



## SURVIVAL AND DEVELOPMENT OF *SPODOPTERA FRUGIPERDA* (J E SMITH) ON PUBLIC SECTOR *Bt* COTTON HYBRIDS IN INDIA

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### ABSTRACT

The fall army worm, *Spodoptera frugiperda* (J E Smith) (Noctuidae; Lepidoptera) is a recently introduced polyphagous pest, poses a potential threat to cotton cultivation in India. Therefore, laboratory assays were conducted on different public sector *Bt* cotton hybrids to study the survival and development of the pest. The results confirmed significantly ( $p=0.05$ ) higher mortality of early larval instars, than the later. The surviving larvae showed adverse effects on the growth and developmental parameters along with low growth and survival indices. *S. frugiperda* reared on leaves (60-80 days old crop) and squares (90-110 days old crop) showed low growth and survival indices values, compared to those reared on young bolls (120-140 days old crop) and mature bolls (150-170 days old crop). Among the different public sector *Bt* cotton hybrids, significantly superior results were exhibited by the PKV Hy-2 BG II when larvae fed on leaves and squares, and the NHH-44 BG II when fed on young and mature bolls.

**Key words:** *Bacillus thuringiensis*, cotton bollworm, public sector, potential pest, invasive pest, mortality, pupation, adult emergence, larval weight, pupal weight, survival indices, growth indices

The fall armyworm, *Spodoptera frugiperda* (J E Smith), is a destructive migratory pest of many crops in the Western Hemisphere (Adamczyk and Gore, 2004). Recently, this pest has been reported from Asian countries and in India, it was first encountered in Karnataka on the maize crop in the year 2018 (Sharanbasappa et al., 2018). The *S. frugiperda* is known to cause considerable damage to the economically important cultivated grasses and cash crops (maize, rice, sorghum, sugarcane, cotton, cabbage, tomato, potato, etc.), with the host range of about 353 larval host plant species belonging to 76 families (Montezano et al., 2018). This pest has been reported to cause serious damage in both conventional cotton and transgenic, *Bt* (*Bacillus thuringiensis* Berliner) cotton cultivation (Adamczyk and Gore, 2004).

The commercial release of transgenic *Bt* cotton hybrids expressing Crystal (*Cry*) toxins has revolutionized cotton production in many countries worldwide, resulting in significant reduction of pesticide use (Lu et al., 2011). The first *Bt* cotton hybrid (Bollgard, Monsanto, St. Louis, MO) was introduced in United States (US) in 1996. In India, it was first approved for commercial cultivation (by the Genetic Engineering Approval Committee (GEAC), Ministry of Environment, Govt. of India) on 26th April 2002 (Likhitha and Bhamare, 2018). Since then, this technology has been found highly effective

against certain lepidopteran pests, mainly against the tobacco budworm, *Heliothis virescens* Fabricius and the pink bollworm, *Pectinophora gossypiella* Saunders (Adamczyk and Gore, 2004; Siebert et al., 2008; Hardke et al., 2015). Subsequently Bollgard II (Monsanto, St. Louis, MO) comprising dual protein gene was introduced in 2002 in the US and 2006 in India, and has been widely commercialized around the world. Many reports have again confirmed the significant reduction in pest survival (often noticed in single protein *Bt* cotton hybrids) by Bollgard II hybrids (Stewart et al., 2000, 2001; Siebert et al., 2008; Greenberg et al., 2010; Likhitha and Bhamare, 2018). Though, several growers and researchers have witnessed the incidence of surviving lepidopteran pest populations on such transgenic genotypes. The  $\delta$ -endotoxin in *Bt* cotton hybrids has been found relatively ineffective for complete management of *S. frugiperda* in laboratory and field conditions (Adamczyk et al., 1998; Henneberry et al., 2001; Greenberg and Adamczyk, 2007; Siebert et al., 2008).

Moreover, many have reported significant variations among the different *Bt* cotton hybrids as well as a decline in *Cry* toxin expressions among the different plant parts with the advancement in crop age (Saini and Dhawan, 2013; Likhitha and Bhamare, 2018). The season-long *Cry* toxin profiling in these hybrids has shown a decrease in the *Cry* toxin expression with the

advancement of plant age and these expression tends to vary among the *Bt* cotton hybrids throughout the season. The plant structures such as leaves, flowers and squares express more  $\delta$ -endotoxin when compared with bolls (Fitt, 1998; Adamczyk et al., 2001, 2008). Despite the fact that, globally, *S. frugiperda* has been reported to incur significant losses in cotton cultivation, still exceptionally rare attempts have been made to evaluate the performance of such transgenic crops against this recently introduced polyphagous pest in Indian agro-ecosystems. Therefore, this research was aimed to evaluate the management efficacy and sublethal effects of different public sector *Bt* cotton hybrids on the survival and development of *S. frugiperda*, which has the potential to pose a serious threat to cotton cultivation in India.

#### MATERIALS AND METHODS

The present studies were conducted at the Post Graduate Laboratory, Department of Agricultural Entomology, College of Agriculture, Latur (Vasant Rao Naik Marathwada Krishi Vidyapeeth, Parbhani), Maharashtra during 2019-20. The different 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) were cultivated on Experimental Farm of Department of Agricultural Entomology by following all the recommended package of practices except the plant protection operations. The fully grown *S. frugiperda* larvae were collected from the surrounding farms and the initial culture was developed and maintained by feeding them on natural diet (leaves, squares, young bolls and mature bolls of non-*Bt* cotton) every day till pupation. After pupation, on the basis of the distance between the two, genital and anal apertures, the sexes were determined as in males it was less, while more in case of females (Luginbill, 1928). The emerged adults, on the same day were released (with proportion of 1:2 female to male) into a standard oviposition cage having cotton swab dipped into honey solution (10%) and a fresh leaves of the host plants were also placed as an oviposition substrate which was examined for the presence of egg masses and replaced with fresh ones after every 24 hours. Once hatched from eggs, the neonate larvae were transferred separately into plastic vials and fed on natural diet till pupation or to obtain different instar larvae which were used for further investigations.

The experiment was carried out in a completely

randomized design (CRD) and replicated thrice using ten larvae per replication. The different plant structures viz., leaves, squares, young bolls and mature bolls of different public sector *Bt* cotton hybrids were collected from the field at pre-determined intervals, and kept separately in labeled plastic bags. The collected samples were cleaned, placed individually in a plastic vial and then the laboratory reared different larval instars of *S. frugiperda* were released in each vial. The periodical replacement of *Bt* cotton plant parts with the fresh ones (the same on which larvae fed) was ensured till pupation. The data on larval mortality was recorded separately for I, II, III, IV, V and VI instars by feeding them on different plant structures of public sector *Bt* cotton hybrids at pre-determined intervals. The weight of the surviving larvae was registered at 24, 48 and 72 hr of exposure and the weight of pupae was also recorded from each treatment soon after pupation. Also, the data on % pupation and adult emergence were recorded from the surviving larvae and pupae of *S. frugiperda*. The growth and survival indices of different treatments were calculated using the formulae given by Vennila et al. (2006): Growth index = % pupation/ larval developmental period (days); Survival index = Number of moths emerged/ total number of neonates tested. The data pertaining to survival and developmental studies were statistically analyzed by the standard 'Analysis of variance' and the null hypothesis tested by 'F' test at 5% level of significance (Gomez and Gomez, 1984).

#### RESULTS AND DISCUSSION

The laboratory bioassay showed significantly ( $p=0.05$ ) maximum larval mortality (30.00 to 100.00%) of *S. frugiperda* early instars (I, II and III), fed on different plant structures (i.e., leaves, squares, young bolls and mature bolls) of public sector *Bt* cotton hybrids at pre-determined intervals, than the later instars. Whereas, the VI instar larvae fed on leaves and squares; the V and VI instar on young bolls and IV, V, and VI instar on mature bolls of *Bt* cotton hybrids exhibited zero per cent mortality (Table 1, 2). Larvae fed on both leaves and squares of PKV Hy-2 BG II showed maximum mortality (100.00%). However, the minimum mortality was observed on leaves (80.00%) of G. COT-08 Hy. BG II and on squares (86.67%) of NHH-44 BG II (Table 1), which still appeared comparatively higher than the larval mortality rate of later instars. Subsequently, significant mortality was recorded when I, II, III and IV instar larvae fed on young bolls (60.00 to 100.00%) and I, II, and III instar larvae on mature bolls (53.33 to 100.00%) of NHH-44 BG II. Whereas,

Table 1. Mortality (%) of *S. frugiperda* fed on leaves and squares of public sector *Bt* cotton hybrids

Treatments	Leaves											
	(60-80 days old crop)						(90-110 days old crop)					
	I	II	III	IV	V	VI	I	II	III	IV	V	VI
	instar	instar	instar	instar	instar	instar	instar	instar	instar	instar	instar	instar
NHH-44 BG II	100.00 (90.00)*	90.00 (71.57)	76.67 (61.12)	60.00 (50.77)	36.67 (37.27)	0.00 (0.00)	86.67 (68.59)	76.67 (61.12)	50.00 (45.00)	40.00 (39.23)	20.00 (26.57)	0.00 (0.00)
PKV Hy-2 BG II	100.00 (90.00)	100.00 (90.00)	83.33 (65.90)	66.67 (54.74)	40.00 (39.23)	0.00 (0.00)	100.00 (90.00)	100.00 (90.00)	86.67 (68.59)	70.00 (56.79)	56.67 (48.83)	0.00 (0.00)
PDKV-JKAL-116 BG II	90.00 (71.57)	83.33 (65.90)	70.00 (56.79)	43.33 (41.17)	30.00 (33.21)	0.00 (0.00)	90.00 (71.57)	80.00 (63.43)	56.67 (48.83)	43.33 (41.17)	30.00 (33.21)	0.00 (0.00)
G. COT-10 Hy. BG II	86.67 (68.59)	76.67 (61.12)	60.00 (50.77)	40.00 (39.23)	30.00 (33.21)	0.00 (0.00)	100.00 (90.00)	86.67 (68.59)	73.33 (58.91)	50.00 (45.00)	40.00 (39.23)	0.00 (0.00)
G. COT-08 Hy. BG-II	80.00 (63.43)	73.33 (58.91)	53.33 (46.91)	20.00 (26.57)	10.00 (18.43)	0.00 (0.00)	100.00 (90.00)	90.00 (71.57)	80.00 (63.43)	60.00 (50.77)	50.00 (45.00)	0.00 (0.00)
NHH-44 (Non- <i>Bt</i> )	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	0.23	0.23	0.19	0.13	-	0.13	0.19	0.23	0.13	0.13	-
CD (p=0.05)	0.41	0.71	0.71	0.58	0.41	-	0.41	0.58	0.71	0.41	0.41	-
CV %	3.09	5.78	7.13	8.69	9.64	-	2.96	4.61	7.06	5.37	7.19	-

\*Figures in parentheses angular transformed values

Table 2. Mortality (%) of *S. frugiperda* fed on young and mature bolls of public sector *Bt* cotton hybrids

Treatments	Young bolls											
	(120-140 days old crop)						(150-170 days old crop)					
	I	II	III	IV	V	VI	I	II	III	IV	V	VI
	instar	instar	instar	instar	instar	instar	instar	instar	instar	instar	instar	instar
NHH-44 BG II	100.00 (90.00)*	90.00 (71.57)	73.33 (58.91)	60.00 (50.77)	0.00 (0.00)	0.00 (0.00)	100.00 (90.00)	86.67 (68.59)	53.33 (46.91)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
PKV Hy-2 BG II	80.00 (63.43)	73.33 (58.91)	50.00 (45.00)	36.67 (37.27)	0.00 (0.00)	0.00 (0.00)	76.67 (61.12)	60.00 (50.77)	40.00 (39.23)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
PDKV-JKAL-116 BG II	90.00 (71.57)	86.67 (68.59)	63.33 (52.73)	50.00 (45.00)	0.00 (0.00)	0.00 (0.00)	70.00 (56.79)	53.33 (46.91)	30.00 (33.21)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
G. COT-10 Hy. BG II	73.33 (58.91)	63.33 (52.73)	40.00 (39.23)	16.67 (24.10)	0.00 (0.00)	0.00 (0.00)	83.33 (65.90)	73.33 (58.91)	46.67 (43.09)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
G. COT-08 Hy. BG-II	90.00 (71.57)	80.00 (63.43)	60.00 (50.77)	40.00 (39.23)	0.00 (0.00)	0.00 (0.00)	90.00 (71.57)	80.00 (63.43)	50.00 (45.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
NHH-44 (Non- <i>Bt</i> )	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	0.23	0.19	0.19	-	-	0.19	0.23	0.19	-	-	-
CD (p=0.05)	0.41	0.71	0.58	0.58	-	-	0.58	0.71	0.58	-	-	-
CV %	3.26	6.22	6.97	9.83	-	-	4.76	6.93	9.09	-	-	-

\*Figures in parentheses angular transformed values

the minimum % mortality was observed on young bolls (40.00 to 73.33%) of G. COT-10 Hy. BG II and on mature bolls (30.00 to 70.00%) of PDKV-JKAL-116 BG II (Table 2).

The data illustrate a similar trend among the different parameters of growth and development of *S. frugiperda*. The larval weights of I, II, III, IV, V and IV instars of *S. frugiperda* survived beyond 24, 48 and 72 hr after exposure were proved to exhibit progressive decline (Table 3). Also, the other parameters such as % pupation, pupal weight and % adult emergence also showed continuing reduction (Table 4-8). The minimum growth index (0.46 and 0.40) and survival index (0.32 and 0.23) values were recorded on leaves and squares of

PKV Hy-2 BG II. Likewise, *S. frugiperda* fed on young bolls and mature bolls of NHH-44 BG II exhibited minimum growth Index (0.60 and 0.78) and survival index (0.42 and 0.57) values (Fig. 1, 2). Among different public sector *Bt* cotton hybrids, the superior results were expressed by PKV Hy-2 BG II when *S. frugiperda* larval instars fed on leaves and squares at 60-80 and 90-110 days old crop, respectively and NHH-44 BG II when fed on young bolls and mature bolls at 120-140 and 150-170 days old crop, respectively.

Among all treatments, per cent larval mortality and other developmental parameters showed affirmative results when *S. frugiperda* fed on different *Bt* cotton hybrids compared to non-*Bt*. Results revealed that the

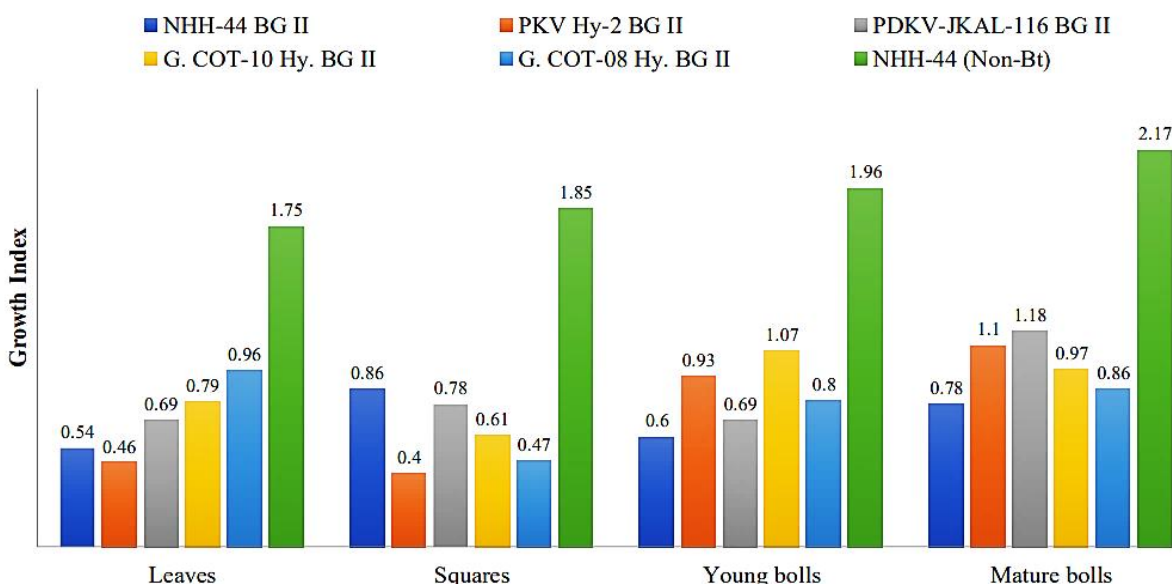


Fig. 1. Growth indices of *S. frugiperda* reared on different plant parts of public sector *Bt* cotton hybrids

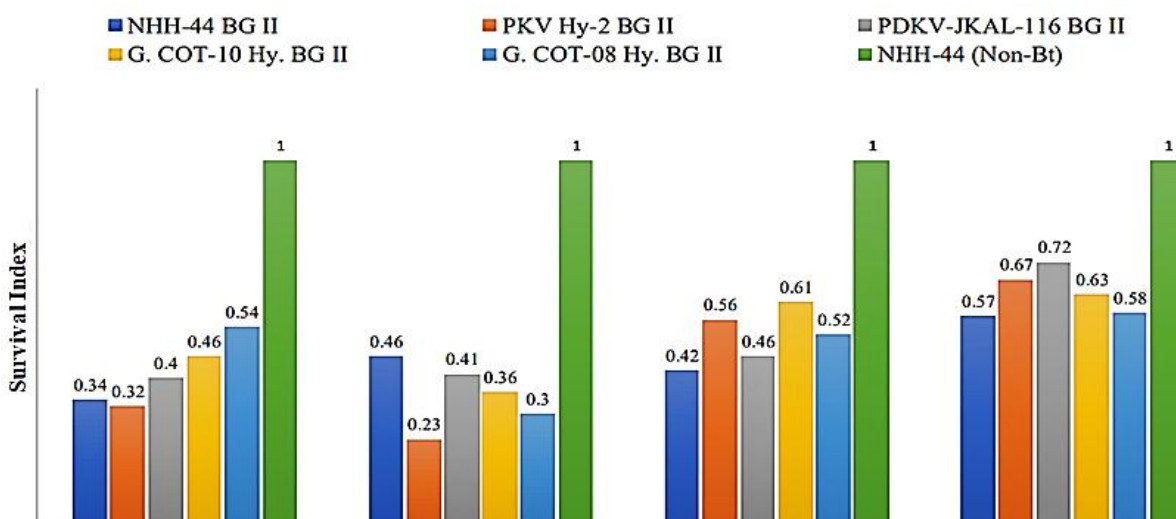


Fig. 2. Survival indices of *S. frugiperda* reared on different plant parts of public sector *Bt* cotton hybrids

Table 3. Effect on larval weight of *S. frugiperda* fed on different plant structures public sector *Bt* cotton hybrids

Treatments	Mean larval weight (mg per larva)																	
	I instar			II instar			III instar			IV instar			V instar			VI 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	24 hr	48 hr	72 hr
	on leaves (60-80 days old crop)																	
NHH-44 BG II	5.63	18.56	38.40	21.86	32.46	46.70	47.19	79.78	86.42	176.13	187.06	205.53	228.73	262.9	285.63	326.95	337.43	361.86
PKV Hy-2 BG II	5.21	16.42	35.23	20.3	26.15	41.15	43.05	75.39	81.59	165.93	176.5	197.86	222.23	252.3	277.1	300.81	312.2	329
PDKV-JKAL-116 BG II	6.81	19.23	39.41	25.77	38.74	50.35	51.22	83.6	98.54	185.9	193.03	210.36	269	290.53	305.46	335.06	352.7	376.86
G. COT-10 Hy. BG II	7.86	21.74	41.08	26.99	42.48	53.64	54.25	98.29	102.18	190.36	200.46	219.33	278.66	300.83	315.36	339.1	355.03	381.88
G. COT-08 Hy. BG-II	9.01	28.80	57.11	29.35	43.79	58.51	56.77	100.96	107.04	197.83	205.7	228.96	294.8	306.13	328.23	350.6	374.23	391.76
NHH-44 (Non- <i>Bt</i> )	10.84	35.83	66.61	32.19	48.81	88.98	63.96	103.43	143.9	206.13	233.83	294.26	318.69	322.2	349.56	365.6	378.23	404.7
SE (m) ±	0.19	0.38	0.78	0.34	0.62	1.04	0.83	0.76	1.32	1.01	0.64	1.33	1.51	1.74	1.57	0.77	1.00	1.33
CD (p=0.05)	0.59	1.16	2.37	1.06	1.89	3.16	2.54	2.32	4.02	3.07	1.96	4.05	4.59	5.27	4.77	2.34	3.03	4.05
CV %	4.47	2.83	2.93	2.32	2.79	3.19	0.45	1.46	2.22	0.15	0.56	1.02	0.97	1.04	0.88	0.39	0.49	0.61
Initial weight	3.00	-	-	20.25	-	-	40.10	-	-	151.09	-	-	251.15	-	-	317.75	-	-
	on squares (90-110 days old crop)																	
NHH-44 BG II	5.63	18.56	38.40	21.86	32.46	46.70	47.19	79.78	86.42	176.13	187.06	205.53	228.73	262.9	285.63	326.95	337.43	361.86
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G. COT-08 Hy. BG-II	9.01	28.80	57.11	29.35	43.79	58.51	56.77	100.96	107.04	197.83	205.7	228.96	294.8	306.13	328.23	350.6	374.23	391.76
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SE (m) ±	0.19	0.38	0.78	0.34	0.62	1.04	0.83	0.76	1.32	1.01	0.64	1.33	1.51	1.74	1.57	0.77	1.00	1.33
CD (p=0.05)	0.59	1.16	2.37	1.06	1.89	3.16	2.54	2.32	4.02	3.07	1.96	4.05	4.59	5.27	4.77	2.34	3.03	4.05
CV %	4.47	2.83	2.93	2.32	2.79	3.19	0.45	1.46	2.22	0.15	0.56	1.02	0.97	1.04	0.88	0.39	0.49	0.61
Initial weight	3.00	-	-	20.25	-	-	40.10	-	-	151.09	-	-	251.15	-	-	317.75	-	-

(contd.)

(contd. Table 3)

Treatments	Mean larval weight (mg per larva)																	
	I instar			II instar			III instar			IV instar			V instar			VI 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	24 hr	48 hr	72 hr
	on young bolls (120-140 days old crop)																	
NHH-44 BG II	5.63	18.56	38.40	21.86	32.46	46.70	47.19	79.78	86.42	176.13	187.06	205.53	228.73	262.9	285.63	326.95	337.43	361.86
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CD (p=0.05)	0.59	1.16	2.37	1.06	1.89	3.16	2.54	2.32	4.02	3.07	1.96	4.05	4.59	5.27	4.77	2.34	3.03	4.05
CV %	4.47	2.83	2.93	2.32	2.79	3.19	0.45	1.46	2.22	0.15	0.56	1.02	0.97	1.04	0.88	0.39	0.49	0.61
Initial weight	3.00	-	-	20.25	-	-	40.10	-	-	151.09	-	-	251.15	-	-	317.75	-	-
	on mature bolls (150-170 days old crop)																	
NHH-44 BG II	5.63	18.56	38.40	21.86	32.46	46.70	47.19	79.78	86.42	176.13	187.06	205.53	228.73	262.9	285.63	326.95	337.43	361.86
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G. COT-08 Hy. BG-II	9.01	28.80	57.11	29.35	43.79	58.51	56.77	100.96	107.04	197.83	205.7	228.96	294.8	306.13	328.23	350.6	374.23	391.76
NHH-44 (Non- Bt)	10.84	35.83	66.61	32.19	48.81	88.98	63.96	103.43	143.9	206.13	233.83	294.26	318.69	322.2	349.56	365.6	378.23	404.7
SE (m) ±	0.19	0.38	0.78	0.34	0.62	1.04	0.83	0.76	1.32	1.01	0.64	1.33	1.51	1.74	1.57	0.77	1.00	1.33
CD (p=0.05)	0.59	1.16	2.37	1.06	1.89	3.16	2.54	2.32	4.02	3.07	1.96	4.05	4.59	5.27	4.77	2.34	3.03	4.05
CV %	4.47	2.83	2.93	2.32	2.79	3.19	0.45	1.46	2.22	0.15	0.56	1.02	0.97	1.04	0.88	0.39	0.49	0.61
Initial weight	3.00	-	-	20.25	-	-	40.10	-	-	151.09	-	-	251.15	-	-	317.75	-	-

Table 4. Pupation (%) of *S. frugiperda* fed on leaves and squares of public sector *Bt* cotton hybrids

Treatments	Leaves (60-80 days old crop)						Squares (90-110 days old crop)					
	I	II	III	IV	V	VI	I	II	III	IV	V	VI
	instar	instar	instar	instar	instar	instar	instar	instar	instar	instar	instar	instar
NHH-44 BG II	00.00 (00.00)*	10.00 (18.43)	23.33 (28.88)	40.00 (39.23)	63.33 (52.73)	100.00 (90.00)	13.33 (21.41)	23.33 (28.88)	50.00 (45.00)	60.00 (50.77)	80.00 (63.43)	100.00 (90.00)
PKV Hy-2 BG II	00.00 (00.00)	00.00 (00.00)	16.67 (24.10)	33.33 (35.26)	60.00 (50.77)	100.00 (90.00)	00.00 (00.00)	00.00 (00.00)	13.33 (21.41)	30.00 (33.21)	43.33 (41.17)	100.00 (90.00)
PDKV-JKAL-116 BG II	10.00 (18.43)	16.67 (24.10)	30.00 (33.21)	56.67 (48.83)	70.00 (56.79)	100.00 (90.00)	10.00 (18.43)	20.00 (26.57)	43.33 (41.17)	56.67 (48.83)	70.00 (56.79)	100.00 (90.00)
G. COT-10 Hy. BG II	13.33 (21.41)	23.33 (28.88)	40.00 (39.23)	60.00 (50.77)	70.00 (56.79)	100.00 (90.00)	00.00 (00.00)	13.33 (21.41)	26.67 (31.09)	50.00 (45.00)	60.00 (50.77)	100.00 (90.00)
G. COT-08 Hy. BG-II	20.00 (26.57)	26.67 (31.09)	46.67 (43.09)	80.00 (63.43)	90.00 (71.57)	100.00 (90.00)	00.00 (00.00)	10.00 (18.43)	20.00 (26.57)	40.00 (39.23)	50.00 (45.00)	100.00 (90.00)
NHH-44 (Non- <i>Bt</i> )	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	0.23	0.23	0.19	0.13	-	0.13	0.19	0.23	0.13	0.13	-
CD (p=0.05)	0.41	0.71	0.71	0.58	0.41	-	0.41	0.58	0.71	0.41	0.41	-
CV %	9.86	13.86	9.54	5.40	3.12	-	11.46	12.00	9.66	4.20	3.50	-

\*Figures in parenthesis angular transformed values

Table 5. Pupation (%) of *S. frugiperda* fed on young and mature bolls of public sector *Bt* cotton hybrids

Treatments	Young bolls (120-140 days old crop)						Mature bolls (150-170 days old crop)					
	I	II	III	IV	V	VI	I	II	III	IV	V	VI
	instar	instar	instar	instar	instar	instar	instar	instar	instar	instar	instar	instar
NHH-44 BG II	00.00 (00.00)*	10.00 (18.43)	26.67 (31.09)	40.00 (39.23)	100.00 (90.00)	100.00 (90.00)	00.00 (00.00)	13.33 (21.41)	46.67 (43.09)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
PKV Hy-2 BG II	20.00 (26.57)	26.67 (31.09)	50.00 (45.00)	63.33 (52.73)	100.00 (90.00)	100.00 (90.00)	23.33 (28.88)	40.00 (39.23)	60.00 (50.77)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
PDKV-JKAL-116 BG II	10.00 (18.43)	13.33 (21.41)	36.67 (37.27)	50.00 (45.00)	100.00 (90.00)	100.00 (90.00)	30.00 (33.21)	46.67 (43.09)	70.00 (56.78)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
G. COT-10 Hy. BG II	26.67 (31.09)	36.67 (37.27)	60.00 (50.77)	83.33 (65.90)	100.00 (90.00)	100.00 (90.00)	16.67 (24.10)	26.67 (31.09)	53.33 (46.91)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
G. COT-08 Hy. BG-II	10.00 (18.43)	20.00 (26.57)	40.00 (39.23)	60.00 (50.77)	100.00 (90.00)	100.00 (90.00)	10.00 (18.43)	20.00 (26.57)	50.00 (45.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
NHH-44 (Non- <i>Bt</i> )	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	0.23	0.19	0.19	-	-	0.19	0.23	0.19	-	-	-
CD (p=0.05)	0.41	0.71	0.58	0.58	-	-	0.58	0.71	0.58	-	-	-
CV %	8.48	11.85	6.38	5.04	-	-	11.11	9.93	5.26	-	-	-

\*Figures in parenthesis angular transformed values

Table 6. Pupal weight of surviving *S. frugiperda* fed on leaves and squares of public sector *Bt* cotton hybrids

Treatments	Pupal weight (mg per pupa)											
	Leaves (60-80 days old crop)						Squares (90-110 days old crop)					
	I instar	II instar	III instar	IV instar	V instar	VI instar	I instar	II instar	III instar	IV instar	V instar	VI instar
NHH-44 BG II	00.00	138.13	165.7	171.83	230.76	257.73	134.2	144.43	167.46	173.5	201.2	203.43
PKV Hy-2 BG II	00.00	00.00	153.03	165.76	227.33	245.96	00.00	00.00	148.4	151.76	166.7	168.33
PKV-JKAL-116 BG II	134.76	140.76	170.56	174.76	235.46	261.45	122.66	141.73	162.16	165.06	193.9	198.53
G. COT-10 Hy. BG II	139.9	146.63	174.03	179.6	239.93	276.26	00.00	135.53	156.36	157.53	183.2	189.2
G. COT-08 Hy. BG-II	152.76	164.76	177.3	195.6	242.91	285.46	00.00	134.46	153.03	154.73	176	181.43
NHH-44 (Non- <i>Bt</i> )	312.86	318.8	323.03	328.86	332.5	344.36	304.03	307.83	310.66	316.75	320.46	322.53
SE (m) ±	0.81	1.50	1.02	1.06	1.99	1.15	0.73	0.64	0.81	0.99	1.17	0.55
CD (p=0.05)	2.46	4.57	3.10	3.22	6.05	3.49	2.23	1.95	2.48	3.00	3.57	1.69
CV %	1.14	1.72	0.91	0.90	1.37	0.71	1.36	0.77	0.77	0.92	0.98	0.46
	Young bolls (120-140 days old crop)						Mature bolls (150-170 days old crop)					
NHH-44 BG II	00.00	211.83	218.2	226.5	243.76	247.44	00.00	185.47	205.16	216.76	224.83	231.83
PKV Hy-2 BG II	201.86	234.53	242.0	252.3	285.83	291.9	207.33	217.6	245.73	267.16	277.6	288.53
PKV-JKAL-116 BG II	180.23	215.33	223.13	233.23	252.33	272.53	213.76	231.53	273.93	276.96	289	304.32
G. COT-10 Hy. BG II	209.76	243.9	255.5	261.73	288.9	303.3	195.28	202.44	234.6	245.4	256.56	262.96
G. COT-08 Hy. BG-II	186.83	224.56	237.93	241.86	262.2	281.03	186.8	189.09	215.26	224.16	228.5	232.8
NHH-44 (Non- <i>Bt</i> )	330.23	337.6	339.26	342.63	344.96	359.53	357.57	363.23	364.93	370.74	371.40	371.74
SE (m) ±	0.98	1.26	1.18	1.13	1.19	1.35	2.10	1.29	1.38	1.03	1.37	1.26
CD (p=0.05)	2.99	3.82	3.59	3.45	3.62	4.11	6.37	3.91	4.19	3.12	4.17	3.85
CV %	0.92	0.89	0.81	0.75	0.74	0.80	1.88	0.96	0.93	0.66	0.86	0.78



Table 7. Adult emergence (%) of *S. frugiperda* fed on leaves and squares of public sector *Bt* cotton hybrids

Treatments	Leaves											
	(60-80 days old crop)						(90-110 days old crop)					
	I	II	III	IV	V	VI	I	II	III	IV	V	VI
instar	instar	instar	instar	instar	instar	instar	instar	instar	instar	instar	instar	
NHH-44 BG II	0.00 (0.00)*	0.00 (0.00)	20.00 (26.57)	30.00 (33.21)	56.67 (48.83)	100.00 (90.00)	0.00 (0.00)	20.00 (26.57)	36.67 (37.27)	50.00 (45.00)	70.00 (56.79)	100.00 (90.00)
PKV Hy-2 BG II	0.00 (0.00)	0.00 (0.00)	10.00 (18.43)	26.67 (31.09)	56.67 (48.83)	100.00 (90.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	20.00 (26.57)	40.00 (39.23)	80.00 (63.43)
PDKV-JKAL-116 BG II	0.00 (0.00)	10.00 (18.43)	26.67 (31.09)	43.33 (41.17)	60.00 (50.77)	100.00 (90.00)	0.00 (0.00)	13.33 (21.41)	30.00 (33.21)	46.67 (43.09)	60.00 (50.77)	100.00 (90.00)
G. COT-10 Hy. BG II	6.67 (14.97)	13.33 (21.41)	36.67 (37.27)	56.67 (48.83)	63.33 (52.73)	100.00 (90.00)	0.00 (0.00)	10.00 (18.43)	20.00 (26.57)	40.00 (39.23)	53.33 (46.91)	93.33 (75.03)
G. COT-08 Hy. BG-II	20.00 (26.57)	20.00 (26.57)	40.00 (39.23)	66.67 (54.74)	80.00 (63.43)	100.00 (90.00)	0.00 (0.00)	0.00 (0.00)	20.00 (26.57)	30.00 (33.21)	43.33 (41.17)	86.67 (68.59)
NHH-44 (Non- <i>Bt</i> )	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	0.27	0.23	-	-	0.13	0.13	0.13	0.19	0.19
CD (p=0.05)	0.41	0.41	0.58	0.82	0.71	-	-	0.41	0.41	0.41	0.58	0.58
CV %	10.34	9.86	8.57	8.74	5.87	-	-	9.86	6.84	4.93	5.45	3.57

\* Figures in parenthesis angular transformed values

Table 8. Adult emergence (%) of *S. frugiperda* fed on young and mature bolls of public sector *Bt* cotton hybrids

Treatments	Young bolls											
	(120-140 days old crop)						(150-170 days old crop)					
	I	II	III	IV	V	VI	I	II	III	IV	V	VI
instar	instar	instar	instar	instar	instar	instar	instar	instar	instar	instar	instar	
NHH-44 BG II	0.00 (0.00)*	3.33 (10.51)	20.00 (26.57)	30.00 (33.21)	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)	10.00 (18.43)	33.33 (35.26)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
PKV Hy-2 BG II	13.33 (21.41)	20.00 (26.57)	46.67 (43.09)	56.67 (48.83)	100.00 (90.00)	100.00 (90.00)	16.67 (24.10)	30.00 (33.21)	56.66 (48.83)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
PDKV-JKAL-116 BG II	0.00 (0.00)	10.00 (18.43)	30.00 (33.21)	40.00 (39.23)	100.00 (90.00)	100.00 (90.00)	30.00 (33.21)	40.00 (39.23)	63.33 (52.73)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
G. COT-10 Hy. BG II	20.00 (26.57)	30.00 (33.21)	50.00 (45.00)	66.67 (54.74)	100.00 (90.00)	100.00 (90.00)	10.00 (18.43)	23.33 (28.88)	46.67 (43.09)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
G. COT-08 Hy. BG-II	10.00 (18.43)	20.00 (26.57)	33.33 (35.26)	53.33 (46.91)	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)	100.00 (90.00)
NHH-44 (Non- <i>Bt</i> )	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	0.23	-	-	0.13	0.13	0.27	-	-	-
CD (p=0.05)	0.41	0.41	0.58	0.71	-	-	0.41	0.41	0.82	-	-	-
CV %	9.86	7.71	7.14	7.06	-	-	9.02	6.62	8.31	-	-	-

\* Figures in parenthesis angular transformed values

*Cry* toxins associated with different *Bt* cotton hybrids exhibited significant mortality and conflicting effect, endorsed with Tindall et al., 2009, Sorgatto et al., 2015, Oliveira et al., 2016 and Yang et al., 2016 on similar studies on surviving *S. frugiperda*. The greater larval mortality was in early instars, when they fed on different plant structures of *Bt* cotton than in later instars. The highest larval mortality was found in newly hatched first instar (neonates) of *S. frugiperda*, which is in correspondence with the findings of Armstrong et al. (2011). These differences in larval mortality and declining growth parameters partially explain the contrasting expression of *Bt* toxins among plant structures as well as different transgenic hybrids. Explicitly, the *Bt* toxin detection by larvae may result in avoidance and apparent feeding preference of the pest (Lu et al., 2011). However, the survival of later instars could eventually lead to the development of resistance in the insect population (Sorgatto et al., 2015 and Horikoshi et al., 2016).

The *Cry* toxin expressions significantly varied among different plant parts as well as with the advancement in crop age. A declining larval mortality rate was found from early crop stages (leaves and squares) to the later stages (young and mature bolls) of cotton, as reported by Siebert et al. (2008). Furthermore, the surviving later larval instars showed contradictory effects on the growth and developmental parameters such as reduction in larval weights, decreased per cent pupation with less pupal weight and reduced adult emergence. These results of growth inhibition and stunting was demonstrated by Ramalho et al. (2011), Sorgatto et al. (2015), Flavio et al. (2014) and Oliveira et al. (2016), where they found larvae and pupae with lesser weight and size when fed on *Bt* cotton, thus supporting our findings. The early instars fed on *Bt* cotton hybrids showed significant failure to reach adult stage. The first larval instar (neonates) exhibited zero per cent survival till adult emergence, which was in consensus with the findings of Armstrong et al. (2011). While the later instars had considerable survival till pupation and adult emergence (Adamczyk et al., 1998). The per cent adult emergence varied among different *Bt* cotton hybrids, where it was maximum on non-*Bt* cotton. These results were in accordance with the findings of Sorgatto et al. (2015) who documented less adult emergence for *S. frugiperda* reared on dual-toxin *Bt* cotton than the single-toxin and non-*Bt* cotton cultivars.

The growth and survival index values showed similar trends, attributed to the prolonged larval

developmental period and reduced pupation among different *Bt* cotton hybrids. In previous investigation, Greenberg et al. (2010) reported that the duration and amount of endotoxin consumption confers reduced survival and growth rate, thus endorsing our results. The *S. frugiperda* fed on leaves and squares had minimal growth and survival indices when compared with young bolls and mature bolls. These results are in accordance with the findings of Greenberg et al. (2010), Soujanya et al. (2011), Armstrong et al. (2011) and others. From India, Naik et al. (2013) reported that *Spodoptera* sp. fed on leaves and squares of *Bt* cotton exhibited lowest growth and survival indices. Therefore, the *Cry* toxins may provide control against majority of the pest population in initial crop stages (Siebert et al., 2008), by conferring mortality mainly in early larval instars (Armstrong et al., 2011).

Our investigations ascertained that the transgenic hybrids producing *Cry* toxins can be an important component of overall management of the pest across a broad range of Indian agro-ecologies. The potential pest incidence at different crop growth stages can be significantly tolerated by *Bt* cotton hybrids. Previously, Khan et al. (2018) documented two types of factors i.e., external and internal factors which confers variation in *Cry* toxin gene expression in cotton. The external factors include temperature, water stress (drought or water logging conditions), Nitrogen availability, plant density, humidity and rainfall. While, internal factors comprise transgene number, its point of insertion, promoter and nucleotide sequences of the gene and overall cell environment. Thus, the sustainable expression of  $\delta$ -endotoxin among *Bt* cotton hybrids is essential for its efficacy against the pest. In India, as in case of many conventional chemical control strategies followed in other crops (Bharadwaj et al., 2020), *S. frugiperda* susceptibility varies among different *Bt* cotton hybrids (Hardke et al., 2015). Among different public sector *Bt* cotton hybrids, the superior results were expressed by PKV Hy-2 BG II when larvae fed on leaves and squares and NHH-44 BG II when fed on young bolls and mature bolls, respectively. Hence, screening and utilization of high *Cry* toxin expressing genotypes has crucial role in enhancing the efficacy against the pest.

As this pest feeds on several crops (Montezano et al., 2018), there is an associated risk factor that the population in India might develop resistance against the *Cry* toxin proteins, present in such transgenic cultivars. The planting of *Bt* cultivars in continuity might extend the exposure period of this lepidopteran pest to *Cry*

proteins (Rosalia et al., 2015). Therefore, to minimize the possibility of selecting resistant individuals by the pest species, to achieve a higher degree of control as well as to elude potential damage from this pest, it will be necessary to maintain effective refuge areas, aiming towards the sustainability of *Bt* technology.

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#### AUTHOR CONTRIBUTION STATEMENT

B A Thakre and V K Bhamare conceived and designed research, B A Thakre conducted experiments, B A Thakre and V K Bhamare analysed data. B A Thakre wrote the manuscript.

#### CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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