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Expression Variability and Comparative Susceptibility of Dual-Toxin Public Sector *Bt* Cotton Hybrids against *Earias vittella* (Fab.) and *Pectinophora gossypiella* (Saunders) in India

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***Abstract*:** In the paradigm of commercially lagging public sector cotton seed corporations, the adoption of novel public sector *Bt* (*Bacillus thuringiensis*) cotton hybrids containing stacked *Bt* genes by Indian growers has not reached the expected scale. To counter the perceived monopolistic dominance of the private sector and address intensified challenges such as plant protection, food and fiber security, and environmental degradation due to indiscriminate application of synthetic pesticides, such research is crucial, particularly for small and marginal rainfed cotton farmers in the country. This study aimed to assess the relative expression of Cry proteins and the biocidal activity of public sector *Bt* cotton hybrids (NHH-44 BG II, PKV Hy-2 BG II, PDKV-JKAL-116 BG II, G. COT-10 Hy. BG II, G. COT-08 Hy. BG II and NHH-44 non-*Bt* as control) against *E. vittella* (Fab.) and *P. gossypiella* (Saunders). Laboratory investigations indicate that the cotton hybrids expressing dual-toxin, Cry1Ac and Cry2Ab genes significantly improved season long expression and contributed to the cessation of bollworm survival. All hybrids demonstrated varying levels of toxins in plant structures at different growth stages of herbivory, resulting in significantly lower survival for early instars compared to later. However, the surviving later instar bollworms flaunted adverse effect on growth and developmental parameters.

***Keywords*:** *Bacillus thuringiensis,* Bollworms, Mortality, Public sector, Transgenic cotton

Cotton (*Gossypium* sp.) is cultivated in more than 80 countries with tropical to temperate agro-climatic conditions (Pathak et al 2023). India hoist its *numero uno* position in cotton cultivation with an area of 125.84 lakh ha under *Bt* cotton and 8.50 lakh ha under non-*Bt*, with production of 360 lakh bales. However, the average productivity of cotton remains low (486 kg per ha) compared to global productivity (CCI 2020). Cotton cultivation alone contributes to the livelihood of 9.9 million farmers (AICCIP 2022) and sustains employment of large labor force in the country, as an industrial commodity. Several factors impede the overall production of cotton, comprised of varied biotic and abiotic stresses (Shuli et al 2018 and Hussain et al 2023). Among biotic factors, globally, this crop shelters over 1326 insect and mite species throughout the growing season (Razaq et al 2013). In India, 162 species have been reported, of which 24 have attained pest status (Arora et al 2011). The lepidopteran pests, spotted bollworm (*Earias vittella* Fabricius) and pink bollworm (*Pectinophora gossypiella* Saunders), are the most vicious constituent of the cotton bollworm complex in India, altering the fitness of cotton produced for textile industries and export (Badiger et al 2011). *E. vittella* is an early to mid-season pest that damages tender growing shoots, bores into stems, and later feeds on squares and bolls (Ahmed et al 2012). Moreover, *P. gossypiella* exhibits most serious threat to cotton production in India, as a borer at boll developing stage of cotton, contributing to considerable reduction of total yield (Likhitha et al 2023). The cotton hybrids exhibit plasticity in getting infested by these insects, where this variation is much pronounced among different *Bt* cotton hybrids (Adamczyk and Gore 2004, Kranthi et al 2006, Dhillon and Sharma 2009, Arshad and Suhail 2010 and Thakre and Bhamare 2023a). Over the years, studies have illustrated the performance of various *Bt* hybrids in different Indian agro-ecological zones, demonstrating broad-spectrum inhibition of bollworm pests on transgenic hybrids (Manjunatha et al 2004, Likhitha and Bhamare 2018 and Thakre and Bhamare 2023a).

In India, these Bollgard cultivars were first approved for commercial cultivation (by the Genetic Engineering Appraisal Committee (GEAC), Ministry of Environment, Forest and Climate Change, Govt. of India) on 26th April, 2002 (Likhitha and Bhamare 2018). Since then, this technology has provided highly effective control against cotton lepidopteran pest complex (Dong and Li 2007 and Knight et al 2016). Over the years, numerous hybrids have been developed simultaneously by private and public sector corporations. Though, the private sector only exhibited monopolistic domination on the cotton seed market, ensuing public seed corporations cast lagging. Keeping this in view, we investigated the effect of season-long expression of Cry toxins in certain public sector *Bt* cotton hybrids on survival of *E. vittella* and *P. gossypiella* during economically critical stages of cotton crop (squares and bolls), grown under rainfed agro-ecological conditions.

MATERIAL AND METHODS

The survival and developmental studies were conducted at the Post Graduate Laboratory, Department of Agricultural Entomology, Latur (Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani), Maharashtra, during *Kharif* 2019-20. The GEAC approved five public sector *Bt* cotton hybrids (NHH-44 BG II, PKV Hy-2 BG II, PDKV-JKAL-116 BG II, G. COT-10 Hy. BG II and G. COT-08 Hy. BG II), as well as a non-*Bt* hybrid (NHH-44 non-*Bt*) as control, were cultivated by following all the recommended operations excluding plant protection practices.

Cry protein expression profiling: The quantification of Cry toxins was performed at Plant Biotechnology Laboratory of Vilasrao Deshmukh College of Agril. Biotechnology, Latur. The expression of Cry toxins was investigated in leaves (60 days old crop), squares (90 days old crop) and bolls (120 and 150 days old crop) of public sector *Bt* cotton hybrids at different crop ages by sandwich enzyme-linked immunosorbent assays (ELISA) using QL 96 quantiplate ELISA kits. The concentration of Cry1Ac proteins was measured using Cry1Ab/Cry1Ac kit (AP 003 QT V50), and Cry2Ab using Cry2A kit (AP 005 QT BC V50) of ENVIROLOGIX 500 Riverside Industrial Parkway Portland, ME, USA, supplied by Amar Immunodiagnostic, Hyderabad, following the manufacturer's instructions (protocol: ENVIROLOGIX 2017).

Bioassay of *E. vittella* and *P. gossypiella*: Larval populations of field-collected *E. vittella* and *P. gossypiella* were reared on a natural diet (non-*Bt* cotton) until pupation to develop initial cultures. After emergence, moths were released into standard oviposition cage (27±1ºC and 70% RH) with cotton swabs dipped in honey solution (10%). The fresh leaves of the host plants were placed as oviposition substrate which was examined for the presence of egg masses and replaced daily with fresh ones. Larvae hatched from eggs were transferred into plastic vials and fed on natural diet to obtain different larval instar (I-V instars for *E. vittella* and I-IV instars for *P. gossypiella*), which were used for further investigations. Plant structures of different public sector *Bt* cotton hybrids were collected from the field in labeled plastic bags, from 90-110 and 120-140 days old crop for *E. vittella* (squares and bolls), and 150-170 days old crop for *P. gossypiella* (bolls). Collected samples were cleaned, placed individually in plastic vials, and then the laboratory-reared larval instars (I-V instars of *E. vittella* and I–IV instars of *P. gossypiella*) were released in each vial. The periodical replacement of *Bt* cotton plant parts with the fresh ones (those on which larvae fed) was ensured till pupation or mortality.

Data analysis: A standard curve was prepared using optical density (OD) values (absorbance at 405 nm) of each calibrator and corresponding concentrations of Cry1Ac and Cry2Ab. The concentration of each sample was determined by its absorbance in an ELISA reader (OD value), and results were multiplied by all dilution factors incurred during extraction, presented as microgram (µg) toxin per gram of fresh tissue. Laboratory bioassays were conducted for each separate instar of bollworms and replicated thrice using ten larvae per replication. The data on per cent mortality, pupation and adult emergence was recorded separately for each instar by feeding them on plant structures of public sector *Bt* hybrids. The weights of surviving instars were registered at 24, 48, and 72 hr of exposure, as well as of pupae soon after pupation. Using the formulae given by Vennila et al (2006), the growth and survival indices were calculated. Concentrations of Cry1Ac and Cry2Ab in different plant structures and the results of bioassays were analyzed in completely randomized design. The data was statistically analyzed by using OPSTAT statistical package by Sheron OP, HAU, Hisar.

RESULTS AND DISCUSSION

Expression of Cry1Ac toxin: The Cry1Ac toxin was estimated using Quantiplate ELISA kit from leaves (60 days old crop), squares (90 days old crop) and bolls (120 and 150 days old crop) of different *Bt* cotton hybrids (Fig. 1). In leaves of 60 days old crop, the highest Cry1Ac expression was observed in PDKV-JKAL-116 BG II (4.71 µg per g fresh tissue), followed by PKV Hy-2 BG II, G. COT-10 Hy. BG II, NHH-44 BG II, and lowest in G. COT-08 Hy. BG II (2.59 µg per g fresh tissue). In squares of 90 days old crop, maximum concentration was recorded in PDKV-JKAL-116 BG II (3.73 µg per g fresh tissue), followed by G. COT-08 Hy. BG II, G. COT-10 Hy. BG II, PKV Hy-2 BG II, and minimum in NHH-44 BG II (2.58 µg per g fresh tissue). Higher Cry1Ac protein expression in bolls of 120 days old crop was found in NHH-44 BG II (1.47 µg per g fresh tissue), followed by G. COT-08 Hy. BG II, G. COT-10 Hy. BG II, PKV Hy-2 BG II and PDKV-JKAL-116 BG II (0.67 µg per g fresh tissue). Whereas, bolls of 150 days old crop registered highest toxin expression with NHH-44 BG II (0.33 µg per g fresh tissue), followed by PKV Hy-2 BG II, PDKV-JKAL-116 BG II, G. COT-10 Hy. BG II, and lowest in G. COT-08 Hy.BG II (0.028 µg per g fresh tissue). The expression profiling indicated a progressive decline in

Cry1Ac concentration from early to later stages of transgenic crop.

Expression of Cry2Ab toxin: The season long expressions of Cry2Ab protein was estimated from leaves (60 days old crop), squares (90 days old crop), and bolls (120 and 150 days old crop) of different public sector *Bt* cotton hybrids (Fig. 2). Among all hybrids, the highest Cry2Ab concentration in leaves was observed in PKV Hy-2 BG II (13.15 µg per g fresh tissue), followed by NHH-44 BG II, PDKV-JKAL-116 BG II, G. COT-10 Hy. BG II and G. COT-08 Hy. BG II (8.18 µg per g fresh tissue). In squares, the maximum expression was found in PKV Hy-2 BG II (15.96 µg per g fresh tissue), followed by G. COT-08 Hy. BG II, G. COT-10 Hy. BG II, PDKV-JKAL-116 BG II, and minimum in NHH-44 BG II (14.32 µg per g fresh tissue). The Cry2Ab protein concentration in bolls of 120 days old crop was highest in NHH-44 BG II (6.40 µg per g fresh tissue), followed by PDKV-JKAL-116 BG II, G. COT-08 Hy. BG II, PKV Hy-2 BG II, and G. COT-10 Hy.BG II (4.25 µg per g fresh tissue). In the bolls of 150 days old crop, higher expression was registered in NHH-44 BG II (4.82 µg per g fresh tissue), followed by G. COT-08 Hy. BG II, G. COT-10 Hy. BG II, PKV Hy-2 BG II, and minimal in PDKV-JKAL-116 BG II (1.77 µg per g fresh tissue). All transgenic cotton hybrids exhibited significant variation in Cry2Ab toxin concentration among plant structures at different stages of crop growth. The highest toxin concentration was detected in early stages of crop growth (leaves and squares), showing progressive decline in later stages (bolls).

Bioassay of *E. vittella* on squares: All treatments showed affirmative results when *E. vittella* fed on different *Bt* cotton hybrids compared to non-*Bt* cotton. Laboratory bioassays indicated significant larval mortality (20.00 to 100.00%) of early instars (I, II and III instar), fed on squares and bolls of *Bt* cotton hybrids at pre-determined intervals than the later instars. However, all larvae from IV and V instars survived till pupation. The first instars fed on squares of PKV Hy-2 BG II and G. COT-08 Hy. BG II, and on bolls of NHH-44 BG II and PDKV-JKAL-116 BG II showed cent per cent mortality. Whereas, the minimum mortality of first instars was observed on squares of NHH-44 BG II (80.00%), and on bolls of G. COT-10 Hy. BG II (76.67%), still higher than the mortality rate of later instars (Table 1). The data followed more or less similar trend for the different growth and developmental parameters of *E. vittella*, when fed on squares and bolls. The larval weights of I-V instars survived beyond 24, 48 and 72 hr after exposure, as well as per cent pupation, pupal weight and per cent adult emergence showed continuing reduction in survival and growth rates (Table 2, 4, 5, 6). The minimum growth index (0.95 and 0.91) and survival index (0.44 and 0.48) values were recorded on squares and bolls of PKV Hy-2 BG II and NHH-44 BG II when fed on transgenic public sector hybrids, respectively (Fig. 3, 4).

Bioassay of *P. gossypiella* on bolls: Larval instars of *P. gossypiella* showed significant survival due to relatively low-toxic reaction against *Bt* cotton hybrids on bolls of 150 days old crop. None of the transgenic hybrids revealed mortality of last larval instar. However, significant mortality rates of 100.00, 86.67 and 60.00 per cent were recorded in I, II and III instars, respectively, when larvae fed on bolls of NHH-44 BG II. Thereafter, mortality rate progressively decreased in G. COT-08 Hy. BG II (100.00, 70.00 and 56.67%), followed by G. COT-10 Hy. BG II, PKV Hy-2 BG II, and minimum in PDKV-JKAL-116 BG II (76.67, 53.33 and 30.00%) (Table 1). Likewise, for other survival and developmental parameters more or less parallel results were evidenced (Table 3, 4, 5, 6). Minimal growth and survival indices of the insect larvae were observed in NHH-44 BG II (0.67 and 0.35), followed by G. COT-08 Hy. BG II, G. COT-10 Hy. BG II, PKV Hy-2 BG II and maximum in PDKV-JKAL-116 BG II (1.33 and 0.55) (Fig. 5).

The dual-toxin expression profiling showed progressive decline in concentrations from early to later stages of crop growth, with higher levels observed at 60 and 90 days (leaves and squares) and lower levels at 120 and 150 days (bolls) of cotton crop. This pattern aligns with previous reports indicating higher expression of Cry proteins in early vegetative and mid-reproductive stages, decreasing in later reproductive stages (Cheema et al 2015 and Zaman et al 2015). Overall, Cry2Ab was found in higher concentration than Cry1Ac, owing to its potential importance in controlling bollworm herbivory at different crop stages (Liu et al 2017 and Manjunatha et al 2017).

The Cry toxins associated with *Bt* hybrids reflected significant mortality and conflicting effect on survival of the bollworms. Among all instars, later instars of bollworms showed higher larval survival than early instars when fed on plant structures of *Bt* cotton. The highest larval mortality was found in newly hatched first instar (neonates) of bollworms, consistent with the findings of Shera and Arora (2016a) and Likhitha and Bhamare (2018). Significant reduction in mortality rate was reported from early crop stages to the later, leading to an incomplete inhibition in bollworm survival, as documented by Kranthi et al (2009), Siebert et al (2009), Hallad et al (2014) and Likhitha et al (2023). *E. vittella* fed on squares showed minimal growth and survival indices when compared with bolls. Therefore, from the illustrated data, these transgenic cotton hybrids may provide control against these pest population in initial crop stages by conferring mortality, especially in early larval instars (Ahmed et al 2012, Hallad et al 2014 and Shera et al 2015). Further, the data showed relatively greater non-toxic response by *P.*

Table 1. Mortality (%) of *E. vittella* on squares and bolls and *P. gossypiella* on bolls of public sector *Bt* cotton hybrids

Treatments	Squares (90-110 days old crop)					Bolls (120-140 days old crop)					Bolls (150-170 days old crop)			
	I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar
NHH-44 BG II	80.00 (63.43)*	60.00 (50.77)	20.00 (26.57)	0.00 (0.00)	0.00 (0.00)	100.00 (90.00)	90.00 (71.57)	50.00 (45.00)	0.00 (0.00)	0.00 (0.00)	100.00 (90.00)	86.67 (68.59)	60.00 (50.77)	0.00 (0.00)
PKV Hy-2 BG II	100.00 (90.00)	86.67 (68.59)	40.00 (39.23)	0.00 (0.00)	0.00 (0.00)	80.00 (63.43)	70.00 (56.79)	30.00 (33.21)	0.00 (0.00)	0.00 (0.00)	83.33 (65.90)	60.00 (50.77)	36.67 (37.27)	0.00 (0.00)
PDKV-JKAL-116 BG II	86.67 (68.59)	66.67 (54.74)	26.67 (31.09)	0.00 (0.00)	0.00 (0.00)	100.00 (90.00)	83.33 (65.90)	43.33 (41.17)	0.00 (0.00)	0.00 (0.00)	76.67 (61.12)	53.33 (46.91)	30.00 (33.21)	0.00 (0.00)
G. COT-10 Hy. BG II	90.00 (71.57)	70.00 (56.79)	30.00 (33.21)	0.00 (0.00)	0.00 (0.00)	76.67 (61.12)	56.67 (48.83)	20.00 (26.57)	0.00 (0.00)	0.00 (0.00)	90.00 (71.57)	63.33 (52.73)	40.00 (39.23)	0.00 (0.00)
G. COT-08 Hy. BG-II	100.00 (90.00)	80.00 (63.43)	40.00 (39.23)	0.00 (0.00)	0.00 (0.00)	90.00 (71.57)	76.67 (61.12)	30.00 (33.21)	0.00 (0.00)	0.00 (0.00)	100.00 (90.00)	70.00 (56.79)	56.67 (48.83)	0.00 (0.00)
NHH-44 (Non-*Bt*)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
SE (m) ±	0.13	0.19	0.13	-	-	0.13	0.23	0.13	-	-	0.19	0.23	0.19	-
CD (p≤0.05)	0.41	0.58	0.41	-	-	0.41	0.71	0.41	-	-	0.58	0.71	0.58	-
CV %	3.09	5.50	9.02	-	-	3.16	6.50	8.15	-	-	4.44	7.34	8.95	-

* Figures in parenthesis angular transformed values

Table 2. Effect on larval weight of *E. vittella* on squares and bolls fed on public sector *Bt* cotton hybrids

Treatments	Mean larval weight (mg/larva)														
	I instar			II instar			III instar			IV instar			V instar		
	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
Larval weight of *E. vittella* fed on squares (90-110 days old crop)															
NHH-44 BG II	3.7	3.84	4.53	4.89	5.12	9.21	24.92	46.41	73.98	65.64	75.64	96.61	96.22	98.38	111.7
PKV Hy-2 BG II	2.91	3.03	3.26	3.89	4.06	7.41	19.69	33.77	55.74	46.47	62.02	68.26	66.34	74.21	94.66
PDKV-JKAL-116 BG II	3.64	3.78	4.09	4.73	4.98	8.50	23.26	44.47	72.36	60.5	71.77	89.42	83.25	94.66	104.06
G. COT-10 Hy. BG II	3.59	3.75	3.87	4.6	4.93	8.15	21.52	43.38	68.56	58.61	68.61	83.51	79.21	88.59	102.03
G. COT-08 Hy. BG-II	3.55	3.62	3.78	4.56	4.82	7.94	20.43	41.63	66.33	53.92	67.44	81.54	74.08	78.27	98.38
NHH-44 (Non-*Bt*)	4.49	4.67	4.73	5.57	6.40	13.5	31.22	57.53	88.17	78.59	94.81	134.8	124.13	157.56	194.83
SE (m) ±	0.01	0.01	0.03	0.04	0.09	0.23	0.52	1.44	0.42	0.54	1.25	0.71	0.89	1.45	1.46
CD (p=0.05)	0.05	0.04	0.09	0.13	0.27	0.72	1.59	4.37	1.28	1.65	3.79	2.17	2.71	4.40	4.43
CV %	0.79	0.69	1.34	1.59	3.10	4.52	3.86	5.60	1.03	1.55	2.95	1.34	1.78	2.55	2.15
Initial weight	2.76	-	-	3.45	-	-	16.22	-	-	52.53	-	-	58.75	-	-
Larval weight of *E. vittella* fed on bolls (120-140 days old crop)															
NHH-44 BG II	2.94	3.10	3.30	3.91	5.92	8.68	21.42	34.47	56.14	47.82	62.93	68.69	68.09	75.75	95.45
PKV Hy-2 BG II	3.72	3.89	4.21	5.13	6.34	9.34	26.76	47.68	74.67	62.30	73.47	90.57	97.36	95.43	106.76
PDKV-JKAL-116 BG II	3.56	3.78	3.80	4.68	6.17	8.82	22.17	41.53	67.37	55.32	66.92	82.51	77.35	78.54	99.95
G. COT-10 Hy. BG II	3.82	3.93	4.54	5.16	6.82	9.58	26.8	49.75	76.25	67.51	76.06	97.63	99.35	100.79	116.2
G. COT-08 Hy. BG-II	3.63	3.83	4.00	4.87	6.25	9.05	25.39	45.50	69.11	58.98	70.07	84.10	81.86	89.09	103.46
NHH-44 (Non-*Bt*)	4.46	4.56	4.77	5.59	7.32	14.28	34.0	57.75	90.47	80.34	97.45	142.27	126.01	155.54	203.71
SE (m) ±	0.02	0.08	0.08	0.05	0.09	0.35	0.70	0.64	0.70	0.61	0.80	1.56	0.71	0.92	1.94
CD (p=0.05)	0.07	0.26	0.26	0.17	0.28	1.08	2.14	1.95	2.12	1.85	2.42	4.76	2.17	2.80	5.89
CV %	1.11	3.99	3.72	0.34	2.47	6.21	4.69	2.41	1.67	1.71	1.86	2.88	1.35	1.61	2.78
Initial weight	2.83	-	-	3.50	-	-	18.94	-	-	54.55	-	-	71.15	-	-

gossypiella larval instars when fed with bolls of public sector *Bt* cotton hybrids. These results are in conformity with the findings of Soujanya et al (2010) and Naik et al (2014), who also reported progressive decline in the survival and development of the pest on late phenological stages of *Bt* cotton. Meanwhile, surviving later larval instars of both bollworms showed waning effects on growth and developmental parameters, such as reduction in larval weights, per cent pupation with lower pupal weight, and reduced adult emergence. These results of growth inhibition and stunting are supported by the findings of Fabrick et al (2015), Likhitha and Bhamare (2018) and Thakre and Bhamare (2023a, 2023b). The growth and survival indices showed similar trend in results, as the duration and amount of endotoxin consumption confers reduced survival and growth rate (Shera and Arora 2016a).

In addition, previous findings (Olsen and Daly 2000, Cheema et al 2015, Manjunatha et al 2017, Khan et al 2018 and Likhitha et al 2023) suggest that different intrinsic and extrinsic attribute to variation in Cry toxin expression. This would also presuppose that the *Bt* toxin detection by larvae may result in avoidance and apparent feeding preference of the pest, resulting into death by starvation of the bollworms (Shera and Arora 2016b). Therefore, sustainable Cry toxin expression among *Bt* hybrids is essential for their efficacy against bollworms. Therefore, screening and adoption of

Table 3. Effect on larval weight of *P. gossypiella* on bolls fed on public sector *Bt* cotton hybrids

Treatments	Mean larval weight of *P. gossypiella* fed on bolls (150-170 days old crop)											
	I instar			II instar			III instar			IV instar		
	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
NHH-44 BG II	1.46	1.57	2.87	10.44	12.94	13.66	25.39	27.57	28.57	38.49	43.36	48.47
PKV Hy-2 BG II	1.77	3.08	5.41	15.01	16.18	18.08	35.61	36.56	37.82	48.66	53.56	58.36
PDKV-JKAL-116 BG II	1.82	3.23	5.79	16.05	17.75	18.37	36.16	38.00	39.39	50.34	55.32	58.79
G. COT-10 Hy. BG II	1.65	2.96	4.63	14.27	15.72	16.41	32.47	33.46	34.56	47.42	50.57	56.98
G. COT-08 Hy. BG-II	1.57	2.87	4.25	12.18	14.60	14.99	27.19	29.38	31.47	41.39	48.47	53.56
NHH-44 (Non-*Bt*)	2.00	3.29	6.42	19.38	27.38	28.05	38.42	41.3	46.76	59.31	74.63	85.37
SE (m) ±	0.04	0.05	0.06	0.42	0.57	0.40	0.80	0.71	1.33	0.79	1.01	0.55
CD (p=0.05)	0.13	0.16	0.19	1.28	1.73	1.22	2.43	2.16	4.04	2.40	3.07	1.69
CV %	4.51	3.36	2.25	5.04	5.69	3.82	4.26	3.59	6.34	2.88	3.23	1.60
Initial weight	1.11	-	-	9.06	-	-	26.88	-	-	37.75	-	-

Table 4. Pupation (%) of *E. vittella* on squares and bolls and *P. gossypiella* on bolls of public sector *Bt* cotton hybrids

Treatments	Squares (90-110 days old crop)					Bolls (120-140 days old crop)					Bolls (150-170 days old crop)			
	I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar
NHH-44 BG II	20.00 (26.57)*	40.00 (39.23)	80.00 (63.43)	100.00 (90.00)	100.00 (90.00)	00.00 (00.00)	10.00 (18.43)	50.00 (45.00)	100.00 (90.00)	100.00 (90.00)	00.00 (00.00)	13.33 (21.41)	40.00 (39.23)	100.00 (90.00)
PKV Hy-2 BG II	00.00 (00.00)	13.33 (21.41)	60.00 (50.77)	100.00 (90.00)	100.00 (90.00)	20.00 (26.57)	30.00 (33.21)	70.00 (56.79)	100.00 (90.00)	100.00 (90.00)	16.67 (24.10)	40.00 (39.23)	63.33 (52.73)	100.00 (90.00)
PDKV-KAL-116 BG II	13.33 (21.41)	33.33 (35.26)	73.33 (58.91)	100.00 (90.00)	100.00 (90.00)	00.00 (00.00)	16.67 (24.10)	56.67 (48.83)	100.00 (90.00)	100.00 (90.00)	23.33 (28.88)	46.67 (43.09)	70.00 (56.79)	100.00 (90.00)
G. COT-10 Hy. BG II	10.00 (18.43)	30.00 (33.21)	70.00 (56.79)	100.00 (90.00)	100.00 (90.00)	23.33 (28.88)	43.33 (41.17)	80.00. (63.43)	100.00 (90.00)	100.00 (90.00)	10.00 (18.43)	36.67 (37.37)	60.00 (50.77)	100.00 (90.00)
G. COT-08 Hy. BG-II	00.00 (00.00)	20.00 (26.57)	60.00 (50.77)	100.00 (90.00)	100.00 (90.00)	10.00 (18.43)	23.33 (28.88)	70.00 (56.79)	100.00 (90.00)	100.00 (90.00)	00.00 (00.00)	30.00 (33.21)	43.33 (41.17)	100.00 (90.00)
NHH-44 (Non-*Bt*)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
SE (m) ±	0.13	0.19	0.13	-	-	0.13	0.23	0.13	-	-	0.19	0.23	0.19	-
CD (p≤0.05)	0.41	0.58	0.41	-	-	0.41	0.71	0.41	-	-	0.58	0.71	0.58	-
CV %	9.86	8.45	3.19	-	-	9.22	10.96	3.31	-	-	13.33	9.18	5.31	-

* Figures in parenthesis angular transformed values

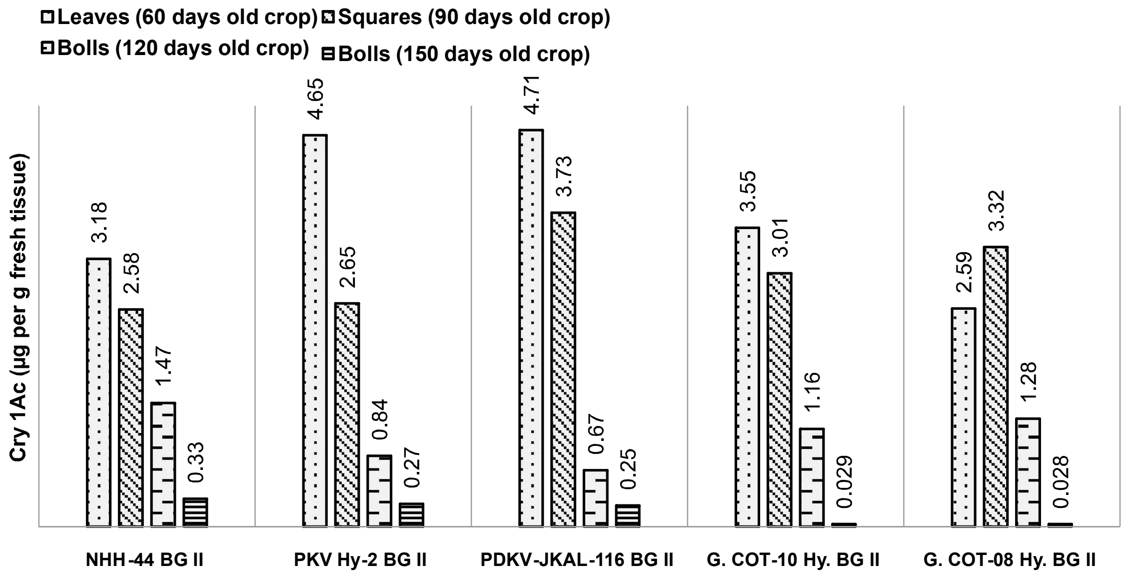

Fig. 1. Expression of Cry 1Ac toxin in different public sector *Bt* cotton hybrids

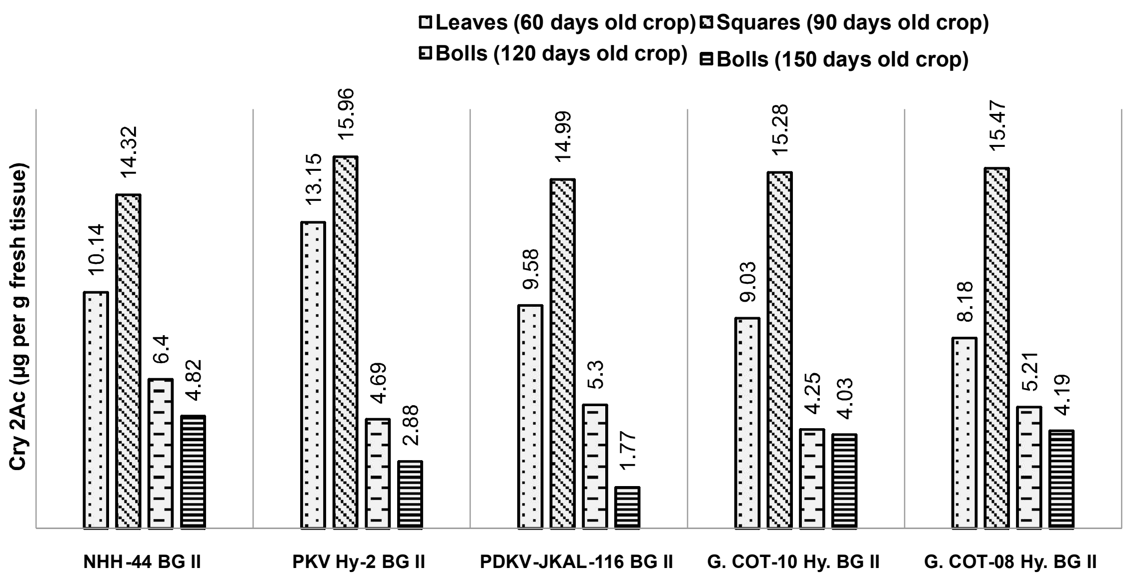

Fig. 2. Expression of Cry 2A toxin in different public sector *Bt* cotton hybrids

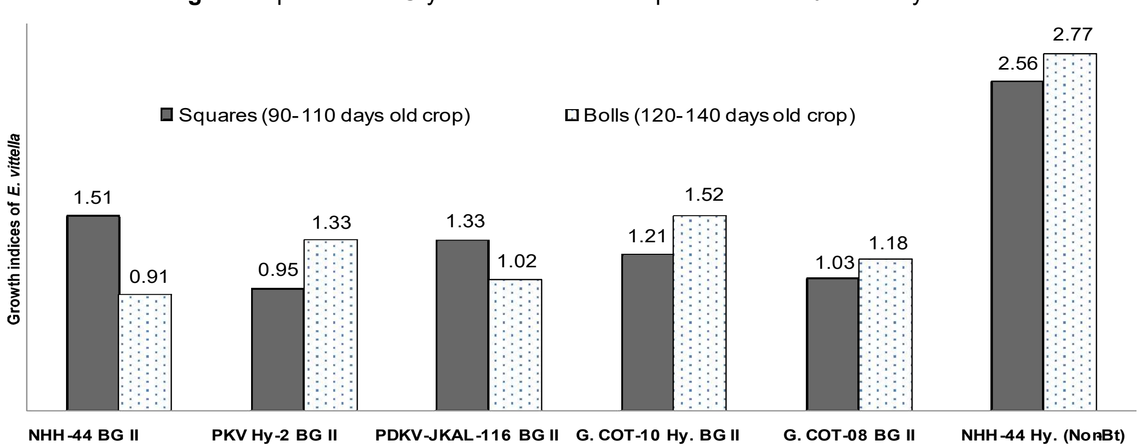

Fig. 3. Growth indices of *E. vittella* reared on different public sector *Bt* cotton hybrids

significant Cry toxin-expressing hybrids play a crucial role in enhancing efficacy against bollworms in specific agro-ecological condition.

This study can be used as baseline notation to detect changes in susceptibility in field populations. Expectedly, in recent past, pink bollworm has developed non-toxic activity against Cry1Ac, first noticed in 2008, and subsequently to Cry2Ab in 2014-15 (Pathak et al 2023). Several factors had

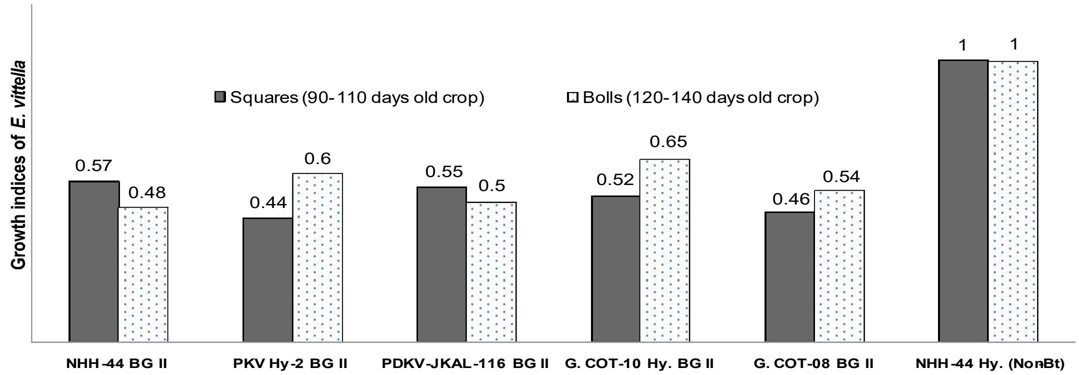

Fig. 4. Survival indices of *E. vittella* reared on different public sector *Bt* cotton hybrids

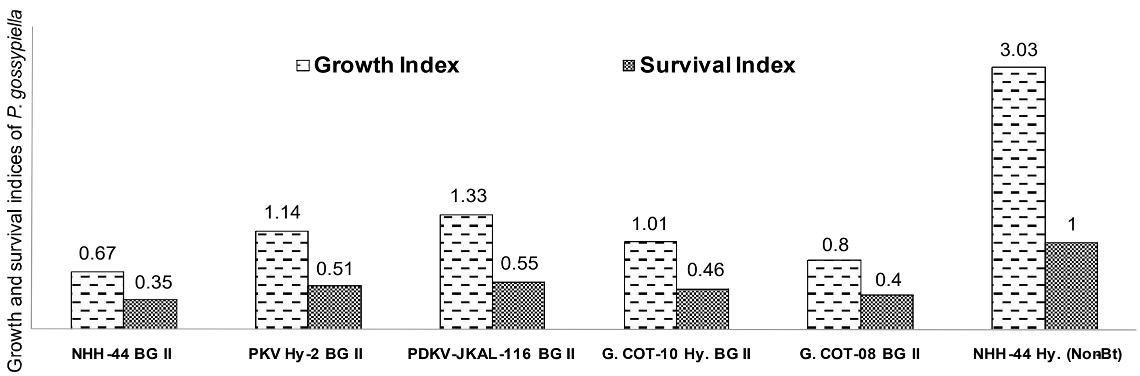

Fig. 5. Growth and survival indices of *P. gossypiella* reared on bolls (150-170 days old crop) sector *Bt* cotton hybrids

Table 6. Adult emergence (%) of *E. vittella* on squares and bolls and *P. gossypiella* on bolls of public sector *Bt* cotton hybrids

Treatments	Squares (90-110 days old crop)					Bolls (120-140 days old crop)					Bolls (150-170 days old crop)			
	I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar
NHH-44 BG II	20.00 (26.57)*	30.00 (33.21)	43.33 (41.17)	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)	10.00 (18.43)	30.00 (33.21)	100.00 (90.00)	100.00 (90.00)	00.00 (00.00)	10.00 (18.43)	33.33 (35.26)	100.00 (90.00)
PKV Hy-2 BG II	0.00 (0.00)	0.00 (0.00)	20.00 (26.57)	100.00 (90.00)	100.00 (90.00)	13.33 (21.41)	30.00 (33.21)	56.67 (48.83)	100.00 (90.00)	100.00 (90.00)	16.67 (24.10)	33.33 (35.26)	56.67 (48.83)	100.00 (90.00)
PDKV-JKAL-116 BG II	13.33 (21.41)	20.00 (26.57)	40.00 (39.23)	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)	10.00 (18.43)	40.00 (39.23)	100.00 (90.00)	100.00 (90.00)	20.00 (26.57)	36.67 (37.27)	63.33 (52.73)	100.00 (90.00)
G. COT-10 Hy. BG II	10.00 (18.43)	20.00 (26.57)	30.00 (33.21)	100.00 (90.00)	100.00 (90.00)	20.00 (26.57)	30.00 (33.21)	70.00 (56.79)	100.00 (90.00)	100.00 (90.00)	10.00 (18.43)	30.00 (33.21)	46.67 (43.09)	100.00 (90.00)
G. COT-08 Hy. BG-II	0.00 (0.00)	6.67 (14.97)	26.67 (31.09)	100.00 (90.00)	100.00 (90.00)	10.00 (18.43)	16.67 (24.10)	43.33 (41.17)	100.00 (90.00)	100.00 (90.00)	00.00 (00.00)	20.00 (26.57)	40.00 (39.23)	100.00 (90.00)
NHH-44 (Non-*Bt*)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
SE (m) ±	0.13	0.13	0.19	-	-	0.13	0.13	0.19	-	-	0.13	0.19	0.36	-
CD (p≤0.05)	0.41	0.41	0.58	-	-	0.41	0.41	0.58	-	-	0.41	0.58	1.09	-
CV %	9.86	8.00	7.69	-	-	9.86	7.19	5.88	-	-	9.64	8.69	11.00	-

* Figures in parenthesis angular transformed values

Table 5. Pupal weight of *E. vittella* on squares and bolls and *P. gossypiella* on bolls of public sector *Bt* cotton hybrids

Treatments	Squares (90-110 days old crop)					Bolls (120-140 days old crop)					Bolls (150-170 days old crop)			
	I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar
NHH-44 BG II	210.91	249.63	253.1	263.16	273.16	00.00	219.46	222.96	231.36	242.53	00.00	145.57	176.36	182.8
PKV Hy-2 BG II	00.00	210.83	226.83	227.23	240.9	210.46	232.73	251.96	258.36	268.73	156.27	167.75	192.94	200.74
PDKV-JKAL-116 BG II	206.7	247.26	251	259.85	270.5	00.00	222.4	242	240.06	250.4	157.35	174.93	198.35	204.25
G. COT-10 Hy. BG II	203.2	229.54	248.56	252.56	265.1	219.83	248.67	260.5	265.43	277.83	145.28	162.44	191.87	198.68
G. COT-08 Hy. BG-II	00.00	216.73	233.46	247.96	254.76	208.52	228.46	248.33	249.38	262.73	00.00	159.46	187.01	191.10
NHH-44 (Non-*Bt*)	319.1	329.5	342.3	340.73	347.43	329.13	334.96	341.3	349.13	362.23	257.57	275.36	296.01	300.74
SE (m) ±	0.99	1.32	1.54	1.17	1.37	0.74	0.83	0.55	1.14	1.34	1.79	0.79	1.04	0.93
CD (p≤0.05)	3.02	4.03	4.67	3.55	4.16	2.25	2.54	1.69	3.47	4.08	5.44	2.39	3.15	2.83
CV %	1.10	0.93	1.03	0.76	0.86	0.80	0.58	0.37	0.74	0.84	2.60	0.75	0.87	0.76

flared up the slowly progressing evolution of bollworm resistance in India (Kranthi et al 1999 and Mahesh and Muralimohan 2023). Apart from that, all public sector *Bt* cotton hybrids in the present investigations showed affirmative results against bollworms survival. In general, critical studies in this regard are made with private sector *Bt* hybrids in the past (Gujar et al 2011, Hallad et al 2014, Shera et al 2015, Naik et al 2016, Shera and Arora 2016a, Likhitha and Bhamare 2018 and Likhitha et al 2023), and seldom with public sector hybrids (Dohare and Tank 2014). This might have caused to set a paradigm of commercially lagging public sector cotton seed corporations. The result of the present investigation illustrates the significant efficacy of public sector hybrids against these bollworms. Furthermore, to sustain food and fiber security and avoid environmental degradation through injudicious synthetic pesticides application, authors suggest investigating more potential combination of stacked *Bt*- toxin genes, having altered membrane insertion or pore formation mechanism. As previous reports summarized by Bravo et al (2007), in case of resistant mosquitocidal-Cry proteins, stacking of Cyt proteins results in synergizing or overcoming the resistance. Thus, for the sustainability of this technology, improved alterations in cultivars and adopting refugee planting are recommended.

CONCLUSIONS

All transgenic cotton hybrids exhibited significant variation in the concentration of Cry toxins at predetermined intervals of crop stages. Superior results were registered with PKV Hy-2 BG II when bollworm were fed on squares, and with NHH-44 BG II when fed on bolls. This underscores the potential of these public sector hybrids with dual-Cry toxins as invaluable assets for the efficient management of bollworms, aiming to enhance crop resilience in conventional cotton cultivation.

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