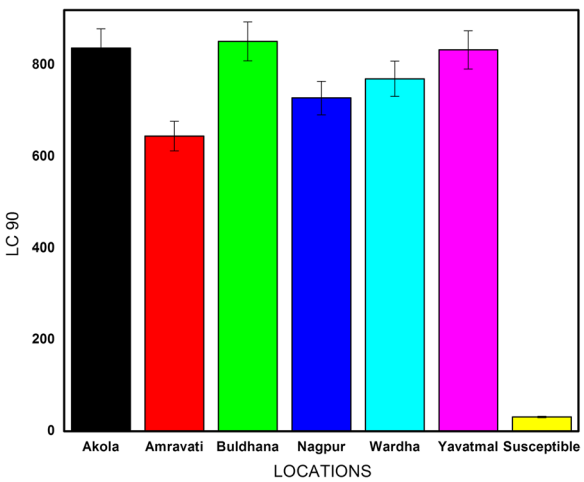

Fig. 8 Median lethal concentration (LC_{90}) of Cry2Ab protein to *Pectinophora gossypiella* neonates

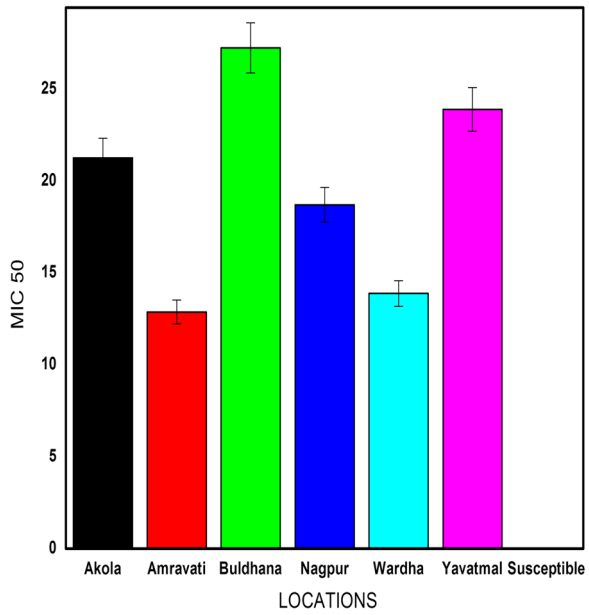

Fig. 9 Molt inhibitory concentration (MIC_{50}) of Cry2Ab protein to *Pectinophora gossypiella* neonates

and Wardha (1323.74) (Fig. 8). The population of Amravati had the lowest resistance ratio (842.88).

Molt inhibitory concentration (MIC_{50} & MIC_{90}) of Cry2Ab protein to PBW neonates

Susceptibility of neonates to Cry2Ab in different populations was as follows: Akola (21.267 µg/ml diet; 9.189–85.301), Amravati (12.881 µg/ml diet; 7.112–30.121), Buldhana (27.258 µg/ml diet; 9.944–175.382), Nagpur (18.717 µg/ml diet; 8.260–66.161), Wardha (13.887 µg/ml diet; 6.857–40.876), Yavatmal (23.906 µg/ml diet; 11.713–72.663) (Fig. 9). The highest MIC_{50} value was recorded in the Yavatmal population (23.906 µg/ml diet; 11.713–72.663), and the lowest MIC_{50} value was recorded in the Amravati population (12.881 µg/ml diet; 7.112–30.121). The MIC_{50} value for the susceptible population reared in the laboratory was 0.015 µg/ml (0.008–0.024) of diet. The highest MIC_{90} value was recorded in the Buldhana population (792.562 µg/ml diet; 756.37–803.83), and the lowest MIC_{90} value was recorded in the Amravati population (342.192 µg/ml diet; 336.72–364.92). MIC_{90} value for the susceptible population reared in a laboratory is (0.931 µg/ml diet; 0.783–1.004). Buldhana (1817.2) had the highest resistance development as measured by the resistance ratio for Cry2Ab for molt inhibitory concentration, followed by Yavatmal (1593.73), Akola (1417.8), Nagpur (1247.8) and Wardha (925.8) (Fig. 10). The population of Amravati had the lowest resistance ratio (858.73).

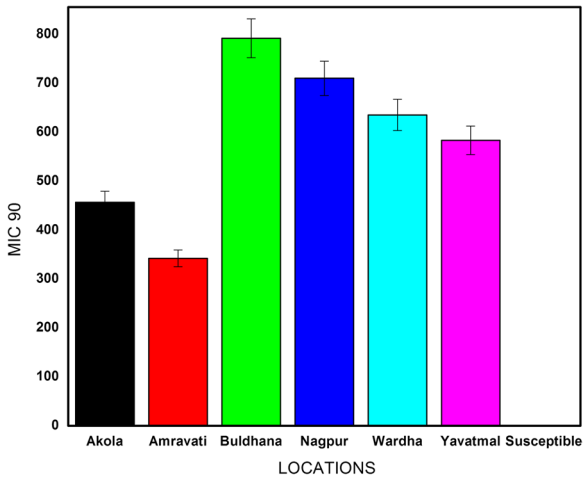

Fig. 10 Molt inhibitory concentration (MIC_{90}) of Cry2Ab protein to *Pectinophora gossypiella* neonates

Inhibitory concentration (IC_{50} & IC_{90}) of Cry2Ab protein to PBW neonates

IC_{50} values ranged from 1.574 to 3.209 µg/ml diet with the highest IC_{50} value recorded in the Yavatmal population (3.209 µg/ml diet; 1.774–5.469) and the lowest IC_{50} value recorded in the Amravati population 1.574 µg/ml (1.113–2.255) of diet (Fig. 11). PBW populations collected in Akola, Buldana, Nagpur and Wardha had IC_{50} values of 3.033 µg/ml (0.175–6.049), 3.559 µg/ml (2.020–6.798), 2.922 µg/ml (1.644–5.112) and 2.527 µg/ml (1.499–4.371) of diet, respectively. The IC_{50} value was 0.002 µg/ml (0.000–0.006) of diet in the susceptible population reared in the laboratory. The highest

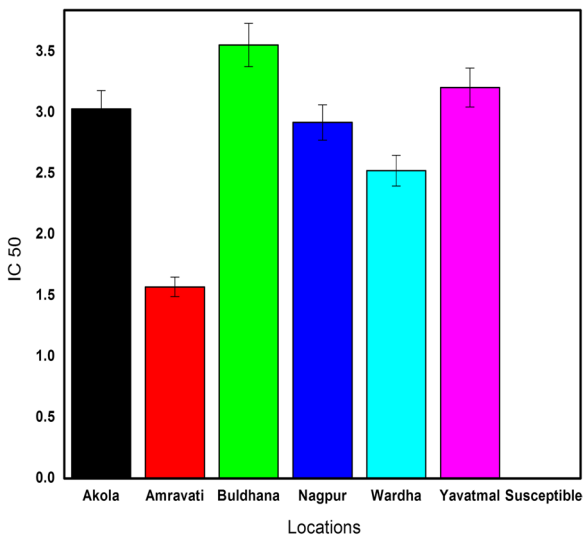

Fig. 11 Inhibitory concentration (IC_{50}) of Cry2Ab protein to *Pectinophora gossypiella* neonates

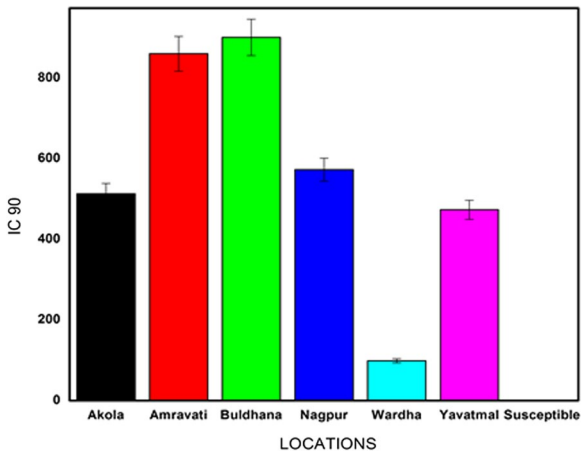

Fig. 12 Inhibitory concentration (IC_{90}) of Cry2Ab protein to *Pectinophora gossypiella* neonates

IC_{90} value was recorded in the Buldhana population (901.352 µg/ml diet; 887.65–912.48), and the lowest IC_{90} value was recorded in the Amravati population (98.912 µg/ml diet; 848.57–874.53) of diet. The IC_{90} value for the susceptible population reared in a laboratory is (0.052 µg/ml diet; 0.024–0.078). The resistance ratio for Cry2Ab for inhibitory concentration was the highest in the population of Buldhana (1779.5), followed by Yavatmal (1604.5), Akola (1516.5), Nagpur (1461.00) and Wardha (1263.50) (Fig. 12). The population of Amravati had the lowest resistance ratio (787.00).

Discussion

Naik et al. (2018) presented findings on the LC_{50} values of Cry1Ac and Cry2Ab toxins in different populations of pests in India which were according to current results. The results indicated that the LC_{50} values of Cry1Ac for the Nanded, Nandurbar, Rahuri and Parbhani populations were 1.012 (0.653–1.658) µg/ml, 0.215 (0.023–3.453) µg/ml, 1.759 (0.279–1.325) µg/ml and 1.77 (1.373–2.298) µg/ml, respectively, and the resistance ratios were 169, 36, 293 and 295, respectively, in 2016. In 2017, the LC_{50} values of Cry1Ac for the Nanded and Rahuri populations were 0.224 (0.077–0.476) µg/ml and 0.677(0.500–0.937) µg/ml, respectively, with resistance ratios of 28 and 85 for the same populations, respectively. Regarding Cry2Ab, the LC_{50} values for the Nanded, Nandurbar, Rahuri, and Parbhani populations were 1.351 µg/ml (0.343–6.878), 2.208 µg/ml (1.379–3.797), 1.347 µg/ml (0.173–106.1), and 11.338 µg/ml (3.798–332.7) of the diet, respectively and the resistance ratios of the populations were 450, 736, 449, and 3779 respectively, in 2016. In 2017, the LC_{50} values of Cry2Ab for the Nanded and Rahuri populations were 3.401 µg/ml (1.753–6.772) and 0.428 µg/ml (0.211–0.77) of the diet, respectively, with resistance ratios of 850 and 107, respectively, for populations. Insect susceptibility to toxins was determined by tests, selection regime and environmental factors. Temperature and host plants had a significant impact on insect susceptibility to cry toxins. Larvae were less susceptible as the crop matured and ambient temperature decreased. Long larval periods at low temperatures resulted in health larvae with offspring developing high tolerance to cry toxins. Insect pre-treatment temperature acclimation in the F_0 generation and susceptibility of their F_1 progeny may have influenced the susceptibility of progenies. Induction of the protease inhibitor gene in crop plants affected insect susceptibility to *Bt* by influencing target insect intake and accumulation of protease inhibitor. Protease inhibitors can inhibit insect growth and development by inhibiting midgut proteases. Secondary metabolites may contribute to variations in toxicity and susceptibility in different locations. The development of resistance leading to control failures in the field depends on multiple factors, including initial allele frequencies, inheritance of resistance, selection pressures and insect behavior over time (Kranthi 2015). The study found that Indian PBW is highly insensitive to the *Bt* proteins in Bollgard and Bollgard II cotton varieties. Identifying the critical factors behind the widespread occurrence of PBW in India in recent years is crucial. PBW was a significant cotton pest in India but declined in importance until its recent resurgence on *Bt* cotton. The introduction of long-duration *Bt* cotton varieties may be a significant cause of the pest's resurgence, as well as factors such as toxin molecule sequestration and the fact that PBW is monophagous and hides in bolls. The cultivation of continuous hosts and a large number of hybrids with varying flowering and fruiting periods provided continuous food for the bollworms, while long-term storage of raw cotton and low *Bt* toxin expression in young bolls also contributed to the pest spread. Segregating seeds in F_1 hybrid plant bolls accelerates resistance development and India is the only country where *Bt* cotton hybrids are grown. The primary strategy for delaying pest resistance to *Bt* crops is the use of 'refuges' of host plants that do not produce *Bt* toxins to promote the survival of susceptible insects. *Bt* cotton hybrid varieties that produce approximately 25% non-Bt seeds in cotton bolls of BG-I and approximately 6.25% non-*Bt* seeds in BG-II bolls, due to the hemizygous condition of the cry gene in the *Bt*-hybrid varieties, were approved. However, PBW survival was higher in *Bt*-hemizygous hybrids compared to *Bt*-homozygous varieties due to low toxin expression and *Bt* toxin segregation in developing bolls. Young developing stages such as square buds, flowers, and developing young bolls were

found to have low *Bt* toxin levels that may have helped the heterozygous-resistant PBW to survive better (Singh et al. 2016). The *Bt*-hemizygous F-$_1$ *Bt* cotton Bollgard II hybrids contain seeds in bolls that segregate in a ratio of 9:3:3:1 for the cry1Ac and cry2Ab genes. The non-compliance of refuge planting, continuous food supply and multiple overlapping generations of PBW exposed to *Bt* toxins due to extended crop, multiplicity of varieties and staggered sowing create an environment where PBW larvae can rapidly develop resistance to *Bt* cotton. PBW has become a major problem in India and Pakistan due to PBW resistance to *Bt* cotton and long season cotton crops, while other countries maintain a low profile as their cotton crops are grown for five to six months, allowing for a long 'closed-season'. Hybrid cotton offers high yield potential, superior traits such as disease resistance and stress tolerance, and genetic uniformity, making it easier to manage and harvest, which maximizes cotton yields and profits for farmers. To manage PBW infestations sustainably in India, it is recommended to restrict the total area-wide cotton season within a state/province to lesser than six months, retain early-formed squares, and implement various management strategies. To manage resistance to *Bt* cotton in a sustainable manner, several strategies can be employed, including the use of stacked *Bt* genes, seed mixtures, trap crops, intercropping, non-crop refuges and habitat manipulation, which can delay resistance, provide refuges and reduce pest pressure on cotton crops. Current resistance studies confirmed the development of resistance to Cry toxins in the PBW due to the aforementioned factors (Prasad Rao et al. 2021).

Conclusion

Neonates of *P. gossypiella* collected from 6 different locations in Vidarbha were exposed to seven different concentrations of Cry1Ac and Cry2Ab. The concentration–response bioassay was conducted by incorporating the insecticidal proteins into the diet of the insects. Data analysis was performed using Probit analysis and the software POLO-PC, and the results were expressed in terms of µg of Cry1Ac or Cry2Ab per ml of diet. The study witnessed that based on the LC_{50}, MIC_{50} and IC_{50} values all of the *P. gossypiella* populations in Vidarbha have developed resistance to the *Bt* proteins found in the transgenic cotton varieties of Bollgard II.

Abbreviations

PBW	Pink bollworm
LC_{50}	Lethal concentration
MIC_{50}	Molt inhibitory concentration
IC_{50}	Inhibitory concentration

Acknowledgements

The first author is thankful to Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola and Mahyco, Jalna, for financing assistance, reinforcement and stabilization protracted during the study period. The first author is beholden to Jawaharlal Nehru Memorial Fund, New Delhi, for a Scholarship award during the tenure of study.

Author contributions

Present work is a component of PL's PhD research work. DBU, USK, AVK and MPM concocted and conceived the experimental paradigm. PL conducted field sampling, executed laboratory procedures, performed data interpretation and drafted and edited the manuscript. All contributors reviewed and concurred with the final manuscript.

Funding

The present research work was supported and funded by Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India-444001.

Availability of data and materials

All results obtained during this research are reported in this article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 24 January 2023 Accepted: 5 June 2023
Published online: 09 June 2023

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