International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 10-01-2026, Accepted: 09-02-2026, Published: 24-02-2026
Volume 11, Issue 1, 2026, Page No. 422-424

Evaluation of transgenic *Bt* cotton hybrids for field incidence, multiplication and ovipositional preference of cotton leafhopper, *Amrasca biguttula biguttula* (Ishida)

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Abstract
The study evaluated the field incidence, survival, development, and ovipositional preference of the cotton leafhopper, *Amrasca biguttula biguttula* (Ishida), on Bt (Cry1Ac), BG II (Cry1Ac + Cry2Ab), and non-*Bt* cotton hybrids (BIO 6488 and RCH 134) grown at CCS HAU, Hisar. Although *Bt* cotton was originally introduced in India in 2002 to control the American bollworm, *Helicoverpa armigera*, concerns arose regarding its impact on sucking pests like leafhopper. Field observations from 40 to 140 days after sowing showed that leafhopper incidence was generally lower on BG II hybrids during early stages, but differences among BG II, *Bt,* and non-*Bt* hybrids were inconsistent and mostly non-significant, especially after peak infestation (90 DAS). Laboratory and cage studies revealed no significant differences in leafhopper survival (61.67–73.33%), though the nymphal developmental period varied slightly, being shortest on RCH 134 *Bt* and longest on BIO 6488 BG II. Ovipositional preference also did not differ significantly, with eggs ranging from 19.83 to 21.17 per leaf across hybrids. Overall, the results indicate that transgenic Bt cotton hybrids do not have a consistent or significant impact on the survival, development, or oviposition behavior of cotton leafhopper populations.

Keywords: Cotton *Bt* hybrids, leafhopper, oviposition, nymphal survival, incidence

Introduction

The *Bt* cotton is under cultivation in India since 2002 primarily for the management of American bollworm, *Helicoverpa armigera* (Hubner) (Arms, N.J., *et al*., 1992; Likhitha, P., *et al.,* 2018) [3, 6]. but later on some other lepidopterous pests like *Spodoptera litura* (Fabricius) were found inflicting severe damage to transgenic hybrid cottons carrying *Bt* toxin regulated by Cry1Ac gene. Therefore, another gene (Cry2Ab) coding for a different toxin was introduced into *Bt* cotton containing Cry1Ac (Bollgard) to develop Bollgard II which improved its efficacy against American bollworm as well as other lepidopteran pests (Stewart, S.D., *et al*., 2001) [13]. But there were conflicting reports about the performance of *Bt* hybrids against sucking insects like whitefly and leafhopper. In some cases build up of leafhopper on *Bt* cotton hybrids was more than non-*Bt* hybrids (Rao, H.N., *et al.,* 2002;Abro, G.H., *et al*., 2003;Aggarwal, N., *et al.,* 2007) [9, 1, 2] whereas some workers reported less population of leafhoppers on *Bt* hybrids than their counterparts (Whitehouse, M.E.A., *et al.,* 2005) [15] However in some experiments, there was no variation with respect to sucking pests population between *Bt* and non-*Bt* hybrids (Kengegowda, N., *et al.,* 2003; Rajanikantha, R*., et al.,* 2004) [5, 8]. There for an experiment was designed to assess the field incidence, development, survival and ovipositional preference of cotton leafhopper, *Amrasca biguttula biguttula* (Ishida) on BGII, *Bt* and non-*Bt* cotton hybrids.

Materials and methods

Field incidence

The experiment was conducted on six cotton hybrids (BIO 6488 non-Bt, BIO 6488 Bt, BIO 6488 BG II, RCH 134 non-Bt, RCH 134 Bt and RCH 134 BG II) grown at the Research Farm, CCS HAU, Hisar. The counting of leafhopper nymphs was initiated after 40 days after sowing. In each plot 10 plants were selected randomly and nymphs were counted on upper three fully expanded leaves. A total of 30 leaves per replication of each hybrid were examined. Population was recorded during morning hours at 10 days interval. A total of eleven observations were recorded till 140 days after sowing.

Survival and development

Two fully expanded leaves were randomly selected in each plot of 80 days old 6 cotton hybrids and the nymphs of leafhopper if any were removed with camel hair brush. Such leaves were caged (20x10 cm muslin cloth cages) as per Singh, R., *et al* (1988) [11] and after 72 hours, the newly hatched nymphs if any were removed. Then 10 first instar nymphs of leafhopper were released on caged leaves with camel hair brush from the susceptible genotype. These nymphs were observed each day after release to record the survival and time taken to turn into adults.

Ovipositional preference

The oviposition preference of leafhopper in six selected cotton hybrids was assessed by adopting the method of Singh, R., *et al.,* (1988) [12]. The nymphs of leafhopper present on third fully expanded leaf on top of selected plants were removed with camel hair brush before caging (20x10cm size muslin cloth cages) the selected leaves. After 72 h of caging, the nymphs, found in these cages, were further removed. Thereafter, thirty adults, captured with an aspirator, were released from the top in such cages for egg laying. After 72 h of release of the adults, these leaves were plucked and brought to the laboratory and processed in lactophenol solution for 10 minutes as suggested by Moffit, H.R. *et al*., (1972) [7]. The leafhopper eggs laid inside the leaf vein become clear and visible after processing in lactophenol solution.

Results and Discussion

Field incidence of leafhopper: Observations recorded on the leafhopper population in different BG II, *Bt* and non *Bt* cotton hybrids have been presented in Table 1. The leafhopper incidence was noticed from 40 days after sowing till 140 days after sowing. At 40 days after sowing leafhopper population was significantly lower on RCH 134 BG II (0.27/ 3leaves) and BIO 6488 BG II (0.30/ 3 leaves) and was higher on RCH 134 non- *Bt* (1.27/ 3leaves) and was on par with BIO 6488 non- *Bt* (0.83/ 3leaves). While the population on *Bt* hybrids of RCH 134 and BIO 6488 was 0.57 / 3leaf and 0.73 / 3leaves, respectively. During peak infestation period (90 days after sowing), incidence recorded on RCH 134 *Bt* (5.0/ 3leaves) and RCH 134 non-*Bt* (4.30/ 3leaves) was significantly higher than BIO 6488 BG II (3.83/ 3leaves), BIO 6488 *Bt* (3.83/ 3leaves), BIO 6488 non-*Bt* (3.80/ 3leaves) and RCH 134 BG II (2.80/ 3leaves).The incidence decreased 90 days after sowing onwards without any significant difference between BG II, *Bt* and non-*Bt* cotton hybrids. Though population of leafhopper on BG II was comparatively less than Bt and non-Bt hybrids but it may not be attributed to the presence of Cry genes as inferred by Vennila, S., *et al.* (2004) [14], Aggarwal, N., *et al.,* 2007) [2] and Dhillon, M., *et al*., (2013) [4] where the variations in leafhopper population between *Bt* and non-*Bt* hybrid cottons were insignificant and inconsistent.

Table 1: Incidence of cotton *Amrasca biguttula biguttula* on *Bt* cotton hybrids at different periods of crop growth

Hybrid	Number of nymphs/ 3 leaves										
	40DAS*	50 DAS	60 DAS	70 DAS	80 DAS	90 DAS	100 DAS	110 DAS	120 DAS	130 DAS	140 DAS
RCH 134 BGII	0.27	0.30	0.97	1.03	1.17	2.80	1.37	0.80	0.47	0.27	0.37
RCH 134 *Bt*	0.57	0.83	2.33	1.87	2.13	5.00	2.23	1.33	0.53	0.37	0.53
RCH 134 non-*Bt*	1.27	1.53	1.73	237	3.70	4.30	1.93	1.30	0.60	0.70	0.50
BIO 6488 BGII	0.30	0.33	0.56	1.30	1.30	3.83	1.93	0.97	0.53	0.57	0.50
BIO 6488 *Bt*	0.73	0.93	0.73	1.50	2.03	3.83	1.53	1.30	0.57	0.57	0.57
BIO 6488 non-*Bt*	0.83	1.20	1.30	1.83	3.23	3.80	1.87	1.00	0.73	0.47	0.73
SEm(±)	0.03	0.07	0.28	0.23	0.16	0.29	0.18	0.23	0.12	0.14	0.10
CD(p=0.05)	0.10	0.22	0.90	0.73	0.50	0.94	N S**	N S	N S	N S	N S

*DAS - Days after sowing
**N S – Non significant

Survival, development and oviposition of leafhopper

Newly hatched first instar nymphs of leafhopper were released and caged on suitable leaves of different cotton hybrids to record the survival (%) and development (days). Nymphs were counted daily till they reached adult stage. The data of the experiment were presented in Table 2. The survival of leafhopper did not show significant variation among the hybrids and ranged from 61.67 to 73.33 percent. The lowest (61.67 %) survival was recorded on BIO 6488 BG II which was at par with 63.33 percent on BIO 6488 non-*Bt*, 66.67 percent on RCH BG II and 68.33 percent on BIO 6488 *Bt*. The survival was highest on RCH 134 non *Bt* (73.33%) and 71.33 percent on RCH 134 *Bt.* The developmental period of leafhopper on different cotton hybrids was significantly lower on RCH134 *Bt* (7.02 days) and was at par with RCH 134 non-*Bt* (7.06 days). The longest nymphal period was found in BIO 6488 BG II (9.10 days) followed by RCH 134 BG II, BIO 6488 *Bt* and BIO 6488 non-*Bt* with 8.32, 8.02 and 7.52 days respectively. These findings are similar to those reported by Shivanna, B.K., *et al.* (2009) [10] where the total nymphal period ranged from 7.00 to 8.50 on BG II hybrid (MRC 7201).

To establish the ovipositional preference of leafhopper on cotton hybrids, studies were carried out under cage condition in the field at 80 days after sowing. The number of eggs laid (Table 2) did not vary significantly among the hybrids and the average number of eggs laid in leaf veins ranged from 19.83 to 21.17 eggs / leaf. It may be inferred that transgenic *Bt* hybrids do not genetically impact leafhopper population.

Table 2: Effect of *Bt* cotton hybrids on survival, development and oviposition of *Amrasca biguttula biguttula* at 80 days after sowing

Hybrid	Nymphal survival (%)	Nymphal duration(days)	Total number of eggs laid/ leaf
RCH 134 BGII	66.67(54.81)*	8.32	20.17
RCH 134 *Bt*	71.33(57.84)	7.02	20.83
RCH 134 non *Bt*	73.33(58.98)	7.06	21.17
BIO 6488 BGII	61.67(51.89)	9.10	20.50
BIO 6488 *Bt*	68.33(56.24)	8.02	19.83
BIO 6488 non *Bt*	63.33(52.88)	7.52	20.33
SEm(±)	8.53(3.79)	0.32	1.11
CD(p=0.05)	N S**	1.01	N S

*Figures in the parentheses are angular transformed values
** N S – Non significant

Conclusion

Leafhopper incidence was generally lower on BG II hybrids during early stages, but differences among BG II, *Bt,* and non-*Bt* hybrids were inconsistent and mostly non-significant, especially after peak infestation (90 DAS). Cage studies revealed no significant differences in nymphal survival (61.67–73.33%), though developmental period varied slightly, being shortest on RCH 134 *Bt* and longest on BIO 6488 BG II. Ovipositional preference also did not differ significantly across hybrids.

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