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Original Research Article

Survival and Development of American Bollworm *H. armigera* on *Bt* Cotton Hybrids of Different Events

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A B S T R A C T

Investigations were carried out to study the survival and development of American bollworm *H. armigera* on *Bt* cotton events with their corresponding one non *Bt* cotton event. During the period of study bioassay was conducted on *Bt* cotton plant parts i.e., leaves, squares and bolls by using different larval instars of *H. armigera*. Results of bio assay studies revealed that mortality percentage was high in early instar larva than late instars and in leaves mortality levels (percentage) was high followed by squares and followed by bolls.in those survived larva of late instars whom survived up to pupation, we observed larval deformities, reduction in pupal weights, pupal malformations, adults with uneven developed body parts, pale coloured body, small size, brittle textured body than those of whom developed from non *Bt* cotton events. NCEH BG-II cotton hybrid resulted well in comparison with remaining hybrids.

Keywords

Transgenic cotton, Bt events, American bollworm, Survival and development

Introduction

Cotton is leading plant fiber crop grown commercially in more than 34 countries across the globe in more than 50,000 hectares. India ranked first in terms of cultivated area (12.7 million ha), occupying over a quarter of the world cotton area, followed by China, USA, and Pakistan. India has the largest share of 36-38 per cent in the total global cotton acreage (Kranthi, 2017) and contributes to 26 per cent of the global cotton produce, currently ranking first in the world. On a global scale, the cost of managing the bollworm plague and its losses in cotton production accounted for more than US$ 5 billion every year. Fifty per cent of pesticides in India are used to control bollworm damage (Crop Life, 2017).

The genes express insecticidal proteins in the plant parts and are generally referred as Cry (crystal) proteins which are toxic to leaf-eating caterpillar pests, more specifically to the three species of cotton bollworms. '*Bt*-cotton' event Mon-531 (cry 1Ac gene) was first approved by the Genetic Engineering Approval Committee (GEAC), Ministry of Environment, for commercial cultivation in India on 26 April 2002. Subsequently in 2006, three new *Bt*-cotton GM events, namely MON-15985 (Bollgard II®, cry 1Ac + cry 2Ab2 genes), event-1 (cry 1Ac gene) of JK seeds and GFM event (fusion gene with cry 1Ab + cry 1Ac sequences) of Nath seeds were approved for commercial cultivation. *Bt*-cotton event

BNLA-601 of UAS Dharwad was approved in 2008 and event MLS-9124 of Meta-Helix Life Sciences was approved in 2009. So far six *Bt* cotton events have been approved for commercial cultivation in India and are being marketed by 49 Indian seed companies under license agreements from Monsanto. Though six different *Bt*-cotton events have been approved thus far in India, currently more than 95 per cent of the cotton area in the country is covered by only Monsanto's two-gene (cry 1Ac + cry 2Ab2) *Bt* event called Mon-15985 (Kranthi, 2016). However, recently many studies proved 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). For the *Bt* cotton to be sustainable, 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). However, studies conducted in India and USA has indicated that the Cry toxin in *Bt* cotton fluctuates during the season (Kranthi *et al.*, 2005 and Greenplate *et al.*, 2001). The first generation transgenic cotton known as Bollgard-I expressed Cry I Ac toxin, the titer of which decline with plant age and often permitted late season survival of larvae (Fitt, 1998). 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 American bollworm *H. armigera* on *Bt* cotton hybrids of different events

Materials and Methods

The legendary *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 American bollworm *H. armigera* 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 2016-17. Package of practices were followed according the recommendations given by V.N.M.K.V Parbhani.

Maintenance of stock culture

The initial cultures of *Helicoverpa armigera* are developed by collecting large number of larvae from the surrounding fields at Post Graduate Laboratory. 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.

Survival and development studies

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 leaves, 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 leaves, squares and bolls of different cotton hybrids were collected randomly in labeled plastic bags at pre-determined interval of 60-80, 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 leaves, squares and bolls were placed individually in a plastic vial. 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 *H. armigera* larvae were studied individually by exposing to different cotton structures *viz*., leaves, squares and bolls of different cotton hybrids at 60-80, 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 *H. armigera*, 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

Effect of different plant parts of *Bt* cotton hybrids of different events on larval mortality of *H. armigera*

Results revealed that none of the first instar of *H.armigera* survived when fed with plant parts of leaves, squares and bolls. Second instar larval mortality was high in leaves in comparison with squares and bolls and it was observed in NCEH-34BGII cotton hybrid with 86.67% which got reduced to 80.00% in squares and 76.67% in bolls (Table 2, 3, 4). Mortality of third and fourth instar larva was also high in leaves then followed by squares and followed by bolls. Fifth instar larva of *H.armigera* survived on all plant parts viz., leaves, squares and bolls of *Bt* cotton hybrids with zero traces of mortality. Mortality percentage of *H. armigera* larva varied with the age of the crop. It was highest in leaves followed by squares and bolls. This clearly indicates that Cry toxin expression varies with the age and plant parts of the crop respectively. Our findings are in close conformity with the findings of Liu *et al.,* (2017) who reported that the bollworm survival 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. 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. Whereas, Knight *et al.,* (2016) elucidated that *H. punctigera* larvae registered close to 100 per cent mortality on Bollgard II cotton throughout the season however, *H. armigera* mortality was close to 100 per cent early in the season, but fell to 65 per cent by mid to late February in the laboratory bioassays. Rosalia *et al.,* (2015) indicated that mortality of *H. armigera* was 100 per cent in all six instars, indicated that the *Bt* soybean expressing the Cry 1Ac protein provided efficient control against all six larval instars. While, Mahalakshmi and Prasad (2013) noticed that among the plant parts tested, mortality of *H. armigera* larvae was higher on leaves compared to squares of both the *Bt* cotton hybrids. The mortality of early larval instars was higher compared to older instar larvae on both leaves and squares of *Bt* hybrids. Similarly, Jayaprakash *et al.,* (2013) revealed that *Bt* cotton leaves expressed higher toxicity than squares and bolls. The toxicity was higher at 60-day-old plant parts, followed by 90, 120 and 150-day-old.Analogously, Naik *et al.,* (2012) revealed that the mortality of early larval instars of *H. armigera* fed on leaves and squares of all *Bt* hybrids of different events was higher than the later instars. The highest Cry protein toxin was observed at 60 DAS with a progressive decrease at 90 DAS and 120 DAS and negligible levels at 150 DAS during both the seasons. Singh *et al.,* (2011) indicated that the expression of Cry toxin in RCH 134 *Bt* cotton hybrid was sufficient till 151 DAS to kill even five larvae of *H. armigera* released per plant. Hallad *et al.,* (2011) documented highest mortality in second instar larvae of *H. armigera* (93.1 and 79.2 per cent) at 80 and 110 DAS, respectively in Tulasi-4 BG-II (MON-15985) however, the mortality of third and fourth instar was 91.10 and 87.10 per cent at 80 DAS, respectively. Gujar *et al.,* (2011) revealed that the *Bt*-F1 cotton hybrids were superior over *Bt*-F2 and non-*Bt* cotton hybrids with zero per cent *H. armigera* survival. Baoqian *et al.,* (2011) revealed that Cry 1Ac and Cry 2Ab expression varied among cotton structures,

but there were no significant differences between the two Bollgard II varieties. The Cry 1 Ac and Cry 2Ab toxin expression was found to be highest levels in unfurled leaves, moderate levels in white flowers and squares and lowest levels in small bolls. Somashekara (2009) documented that average larval mortality of *H. armigera* was 98.53 and 98.08 per cent in RCH-2 and Bunny BG-II as compared to RCH-2 *Bt* and Bunny *Bt* (95.13 and 95.40 per cent) from 60 to 120 DAS. Siebert *et al.,* (2009) reported that Cry 1Ac expression in cotton bolls was lowest compared with terminal leaves, squares, flowers and mature leaves. Arshad *et al.,* (2009) indicated significantly higher mortality (100 per cent) in neonates fed on *Bt* cotton leaves than those fed on *Bt* flower-bolls (93 per cent). SrinivasaRao and ArjunaRao (2008) observed that all the first and second instar bollworm larvae fed with *Bt* cotton flower buds died before pupation. Vennila *et al.,* (2006) noticed that larval mortality of *H. armigera* on *Bt* and non-*Bt* cotton was 58.7 and 43.5 per cent, respectively.

Effect of different plant parts of *Bt* cotton hybrids of different events on larval weight of *H. armigera*

Mean weight of first instar larva was not recorded due to mortality observed in *Bt* cotton hybrids. Results on mean weight of second instar larva revealed that minimum weight was recorded on NCEH BG II in comparison with remaining *Bt*cotton hybrids. Minimum weight recorded on second instar larva was 14.80, 20.23, 27.33 mg/larva on leaves respectively after 24, 48 and 72 hrs after feeding which was low in comparison with squares and bolls which were 18.50, 19.17, 21.67 and 20.70, 21.17, 23.20 mg/larva respectively after 24,48 and 72 hrs after feeding (Table 5, 6, 7). Same trend of increase in mean larval weight (mg/larva) was observed in third instar and fourth instar of *H. armigera* in comparison of leaves to squares and squares to bolls. Our resultsare in accordance with the findings of 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. However, 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, SrinivasaRao and ArjunaRao (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 *H. armigera*

Pupation percentage was zero percent in all *Bt* cotton hybrids in first instar larva on

leaves, squares and bolls i.e., none of the first instar larva survived up to pupation. Percent pupation in second instar larva was minimum in NCEH BG-II cotton hybrid (13.33%) which was low in contrast to squares and bolls (Table 8, 9, 10). We observed minimum of 20.00 and 56.67 per cent pupation in third instar larva when fed with leaves and squares in which 100 per cent pupation percentage was observed in third instar larva when fed with bolls. we observed minimum percentage of pupation of 63.33% in fourth instar larva whose fed on leaves, in which it was 100 per cent in the same fourth instar larva whose were fed with squares and bolls i.e., 100 per cent pupation was observed in fourth larva. We observed 100% pupation in fifth instar larva which was fed with plant parts of leaves, squares and bolls. Our results are inare parallel to 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. SrinivasaRao and ArjunaRao (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. It was found that in leaves percentage of pupation was less in comparison with squares and bolls.

Effect of different plant parts of *Bt* cotton hybrids of different events on pupal weight of *H. armigera*

Minimum pupal weight was recorded in *Bt* cotton varieties whereas maximum pupal weight was recorded on non *Bt* cotton variety. Over all minimum weight was observed in NCEH BG –II variety (Table 11, 12, 13). In comparison of leaves, squares and bolls it was observed that minimum pupal weight was observed on leaves followed by squares and bolls. Our findings coincide 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 Cry1A toxins differed significantly among *Bt* cultivars and plant structures, but the interaction between these

factors was not significant. 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.

While, 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 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. SrinivasaRao and ArjunaRao (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 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**)**. Those pupa that were reared on *Bt* events resulted with less weight, malformations, lesser in size than pupa that were reared on non *Bt* events.

Effect of different plant part of *Bt* cotton hybrids of different events on adult emergence of *H. armigera*

We observed 100 per cent adult emergence in fifth instar larva of *H. armigera* on leaves, squares and bolls. As we observed that fifth instar larva had successfully survived with zero traces of mortality on all plant parts i.e., leaves, squares and bolls. Further we observed 100 per cent adult emergence from fourth instar larva which were fed with squares and bolls(Table 14,15,16). Those emerged adults from *Bt* events were identified with several deformities mainly sick appearance, small wings, pale coloured body. our results supported by the work of Liu *et al.*, (2017) who reported that the bollworm survival 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. 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. While, Mahalakshmi and Prasad (2013) 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 the growth and development such as reduction in adult emergence. SrinivasaRao and ArjunaRao (2008) observed that all the first and second instar bollworm larvae fed with *Bt* cotton flower buds died before pupation. However, the 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).In keeping view of the above obtained results we observed that the events which we tested were giving protection to *H. armigera* up to maximum third instar only, fourth and fifth instar larva of *H. armigera* were happily feeding on *Bt* cotton.

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

Low growth and survival attributes were recorded on leaves followed by squares followed by bolls(Table17,18). Our findings are in close agreement with the findings of 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.

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