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Vrushali Pawade
Department Of Entomology,
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. 444 104 (M.S.)

S. M. Thakare
Department Of Entomology,
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. 444 104 (M.S.)

B.S. Ghodaki
Department Of Entomology,
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. 444 104 (M.S.)

Correspondence:
Vrushali Pawade
Department Of Entomology,
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. 444 104 (M.S.).

Bio-efficacy of transgenic cotton hybrids against different instars of *Helicoverpa armigera* (Hubner) Hardwick

Vrushali Pawade, S. M. Thakare, B.S. Ghodaki

Abstract
The bioassays on *Helicoverpa armigera* were conducted against leaves, squares, flowers and bolls from 15 to 135 days after emergence (DAE) on first, second and third instars of *H. armigera*, separately for two seasons during 2007-08 and 2008-09. Cent per cent mortality was observed in all Bt hybrids on first and second instar of *H. armigera*. However, in third instar larvae the average mortality was between 58.33 to 96.25 per cent. There was inverse relationship between amounts of Cry1Ac toxin in the plant parts versus the weight of larvae. Amongst Bt cotton hybrids Tulshi-9 recorded highest average mortality (80.00 to 96.25%) followed by RCH-Alto and JK-99 (75.00 to 92.50% and 71.67 to 90.00%, respectively) and lowest in Mallika and MECH-162 (both 58.33 to 80.00%). The non-Bt cotton hybrid PKV Hy-2 recorded lowest average per cent morality against first, second and third instars of *H. armigera* and all the Bt hybrids were found superior over non-Bt cotton hybrid.

Keywords: Bt cotton, Bio efficacy, *H. armigera*, Hybrids

1. Introduction
Cotton (*Gossypium* spp.) popularly known as 'White Gold' plays a prominent role in Indian economy. It is grown chiefly for its fibre used in the manufacture of cloth for the mankind. The area under cotton in India during 2007-08 was 9.3 million ha with average productivity of 599 kg lint/ha. However, the productivity of Maharashtra was only 320 kg lint/ha as compare to national productivity [1]. *Bacillus thuringiensis* i.e., Bt is a gram positive, spore forming bacterium, which synthesize crystalline protein during sporulation. Bt kills certain insects due to an insecticidal protein called delta endotoxin, which disrupts the function of digestive system of these insects.

The crystals, upon ingestion by the insect larva, are solubilized in the highly alkaline midgut into individual protoxins which vary from 133 to 138 KDa in molecular weight, depending upon the type of protoxin. The protoxins are acted upon by midgut proteases which cleave them into two halves, the N-terminal half them into two halves, which is usually of 65-68 KDa in the toxin protein. The toxin protein fragment can be divided into three domains. The first is involved in pore formation, the second determines receptor binding and the third is involved in protection to the toxin from proteases. The toxin protein binds to specific receptors present in the midgut epithelial membranes. Upon receptor binding, the domain I inserts itself into the membrane leading to the pore formation. The disturbances in osmotic equilibrium and cell lysis lead to insect paralysis and death. [6] Bt-cotton hybrids developed by using Indian parent varieties into which the Cry1Ac gene was introgressed from a transgenic Bt cotton variety, cocker 312. Current Bollgard cotton hybrids have descended from a common parent with a single genetic transformation event 'Monsanto – 531', which was transformed with a vector containing a full length Cry1Ac coding sequence driven by an enhanced 35 S promoter that enables the production of Cry1Ac protein in almost all parts of the plants. The novel transgenic technology was found to be highly beneficial in almost all parts of the world in terms of its capabilities to keep the target pests such as bollworms under check. However, for the Bt-transgenic technology to be sustainable, it is important that the toxin expression levels be expressed in adequate quantities in appropriate plant parts at the requisite time of the season to afford protection against major target insect pests, which primarily include the bollworms. Hence the present studies were carried out to determine the effect of different Bt cotton cultivars on growth of *H. armigera*.

2. Material and Methods

Eight Bt-cotton Bollgard hybrids, i.e., Bunny, Mallika, MECH-162, RCH-2, Ankur-651, JK-99, RCH-Alto and Tulshi-9 Bt along with non-Bt check, PKV Hy-2 were sown in Randomized Block design (RBD) with three replications in the field of Department of Entomology, Dr. PDKV, Akola, during *kharif* 2007-08 and 2008-09. Laboratory bioassays on first, second and third instars of *H. armigera* were conducted separately on upper canopy, leaves, squares, flowers and green bolls at 15 days interval from 15 DAE till the end of crop season using 30 larvae per treatment in unreplicated trials. First, second and third instars of *H. armigera* were fed on the different plant parts collected treatment wise from the field. The plant parts were changed every day until the end of the bioassay. Percent mortality observations and individual weight of surviving larvae were recorded on the seventh day after larval release.

$$\text{Mortality} = \frac{\text{Dead}}{\text{Number}} \times 100$$

However the ± **SE** were calculated for weight of surviving larvae using three replication of 10 larvae each using the formula

$$SE = SD / \sqrt{r}$$

Whereas,

SE = Standard Error,
SD = Standard Deviation,
r = Number of replication

3. Results and Discussion

3.1 Bioassay against various instars of *H. armigera* on different plant parts of Bt transgenic cotton cultivars

The average larval mortality in bioassays with first and second instar *H. armigera* on different plant parts i.e., leaves, squares, flowers and bolls was observed to be 100 per cent (Table 1 and 2). However, it was in the range of 0.42 to 3.33 per cent in non-Bt cotton hybrid PKV Hy-2.

3.2 Bioassays against third instar *H. armigera* on leaves

The bioassay (Table 3) against third instar *H. armigera* was conducted on leaves at fortnightly interval form 15 DAE to 135 DAE. Amongst, the treatments the Bt cotton hybrid Tulshi-9 recorded highest average mortality (96.25%) followed by RCH-Alto (92.50%), JK-99 (90.00%), RCH-2 and Ankur-651 (88.75%), Bunny (83.75%), MECH-162 and Mallika (80.00%) as compared to non-Bt hybrid PKV Hy-2 in which only 2.04 per cent mortality was recorded.

There was inverse relationship between amount of Cry1Ac versus the weight of larvae. The average weight of surviving larvae was in range of 1.07 ± 0.35 mg to 6.90 ± 0.66 mg. While highest average larval weight 32.6 ± 1.39 mg was recorded in non-Bt hybrid PKV Hy-2. Similar result was observed in case of per cent weight reduction, i.e., 78.80% to 96.70% over non-Bt hybrid PKV Hy-2.

3.3 Bioassays against third instar *H. armigera* on squares

In the bioassay (Table 3) conducted on squares at fortnightly interval from 60 DAE to 135 DAE. The Bt hybrid Tulshi-9 recorded highest average mortality (94.45%) followed by RCH-Alto (90.28%), JK-99 (87.50%), RCH-2 and Ankur-651 (86.11%), Bunny (80.56%), MECH-162 and Mallika (76.39%) as compared to non Bt hybrid PKV Hy-2 in which only 2.78 per cent mortality was recorded. There was inverse relationship between amount of Cry1Ac versus the weight of larvae. The average weight of surviving larvae was in range of 1.47 ± 0.38 mg to 7.17 ± 0.43 mg. While highest average larval weight 33.76 ± 1.19 mg was recorded in non-Bt hybrid. In case of average per cent larval weight reduction, it was in range of 78.82% to 95.76% over non Bt hybrid PKV Hy-2.

3.4 Bioassays against third instar *H. armigera* on flowers

The bioassay (Table 3) against 3rd instar *H. armigera* was conducted on flowers at fortnightly interval from 75 DAE to 135 DAE. The Bt hybrid Tulshi-9 recorded highest average mortality (85.00%) and proved better followed by RCH-Alto (80.00%), JK-99 (76.67%), RCH-2 and Ankur-651 (75.00%), Bunny (68.33%), MECH-162 and Mallika (63.33%) as compared to non-Bt hybrid PKV Hy-2 in which only 2.67 per cent mortality was recorded. There was inverse relationship between amount of Cry1Ac versus the weight of larvae. The average weight of surviving larvae was in range of 8.80 ± 0.63 mg to 3.35 ± 0.45 mg. While highest average larval weight 33.41 ± 0.87 mg was recorded in non-Bt hybrid. While, the average per cent larval weight reduction was in range of 72.32% to 89.92% over non-Bt hybrid PKV Hy-2.

3.5 Bioassays against third instar *H. armigera* on bolls

The bioassay (Table 3) conducted on bolls at fortnightly intervals from 90 DAE to 135 DAE, recorded the mortality of 53.33 to 85 per cent. Whereas, the Bt hybrid Tulshi-9 recorded highest average mortality (80.00%) followed by RCH-Alto (75.00%), JK-99 (71.67%), RCH-2 and Ankur-651(70.00%), Bunny (63.33%), MECH-162 and Mallika (58.33%) as compared to non-Bt hybrid PKV Hy-2 in which only 6.67 per cent mortality was recorded. There was inverse relationship between amount of Cry1Ac versus the weight of larvae. The average weight of surviving larvae was in range of 4.36 ± 0.59 mg to 10.33 ± 0.48 mg as against 31.97 ± 1.26 mg in non Bt hybrid. Similarly the average per cent larval weight reduction was in range of 67.45% to 86.23% over non-Bt hybrid PKV Hy-2.

All the Bt hybrids were found superior over non-Bt variety in recording maximum mortality. The previous workers like, Mandaokar *et al*. (2000) [5] also found that the leaves and fruits of the seven transgenic plants were protected from the insect damage. Some studies have indicated an inverse relationship between the amount of Cry1Ac among cultivars versus the weight of bollworm larva. The lower concentrations provided low levels of mortality, whereas the higher concentrations provided high levels of mortality (Adamczyk and Gore 2004 [2], Gore *et al*. 2005) [3]. Furthermore, Kranthi *et al*. (2005) [4] reported increasing levels of *H. armigera* survival, correlated with the toxin levels decreasing below 1.8 µg/g in the plant parts. Whereas, Sivasupramaniam *et al*. (2008) [7] observed feeding of leaf discs from Cry1Ac/ Cry2Ab2 cotton resulted in mortality of second instars of *S. frugiperda* ranging from 69 to 93 per cent depending on plant age. The findings at the present investigation in case of first and second instars mortality and reduction in weight of survived larvae are similar to the findings discussed above which confirms the present results.

4. Conclusion

Data obtained from the present investigation concluded that all Bt-cotton hybrids tested was found to be most effective in controlling 100 percent first and second instar larvae of *H. armigera*. The highest larval mortality of third instar on leaves and fruiting parts was observed in Tulshi-9. There was inverse relationship between amount of Cry 1AC verses the weight of larvae. All the Bt hybrids were found superior over non-Bt hybrid in recording minimum mortality.

Table 1: Bioassay against first instar *H. armigera* on different plant parts of Bt cotton hybrids

Hybrids	Average Percent mortality of *H. armigera* on different plant parts			
	Leaves	Squares	Flowers	Bolls
Bunny	100	100	100	100
Mallika	100	100	100	100
MECH-162	100	100	100	100
RCH-2	100	100	100	100
Ankur-651	100	100	100	100
JK-99	100	100	100	100
RCH-Alto	100	100	100	100
Tulshi-9	100	100	100	100
PKV-Hy2	3.33	0.56	1.00	0.42

Table 2: Bioassay against second instar *H. armigera* on different plant parts of Bt cotton hybrids

Hybrids	Average Percent mortality of *H. armigera* on different plant parts			
	Leaves	Squares	Flowers	Bolls
Bunny	100	100	100	100
Mallika	100	100	100	100
MECH-162	100	100	100	100
RCH-2	100	100	100	100
Ankur-651	100	100	100	100
JK-99	100	100	100	100
RCH-Alto	100	100	100	100
Tulshi-9	100	100	100	100
PKV-Hy2	0.74	1.39	0.67	0.42

Table 3: Bioassay against third instar *H. armigera* on different plant parts of Bt cotton hybrids

Hybrids	Leaves			Squares			Flowers			Bolls		
	Avg %Mortality	Avg Wt of surviving larvae (mg±SE)	Avg % larval Wt reduction	Avg %Mortality	Avg Wt of surviving larvae (mg±SE)	Avg % larval Wt reduction	Avg %Mortality	Avg Wt of surviving larvae (mg±SE)	Avg % larval Wt reduction	Avg %Mortality	Avg Wt of surviving larvae (mg±SE)	Avg %larval Wt reduction
Bunny	83.75	5.35 ±0.54	83.56	80.56	5.73 ± 0.53	83.14	68.33	7.76 ± 0.58	76.66	63.33	8.88 ±0.52	71.99
Mallika	80.00	6.90 ±0.66	78.80	76.39	7.17 ± 0.43	78.88	63.33	8.80 ± 0.63	72.32	58.33	10.33 ±0.48	67.45
MECH-162	80.00	6.76 ±0.48	79.24	76.39	7.19 ± 0.58	78.82	63.33	8.98 ± 0.84	72.96	58.33	10.16 ±0.77	67.99
RCH-2	88.75	3.46 ±0.49	89.36	86.11	3.88 ± 0.59	88.62	75.00	5.91 ± 0.46	82.25	70.00	6.95 ±0.54	78.07
Ankur-651	88.75	3.54 ±0.64	89.12	86.11	3.90 ± 0.51	88.56	75.00	5.88 ± 0.44	82.31	,70.00	7.11 ±0.54	77.60
JK-99	90.00	3.07 ±0.49	90.57	87.50	3.49 ± 0.69	89.76	76.67	5.50 ± 0.51	83.45	71.67	6.56 ±0.43	79.31
RCH-Alto	92.50	2.22 ±0.35	93.16	90.28	2.66 ± 0.51	92.21	80.00	4.77 ± 0.46	85.96	75.00	5.77 ±0.38	81.79
Tulshi-9	96.25	1.07 ±0.35	96.70	94.45	1.47 ± 0.38	95.76	85.00	3.35 ± 0.45	89.92	80.00	4.36 ±0.59	86.23
PKV-Hy2	2.04	32.6 ±1.39	0.00	2.78	33.76 ± 1.19	0.00	2.67	33.41 ± 0.87	0.00	6.67	31.97 ±1.26	0.00

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