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# Seasonal variation in expression of Cry1Ac and Cry2Ab gene regulated endotoxins in transgenic cotton hybrids against neonates of *Helicoverpa armigera* (Hubner)

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**Abstract**
Three variants (non-BT, BT, and BG II) of the two cotton hybrids (RCH 134 and BIO 6488) were evaluated for insecticidal efficacy against *Helicoverpa armigera* (Hubner) at CCS Haryana Agricultural University, Hisar. BG II consistently exhibited superior efficacy against first instar larvae of *H. armigera* compared to single-gene BT and non-BT hybrids. Toxin expression was highest in leaves, followed by squares and bolls, at all crop stages. However, overall insecticidal efficacy declined with advancing crop age, being highest at 80 DAS (days after sowing), intermediate at 120 DAS, and lowest at 140 DAS. Differences between squares and bolls at similar crop ages were generally non-significant. The study confirms that dual-gene (BG II) cotton hybrids provide more stable and sustained protection against *H. armigera*, although efficacy decreases as the crop matures.

**Keywords:** BT cotton, efficacy, *Helocoverpa armigera*, neonates, Cry1Ac, Cry2Ab

## Introduction
The American bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae), is a major pest of cotton in India (Murugan, M., *et al*.,2003) [9]. It typically occurs during the early to mid-crop season and damages tender terminal shoots, squares, and green bolls. To manage this pest, transgenic BT cotton expressing the Cry1Ac gene was introduced in India in 2002 (Arms, N.J., *et al*., 1992; Qaim, M., *et al.,* 2003; Rao, N.H.P., *et al.,* 2002; Venugopal, K., *et al.,* 2002) [2, 12, 13, 16]. This technology primarily targeted *H. armigera*. However, other lepidopteran pests such as *Spodoptera litura* and *Pectinophora gossypiella* were not effectively controlled by Cry1Ac toxin alone. To enhance insecticidal efficacy and broaden the spectrum of control, a second gene, Cry2Ab, encoding a different BT toxin, was incorporated into BT cotton already expressing Cry1Ac (Bollgard), resulting in Bollgard II (BG II). This dual-gene cultivars have shown more consistent and superior activity against bollworms compared to single-gene cultivars (Udikeri, S.S., *et al.*, 2006; Bheemanna, M, *et al.,* 2008) [15, 4].

In general Cry protein expression in bolls is lower than in squares or leaves. However, Rui, C.H., *et al.,* (2002) [14] observed decreased insecticidal activity in BT cotton against *H. armigera* with plant age. Insecticidal activity in leaves was highest in the early growing season (June and July), but bolls and squares showed higher activity in the middle or late growing season (August and September). Reduction in insecticidal efficacy against *H. armigera* in leaves, squares and bolls of transgenic BT cotton hybrids with advancing crop age was reported at several locations in India (MahaLakshmi, M.S., *et al.*, 2014; Likhitha, P., *et al.,* 2018) [7, 6]. The present study evaluated the age-specific efficacy of BT, non-BT, and BG II cotton hybrids in leaves, squares, and bolls against first instar larvae of *H. armigera*.

## Materials and methods
### Stock culture of *Helicoverpa armigera*
Larvae of *H. armigera* were collected from okra fields at the Research Farm of CCS Haryana Agricultural University, Hisar, India. The larvae were individually reared on fresh okra fruits in plastic vials until pupation under controlled laboratory conditions (28 ± 1°C) in a BOD incubator. One-day-old pupae were sexed under a binocular microscope based on the position of genital and anal openings. In male pupae, the genital and anal openings are located mid-ventrally on the 9th and 10th abdominal segments, respectively, whereas in females they are located on the 8th and 10th segments (Paul, A.V.N., *et al.,* 1978) [11]. Female pupae also exhibit a characteristic inverted 'V'-shaped depression extending to the 10th segment. Male and female pupae were placed in glass jars (21 × 15 cm) for adult emergence. Emerging moths were provided with a 15% sucrose solution using cotton swabs. Muslin cloth was used as an oviposition substrate. Eggs laid on the cloth were transferred to separate jars for hatching, and the neonates oBTained were used for bioassays.

### Laboratory bioassay for efficacy and persistence
Three plant parts—fully expanded third top leaf, young squares, and young bolls—were collected from six cotton hybrids grown at the Research Farm, CCS HAU, Hisar. Samples were taken at 80, 120, and 140 days after sowing (DAS). The hybrids evaluated were: BIO 6488 non-BT, BIO 6488 BT, BIO 6488 BG II, RCH 134 non-BT, RCH 134 BT and RCH 134 BG II. Plant parts were brought to the laboratory and placed individually in Petri dishes. Ten first instar larvae (neonates) of *H. armigera* were released per plant part per hybrid, with three replications. Larval survival was recorded after five days. Food was replaced on alternate days. All bioassays were conducted at 28 ± 1°C in a BOD incubator. Leaf petioles were wrapped with water-soaked cotton swabs to maintain turgidity.

## Results and Discussion
The data on survival of first instar larvae of *Helicoverpa armigera* (Hubner) on leaves, squares and bolls of 3 events (BG II, BT and non-BT) of each of two recommended cotton hybrids (RCH 134 and BIO 6488) after 80, 120 and 140 days of sowing have been summarized in Table 1

**Table 1:** Survival of first instar *Helicoverpa armigera* larvae on leaves and fruiting bodies of different BT cotton hybrids at various periods of crop growth

| Hybrid | Larval survival (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Leaves | | | Squares | | | Bolls | | |
| | 80 DAS* | 120 DAS | 140 DAS | 80 DAS | 120 DAS | 140 DAS | 80 DAS | 120 DAS | 140 DAS |
| RCH 134 BG II | 10.00(14.99) | 20.00(26.06) | 60.00(50.83) | 6.67 (12.28) | 30.00(32.99) | 66.67(55.05) | 13.33(21.14) | 30.00(32.98) | 50.00(44.98) |
| RCH 134 BT | 20.00(26.06) | 66.67(54.76) | 90.00(74.98) | 30.00(32.99) | 76.67(61.19) | 90.00(74.98) | 33.33(35.20) | 83.33(70.06) | 93.33(77.49) |
| RCH 134 non BT | 76.67(61.69) | 86.67(72.76) | 93.33(77.69) | 83.33(66.12) | 86.67(68.83) | 96.67(83.84) | 86.67(68.82) | 90.00(74.98) | 96.67(83.84) |
| BIO 6488 BG II | 0.00(1.81) | 23.33(28.27) | 46.67(42.98) | 3.33(6.14) | 20.00(26.06) | 46.67(43.06) | 3.33 (6.14) | 16.67(23.84) | 46.67(42.97) |
| BIO 6488 BT | 20.00(26.06) | 43.33(41.13) | 53.33(46.90) | 23.33(28.77) | 46.67(42.98) | 53.33(46.99) | 26.67(30.98) | 46.67(43.06) | 50.00(44.98) |
| BIO 6488 non BT | 80.00(63.90) | 83.33(66.11) | 83.33(66.12) | 83.33(66.61) | 83.33(66.61) | 80.00(63.90) | 83.33(66.61) | 86.67(72.77) | 80.00(63.90) |
| SEm(±) | (4.84) | (4.99) | (4.98) | (4.57) | (3.97) | (5.44) | (3.77) | (6.85) | (4.88) |
| CD(p=0.05) | (15.08) | (15.57) | (15.53) | (14.24) | (12.38) | (16.94) | (11.76) | (21.35) | (15.20) |

**Effect of 80-day-old crop:** Significant differences in larval survival were observed among BT, BG II, and non-BT hybrids. On leaves of 80-day-old plants: survival ranged from 0–10% in BG II hybrids, 20% in BT hybrids and 76.67–80% in non-BT hybrids. The lowest survival (0%) was recorded in BIO 6488 BG II, followed by 10% in RCH 134 BG II. Both BT hybrids recorded 20% survival. Among non-BT hybrids, survival was highest (76.67–80%). On squares: survival ranged from 3.33–30% in BT and BG II hybrids but 83.33% in non-BT hybrids. On bolls: it ranged from 3.33–33.33% in BT and BG II hybrids, and 83.33–86.67% in non-BT hybrids. These results indicate strong efficacy of BT and especially BG II hybrids at the early crop stage.

**Effect of 120-day-old crop:** At 120 DAS, larval survival increased across all hybrids. On leaves: survival ranged from 20–23.33% in BG II, 43.33–66.67% in BT and 83.33–86.67% in non-BT hybrids. On squares: 20–30% survival in BG II, 46.67–76.67% in BT and 83.33–86.67% in non-BT hybrids. A similar trend was observed in bolls. This indicates a decline in toxin efficacy with advancing crop age.

**Effect of 140-day-old crop:** At 140 DAS, larval survival further increased. On leaves: maximum survival (93.33%) was recorded on RCH 134 non-BT. BIO 6488 BG II showed the lowest survival (46.67%), though it was statistically at par with BIO 6488 BT (53.33%) and RCH 134 BG II (60%). On squares: lowest survival was recorded on BIO 6488 BG II (46.67%). Highest survival (96.67%) was observed on RCH 134 non-BT. On bolls: minimum survival (46.67%) occurred on BIO 6488 BG II and maximum survival (96.67%) occurred on RCH 134 non-BT. These findings clearly demonstrate a progressive decline in efficacy of BT toxins with crop maturity.

These findings are consistent with earlier reports indicating higher mortality of *H. armigera* on dual-gene BT hybrids compared to single-gene hybrids, greater susceptibility of first instar larvae compared to later instars and reduced Cry toxin expression and efficacy in older cotton plants. In earlier studies (Basavaraja, H., *et al*., 2008 [3]; Onkaramurthy, S.G., *et al.,* 2008 [10]) higher mortality of *H. armigera was* reported on RCH-2 BG II as compared to RCH-2 BT. Murugan, M., *et al.,* (2003) [9] also noticed that the first instar larvae suffered high mortality (92.8 %) as compared to third instar larvae (51.7 %) when fed with BT leaves, squares and bolls. Reduction in mortality of early instar *H. armigera* larvae with advancing age of transgenic cotton hybrids in leaves and fruiting bodies was observed in several locations in India (Mahon, R.J., *et al*., 2009 [8] ; MahaLakshmi, M.S., *et al*., 2014 [7] ; Likhitha, P., *et al*., 2018 [6] ), hence, in conformity with the present findings.

## Conclusion

It may be inferred from the present studies that among 3 variants of two hybrids (RCH 134 and BIO 6488), BGII was more effective against larvae than BT and non-BT events. The expression of toxin was more in leaves as compared to squares and bolls at all stages of crop age, although efficacy in fruiting bodies was more in 80 days old crop as compared to 140 days old crop. The response was intermediate at 120 days old crop. The differences in efficacy of squares and bolls of similar crop age were non-significant.

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