Int.J.Curr.Microbiol.App.Sci (2018) Special Issue-6: 1393-1407

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Special Issue-6 pp. 1393-1407**
Journal homepage: http://www.ijcmas.com

Original Research Article

Survival and Development of Spotted Bollworm *Eariasvittella* (Fabricius) on Bt Cotton Hybrids of Different Events

P. Likhitha* and V. K. Bhamare

Department of Agriculture Entomology, College of Agriculture-Latur 413512, Maharashtra(s), India
**Corresponding author*

A B S T R A C T

In the present investigation four *Bt* cotton hybrids of RCH-799 BG II, NCEH-34 BG-II, YRCH-31 BG-II, JKCH-1947 BG-I and along with one NCEH-34 non *Bt* cotton hybrid were evaluated to study the survival and development of spotted bollworm *E.vittella*. Laboratory bioassays were conducted on squares and bolls of above mentioned *Bt* and non-*Bt* cotton hybrids. Larval mortality was more on squares in comparison to bolls and in that mortality levels varied among instars i.e. late instar larvae survived successfully on squares and bolls of both 90-110 and 120-140 old days crop. The vulnerability equality of *E.vittella* to NCEH-34 BG-II speckled with instar wise and the late instar larva had verypetite effect on the survival and developmental biology of the insect. Low growth and survival indices were verified on squares in comparison to bolls representing that development of *E.vittella* was radically exaggerated. In keeping the view on the results we witnessed that survival(growth) and development of *E.vittella* was less on 90-110 days old crop in comparison to 120-140 days old crop signifying that., decreased expression levels of *Bt* toxin in plant parts with increased time (days) of crop.

Keywords

Bollworm, *Eariasvittella*, cotton

Introduction

Cotton (*Gossypiumspp*) (Malvaceae) is the most important industrial crop worldwide with an area of 12.7 million hectares planted in 2016-17 (Kranthi *et al.*, 2017). Area wise, India rank first in global scenario contributing about 26% of the world cotton area with a production of 40.0 million bales (170 kg lint/bale) and average lint yield of 537 kg ha-1 in 2014-15(Anonymous 2015). India's National average yield was less compared to the 'rest of world's average yield of 931 kg per hectare. The yields in a few countries *viz.*, Australia (2619 kg per ha), China (1508 kg per ha), Brazil (1601 kg per ha), Turkey (1574 kg per ha) and Mexico (1577 kg per ha) were 1500 to 2600 kg lint per hectare which is 3 to 5 times higher than India (Kranthi, 2017). Yield losses caused by *E. vittella* and *E. insulana* were 44% in *G. hirsutum* (Dhawan *et al.*, 1988) and 48.2% in *G. arboreum* (Shera 2009) varieties in India. Genetic Engineering Approval Committee (GEAC), Ministry of Environment and Forests, Government of India approved for the first commercial cultivation of Transgenic *Bt* cotton expressing genes from soil inhabiting spore forming bacterium, *Bacillus thuringiensis* Berliner (*Bt*) toxins for bollworm management in Central and South

India in 2002 and in North India in 2005. So far, six transgene events, viz., MON 531 (cry1Ac; Monsanto-Mahyco, Maharshtra), Event 1 (modified cry1Ac; IIT Kharagpur/ JK Agri-Genetics Ltd., Andhra Pradesh), GFM event (fusion cry1Ab/cry1Ac; CAS, China/ Nath Bio-Genes Ltd., Maharashtra), BNLA-601 (cry1Ac; UAS Dharwad/CICR Nagpur), MLS-9124 (cry1C gene; Metahelix Pvt. Ltd., Bangalore), MON 15985 (cry1Ac and cry2Ab/Monsanto-Mahyco, Maharashtra) have been approved by GEAC in India (Navarro and Hautea 2014).Recently many studies demonstrated that bollworm can survive and develop on some of the elite *Bt* cotton hybrids (Mahalakshmi *et al.*, 2013, Naik *et al.*, 2012, Soujanya *et al.*, 2010, Naik *et al.*, 2014 and Shera and Arora, 2016a). It is important that the toxin protein be expressed in adequate quantity in appropriate plant parts for protection against target pests (Bhullar and Gill, 2015).

Therefore, BG-I cotton expressing Cry 1Ac is being replaced by BG-II expressing dual genes (Cry 1Ac + Cry 2Ab) and throughout the season Cry 2Ab is expressed at higher level than Cry 1Ac (Adamczyk *et al.*, 2001). However, significant variation in the quantum of expression of Cry 2Ab toxin in plant parts and significant decline with the age of the crop has been reported from BG-II hybrids in India (Saini and Dhawan, 2013).

Under this background, in view of the availability of numerous transgenic cotton hybrids of different events for farmers in the open market and the variability in the performance of the Cry 1Ac and Cry 2Ab toxins among the plant parts and different stages of crops, the present investigation was planned to study the survival and development of spotted bollworm on *Bt* cotton hybrids of different events.

Materials and Methods

The renowned *Bt* cotton hybrids of different events were selected and cultivated on Experimental Farm of Department of Agricultural Entomology and the laboratory studies on the survival and development of bollworms on field collected *Bt* cotton structures or parts of different events at pre-determined intervals were conducted at Post Graduate Laboratory, Department of Agricultural Entomology, College of Agriculture, Latur (Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra) during *Kharif* 2016-17. The production technology was monitored as recommendations given by VNMKV, Parbhani excluding plant protection operations.

Maintenance of stock culture

The initial cultures of *Earias vittella* (Fabricius) were developed by collecting large number of larvae from the surrounding fields at Post Graduate Laboratory, Dept. of Agril. Entomology, College of Agriculture, Latur during 2016-17.The rearing was conducted at ambient room temperature.

The collected larvae were individually reared in round plastic vials (measuring 4 cm diameter and 5 cm height) by feeding them on natural diet (squares, flowers and bolls of non-*Bt* cotton) every day till pupation. Pupae were transfer to round clean plastic containers covering top with muslin cloth secured firmly with rubber band. The sexes were determined in pupal stages on the basis of distance between genital and anal apertures. It is less in the case of male and more in the case of female. The freshly emerged adults were released into standard oviposition cage (measuring 50 cm x 30 cm) covered with black muslin cloth. The oviposition cage was placed over the water trough in order to create humidity. The proportion of female and male in the cage was 1:5 in order to get fertilized eggs. Cotton swab dipped into 10 per cent honey solution was provided to serve as food for the adults. A strip of cotton cloth toweling (6×17 cm) was hung vertically inside each oviposition cage as oviposition substrate. The eggs on the toweling were kept in a transparent polythene box (measuring 26 cm L × 17 cm W x 6 cm H). The eggs from each pair were kept separately. After hatching, neonate larvae were transferred separately into plastic vials (diameter 15 cm) to avoid cannibalism. Daily the larvae were fed on natural diet till pupation. The different instar larvae obtained were used for further investigations.

Study of survival and development of spotted bollworm on *Bt* cotton hybrids of different events

The experiment was conducted in completely randomized design (CRD) with three replications using ten larvae per replication. The experiment was conducted with different larval instars by feeding on squares and bolls of three BG-II cotton hybrids (RCH-799, NCEH-34 and YRCH-31,) one BG-I cotton hybrid (JKCH-1974) and one non-*Bt* cotton (Jagannath) as control.

The cotton parts or structures mainly squares and bolls of different cotton hybrids were collected randomly in labeled plastic bags at pre-determined interval of 90-110 and 120-140 days old crop, respectively. Immediately, plant parts were brought to the laboratory with due care to avoid any contamination and drying. Collected samples were cleaned with sterile water and wiped with blotting paper to remove excess moisture from the plant parts. The squares and bolls were placed individually in a plastic vials. Later laboratory reared different instar larvae of bollworms were released on different cotton structures at the rate of 10 larvae per replication.

The vial was covered with a plastic lid for avoiding escape of larvae. The plant parts were replaced daily with fresh *Bt* plant parts (same on which larvae fed) till pupation to avoid death or growth reduction due to tissue drying or nutritional deterioration in the experimental treatment. The data on mortality of larvae was recorded separately for each instar.

The mortality in all the instars of *E. vittella* larvae were studied individually by exposing to different cotton structures *viz*., squares and bolls of different cotton hybrids at 90-110 and 120-140 days old crop, respectively. Weight of the surviving larvae was recorded after 24, 48 and 72 h of exposure and weight of pupae was also recorded from each treatment. In addition, other parameters *viz*., per cent pupation and adult emergence were observed from the larvae. The growth index and survival index were calculated for *E. vittella* population on different treatments using the formulae given by Vennila *et al*., (2006).

$$\text{Growth index} = \frac{\text{Per cent pupation}}{\text{Larval developmental period (days)}}$$

$$\text{Survival index} = \frac{\text{Number of moths emerged}}{\text{Total number of neonates tested}}$$

Statistical analysis

The data recorded on survival and development of bollworms on *Bt* cotton hybrids of different events were subjected to completely randomized design (CRD) (Gomez and Gomez, 1984). The CRD analysis was done by using the statistical programme OPSTAT.

Results and Discussion

The survival and development of *E. vittella* larvae was observed instar wise i.e., first to fifth instar larvae on squares and bolls of *Bt* cotton hybrids of different events (RCH-799 BG II, NCEH-34 BG-II, YRCH-31 BG-II, JKCH-1947 BG-I and NCEH-34 non-*Bt* as control) at 90-110 and 120-140 days old crop.

Effect of different plant parts of *Bt* cotton hybrids of different events on larval mortality of *E. vittella*

Squares

Mortality of first instar larvae of *E. vittella* was hundred per cent on squares of *Bt* cotton hybrids of RCH-799 BG-II, NCEH-34 BG-II, YRCH-31 BG-II and JKCH-1947 BG-I 9(Table 2). Nevertheless, no mortality was noticed in first instar larvae when fed on squares of NCEH-34 non-*Bt* cotton hybrid.

Mortality of second instar larvae of *E. vittella* varied from 66.67 to 86.67 per cent on squares of *Bt* cotton hybrids of different events. Significantly highest mortality of second instar larvae was observed on squares of NCEH-34 BG-II (86.67 per cent) followed by RCH-799 BG-II (76.67 per cent), YRCH-31 BG-II (73.33 per cent) and JKCH-1947 BG-I (66.67 per cent).

Mortality of third instar larvae of *E. vittella* ranged from 26.67 to 46.67 per cent on squares of *Bt* cotton hybrids of different events. Significantly maximum mortality of third instar larvae was exhibited to the tune of 46.67 per cent on squares of NCEH-34 BG-II followed by RCH-799 BG-II (36.67 per cent), YRCH-31 BG-II (30.00 per cent) and JKCH-1947 BG-I (26.67 per cent). The fourth and fifth instar larvae of *E. vittella* were resistant to *Bt* and non-*Bt* cotton hybrids and there was no mortality in all the test hybrids.

The mortality of *E. vittella* larvae was evidenced during first, second and third instars only, when fed on squares of *Bt* cotton hybrids of different events. Among all the *Bt* cotton hybrids of different events, BG-II cotton hybrids expressed better than BG-I. The first, second and third instar larvae of *E. vittella* fed on squares of NCEH-34 BG-II noticed maximum mortality. The per cent larval mortality varied with the instars. The mortality of *E. vittella* decreased gradually with advance in the age of the larvae.

Bolls

The data on per cent mortality of *E. vittella* larval instars when fed on bolls of *Bt* cotton hybrids of different events at 120-140 days old crop evidenced that maximum larval mortality was recorded on all *Bt* cotton hybrids of different events under investigation compared to non-*Bt* cotton hybrid (Table 3).

The mortality of first instar larvae of *E. vittella* was cent per cent on bolls of *Bt* cotton hybrids *viz*., RCH-799 BG-II, NCEH-34 BG-II, YRCH-31 BG-II and JKCH-1947 BG-I. Whereas, no mortality was noticed in first instar larvae when fed on bolls of NCEH-34 non-*Bt* cotton hybrid.

The mortality of second instar larvae of *E. vittella* varied from 63.33 to 80.00 per cent on bolls of *Bt* cotton hybrids of different events. The significantly maximum mortality of second instar larvae was observed to the tune of 80.00 per cent on bolls of NCEH-34 BG-II followed by RCH-

799 BG-II (76.67 per cent), YRCH-31 BG-II (63.33 per cent) and JKCH-1947 BG-I (66.33 per cent).

The third, fourth and fifth instar larvae of *E. vittella* registered no mortality when fed on bolls of *Bt* and non-*Bt* cotton hybrids.

The larval mortality of *E. vittella* was less on green bolls, compared to squares. The mortality of *E. vittella* larvae when fed on bolls of *Bt* cotton hybrids of different events was noticed during first and second instars only. Among all the *Bt* cotton hybrids of different events, BG-II cotton hybrids revealed superior results over BG-I. The first and second instar larvae of *E. vittella* fed on bolls of NCEH-34 BG-II noticed maximum mortality. The per cent larval mortality varied with the instars. The mortality of *E. vittella* decreased gradually with progress in the age of the larvae.

Of all the tested test hybrids NCEH-34 BGII cotton hybrid expressed well with highest mortality percentage in comparison to remaining hybrids.

The results on the per cent mortality of different larval instars of *E. vittella* fed on squares and bolls of *Bt* cotton hybrids of different events at pre-determined intervals are in accordance with the results of Shera and Arora (2016b) who indicated that third instar larvae of *E. vittella* had greater preference for bolls (7.29-7.50 per cent) than for the squares (5.0-5.21 per cent) and reverse was true for the first instar larvae which showed greater preference for squares (7.08-7.29 per cent) than for the bolls (5.21-5.42 per cent), in a multiple-choice test. *E. vittella* larvae showed significant difference in preference for different cotton genotypes. Whereas, Shera and Arora (2016a) noticed that none of *E. vittella* neonates were able to complete their larval period and reach pupal stage on squares and bolls of 90, 120 and 150 days old crop of all *Bt* hybrids. The concentration of Cry toxin varied significantly in squares and bolls and also among the crop ages.

The amount of Cry toxin in squares and bolls had significant negative correlation with average survival period of *E. vittella* larvae. Bhullar and Gill (2015) showed that significantly higher Cry 1Ac and Cry 2Ab were estimated from leaves (4.04 and 20.34 µg) followed by squares (3.97 and 20.01µg) and bolls (3.51 and 18.94 µg). Higher expression of Cry 1Ac and Cry 2Ab were estimated from 105 days old plants (4.03 and 20.43 µg) as compared to 120 days old plants (3.78 and 19.81 µg) followed by 135 days old plants (3.56 and 19.04 µg).

Shera *et al.*, (2015) found that all *Bt* cotton hybrids recorded significantly reduced damage of *Earias spp*. in shoots, squares, green bolls and freshly shed fruiting bodies as compared to the isogenic non-*Bt* genotypes. Ahmed *et al.,* (2012) revealed that the seasonal infestation of squares, flowers and bolls due to *E. vittella* was highest on FH 2015 non-*Bt* followed by FH 113 *Bt* and FH 114 *Bt*.

Mann *et al.*, (2010) revealed that both Bollgard II and Bollgard cultivars were infested with larvae of spotted bollworm, *E. vittella* and spiny bollworm, *E. insulana*, at later crop growth stages. However, Channakeshava and Patil (2009) stated that incidence of *E. vittella* was nil in MECH-184 *Bt* cotton. Udikeri (2006) observed that larval mortality of *E. vittella* was maximum (>99 per cent) between 40 and 80 DAS in RCH-2 Bollgard-II with Cry 1 Ac + Cry 2 Ab genes. Kranthi *et al.,* (2000) indicated that spotted bollworm larvae were found to be extremely susceptible to all the three Cry 1A toxins (Cry 1Aa, Cry 1Ab and Cry 1Ac).

Effect of different plant parts of *Bt* cotton hybrids of different events on larval weight of *E. vittella*

The data pertaining to mean weight of *E. vittella* larvae that survived beyond 24, 48 and 72 h after exposure on squares and bolls of *Bt* cotton hybrids of different events at 90-110 and 120-140 days old crop, respectively was recorded for II, III and IV instars

Squares

Minimum larval weight was recorded on all *Bt* cotton hybrids of different events under investigation compared to non-*Bt* cotton hybrid (Table 4). The weight of first instar larvae of *E. vittella* was not recorded due to the death of neonate larvae on squares of all *Bt* cotton hybrids of different events.

The minimum weight of second instar larvae of *E. vittella* was recorded to the extent of 3.88, 4.02 and 4.21 mg per larva on squares of NCEH-34 BG-II at 24, 48 and 72 h after feeding, respectively. While, maximum weight of second instar larvae was recorded to the tune of 5.24, 5.40 and 5.59 mg per larva on squares of NCEH-34 non-*Bt* at 24, 48 and 72 h after feeding, respectively.

Among the *Bt* cotton hybrids of different events, NCEH-34 BG-II evidenced lowest weight of third instar larvae of *E. vittella* to the extent of 16.11, 31.41 and 53.00 mg per larva when fed on squares at 24, 48 and 72 h, respectively. While, maximum weight of third instar larvae was recorded to the tune of 29.16, 52.74 and 87.33 mg per larva on squares of NCEH-34 non-*Bt* at 24, 48 and 72 h after feeding, respectively.

Analogously, minimum weight of fourth instar larvae of *E. vittella* was observed to the tune of 45.93, 59.00 and 63.67 mg per larva on the squares of NCEH-34 BG-II at 24, 48 and 72 h after feeding, respectively. However, NCEH-34 non-*Bt* registered maximum weight of fourth instar larvae to the extent of 76.33, 140.67 and 193.00 mg per larva at 24, 48 and 72 h after feeding on squares, respectively.

The larvae feeding on squares of *Bt* cotton hybrids of different events revealed lowest weight as compared to non-*Bt* cotton hybrid. The NCEH-34 BG-II hybrid was less palatable to *E. vittella* as the larval weight was very less compared to other *Bt* and non-*Bt* cotton hybrid.

Bolls

Minimum larval weight was recorded on all *Bt* cotton hybrids of different events under investigation compared to non-*Bt* cotton hybrid (Table 5).

The weight of first instar larvae of *E. vittella* was not recorded due to the death of neonate larvae on bolls of all *Bt* cotton hybrids of different events.

The minimum weight of second instar larvae of *E. vittella* was recorded to the extent of 4.84, 4.59 and 5.47 mg per larva on bolls of NCEH-34 BG-II at 24, 48 and 72 h after feeding, respectively. While, maximum weight of second instar larvae was recorded to the tune of 7.48, 8.00 and 9.32 mg per larva on squares of NCEH-34 non-*Bt* at 24, 48 and 72 h after feeding, respectively.

Among the *Bt* cotton hybrids of different events, NCEH-34 BG-II evidenced minimum weight of third instar larvae of *E. vittella* to the extent of 17.04, 18.36 and 19.60 mg per larva when fed on bolls at 24, 48 and 72 h, respectively. While, maximum weight of third instar larvae was recorded to the tune of 31.97, 32.79 and 33.79 mg per larva on bolls of NCEH-34 non-*Bt* at 24, 48, and 72 h after feeding, respectively.

Analogously, minimum weight of fourth instar larvae of *E. vittella* was observed to the tune of 46.55, 61.00 and 63.33 mg per larva on the bolls of NCEH-34 BG-II at 24, 48 and 72 h after feeding, respectively. However, NCEH-34 non-*Bt* registered maximum weight of fourth instar larvae to the extent of 86.01, 152.09 and 159.48 mg per larva at 24, 48 and 72 h after feeding on bolls, respectively.

The larvae feeding on bolls of *Bt* cotton hybrids of different events revealed lowest weight as compared to non-*Bt* cotton hybrid. The NCEH-34 BG-II hybrid was less palatable to *E. vittella* as the larval weight was very less compared to other *Bt* and non-*Bt* cotton hybrid.

More or less similar findings are reported by Mahalakshmi and Prasad (2013) who noticed that later instar larvae of *H. armigera* able to survive when exposed continuously to *Bt* cotton plant parts, but reduction in larval weight was observed compared to their corresponding non-*Bt* hybrids and check hybrid. Analogously, Naik *et al.,* (2012) revealed that exposure of later instar larvae of *H. armigera* to plant parts of *Bt* event hybrids exhibited adverse effects on the larval weight.

Arshad *et al.,* (2009) indicated that pupal weight of *H. armigera* was significantly higher for larvae feed on leaves and flowers-bolls of non-*Bt* cotton compared with *Bt* cotton plant parts. Similarly, Srinivasa Rao and Arjuna Rao (2008) observed that few third and many fourth and fifth instar larvae of *H. armigera* fed with *Bt* cotton flower buds pupated but, they were small in size. Analogously, Men *et al.,* (2005) showed that pupal weight of *H. armigera* decreased by 48.60 per cent when larvae fed with flowers and bolls of *Bt* cotton compared with non-*Bt*. Deterrence index (DI) of *H. armigera* larvae decreased in later instars, which indicated that the *Bt* toxin decreased with age of the crop. However, Jayaprakash *et al.,* (2013), Baoqian *et al.,* (2011), Siebert *et al.,* (2009) and Kranthi *et al.,* (2005) revealed that Cry toxin expression was found to be highest in leaves followed by squares, flowers and bolls. Cry toxin levels were changed significantly as the season progressed (Akin *et al.,* 2004**)**.

Effect of different plant parts of *Bt* cotton hybrids of different events on pupation of *E. vittella*

Squares

The data on per cent pupation of surviving larval instars of *E. vittella* when fed on squares of *Bt* cotton hybrids of different events at 90-110 days old crop revealed that lowest pupation was noticed on all *Bt* cotton hybrids of different events under investigation compared to non-*Bt* cotton hybrid (Table 6).

The pupation of first instar larvae of *E. vittella* was 0.00 per cent on squares of *Bt* cotton hybrids of RCH-799 BG-II, NCEH-34 BG-II, YRCH-31 BG-II and JKCH-1947 BG-I. However, cent per cent pupation was noticed in first instar larvae when fed on squares of NCEH-34 non-*Bt* cotton hybrid.

The pupation of second instar larvae of *E. vittella* varied from 13.33 to 33.33 per cent on squares of *Bt* cotton hybrids of different events. The second instar larvae fed on squares of NCEH-34 BG-II exhibited significantly lowest pupation to the tune of 13.33 per cent followed by RCH-799 BG-II (23.33 per cent), YRCH-31 BG-II (26.67 per cent) and JKCH-1947 BG-I (33.33 per cent).

The pupation of third instar larvae of *E. vittella* ranged from 53.33 to 73.33 per cent on squares of *Bt* cotton hybrids of different events. The significantly minimum pupation of third instar larvae was observed on squares of NCEH-34 BG-II (53.33 per cent) followed by RCH-799 BG-II (63.33 per cent), YRCH-31 BG-II (70.00 per cent) and JKCH-1947 BG-I (73.33 per cent).

However, cent per cent pupation was observed when fourth and fifth instar larvae of *E. vittella* fed on squares of *Bt* cotton hybrids of different events.

Among all the *Bt* cotton hybrids of different events, BG-II cotton hybrids showed better results over BG-I. The first, second and third instar larvae of *E. vittella* when fed on squares of NCEH-34 BG-II registered lowest pupation. The per cent pupation varied with the instars. The gradual increase in per cent pupation of *E. vittella* was recorded in II and III instar larvae fed on squares of *Bt* cotton hybrids of different events.

Bolls

The data on per cent pupation of surviving larval instars of *E. vittella* when fed on bolls of *Bt* cotton hybrids of different events at 120-140 days old crop revealed that lowest pupation was noticed on all *Bt* cotton hybrids of different events under investigation compared to non-*Bt* cotton hybrid (Table 7).

The pupation of first instar larvae of *E. vittella* was 0.00 per cent on bolls of *Bt* cotton hybrids of RCH-799 BG-II, NCEH-34 BG-II, YRCH-31 BG-II and JKCH-1947 BG-I. However, cent per cent pupation was noticed in first instar larvae when fed on bolls of NCEH-34 non-*Bt* cotton hybrid.

The pupation of second instar larvae of *E. vittella* varied from 20.00 to 36.67 per cent on bolls of *Bt* cotton hybrids of different events. The second instar larvae fed on bolls of NCEH-34 BG-II exhibited significantly lowest pupation to the tune of 20.00 per cent followed by RCH-799 BG-II (23.33 per cent), YRCH-31 BG-II (33.33 per cent) and JKCH-1947 BG-I (36.67 per cent).

The cent per cent pupation was noticed when third, fourth and fifth instar larvae of *E. vittella* fed on bolls of *Bt* cotton hybrids of different events.

Among all the *Bt* cotton hybrids of different events, BG-II cotton hybrids showed better results over BG-I. The first and second, instar larvae of *E. vittella* when fed on bolls of NCEH-34 BG-II registered lowest pupation. The per cent pupation varied with the instars. The gradual increase in per cent pupation of *E. vittella* was recorded when II instar larvae fed on bolls of *Bt* cotton hybrids of different events.

The results on the per cent pupation of surviving larvae of different instars of *E. vittella* fed on squares and bolls of *Bt* cotton hybrids of different events at pre-determined intervals are more or less similar with the findings of Liu *et al.*, (2017) who reported that the bollworm survival to pupation was significantly affected by strain, cultivar and the interaction between these factors.

The concentration of Cry 1A toxins differed significantly among *Bt* cultivars and plant structures, but the interaction between these factors was not significant. Overall, the concentration of Cry 1A toxins was highest in leaves, intermediate in buds and smallest in bolls. The concentration of Cry 2Ab did not differ significantly among plant structures. Mahalakshmi and Prasad (2013) noticed that later instar larvae of *H. armigera* were able to survive when exposed continuously to *Bt* cotton plant parts, but exhibited malformation in pupae. Naik *et al.,* (2012) revealed that exposure of later instar larvae to plant parts of *Bt* event hybrids exhibited adverse effects on the growth and development such as reduced pupation, formation of small pupae with less weight.

Srinivasa Rao and Arjuna Rao (2008) observed that all the first and second instar bollworm larvae fed with *Bt* cotton flower buds died before pupation. However, a few third and many fourth and fifth instar larvae of *H. armigera* fed with *Bt* cotton flower buds pupated but, they were small in size. Men *et al.,* (2005) showed that 8.3 per cent *H. armigera* fed with flowers and bolls of GK-12 (with Cry l Ac toxin) could develop from neonate to pupa. Jayaprakash *et al.,* (2013), Baoqian *et al.,* (2011), Siebert *et al.,* (2009), Kranthi *et al.,* (2005) revealed that Cry toxin expression was found to be highest levels in unfurled leaves, moderate levels in white flowers and squares and lowest levels in small bolls. Cry toxin expression decreased consistently as the plant aged. Akin *et al.,* (2004**)** revealed that in single-toxin and double-toxin *Bt* cotton, toxin levels were changed significantly as the season progressed. Stewart *et al.,* (2001) revealed that survival and growth of bollworm and tobacco budworm was reduced by *Bt* cotton, particularly the dual-toxin cultivar.

Effect of different plant parts of *Bt* cotton hybrids of different events on pupal weight of *E. vittella*

Squares

The data on mean pupal weight of *E. vittella* reared on squares of *Bt* cotton hybrids of different events at 90-110 days old crop evidenced that minimum pupal weight was recorded on all *Bt* cotton hybrids of different events under investigation compared to non-*Bt* cotton hybrid (Table 8).

The mean pupal weight of first instar larvae of *E. vittella* was not recorded due to the death of first instar larvae fed on squares of *Bt* cotton hybrids of different events before pupation. The mean pupal weight of surviving first instar larvae on squares of NCEH-34 non-*Bt* was 325.58 mg per pupa.

The mean pupal weight of surviving second instar larvae of *E. vittella* fed on squares of *Bt* cotton hybrids of different events varied from 215.84 to 253.52 mg per pupa. The significantly lowest mean pupal weight was recorded with surviving second instar larvae reared on squares of NCEH-34 BG-II (215.84 mg per pupa) followed by RCH-799 BG-II (240.39 mg per pupa), YRCH-31 BG-II (248.10 mg per pupa) and JKCH-1947 BG-I (253.52 mg per pupa).

The mean pupal weight of surviving third instar larvae of *E. vittella* fed on squares of *Bt* cotton hybrids of different events varied from 219.53 to 257.04 mg per pupa. The third instar larvae registered significantly minimum mean pupal weight of 219.53 mg per pupa on squares of NCEH-34 BG-II followed by RCH-799 BG-II (245.53 mg per pupa), YRCH-31 BG-II (250.75 mg per pupa) and JKCH-1947 BG-I (257.04 mg per pupa).

The mean pupal weight of fourth instar larvae of *E. vittella* fed on squares of *Bt* cotton hybrids of different events ranged from 225.23 to 269.90 mg per pupa. The significantly minimum mean pupal weight (225.23 mg per pupa) was registered with fourth instar larvae reared on squares of NCEH-34 BG-II followed by RCH-799 BG-

II (250.59 mg per pupa), YRCH-31 BG-II (263.82 mg per pupa) and JKCH-1947 BG-I (269.90 mg per pupa).

The mean pupal weight of fifth instar larvae of *E. vittella* fed on squares of *Bt* cotton hybrids of different events varied from 230.99 to 270.63 mg per pupa. The fifth instar larvae showed significantly lowest mean pupal weight of 230.99 mg per pupa on squares of NCEH-34 BG-II followed by RCH-799 BG-II (255.61 mg per pupa), YRCH-31 BG-II (266.28 mg per pupa) and JKCH-1947 BG-I (270.63 mg per pupa).

The pupal weight was significantly lower on all *Bt* cotton hybrids of different events compared to non-*Bt* cotton hybrid. The pupal weight was less on squares compared to bolls.

Bolls

The data on mean pupal weight of *E. vittella* reared on bolls of *Bt* cotton hybrids of different events at 120-140 days old crop evidenced that minimum pupal weight was recorded on all *Bt* cotton hybrids of different events under investigation compared to non-*Bt* cotton hybrid (Table 9).

The mean pupal weight of first instar larvae of *E. vittella* was not recorded due to the death of first instar larvae fed on bolls of *Bt* cotton hybrids of different events before pupation. The mean pupal weight of surviving first instar larvae on bolls of NCEH-34 non-*Bt* was 357.67 mg per pupa.

The mean pupal weight of surviving second instar larvae of *E. vittella* fed on bolls of *Bt* cotton hybrids of different events varied from 219.16 to 257.16 mg per pupa. The significantly lowest mean pupal weight was recorded with surviving second instar larvae reared on bolls of NCEH-34 BG-II (219.16 mg per pupa) followed by RCH-799 BG-II (244.98 mg per pupa), YRCH-31 BG-II (251.14 mg per pupa) and JKCH-1947 BG-I (257.16 mg per pupa).

The third instar larvae registered significantly minimum mean pupal weight of 222.97 mg per pupa on bolls of NCEH-34 BG-II followed by RCH-799 BG-II (248.75 mg per pupa), YRCH-31 BG-II (253.94 mg per pupa) and JKCH-1947 BG-I (258.57 mg per pupa).

Significantly minimum mean pupal weight (224.61 mg per pupa) was registered with fourth instar larvae reared on bolls of NCEH-34 BG-II followed by RCH-799 BG-II (252.52 mg per pupa), YRCH-31 BG-II (255.27 mg per pupa) and JKCH-1947 BG-I (260.51 mg per pupa).

The mean pupal weight of fifth instar larvae of *E. vittella* fed on bolls of *Bt* cotton hybrids of different events ranged from 230.71 to 269.84 mg per pupa. The fifth instar larvae showed significantly lowest mean pupal weight of 230.71 mg per pupa on bolls of NCEH-34 BG-II followed by RCH-799 BG-II (256.88 mg per pupa), YRCH-31 BG-II (261.59 mg per pupa) and JKCH-1947 BG-I (269.84 mg per pupa).

The weight of pupae was significantly lower on all *Bt* cotton hybrids of different events compared to non-*Bt* cotton hybrid. The pupal weight was less on squares compared to bolls.

The results on mean pupal weight of *E. vittella* reared on squares and bolls of *Bt* cotton hybrids of different events at pre-determined interval are in match with the findings of Mahalakshmi and Prasad (2013) who noticed that later instar larvae of *H. armigera* were able to survive when exposed continuously to *Bt* cotton plant parts, but exhibited malformed pupae and reduction in pupal weight. Analogously, Naik *et al.,* (2012) revealed that exposure of later instar larvae to plant parts of *Bt* event hybrids exhibited adverse effects on the growth and development such as formation of small pupae with less weight. Arshad *et al.,* (2009) indicated that pupal weight of *H. armigera* was significantly higher for larvae feed on leaves and flowers-bolls of non-*Bt* cotton compared with *Bt* cotton plant parts.Srinivasa Rao and Arjuna Rao (2008) observed that few third and many fourth and fifth instar larvae of *H. armigera* fed with *Bt* cotton flower buds pupated but, they were small in size. Men *et al.,* (2005) showed that pupal weight of *H. armigera* fed with flowers and bolls of *Bt* cotton decreased by 48.60 per cent compared with those of non-*Bt* SI-3.

Jayaprakash *et al.,* (2013), Baoqian *et al.,* (2011), Siebert *et al.,* (2009), Kranthi *et al.,* (2005) revealed that Cry toxin expression was found to be highest levels in unfurled leaves, moderate levels in white flowers and squares and lowest levels in small bolls. Cry toxin levels were changed significantly as the season progressed (Akin *et al.,* 2004).

Effect of different plant part of *Bt* cotton hybrids of different events on adult emergence of *E. vittella*

Squares

The adult emergence of first instar larvae of *E. vittella* was not recorded due to the death of first instar larvae fed on squares of *Bt* cotton hybrids of different events before emergence of adults. Maximum adult emergence (96.67 per cent) was registered in first instar larvae when fed on squares of NCEH-34 non-*Bt* cotton hybrid (Table10). The second instar larvae fed on squares of NCEH-34 BG-II exhibited significantly lowest adult emergence to the tune of 6.67 per cent followed by RCH-799 BG-II (16.07 per cent), YRCH-31 BG-II (18.00 per cent) and JKCH-1947 BG-I (20.00 per cent). Significantly minimum adult emergence of third instar larvae was observed on squares of NCEH-34 BG-II (20.00 per cent) followed by RCH-799 BG-II (26.17 per cent), YRCH-31 BG-II (30.00 per cent) and JKCH-1947 BG-I (36.00 per cent). The cent per cent adult emergence was observed when fourth and fifth instar larvae of *E. vittella* fed on squares of *Bt* cotton hybrids of different events.

Among all the *Bt* cotton hybrids of different events, BG-II cotton hybrids expressed superior results over BG-I. The first, second and third instar larvae of *E. vittella* when fed on squares of NCEH-34 BG-II registered lowest adult emergence. The per cent adult emergence varied with the instars. The gradual increase in per cent adult emergence of *E. vittella* was recorded in II and III instar larvae fed on squares of *Bt* cotton hybrids of different events.

Bolls

The adult emergence of first instar larvae of *E. vittella* was not recorded due to the death of first instar larvae fed on bolls of *Bt* cotton hybrids of different events before emergence of adults. The adult emergence of second instar larvae of *E. vittella* varied from 16.67 to 26.67 per cent on bolls of *Bt* cotton hybrids of different events. The second instar larvae fed on bolls of NCEH-34 BG-II exhibited significantly lowest adult emergence to the tune of 16.67 per cent followed by RCH-799 BG-II (20.00 per cent), YRCH-31 BG-II (23.33 per cent) and JKCH-1947 BG-I (26.67 per cent) (Table11). The adult emergence of third instar larvae of *E. vittella* ranged from 73.33 to 100.00 per cent on bolls of *Bt* cotton hybrids of different events. The significantly lowest adult emergence (73.33 per cent) was observed when third instar larvae fed on bolls of NCEH-34 BG-II.

However, RCH-799 BG-II, YRCH-31 BG-II and JKCH-1947 BG-I evidenced non-significant difference with NCEH-34 non-*Bt* in recording adult emergence. Cent per cent adult emergence was exhibited when fourth and fifth instar larvae of *E. vittella* fed on bolls of *Bt* cotton hybrids of different events.

Among all the *Bt* cotton hybrids of different events, BG-II cotton hybrids expressed superior results over BG-I. The first, second and third instar larvae of *E. vittella* when fed on bolls of NCEH-34 BG-II registered lowest adult emergence. The per cent adult emergence varied with the instars. The gradual increase in per cent adult emergence of *E. vittella* was recorded in II and III instar larvae fed on bolls of *Bt* cotton hybrids of different events.

Similar trend in results were observed by Mahalakshmi and Prasad (2013) who documented that later instar larva of *H. armigera* when exposed continuously to *Bt* cotton plant parts showed reduction in adult emergence and formation of malformed adults. Analogously, Naik *et al.,* (2012) revealed that exposure of later instar larvae of *H. armigera* to plant parts of *Bt* event hybrids exhibited adverse effects on adult emergence. Srinivasa Rao and Arjuna Rao (2008) observed that late instar (third, fourth and fifth instar) larvae of *H. armigera* could survive and successfully develop into adults even on *Bt* cotton but in less proportion.

Jayaprakash *et al.,* (2013), Baoqian *et al.,* (2011), Siebert *et al.,* (2009), Kranthi *et al.,* (2005) revealed that Cry toxin expression was found to be highest levels in unfurled leaves, moderate levels in white flowers and squares and lowest levels in small bolls. Cry toxin levels were changed significantly as the season progressed (Akin *et al.,* 2004).Thus the present findings are in line with these findings.

Growth and survival indices of *E. vittella* reared on different plant part of *Bt* cotton hybrids of different events

Growth indices

The data on growth indices of *E. vittella* reared on squares and bolls of *Bt* cotton hybrids of different events evidenced that minimum growth index was observed on all *Bt* cotton hybrids of different events under investigation compared to non-*Bt* cotton hybrid (Table 12). The growth index values for *E. vittella* larvae reared on squares of *Bt* cotton hybrids of different events were ranged from 2.63 to 4.20. Minimum growth index value for *E. vittella* larvae was registered on NCEH-34 BG-II (2.63) followed by RCH-799 BG-II (3.33), YRCH-31 BG-II (3.57) and JKCH-1947 BG-I (4.20).

The highest growth index value to the extent of 8.16 was exhibited for *E. vittella* larvae reared on squares of NCEH-34 non-*Bt*. The growth index values for *E. vittella* larvae reared on bolls of *Bt* cotton hybrids of different events were ranged from 4.5 to 7.6. The lowest growth index value for *E. vittella* larvae was observed on NCEH-34 BG-II (4.5) followed by RCH-799 BG-II (7.7), YRCH-31 BG-II (6.8) and JKCH-1947 BG-I (7.6). However, NCEH-34 non-*Bt* exhibited highest growth index value (9.0) for *E. vittella* larvae reared on bolls.

Survival indices

The data on survival indices of *E. vittella* reared on squares and bolls of *Bt* cotton hybrids of different events evidenced that lowest survival index was recorded on all *Bt* cotton hybrids of different events under investigation compared to non-*Bt* cotton hybrid (Table13). Survival index values for *E. vittella* larvae reared on squares of *Bt* cotton hybrids of different events were ranged from 0.15 to 0.17. The minimum survival index value for *E. vittella* larvae was registered on NCEH-34 BG-II (0.15) followed by RCH-799 BG-II (0.16), YRCH-31 BG-II (0.16) and JKCH-1947 BG-I (0.17). However, the highest survival index value to the extent of 0.33 was exhibited for *E. vittella* larvae reared on squares of NCEH-34 non-*Bt*.

The survival index values for *E. vittella* larvae reared on bolls of *Bt* cotton hybrids of different events were ranged from 0.19 to 0.22. The lowest survival index value for *E. vittella* larvae was observed on NCEH-34 BG-II (0.19) followed by RCH-799 BG-II (0.21), YRCH-31 BG-II (0.21) and JKCH-1947 BG-I (0.22). However, NCEH-34 non-*Bt* exhibited highest survival index value (0.34) for *E. vittella* larvae reared on bolls.

The growth and survival indices for *E. vittella* were very low when reared on squares and bolls *Bt* cotton hybrids of different events compared to non-*Bt* cotton hybrid. Both the growth index and survival index were high for the larvae reared on bolls followed by squares. The low growth and survival indices can be attributed to longer developmental period, low per cent pupation and less adult emergence on *Bt* hybrids.

More or less analogous results were documented by Mahalakshmi and Prasad (2013) who revealed that low growth and survival indices of *H. armigera* was observed on *Bt* cotton hybrids compared to their corresponding non *Bt* hybrids and check hybrid. Analogously, Naik *et al.,* (2012) revealed that the growth index values were low for the larvae reared on leaves compared to those reared on squares of *Bt* cotton hybrids. Vennila *et al.,* (2006) noticed that slow growth rate induced by the action of *Bt* insecticidal protein led to more number of days to mortality in *H. armigera* over non-*Bt* cotton. Survival index for *H. armigera* on *Bt* and non-*Bt* cotton was 20 and 47.7 per cent and 84.6 and 82.3 per cent, respectively.

Stewart *et al.,* (2001) revealed that survival and growth of bollworm and tobacco budworm was reduced by *Bt* cotton, particularly the dual-toxin cultivar. Gore *et al.,* (2001) revealed that bollworm survival was higher on square and flower anthers than on other floral structures. Survival was lower on all structures of bollgard II than on corresponding structures of bollgard and conventional cotton.

References

Ahmed, S., Iqbal, M.K., Shahid, M and Khan, R. 2012. Comparison of antibiosis of spotted bollworm, *Earias vittella* (Fab.), on two *Bt* and one non *Bt* cotton varieties. *Pakistan. J. Zool.*, 44 (2): 463-468.

Akin, D.S., Layton, M.B., Stewart, S.D and Adamczyk, J. 2004. Temporal expression profiles and bioactivity of single (Bollguard) and dual toxin (Bollguard II) *Bt* cotton.Belt-wide cotton conferences, San Antonio, TX, Jan. 5-9 2004: 1422-1429.

Anonymous, 2015. Annual Report of AICRPC on cotton 2014-15. CICR, Coimbatore, India.

Arshad, M., A. Suhail, M.J. Arif and M.A. Khan. 2009. Transgenic-*Bt* and non-transgenic cotton effects on survival and growth of *Helicoverpa armigera*.

Int. J. Agric. Biol., 11: 473-476.

Baoqian, L., Sharon, D., Lewis, W., Peter, G., Kristen, K., Greg, K and Bruce, M. 2011. Preferences of field bollworm larvae for cotton plant structures: Impact of *Bt* and history of survival on *Bt* crops. *Entomologia Experimentalis et Applicata,* 140: 17-27.

Bhullar, H.S. and Gill, R.S.2015. Cry toxin expression on different plant parts and crop stages among transgenic *Bt* cotton hybrids. *Pesticide Res. J.*, 27 (2): 155-159.

Bhullar, H.S. and Gill, R.S.2015. Cry toxin expression on different plant parts and crop stages among transgenic *Bt* cotton hybrids. *Pesticide Res. J.*, 27 (2): 155-159.

Channakeshava, R and Patil, B.V. 2009. Seasonal incidence and management of bollworm complex in *Bt* cotton under irrigated ecosystem. *Ann. Pl. Prot. Sci.,* 17 (2): 275-278

Dhawan, A. K., Sidhu, A. S., & Simwat, G. S. 1988. Assessment of avoidable loss in cotton (*Gossypium hirsutum* and *G. arboreum*) due to sucking pests and bollworms. *Ind. J. Agri* Sci, 58: 290–292.

Gomez, K.A., and Gomez, A.A. 1984. Statistical procedures for agricultural and research (2 Edn.). A John Wiley and Sons Intersciences Publications. An International Res. Institute, Philippines: 680.

Gore, J., Leonard, B.R., and Adamczyk J.J. 2001. Bollworm (Lepidoptera: Noctuidae) survival on Bollgard and Bollgard II cotton flower bud and flower components. *J. Econ. Entomol*, 94 (6): 1445-1451.

Jayaprakash S.A., Mohan, S. and Kannan, M. 2013. Temporal and Spatial variation in susceptibility of *Helicoverpa armigera* (Hubner) populations to Bollgard II *Bt* Cotton in Tamil Nadu. *Inter. Tropical Agri. Res*., 24 (3): 249-257.

Kranthi, K. R., Naidu, S., Dharwad, C. S., Tatwawadi, A., Mate, K., Patil, E., Bharose, A. A., Behera, G. T., Wadaskar, R. M. and Kranthi, S. 2005. Temporal and intra plant variability of Cry 1Ac expressing in *Bt* cotton and its influence on the survival of cotton bollworm, *Helicoverpa armigera* (Noctuidae: Lepidoptera). *Current Science,* 89: 291-298.

Kranthi, K.R., 2017. Unlearn a few and learn some new (Part-1). *Cotton Statistics and News*, 43:1-4.

Kranthi. S., K. Kranthi R. and Lavhe, N.V. 2000. Baseline toxicity of Cry 1A toxins to the spotted bollworm, *Earias vittella*. *Crop. Prot.,* 18 : 551-555.

Liu, Laipan, Meijing Gao, Song Yang, Shaoyan Liu, Yidong Wu, Yves Carrière, and Yihua Yang. 2017. Resistance to *Bacillus thuringiensis* toxin Cry 2Ab and survival on single-toxin and pyramided cotton in cotton bollworm from China. *Evolutionary Applications*, 10: 170-179 (DOI: 10.1111 /eva. 12438).

Mahalakshmi, M.S, and Prasad, N.V.V.S.D. 2013. Survival and development of American Bollworm *(Helicoverpa armigera)* on transgenic *Bt* cotton. *Indian J. Agric. Res.*, 48 (3): 177-184.

Mann, R.S., Gill, R.S., Dhawan, A.K. and Shera, P.C. 2010. Relative abundance and damage by target and non-target insects on Bollgard and BollgardII cotton cultivars. *Crop Prot*., 29: 44-45.

Men, X.Y., Ge, F., Yardim, E.N., and Parajulee, M.N. 2005. Behavioural response of *Helicoverpa armigera* (Lepidoptera: Noctuidae) to cotton with and without expression of the Cry l Ac δ-endotoxin Protein of *Bacillus* *thuringiensis* Berliner. *J. Insect Behavior*, 18 (1): 33-50.

Naik, V. C., Prasad, NVVSD, Rao, Ramachandra G., Raghavaiah, G. and Kranthi S. 2012. Survival and development of American bollworm, *Helicoverpa armigera* on *Bt* cotton hybrids of different *Bt* events. *Indian J. Pl. Prot*., 41 (1): 16-24.

Navarro, M. J., & Hautea, R. A. 2014. Adoption and uptake pathways of GM/Biotech crops by small-scale, resource-poor farmers in China, India, and the Philippines. Ithaca: ISAAA Brief No. 48. ISAAA.

Saini, M.K., and Dhawan, A.K. 2013. Variability of delta-endotoxin expression in dual and single-toxin cotton genotypes and on survival of *Helicoverpa armigera* and *Spodoptera litura*. *Ann. Pl. Prot, Sci.*, 21: 1-8.

Shera, P. S. 2009. Extent of avoidable losses due to spotted bollworms (*Earias spp)* in Asiatic cotton *Gossypium arboreum* L. *Pest Manag and Eco Zoology*, 17: 101–104.

Shera, P. S. and Arora, Ramesh. 2016a. Survival and development of spotted bollworm, *Earias vittella* (Fabricius) (Lepidoptera: Nolidae) on different transgenic *Bt* and isogenic non-*Bt* cotton genotypes. *Phytoparasitica*, 44: 99-113.

Shera, P.S. and Arora, Ramesh. 2016b. Comparative study on oviposition and larval preference of spotted bollworm, *Earias vittella* on *Bt* and non-*Bt* cotton. *J. Environ. Biol*., 37:121-127.

Shera, P.S., Arora, R., and Singh, P. 2015. Comparative susceptibility of transgenic *Bt* cotton hybrids to *Earias spp*. and other non-target insects. *Crop Prot*., 71: 51-59.

Siebert, M. Willrich, T.G. Patterson, G.J. Gilles, S.P. Nolting, L.B. Braxton, B.R. Leonard, J.W. Van Duyn and R.B. Lassiter. 2009. Quantification of Cry 1Ac and Cry 1F *Bacillus thuringiensis* insecticidal proteins in selected transgenic cotton plant tissue types. *J. Econ. Entomol*, 102 (3): 1301-1308.

Srinivasa Rao, N and Arjuna Rao, P. 2008. Behavioural and physiological effects of *Bt* cotton bollworm, *Helicoverpa armigera* (Hub.). *J. Entomol. Res*., 32 (4): 273-277.

Stewart, S. D, Adamczyk, J. J, Knighten, K. S and Davis, F. M. 2001. Impact of *Bt* cottons expressing one or two insecticidal proteins of *Bacillus thuringiensis* on growth and survival of Noctuid larvae. *J. Econ. Entomol*, 94:752-760.

Udikeri, S.S. 2006. Evaluation of new generation *Bt* cotton genotypes, sustainability of Cry protein expression, computation of ETL, effect on aphid predators and development of IPM module for *Bt* Cotton under rainfed conditions. Ph.D. Thesis, University of Agricultural Science, Dharwad, Karnataka, India.

Vennila, S., Panchbhai, P.R and Biradar, V.K. 2006. Growth and survival of *Helicoverpa armigera* (Hubner) and *Spodoptera litura* (Fab.) on transgenic *Bt* cotton. *J. Cotton Res. Dev.*, 20 (1): 131-133.