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Impact of transgenic *Bt* cotton hybrids expressing Cry1Ac and Cry2Ab endotoxins and non-*Bt* on biological attributes of American bollworm, *Helicoverpa armigera* (Hubner)

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Abstract
The American bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae), is a destructive pest of cotton causing substantial yield losses. The deployment of Bt cotton expressing Cry1Ac toxin provided effective control; however, its limited spectrum and declining efficacy with crop age necessitated the development of dual-gene Bollgard II (Cry1Ac + Cry2Ab) hybrids. The present investigation evaluated the age-specific efficacy and persistence of Bt, non-Bt, and BG II cotton hybrids against third instar larvae of *H. armigera* under laboratory conditions at CCS HAU, Hisar, India. Leaves, squares, and bolls were collected at 85, 125, and 145 days after sowing (DAS) from six hybrids and assessed for their impact on larval survival, duration, weight, pupation, pupal period, and adult emergence. Results revealed that BG II hybrids recorded significantly lower larval survival, larval weight, pupal weight, and adult emergence compared to *Bt* and non-*Bt* hybrids across all crop stages. At 85 DAS, complete larval mortality was observed on BG II plant parts, whereas survival increased at later stages (125 and 145 DAS), indicating a decline in toxin efficacy with crop maturity. Larval and pupal durations were prolonged on BG II hybrids, reflecting growth inhibition effects. In contrast, non-Bt hybrids consistently supported higher survival, faster development, and greater adult emergence. The findings demonstrate the superior efficacy of BG II hybrids over single-gene *Bt* cotton, although a gradual reduction in effectiveness with advancing crop age was evident. These results highlight the importance of monitoring toxin persistence and adopting integrated pest management strategies for sustainable control of H. armigera

Keywords: *Helicoverpa armigera,* third instar, cotton *Bt* hybrids, biology

Introduction
The American bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae), is a major pest of cotton in India (Murugan, M., *et al*.,2003) [12]. Transgenic Bt cotton expressing the Cry1Ac gene was introduced in India in 2002 for effective management of *H. armigera* (Arms, N.J., *et al*.,1992; MahaLakshmi, M.S., *et al.*, 2014; Likhitha, P., *et al.,*2018) [2,10,9]. However, Cry1Ac toxin alone in *Bt* cotton could not effectively control other lepidopteran pests such as *Spodoptera litura* and *Pectinophora gossypiella.* 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). These dual-gene cultivars have shown more consistent and superior activity against bollworms and defoliators compared to single-gene cultivars (Bheemanna, M, *et al.,* 2008) [4]. However, gradual reduction in insecticidal efficacy in leaves, squares and bolls of transgenic *Bt* cotton hybrids against *H. armigera* was reported with advancing crop age and larval stage (MahaLakshmi, M.S., *et al.*, 2014; Likhitha, P., *et al.,* 2018) [10, 9].The age-specific efficacy of *Bt*, non-*Bt*, and BG II cotton hybrids in leaves, squares, and bolls was evaluated against third instar larvae of *H. armigera* and the impact is reported hereunder.

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. 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 were reared individually on dissected young green bolls of non-*Bt* hybrid till moulting to third instar which were subsequently used in present 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 85, 125, and 145 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 to rear third instar larvae of *H armigera* on 3 crop stages. In the experiment, each replication consisted of 10 larvae and the food was replenished 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. The larval period was worked out by adding the larval period of first two instars to the subsequent durations (3rd instar onwards). Biological attributes like larval weight (mg), larval survival (%), pupal weight (mg), pupal period (days) and adult emergence (%) were recorded and analysed.

Results and Discussion

The effect of *Bt* cotton leaves, squares and bolls of plants of different age (85, 125 and 145 day old) of 6 cotton hybrids on biological parameters of third instar larvae of *H. armigera* has been reported in the present studies.

Impact on larval survival: The survival of third instar larvae was significantly different among the BG II, *Bt* and non *Bt* hybrids at 85, 125 and 145 days after sowing (Table 1). At 85 days after sowing the minimum survival (0.00%) was observed on leaves, squares and bolls of BG II hybrids, whereas the survival on *Bt* and non *Bt* hybrids ranged from 33.33 to 40.00 percent and 73.33 to 76.67 percent respectively. At 125 days after sowing the larval survival increased as compared to survival observed at 85 days after sowing. The survival on leaves, squares and bolls ranged from 23.33 to 26.67percent on BIO 6488 BG II and 36.67 percent on RCH 134 BG II, while on both the *Bt* hybrids it ranged from 46.67 to 56.67 percent. At 145 days after sowing the survival on leaves squares and bolls of BG II hybrids ranged from 40.00 to 43.33 percent and 53.33 to 56.67 percent on *Bt* hybrids, while on non *Bt* survival ranged from 76.67 to 86.67 percent. Similar observations were noticed by several workers (Fitt, G.P. *et al.*, 1994; Greenplate, J.T., *et al*.,1999; Basavaraja, H., *et al.,* 2008) [5, 6, 3] who reported that efficacy of *Bt* starts declining as the crop matures. Similar results were reported by Akin, D.S., *et al.* (2011) [1] and Mahon, R.J., *et al*., (2009) [11] who found BG II hybrids enhanced the mortality as compared to *Bt* hybrids which are in conformity with the present findings.

Impact on larval duration: Duration of third instar larvae of *H. armigera* also differed significantly among the BG II, *Bt* and non *Bt* hybrids at 85, 125 and 145 days after sowing (Table 2). At 85 days after sowing the larval duration on BG II hybrids ranged from 13.03 to 14.27 days whereas larval period was 23.13 to 23.83 days on *Bt* hybrids, while on non *Bt* hybrids it ranged from 15.52 to 16.23 days. At 125 days after sowing the larval period was observed maximum (25.88 to 27.23 days) on BG II hybrids and 15.37 to 17.56 days on *Bt* and non *Bt* hybrids. At 145 days after sowing larval period shows similar trend. Similar results were found by Mahon, R.J., *et al*., (2009) [11] who found larvae grew slowly on BG II hybrids as compared to other conventional hybrids. Similarly, Fitt, G.P. *et al.*, (1994) [5] and Basavaraja, H., *et al.,* (2008) [3] reported that larvae took more time to complete larval period in transgenic cotton as compared to non-transgenic cotton. These results strongly support the present findings.

Impact on larval weight: The larval weight of *H. armigera* was found significantly lower (43.66 to 51.30 mg) on leaves, squares and bolls of BG II hybrids as compared to *Bt* and non *Bt* hybrids which was ranged from 278.67 to 320.06 mg at 85 days after sowing (Table 3). At 125 and 145 days after sowing the larval weight found almost similar (324.80 to 366.59 mg) on *Bt* and non *Bt* hybrids and on BG II hybrids it ranged from 197.17 to 221.97 mg. The larval weight found higher on bolls of cotton hybrids followed by squares and leaves. Murugan, M., *et al*., (2003) [12] and Gujar, G.T., *et al*., (2000) [7] reported that feeding *Bt* cotton to *Helicoverpa armigera* (Hubner) reduced weight of each instar of larvae.

Impact on pupation: None of the larvae pupated on leaves, squares and bolls of both the BG II hybrids at 85 days after sowing, while on *Bt* and non *Bt* the pupal weight ranged from 181.72 to 185.60 mg and 215.33 to 219.23 mg respectively (Table 4). At 125 days after sowing the pupal weight was lower (159.43 to 169.18 mg) on BG II hybrids as compared to 205.93 to 220.20 mg on *Bt* and non *Bt* hybrids. Similar trend was followed at 145 days after sowing. Similarly, Li, Y.X., *et al.* (2007) [8] reported that pupae developed from larvae of *Trichoplusia ni* (Hubner) fed on *Bt* cotton (Cry1Ac and Cry2Ab) were smaller in size compared with non-*Bt*. Mahon, R.J., *et al*., (2009) [11] also reported that survivors of *H. armigera* larvae on Bollgard II genotypes produced smaller pupae. Zhang, G.F., *et al.* (2005) [14] and Basavaraja, H., *et al.,* (2008) [3] also strongly favoured the present investigations.

Impact on pupal duration: Pupal duration was significantly different among the BG II, *Bt* and non *Bt* hybrids at 85 days after sowing (Table 5). At 85 days after sowing none of the larvae pupated on BG II hybrids and the pupal period on *Bt* hybrids ranged from 16.23 to 17.00 days followed by non *Bt* hybrids 12.67 to 12.90 days. At 125 days after sowing pupal period on of BG II hybrids was higher (17.33 to 18.44 days) than *Bt* hybrids (13.56 to 14.93 days) and non *Bt* hybrids (122.66 to 13.04 days). Pupal period at 145 days after sowing was higher (16.33 to 18.37 days) on BG II hybrids than on non *Bt* hybrids (12.59 to 12.88 days) and *Bt* hybrids (13.21 to 14.88 days). Akin, D.S., *et al.,* (2011) [1] observed BG II cotton caused growth inhibition of *Helicoverpa zea* (Boddie), *Heliothis virescens* (F.), *Spodoptera frugiperda* (J.E. Smith), *S. exigua* (Hubner), *Pseudoplusia includens* (Walker), and *Estigmene acrea* (Drury) than Cry1Ac only and non-Bt cotton. Similar results were found by Mahon, R.J., *et al*., (2009) [11]; Zhang, G.F., *et al.* (2005) [14] and Basavaraja, H., *et al.,* (2008) [3] which are in conformity with present findings.

Impact on adult emergence: The adult emergence on leaves, squares and bolls was found significantly different among BG II, *Bt* and non *Bt* hybrids (Table 6). At 85 days after sowing none of the larvae pupated on BG II hybrids so the adult emergence on BG II hybrids was nil. On *Bt* and non *Bt* hybrids adult emergence ranged from 26.67 to 33.33 percent and 63.33 to 70.00 percent respectively. At 125 days after sowing the adult emergence was lower on BG II hybrids (20.00 to 23.33 %) as compared to *Bt* hybrids (43.33 to 50.00 %) and non *Bt* hybrids (66.67 to 76.67 %). The adult emergence at 145 days after sowing also found lower 33.33 to 36.67 percent on BG II hybrids than *Bt* and non *Bt* hybrids 46.67 to 53.33 percent and 70.00 to 80.00 percent respectively. Similarly, Mahon, R.J., *et al*., (2009) [11] also reported that the adults showed reduced longevity and fecundity on Bollgard II. Similarly, Li, Y.X., *et al.,* (2007) [8] reported that *Trichoplusia ni* (Hubner) fed on *Bt* cotton (Cry1Ac and Cry2Ab) only 17.9 per cent larvae could become adults. Basavaraja, H., *et al.,* (2008) [3] also found results in accordance with the present results achieved.

Conclusion

The present study clearly establishes that Bollgard II cotton hybrids expressing dual Bt toxins (Cry1Ac + Cry2Ab) provide significantly enhanced suppression of *Helicoverpa armigera* compared to single-gene *Bt* and non-*Bt* hybrids.

BG II hybrids effectively reduced larval survival, larval and pupal weight, and adult emergence while prolonging developmental periods, thereby exerting strong antibiosis effects on third instar larvae. However, a progressive decline in efficacy was observed with increasing crop age, as reflected by higher larval survival and adult emergence at 125 and 145 DAS compared to 85 DAS. These findings underscore that although BG II hybrids remain more potent than first-generation *Bt* cotton, toxin expression and insecticidal activity diminish as the crop matures. Therefore, reliance solely on transgenic technology may not ensure season-long protection. Integration of *Bt* cotton with appropriate resistance management and complementary pest control strategies is essential to sustain its long-term effectiveness against *H. armigera*.

Table 1: Survival of third 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		
	85 DAS*	125 DAS	145 DAS	85 DAS	125 DAS	145 DAS	85 DAS	125 DAS	145 DAS
RCH 134 BG II	0.00 (1.81)	36.67(37.21)	40.00(39.13)	0.00 (1.81)	36.67(37.21)	43.33(41.14)	0.00(1.81)	36.67(37.21)	43.33(41.14)
RCH 134 *Bt*	36.67 (37.21)	53.33(46.90)	56.67(48.83)	36.67(37.21)	50.00(44.98)	53.33(46.90)	40.00(39.13)	46.67(43.06)	53.33(46.90)
RCH 134 non *Bt*	76.67 (61.19)	80.00(63.90)	83.33(66.61)	73.33 (58.98)	76.67(61.69)	76.67(61.69)	76.67(61.89)	86.67(68.83)	86.67(68.83)
BIO 6488 BG II	0.00 (1.81)	26.67(30.98)	43.33(41.14)	0.00 (1.81)	23.33(28.769)	40.00(39.21)	0.00(1.81)	23.33(28.77)	43.33(41.14)
BIO 6488 *Bt*	33.33 (35.20)	56.67(48.91)	56.67(49.91)	33.33 (35.20)	56.67(48.83)	56.67(48.83)	33.33(35.20)	53.33(46.98)	56.67(48.83)
BIO 6488 non *Bt*	73.33(58.98)	83.33(66.12)	83.33(66.12)	73.33 (58.98)	86.67(68.83)	86.67(68.83)	73.33(58.98)	83.33(66.12)	83.33(66.12)
SEm(±)	(1.72)	(3.28)	(3.58)	(1.72)	(3.03)	(2.67)	(3.08)	(2.65)	(2.22)
CD(p=0.05)	(5.37)	(10.23)	(11.16)	(5.37)	(9.46)	(8.31)	(9.61)	(8.25)	(6.90)

*DAS – days after sowing.
Figures in parentheses are angular transformed values.

Table 2: Larval duration (from third instar and onwards) of *Helicoverpa armigera* on leaves and fruiting bodies of different *Bt* cotton hybrids

Hybrid	Larval duration (days)								
	Leaves			Squares			Bolls		
	85 DAS*	125 DAS	145 DAS	85 DAS	125 DAS	145 DAS	85 DAS	125 DAS	145 DAS
RCH 134 BG II	14.26**	25.88	21.85	14.17**	26.33	26.33	14.27**	26.82	25.15
RCH 134 *Bt*	23.85	16.55	15.21	23.15	16.74	16.74	23.83	16.45	16.45
RCH 134 non *Bt*	15.92	15.77	15.44	15.59	16.07	15.07	15.87	15.55	14.88
BIO 6488 BG II	13.33**	27.23	25.23	14.00**	26.65	25.31	13.03**	26.56	25.22
BIO 6488 *Bt*	23.13	17.50	16.17	23.33	17.56	16.90	22.87	17.55	16.88
BIO 6488 non *Bt*	16.20	15.96	15.30	15.52	15.37	15.04	16.23	15.90	15.23
SEm(±)	0.98	0.70	0.72	0.73	0.79	0.64	0.89	0.73	0.61
CD(p=0.05)	3.07	2.18	2.25	2.29	2.44	2.00	2.78	2.27	1.89

*DAS – days after sowing.
** Days for which larvae survived

Table 3: Larval weight of *Helicoverpa armigera* (released in third instar) fed on leaves and fruiting bodies of different *Bt* cotton hybrids

Hybrid	Larval weight (mg)								
	Leaves			Squares			Bolls		
	85 DAS*	125 DAS	145 DAS	85 DAS	125 DAS	145 DAS	85 DAS	125 DAS	145 DAS
RCH 134 BG II	48.90**	213.20	215.53	51.30**	213.87	219.20	46.08**	213.97	221.97
RCH 134 *Bt*	291.40	335.13	335.13	293.33	345.22	345.22	295.18	364.33	364.33
RCH 134 non *Bt*	317.50	335.40	340.07	317.00	366.19	364.85	320.06	365.93	366.59
BIO 6488 BG II	45.48**	197.17	207.17	43.66**	197.82	203.67	50.76**	197.33	206.87
BIO 6488 *Bt*	278.67	324.80	324.80	280.67	357.58	357.58	278.67	358.40	358.40
BIO 6488 non *Bt*	311.00	329.30	339.63	312.33	362.95	363.28	311.97	364.19	364.52
SEm(±)	4.78	4.62	6.59	4.87	8.57	8.52	4.02	3.73	3.71
CD(p=0.05)	14.91	14.39	20.54	15.16	26.69	26.54	12.53	11.63	11.57

*DAS – days after sowing.
**Larval weight upto 13 days

Table 4: *Pupal weight of *Helicovepa armigera* on leaves and fruiting bodies of different *Bt* cotton hybrids

Hybrid	Pupal weight (mg)								
	Leaves			Squares			Bolls		
	85 DAS**	125 DAS	145 DAS	85 DAS	125 DAS	145 DAS	85 DAS	125 DAS	145 DAS
RCH 134 BG II	0.00	169.18	167.52	0.00	167.12	167.52	0.00	165.82	168.48
RCH 134 *Bt*	183.46	209.41	209.41	181.72	208.12	209.41	182.43	207.88	207.88

RCH 134 non *Bt*	218.33	219.10	219.10	219.23	220.20	219.10	217.97	218.50	222.10
BIO 6488 BG II	0.00	159.87	161.20	0.00	160.16	161.20	0.00	159.43	163.10
BIO 6488 *Bt*	184.67	206.67	209.67	185.54	206.55	209.67	185.60	205.93	211.27
BIO 6488 non *Bt*	215.33	215.50	218.17	216.42	216.15	218.17	215.63	215.66	219.67
SEm(±)	1.47	3.87	4.01	1.55	3.51	3.15	1.46	3.29	2.40
CD(p=0.05)	4.57	12.05	12.50	4.84	10.96	9.82	4.54	10.24	7.49

* Pupae produced from the released third instar larvae.
** DAS – days after sowing

Table 5: *Pupal duration of *Helicoverpa armigera* on leaves and fruiting bodies of different *Bt* cotton hybrids

Hybrid	Pupal duration (days)								
	Leaves			Squares			Bolls		
	85 DAS**	125 DAS	145 DAS	85 DAS	125 DAS	145 DAS	85 DAS	125 DAS	145 DAS
RCH 134 BG II	0.00	17.33	16.33	0.00	17.50	17.17	0.00	17.66	17.66
RCH 134 *Bt*	16.38	13.88	13.21	17.00	13.98	13.31	16.53	13.56	13.23
RCH 134 non *Bt*	12.67	12.93	12.59	12.67	12.77	12.77	12.90	12.66	12.66
BIO 6488 BG II	0.00	18.44	17.11	0.00	18.22	18.22	0.00	18.37	18.37
BIO 6488 *Bt*	16.66	14.88	14.21	16.28	14.93	14.26	16.23	14.88	14.21
BIO 6488 non *Bt*	12.67	12.73	12.73	12.67	13.04	13.04	12.80	12.88	13.21
SEm(±)	0.43	0.39	0.67	0.53	0.39	0.58	0.50	0.49	0.52
CD(p=0.05)	1.33	1.24	2.09	1.65	1.24	1.82	1.57	1.53	1.61

*Pupae produced from the third instar larvae
**DAS – days after sowing.

Table 6: Effect of feeding of third instar *Helicoverpa armigera* larvae on leaves and fruiting bodies of different *Bt* cotton hybrids on adult emergence

Hybrid	Adult emergence (%)								
	Leaves			Squares			Bolls		
	85 DAS*	125 DAS	145 DAS	85 DAS	125 DAS	145 DAS	85 DAS	125 DAS	145 DAS
RCH 134 BG II	0.00(1.81)	23.33(28.78)	33.33(35.20)	0.00(1.81)	23.33(28.77)	36.67(37.21)	0.00(1.81)	20.00(26.55)	33.33(35.20)
RCH 134 *Bt*	30.00(32.99)	43.33(41.14)	53.33(46.90)	26.67(30.98)	50.00(44.98)	50.00(44.98)	33.33(35.20)	46.67(43.06)	53.33(46.90)
RCH 134 non *Bt*	70.00(56.97)	66.67(54.76)	70.00(56.97)	70.00(56.96)	70.00(56.98)	73.33(58.98)	66.67(54.76)	70.00(56.97)	73.33(58.98)
BIO 6488 BG II	0.00(1.81)	23.33(28.77)	36.67(37.21)	0.00(1.81)	20.00(26.55)	33.33(35.20)	0.00(1.81)	20.00(26.55)	33.33(35.20)
BIO 6488 *Bt*	26.67(30.98)	46.67(4298)	50.00(44.98)	30.00(32.99)	50.00(44.98)	53.33(46.90)	26.67(30.98)	46.67(43.06)	46.67(43.06)
BIO 6488 non *Bt*	66.67(54.76)	73.33(58.98)	76.67(61.19)	66.67(54.76)	76.67(61.19)	80.00(63.41)	63.33(52.75)	76.67(61.19)	76.67(61.19)
SEm(±)	(2.44)	(2.86)	(2.62)	(2.44)	(2.75)	(1.67)	(1.68)	(2.07)	(2.05)
CD(p=0.05)	(7.59)	(8.91)	(8.15)	(7.57)	(8.56)	(5.19)	(5.24)	(6.44)	(6.39)

*DAS – days after sowing.
Figures in parentheses are angular transformed values.

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