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BASELINE SUSCEPTIBILITY OF *LEUCINODES ORBONALIS* TO CRY1AC TOXIN USING A DIET-BASED BIOASSAY

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ABSTRACT

Baseline susceptibility of Brinjal shoot and fruit borer *Leucinodes orbonalis* to Cry1Ac was elucidate by using diet incorporated bioassay. The results show that, LC_{50} values during *kharif* ranged from 0.0326 to 0.0369 mg/mL of diet. The LC_{50} values from Akola, Amaravati, Washim and Buldhana populations were 0.0369, 0.0363, 0.0349 and 0.0326 mg/ml of diet respectively. The highest LC_{50} values were observed in Akola population followed by Amaravati and Washim, the lowest LC_{50} value was observed in Buldhana population. LC_{90} values of the populations ranges within the baseline limits at 0.0440 to 0.0480 mg/mL of diet. Similarly, LC_{50} values during *Rabi* ranged from 0.0322 to 0.0369 mg/mL of diet. The LC_{50} values from Akola, Amaravati, Washim and Buldhana populations were 0.0349, 0.0369, 0.0339 and 0.0322 mg/mL of diet respectively. The highest LC_{50} values were observed in Amaravati population followed by Akola and Washim, the lowest LC_{50} value was observed in Buldhana population and LC_{90} values of the populations ranges within the baseline limits at 0.0458 to 0.0483 mg/mL of diet.

INTRODUCTION

Brinjal shoot and fruit borer (BSFB) is one of the major constrains in brinjal production and cause heavy losses. The losses range from 48.30 % (Singh *et al.,* 2000), 55.66 % to 80 % (Lal *et al.,* 2004), up to 70 % (Singh *et al.,* 2008).

Management of BSFB is very difficult because of its internal feeding habit similarly; development of resistance to conventional insecticides is also a limiting factor for its effective control. Bt brinjal is suppose to be the sustainable alternative to mitigate the losses impose by BSFB and thus prompt an international efforts for its development. Bt brinjal is instrumental in decreasing the use of insecticides by 70% and insect population 70-80% (Sharma, 2004; Anonymous, 2006).

Mahyco-Monsanto Research Foundation has developed Bt brinjal by using cry1Ac gene and conducted RCGM Bt trial on 29 locations in India in collaboration with Tamil Nadu Agriculture University, Coimbatore and University of Agriculture Sciences, Dharwad (Anonymous, 2006).

Baseline data can be used in monitoring resistance development in future and also used in insecticide resistance management strategies. Regular monitoring for resistance development helps to detect the emergence of resistant phenotypes in order to initiate timely remedial measures. In this way this could be useful for environmentally management of insect pest.

The main purpose of a calculation of baseline toxicity is to distinguish between resistant and susceptible phenotypes. The present investigation was carried before the development of any selection pressure hence it is important. In future the field application rates of Bt toxin could be determined by commercial considerations and can be several times more or less than the equivalent of the diagnostic dose at the point of delivery to the insect. Hence the investigation was conducted prior to and after release of Bt brinjal expressing major toxin Cry1Ac to avoid development of resistance in BSFB, *Leucinodes orbonalis* to Cry1Ac.

MATERIALS AND METHODS

Insect population

Brinjal shoot and fruit borer infested fruits were collected from different Brinjal fields during kharif and rabi 2010-11 from four district of Vidarbha viz. Akola, Buldhana, Amaravati and Washim. Field collected population were reared on semi-synthetic diet using standard rearing technique in laboratory (Talekar, 1999) in the laboratory. Eggs collected from mating cages were allowed to hatch and neonate larvae of these field collected parents were used in bioassays to determine their Cry1Ac susceptibility.

Before generating the baseline, the diet was tested for its suitability by rearing Brinjal shoot and fruit borer larvae continuously for at least three generations. The bioassay

method reported herein was standardized by replicated bioassays repeated on laboratory strains and its subsequent validation on sub-sets of F1 larvae from field populations (Kranthi *et al.,* 2004).

Bioassays

Bioassays involved exposure of neonate larvae to various concentrations of diet incorporated Cry1Ac protein that produced 0-100% mortality. The protocol used for diet incorporation of Bt toxin to test their toxicity (Kranthi, 2005).

Method of preperation of artificial semi-synthetic diet

Field-collected larvae were reared on semi-synthetic diet (Salunke, 2011) and the constituents of which are mentioned in Table 1.

One fraction was prepared by boiling agar upto boiling point in half quantity of water, then yeast was added and mixture was again boiled. The second fraction containing other ingredients except sorbic acid, vitamin suppliment and sreptomycin were weighed as per recipe and were homogenously mixed in mixer. These two fractions were thoroughly mixed together after adding remaining ingredients. The semiliquid diet was poured in multi-cell 12-well plates and larvae were reared until pupation.

Isolation of *Bt* Toxin

Bt toxin i.e. Cry1Ac was produced according to protocol given by Dulmage et al., 1970 from the Bacterial strain HD-73 which was obtained from Biotechnology center, Dr. P.D.K.V., Akola

Quantification of Protein and Toxin

Table 1: Diet recipe for rearing of *Leucinodes orbonalis* larvae

Sr. No.	Ingredients	Quantity
1	Water	1000 mL
2	Agar agar	16 g
3	Chickpea flour	112 g
4	Brinjal fruit powder	48 g
5	Wheat germ	60 g
6	Methyl parahydroxy benzoate	3.3 g
7	Ascorbic acid	5.3 g
8	Sorbic acid	1.7 g
9	Yeast	53 g
10	Wesson salt	10 g
11	Formaldehyde 10%	10 mL
12	Ciprofoxacin	250 mg
13	Vitamin E	400 mg
14	Vit B complex	1 tab

Protein was quantified according to the method by Bradford (1976) and the toxin was quantified on SDS–PAGE of stacking (6%) and resolving gels (10%).

RESULTS AND DISCUSSION

Estimation of Protein and Toxin

Isolated crude protein was estimated (0.03 μg/mg) according to Bradford (1976) method and further analyzed using SDS–PAGE with standard molecular weight marker from MBI fermentas (116, 62.2, 45, 35, 25, 18.4 and 14.4 kDa). The protein showed the molecular weight of Cry1Ac (~130 kDa), the LC_{50} and LC_{90} values were determined as μg Cry1Ac per ml diet.

Log dose probit assays

The median lethal concentration (LC_{50}) values of Cry1Ac on the field populations of *L. orbonalis* were determined through log dose probit assays. The population collected from different geographical location of vidharbha.

The LC_{50} values ranged from 0.0326 to 0.0369 mg/ml of diet (Table 2). The LC_{50} values from four district of vidarbha *viz*. Akola, Amaravati, Washim and Buldhana populations were 0.0369, 0.0363, 0.0349 and 0.0326 mg/ml of diet respectively. The highest LC_{50} values were observed in Akola population followed by Amaravati and Washim, the lowest LC_{50} value was observed in Buldhana population. Similarly the LC_{90} values of the populations ranges within the baseline limits at 0.0440 to 0.0480 mg/ml of diet.

The LC_{50} values ranged from 0.0322 to 0.0369 g/ml of diet (Table 3). The LC_{50} values from four district of vidharbha viz Akola, Amaravati, Washim and Buldhana populations were 0.0349, 0.0369, 0.0343 and 0.0322 mg/ml of diet respectively. The highest LC_{50} values were observed in Amravati population followed by Akola and Washim, the lowest LC_{50} value was observed in Buldhana population. Similarly the LC_{90} values of the populations ranges within the baseline limits at 0.0458 to 0.0483 mg/mL of diet.

The susceptibility to the Cry1Ac protein among various populations of the fruit and shoot borer observed in this study indicates natural variability in this insect. The baseline data generated in this study could be used to monitor the susceptibility in Brinjal fruit and shoot borer to Cry1Ac protein, prior to and after the commercial release of Bt Brinjal expressing the Cry1Ac protein.

Table 2: Baseline data of Cry1Ac (µg/ml) against *L. orbonalis* (Kharif 2011)

District	N	LC_{50} (50 % FL)	LC_{90} (95 % FL)	Slope + SE	X^2
Akola	60	0.0369(0.0353-0.0386)	0.0465(0.0434-0.0497)	12.77+1.75	5.07
Amravati	60	0.0363(0.0344-0.0382)	0.0474(0.0436-0.0515)	11.00+1.70	1.33
Washim	60	0.0349(0.0325-0.0374)	0.0480(0.0436-0.0529)	9.19+1.68	5.73
Buldhana	60	0.0326(0.0304-0.0350)	0.0440(0.0405-0.0478)	9.90+1.73	0.15

Table 3: Baseline data of Cry1Ac (µg/mL) against *L. orbonalis* (Rabi 2011)

District	N	LC_{50} (50 % FL)	LC_{90} (95 % FL)	Slope + SE	X^2
Akola	60	0.0349(0.0331-0.0369)	0.0458(0.0423-0.0495)	10.94+1.71	4.60
Amravati	60	0.0369(0.0353-0.0386)	0.0466(0.0434-0.0500)	12.65+1.79	0.44
Washim	60	0.0339(0.0317-0.0362)	0.0462(0.0422-0.0507)	9.50 +1.64	1.14
Buldhana	60	0.0322(0.0292-0.0355)	0.0483(0.0426-0.0548)	7.27 +1.54	1.16

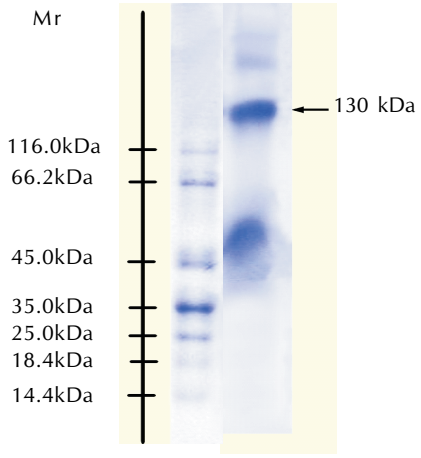

Figure 1: Quantification of Cry Ac toxin on SDS- PAGE

Kranthi *et al.* (2004) determined the Baseline toxicity of Cry1Ac for the spotted bollworm *E. vitella* in India using a semi-synthetic diet. A total of 27 sites from 19 cotton growing district of north, central and south India were sampled during the 2002 and 2003 cropping season and Cry1Ac diet incorporation bioassays were carried out to understand the geographical variability in the baseline susceptibility. The LC_{50} of Cry1Ac ranged from0.006 to 0.105 mg/ml of diet with majority of strains showing response of LC_{50} at 0.01 to 0.03 mg/mL.

Kranthi and Kranthi (2005) studied the median lethal concentration (LC_{50}) values of Cry1Ac on the field populations of *H. armigera* were determined through log dose probit assays. The LC_{50} values ranged from 0.063 to 0.73 mg Cry1Ac/mL of diet. The variability in LC_{50} values from north Indian populations was less at 0.12 to 0.23 mg Cry1Ac/mL of diet, except in the case of Sriganganagar, where it was 0.063 mg Cry1Ac/mL of diet. The LC_{50} values from central and south Indian populations ranged between 0.065 to 0.732 and 0.33 to 0.56 mg Cry1Ac/mL of diet respectively.

Annonymous, (2006) conducted the baseline susceptibility of Cry1Ac to *Leucinodes orbonalis* and data was generated for nine populations during RCGM trial kharif 2004, the LC_{50} values ranged from 0.022 mg/mL (Dharwad, KA) to 0.095 mg/mL (Ahmednagar, MS) and during RCGM trial kharif 2005, the LC_{50} values ranged from 0.028 mg/mL (Kolar, KA) to 0.081 mg/mL (Jaipur, RJ).

Yashodha and Kuppusamy (2008) studied the median lethal concentration (LC_{50}) value of Dipel was lesser with 83.93, 190.0 and 310.42 ppm against II, III and IV instar larvae than Delfin which had 245.24, 372.32 and 536.34 ppm against II, III and IV instar *Leucinodes orbonalis* larvae respectively.

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