ISSN: 0974 - 0376

The Ecoscan : Special issue, Vol. IX: 25-31: 2016
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES
www.theecoscan.com

EFFICACY OF CRY TOXINS ON DIFFERENT AGRO-ECOLOGICAL POPULATIONS OF *SPODOPTERA LITURA* USING IN PLANTA (COTTON PLANT) BIOASSAYS

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KEYWORDS

Spodoptera litura
Cry toxins
Cotton

Proceedings of National Conference on Harmony with Nature in Context of Resource Conservation and Climate Change
(HARMONY - 2016)
October 22 - 24, 2016, Hazaribag,
organized by
Department of Zoology, Botany, Biotechnology & Geology
Vinoba Bhave University,
Hazaribag (Jharkhand) 825301
in association with
National Environmentalists Association, India
www.neaindia.org

The Ecoscan
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES

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ABSTRACT

An experiment was conducted to study the efficacy of cry toxins towards different populations of *Spodoptera litura* at CICR, Nagpur during 2014-15. *Spodoptera litura* population was collected from four locations representing three different states. The laboratory assays showed that Bollgard II expressing Cry1Ac+ Cry2Ab toxins was more toxic to *S. litura* as compared to Cry1Ac and non Bt cotton. Considering leaf and square bioassays with Bollgard and Bollgard II cotton, intraspecific variation existed between populations with the Rangareddy population being most susceptible. The Parbhani strain and Raipur strain of *S. litura* were not affected by Cry1Ac expression in leaves of BG. The study revealed the superior bioefficacy of Cry1Ac + Cry2Ab over Cry1Ac squares and leaves.The mortality of *S. litura* in Bollgard II genotypes was more on squares followed by leaves. At 30 DAS, the square bioassays of Bollgard and Bollgard II were not significantly different whereas bioassays carried out at 60 and 90 DAS were significantly different. Mortality on square is by and large higher as compared to mortality when fed With leaves of Bollgard and Bollgard II. Bollgard II square expressing Cry1Ac + Cry2Ab is more effective than Bollgard square expressing only Cry1Ac against *S. litura* larvae.

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INTRODUCTION

Cotton is the most important commercial cash crop of India. The textile industry is nourished by cotton for over a century. Today, the textile industry has grown to be the largest industry in India. Among the insects, cotton bollworms are the most serious pests of cotton in India causing annual losses of at least US$300 million. American bollworm (*Helicoverpa armigera*), spotted bollworms (*Earias spp*.), pink bollworm (*Pectinophora gossypiella*), tobacco caterpillar (*Spodoptera litura*), whitefly (*Bemisia tabaci*) and leafhopper (*Amrasca biguttula biguttula*) are the most important and have been reported to cause serious quantitative and qualitative losses (Dhaliwal *et al.,* 2006). *Bacillus thuringiensis* is an effective insecticide, relatively harmless to natural enemies, safe to the higher animals and is environmentally acceptable. Insecticides based on *B. thuringiensis* are widely used as natural insecticide against Lepidopteran pests. Mode of action of *B. thuringiensis* in the gut is a complex process, involving many steps in the conversion of insecticidal crystal proteins (ICPs) to toxins (Gill *et al*., 1992). Leaf worm, *Spodoptera litura* (Fab.) (Noctuidae: Lepidoptera) is one of the most destructive pests of cotton, which feeds on foliage and sometimes young green bolls. It is a secondary pest of cotton (Allen *et al.,* 2000). Devastates a large host range of more than 120 host plants (Ramana *et al.,* 1988). The technology is effective in the management of bollworms but not for tobacco caterpillar, *Spodoptera litura,* beet armyworm, *Spodoptera exigua* and soybean looper, *Pseudoplusia includes* which are reported to be tolerant to Cry1Ac. It has been found that *S. litura* has a greater potential to survive in the presence of Bt toxins when compared to other bollworms. In the present study the different plant parts of Bt and non Bt cotton hybrids were tested for their effect on larvae of *S. litura* (Chitkowski *et al*., 2003). In the present study precipitated mixture of saponins extracted from *G. sylvestre* showed very good antifeedant activity, which could be used for the ecofriendly management of lepidopteran insect pests (Choudhary *et al.,* 2014). Various dose levels were applied in the field of explore larvicidal potential of phytoextracts. Percentage mortality for various concentrations proved that Acacia concinna has effective potential with 85.2% mortality at 50 ppm dose level. (Patil D. S and Chavan N. S. 2010)

Bollgard II hybrids which express Cry1Ac + Cry2Ab toxins are toxic to bollworms as well as *S. litura*. Cry1Ac and Cry2Ab protein have different binding sites in the midgut and may thus enhance the host spectrum. This prompted scientists to develop new genetically modified cotton hybrids *i.e.* dual toxin *Bacillus thuringiensis* (Bt) cultivar expressing both Cry1Ac and Cry2Ab endotoxins (Greenplate *et al*., 2000). Cry2Ab protein was added to provide greater insecticidal activity against target pests to broad spectrum of kill. A three to six fold increase was observed in bioactivity of dual toxin Bt cultivars compared with single toxin. Cry2Ab, Cry1Ac, Cry1Ac + Cry2Ab toxins have been studied on geographic populations of *S. litura* with a view of monitor the effects of these proteins or growth mortality.

MATERIALS AND METHODS

Comparing the relative efficacy of Cry1Ac, Cry2Ab and Cry1Ac + Cry2Ab on

different agro ecological populations of *Spodoptera litura* using in planta (cotton plant) bioassays conducted at Central Institute for Cotton Research, (CICR), Nagpur (Maharashtra) during season 2014-2015.

Larvae were collected from the different agro-ecological zones viz., Nagpur (Maharashtra), Raipur (Chhattisgarh), Parbhani (Maharashtra) and Rangareddy district of Telangana from different hosts such as cotton, soybean, chickpea and castor, respectively during kharif season 2014-2015 for study the efficacy of Cry toxins on agro-ecological populations of Tobacco leaf eating caterpillar *Spodoptera litura* (Fabricius). (Fig.1)

Mass rearing of *Spodoptera litura* on natural diet

The larvae collected from the 4 agro-ecological zones were reared in the laboratory under controlled conditions of temperature 25 ± 2ºC, 75% RH and photo period of 13 hrs light: 11 hrs dark and were reared on diet of fresh castor leaves. Larvae were reared in plastic jars on fresh castor leaves washed with water and fed up to final instars. The leaves were changed every alternate day and fresh leaves were provided. Pupae were sexed and maintained. The pupae were transferred to plastic cups containing sand. Adults that emerged were transferred into mating chamber by maintaining male female ratio (1:1) and were provided with adult diet. Neonates that hatched from the eggs were transferred on fresh castor leaves. Continuous rearing was done up to the subsequent generation for bioassays studies. According to the procedure outlined by Kranthi (2005).

Leaf and square bioassays

The bioassay was conducted on *Spodoptera litura* larval population with Bt cotton expressing toxins, Cry1Ac, Cry1Ac + Cry2Ab along with their corresponding non Bt at Insectary and Biocontrol Laboratory of Central Institute for Cotton Research, (CICR) Nagpur, Maharashtra during 2014-2015. Field plots were maintained by standard agronomic practices. This experiment was conducted with first to fifth instars by feeding on leaves and squares of Bt cotton hybrid Bunny BG II expressing toxins Cry1Ac, and Cry1Ac + Cry2Ab along with their corresponding non Bt hybrid. According to the procedure outlined by Kranthi (2005).

The Bt cotton plant parts were excised along with their petiole from the node and brought in to the laboratory in cool boxes. Plant parts were rinsed under tap water and wiped off with blotting paper to remove excess moisture and were air dried. The bioassays were carried out in cups having a 4 cm diameter. Then plant parts were placed in plastic cups on a moist layer of blotting paper. Ten first instar larvae were released on the plant parts in each cup. Cups were closed with finely perforated lids with parafilm to avoid the escape. The cups were transferred to BOD incubator at 25 ± 1ºC, and 70 ± 5% relative humidity. Plant parts were changed every day to avoid mortality. After the larvae attained 2nd instar these were transferred into single cups, to avoid cannibalism. Larval mortality was recorded every day up to the seventh day, and individual weight (mg) of each surviving larva was recorded on the seventh day.

Corrected mortality was calculated as per Abbott's formula (Abbott, 1925).

$$\text{Corrected mortality} = \frac{\%\text{ kill in treated} - \%\text{ kill in control}}{100 - \%\text{ kill in control}} \times 100$$

$$\%\text{ mortality} = \frac{\text{No. of dead larvae}}{\text{Total no. of larvae}} \times 100$$

Statistical analysis

Mean of per cent mortality data and 'F' test of two values for variance was calculated by using Data Analysis Microsoft Excel® software.

RESULTS AND DISCUSSION

Intraspecific variability within *S. litura* populations as evidenced by leaf bioassays

S. litura leaf bioassay were carried out using tender terminal leaves of Bollgard, Bollgard II and non Bt at 30 DAS, 60 DAS and 90 DAS. Bioassays were carried out over a period of 7

Table 1: Efficacy of Cry toxins on populations of *Spodoptera litura* different agro-ecological zones using plant part bioassay

Locations	Toxins	Days after sowing					
		30 days		60 days		90 days	
		Leaf bioassay	Square bioassay	Leaf bioassay	Square bioassay	Leaf bioassay	Square bioassay
Raipur	Cry1Ac	13.33 ± 0	13.34 ± 6.67	8.89 ± 4.44	23.34 ± 2.73	11.11 ± 5.88	24.45 ± 2.23
(Chhattisgarh)	Cry1Ac + Cry2Ab	22.22 ± 5.88	42.22 ± 14.59	31.11 ± 11.11	57.78 ± 8.02	42.22 ± 16.02	46.67 ± 7.71
	NBT	4.44 ± 4.44	4.44 ± 4.45	2.22 ± 2.22	0.00 ± 0.00	4.45 ± 2.22	2.22 ± 2.23
	Control	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Nagpur	Cry1Ac	8.89 ± 4.45	11.11 ± 4.44	20 ± 0.00	22.22 ± 4.45	8.89 ± 2.22	17.78 ± 4.45
(Maharashtra)	Cry1Ac + Cry2Ab	31.11 ± 8.02	31.11 ± 4,45	24.44 ± 4.45	44.45 ± 2.23	35.56 ± 4.45	53.33 ± 10.19
	NBT	0.00 ± 0.00	2.22 ± 2.23	4.45 ± 2.23	0.00 ± 0.00	2.22 ± 2.23	2.22 ± 2.22
	Control	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Parbhani	Cry1Ac	15.56 ± 5.89	20 ± 3.86	6.67 ± 6.67	20.00 ± 6.67	6.67 ± 0.00	15.55 ± 2.23
(Maharashtra)	Cry1 Ac + Cry2Ab	13.33 ± 0.00	44.45 ± 2.23	8.89 ± 2.22	44.44 ± 5.89	11.11 ± 2.22	53.33 ± 7.71
	NBT	2.22 ± 2.23	2.22 ± 2.23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.22 ± 2.23
	Control	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Rangareddy	Cry1Ac	36.67 ± 3.34	36.67 ± 6.67	30 ± 5.78	33.33 ± 6.67	30 ± 5.78	30 ± 5.78
Dist.(Telangana)	Cry1Ac + Cry2Ab	43.33 ± 3.34	46.67 ± 3.34	50 ± 11.56	56.67 ± 3.34	50 ± 5.78	66.67 ± 8.83
	NBT	3.33 ± 3.34	3.33 ± 3.34	3.33 ± 3.34	3.33 ± 3.34	0.00 ± 0.00	3.33 ± 3.3
	Control	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
F- TEST		Sig	NS	Sig	Sig	Sig	Sig

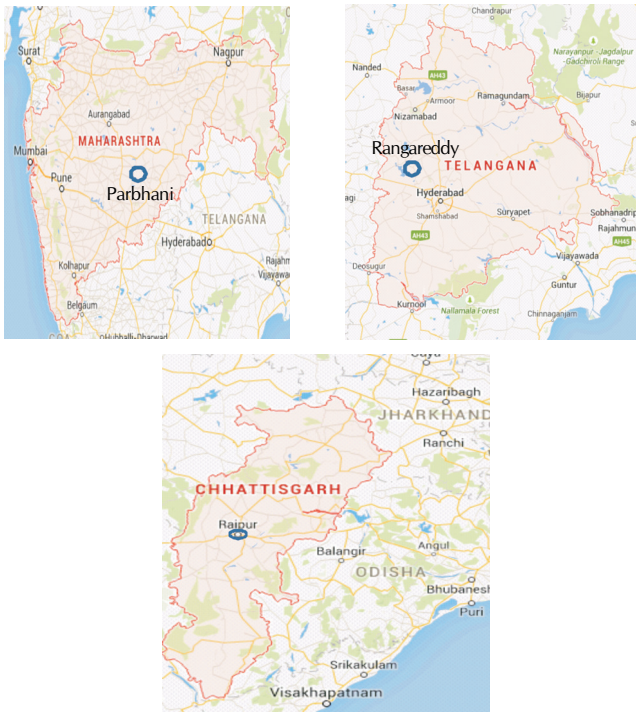

Figure 1: Population of *S. litura* collected from 4 different agro-ecological zones

days and leaves were changed every day. Similar bioassays using leaves of Bt transgenic against 2 day old larvae of lepidopteran insect pests have been carried out by Henneberry *et al.,* (2001) who studied the effects of transgenic cotton on cabbage looper, tobacco budworm, beet armyworm and the mortality was 95%, 100% and 57%, respectively.

The toxin expression declined with age of crop is well known and was reported by Kranthi *et al.,* (2005) While Cry toxin expression was highest in leaves it declined in the reproductive part especially as the crop age. According to Adamczyk *et al.,* (2000) and Arshad *et al.,* (2009) lower expression of Cry1Ac toxin in the reproductive parts (flowers and bolls) than critical level required for its effective control is responsible for less than desirable control. Thus the mortality recorded was not entirely unexpected that in leaf bioassays till 90 DAS ranged from 11.11% to 50% (Table 1, Fig. 2 and 3). Mortality in populations of *S. litura* ranged between 11.11 % to 50 % as in Parbhani and Rangareddy respectively on Cry1Ac + Cry2Ab in Bollgard II leaves. At 30 DAS Cry1Ac + Cry2Ab expressing leaves recorded percent mortality between 13.33 % to 43.33 % and at 60 DAS 2 gene expressing leaves recorded percent mortality between 8.89% to 50% (Table 1, Fig. 2 and 3). The intraspecific variability between populations existed when leaf bioassay was carried out with leaves expressing Cry1Ac + Cry2Ab at all ages of the crop.

The present findings derive support from Dong Lin *et al.,* (2006) who revealed that effect of *S. litura* on dual toxin Bt cotton, non Bt cotton and found higher mortality on dual toxin *Bt* cotton over non Bt cultivars. Populations of Parbhani were relatively more tolerant showing least mortality on BG II leaves at 30, 60 and 90 DAS (Table 1, Fig. 2). Leaf bioassays were not extended beyond at 90 DAS on *S. litura* as it generally occurs at peak vegetation stage.

Higher tolerance of the Parbhani strain to Bollgard II could be attributed to the host plant on which the larvae were collected. Parbhani populations of *S. litura* were collected from Chickpea while other populations were collected from Soybean, Castor and Cotton. Bioassays were carried out using Cry1Ac expression Bollgard cotton intraspecific variation existed between population with the Rangareddy population being most susceptible recording mortality of 36.67%, 30% and 30% at 30, 60 and 90 DAS of the crop respectively (Table 1, Fig. 3). The Parbhani strain and Raipur strain of *S. litura* were not affected by Cry1Ac expression in leaves of BG (Table 1, Fig. 4). Arshad *et al*. (2009) reported significantly higher mortality in neonates fed on Bt cotton leaves than those fed on Bt bolls. The results are in conformity with Badigar (2010) reported that 35.00 percent corrected mortality of *S. litura* when fed on leaves of MRC 6918 BG-I expressing single gene (Cry1Ac).

When the mortality data across the strains fed on leaves from plants at different days after sowing and toxins Cry1Ac and Cry1Ac + Cry2Ab was compared, the differences were significant at 30, 60 and 90 DAS with leaves expressing Cry1Ac + Cry2Ab being significantly more toxic to *S. litura* as compared to Cry1Ac alone. Chitkowski *et al*. (2003) Sivasupramaniam *et al*. (2008) and Akin *et al*. (2011) reported that the mortality of both *S. exigua* and *S. frugiperda* were significantly greater on Bollgard II plant leaves than on either Bollgard or conventional cotton. The present study revealed the superior bioefficacy of Cry1Ac + Cry2Ab over Cry1Ac in squares and leaves. The Nagpur populations of *S. litura* show maximum mortality on leaf of BG-II than BG in 30, 60 and 90 DAS of the crop as did the Raipur strain (Table 1, Fig. 5).

The mortality of neonates in Cry1Ac + Cry2Ab was significantly higher as compared to the mortality caused by Cry1Ac which agree with the finding of Udikeri (2006), Omkarmurthy (2008) and Somashekara (2009). According to Soujanya *et al.* (2011) neonate larvae of *S. litura* were more susceptible on leaves and squares of dual toxin hybrids than those from single toxin Bt hybrids or non Bt cotton after 72 hrs of feeding. With regard to 1st instar, the mortality was percent when fed on leaves of 60-90 days old crop of both the dual toxin Bt cotton cultivars, while it was 15% only in single toxin Bt cultivars as well as on non Bt. Unlike on leaves, the 1st instar recorded 73.33 and 76.66% mortality when fed on squares

Table 2: F test values calculated across the locations (Leaf bioassay)

Sr.No.	F value	30 DAS	60 DAS	90 DAS
1	F tab.	0.10	0.10	0.10
2	F cal.	0.92	0.40	0.40
3	Significant	Sig	Sig	Sig

Table 3: F test values calculated across the locations (Square bioassay)

Sr. No.	F value	30 DAS	60 DAS	90 DAS
1	F tab.	9.27	0.10	0.10
2	F cal.	2.79	0.63	0.61
3	Significant	NS	Sig	Sig

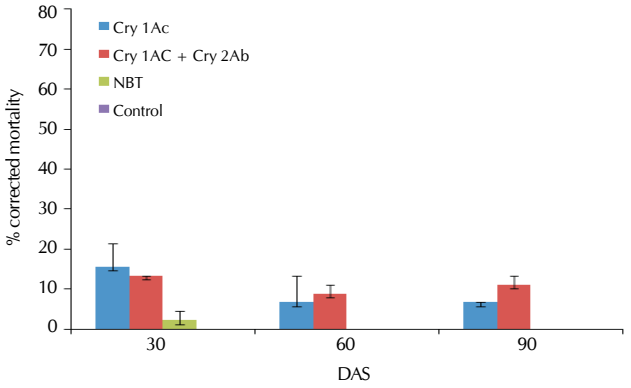

Figure 2: Cry toxin bioassay with *S. litura* Parbhani population (leaf)

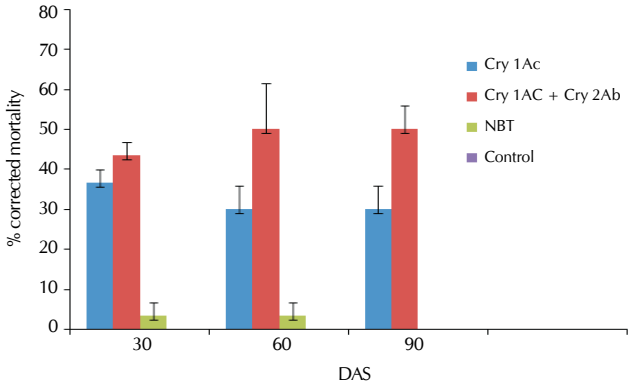

Figure 3: Cry toxin bioassay with *S. litura* Rangareddy population (leaf)

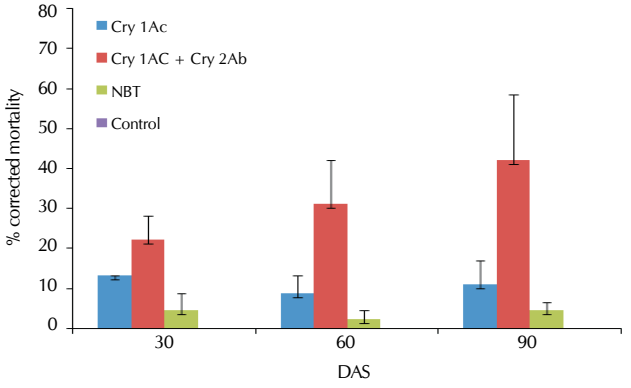

Figure 4: Cry toxin bioassay with *S. litura* Raipur population (leaf)

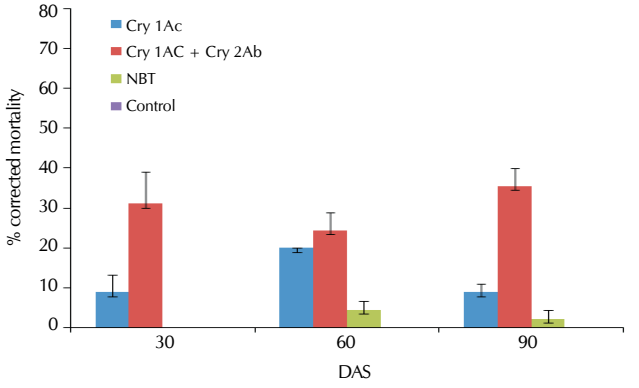

Figure 5: Cry toxin bioassay with *S. litura* Nagpur population (leaf)

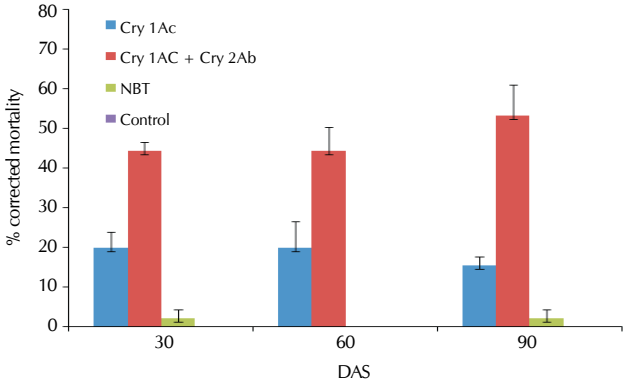

Figure 6: Cry toxin bioassay with *S. litura* Parbhani population (square)

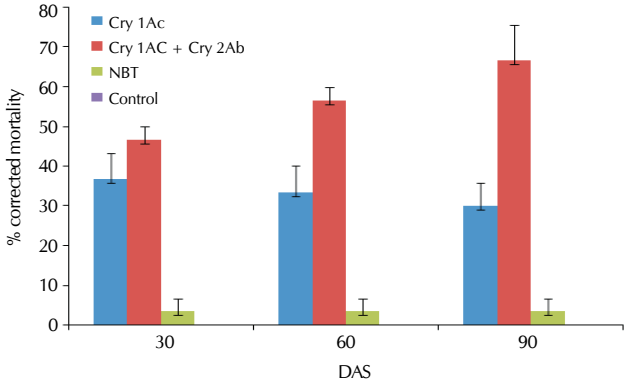

Figure 7: Cry toxin bioassay with *S. litura* Rangareddy population (square)

expressing Cry1Ac and Cry2Ab respectively as against 20% on single toxin Bt hybrids and 10% on non Bt hybrids. According to Soujanya *et al*. (2011) a higher survival rate was observed for larvae fed on leaves and squares of single toxin Bt cultivars and non Bt cotton as compared with the treatment of feeding on dual toxin Bt cotton cultivars as the mortality was nil both on leaves and squares of single toxin Bt and non Bt cultivars. It is certainly true that Cry1Ac + Cry2Ab is more effective against *S. litura* in leaf bioassays in most of cases, but our study shows that population of *S. litura* for Parbhani showed least tolerance to Cry1Ac + Cry2Ab.

Intraspecific variability between *S. litura* populations as evidenced by Square bioassays

Using freshly detached squares of Bollgard, Bollgard II and non Bt, square bioassays were carried out with *S. litura* to understand the variability recorded in leaf bioassays extended to square bioassay.

Square bioassay revealed that squares expressing Cry1Ac + Cry2Ab recorded higher mortality as compared to squares

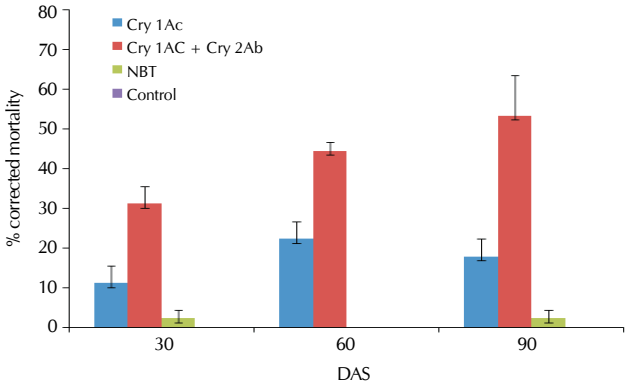

Figure 8: Cry toxin bioassay with *S. litura* Nagpur population (square)

expressing only Cry1Ac. It must be mentioned here that the mortality of larvae of Parbhani strain in square bioassay were similar with Bollgard and Bollgard II leaves (Table 1, Fig.6). However the differences in mortality recorded in bioassays involving squares of Bollgard and Bollgard II at 30, 60 and 90 DAS were high. The mortality of *S. litura* in Bollgard II genotypes was more on squares followed by bolls and leaves as the expression of Cry2Ab was more in squares and this high expression in squares were Anon., (2003).

The Rangareddy population of *S. litura* was more susceptible to Cry1Ac and Cry1Ac + Cry2Ab with mortality in the latter extending to nearly 70% in square bioassays (Table 1, Fig 7). Govindan *et al*., (2009) recorded that 1st instar larva of *S. litura*, showed highest per cent mortality (72.7%) when fed on RCH2 Bt young green bolls, 61.67 per cent on top fully opened young leaves 61.0 per cent on squares and 45.8% on middle leaves after 7 days. Naik *et al*., (2013) where the mortality of larvae on squares of RCH 2 BG II hybrid was tested and maximum mortality of 71.66 % was recorded in first instar larvae. Similar findings were reported by Anon., (2003) where, the mortality of *S. litura* in Bollgard II genotypes was more on squares followed by bolls and leaves as the expression of Cry2Ab was more in squares. Mortality of larvae on non Bt leaves and square did not extend beyond 5% in bioassays.

The Nagpur populations of *S. litura* show maximum mortality on Square of BG-II than BG in 30, 60 and 90 DAS of the crop. (Table 1, Fig.8)

This study shows that

Spodoptera litura larvae die on feeding on Bollgard and Bollgard II square.

Mortality on square is by and large higher as compared to mortality when fed with leaves of Bollgard and Bollgard II.

Bollgard II square expressing Cry1Ac + Cry2Ab is more effective than Bollgard square expressing only Cry1Ac against *S. litura* larvae.

The mortality on non Bt square was negligible indicating the robustness of the assay.

F test carried out to detect the level of significance in mortality data obtained from bioassays involving Bollgard and Bollgard II squares showed that at 30 DAS, the Bollgard and Bollgard II square bioassays were not significantly different (Table 3) whereas bioassays carried out at 60 and 90 DAS were significantly different (Table 2).The findings of the present study are in tune with the findings of Chitkowski *et al*. (2003). Sivasupramaniam *et al*. (2008) and Akin *et al*. (2011) who reported that the mortality of both *S. exigua* and *S. frugiperda* were significantly greater on Bollgard II plant leaves than on either Bollgard or conventional cotton.

However this needs confirmation through systematic simultaneous experiment involving in Cry1Ac and Cry1Ac + Cry2Ab expressing in Bollgard and Bollgard II, across the season.

ACKNOWLEDGEMENT

The authors are highly thankful to Dr. Sandhya Kranthi, Head, Division of Crop Protection, Central Institute For Cotton Research, (CICR) Nagpur, (Maharashtra) for providing necessary facilities for carrying out this research work and finally thanks to IGKV, Raipur for financial support.

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