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Survival and Development of Spotted Bollworm *E. vittella*on different *Bt* Cotton Hybrids

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Abstract

Bt-cotton contains one or more genes derived from the soil bacterium *Bacillus thuringiensis* and introduced into the cotton genome through genetic modification (GM). 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. The current study aimed to study the survival and development of Spotted Bollworm *E. vittella*on different *Bt* cotton hybrids. This study was done at the Post Graduate Laboratory, Department of Agricultural Entomology, College of Agriculture, Latur, Maharashtra-India during 2018-19. During the period of study bioassay was conducted on *Bt*cotton plant parts i.e., squares and bolls by using different larval instars of *E. vittella* by feeding them on squares and bolls of six BG-II cotton hybrids (AJEET-155, BHAKTI NCS-245, JAY, YUVA 7215-2, RCH-2, Dr. BRENT MRC-7347) and one non-*Bt*cotton hybrid (NHH-44)as a control. Results of bio assay studies revealed that the mortality of early larval instars of *E. vittella* fed on squares and bolls of different *Bt* cotton hybrids was higher than the later larval instars. Exposure of later instar larvae to different plant parts of different *Bt* cotton hybrids also showed adverse effects on the growth and development such as reduced larval weights, prolonged larval developmental period, reduced pupation, formation of small pupae with less weight, reduction in adult emergence with low growth and survival indices for *E. vittella*. The growth and survival index values were also low for the larvae reared on squares compared to those reared on bolls of different *Bt* cotton hybrids.

Key words : *Transgenic cotton, spotted bollworm, survival and development, genotype.*

Introduction

Cotton is an international crop grown commercially in more than 80 countries on 33.19 million hectares area with 25.88 million tonnes of production and 780 kg per ha average productivity across the globe (USDA, 2019). In India cotton was cultivated on 12.25 million ha area with 5.66 million tonnes of production and 462 kg per ha of average productivity during 2018-19 (USDA, 2019). Maharashtra is leading cotton producing state which cultivates cotton in about 4.12 million ha area with 1.38 million tonnes of production having average productivity of 334 kg per ha during 2018-19 (CAB, 2019).

The yield of cotton mainly depends on weather, pests, diseases and management practices. Unfortunately, cotton is damaged by more than 160 species of insect-pests right from emergence till the final picking (Manjunath, 2004). Among which bollworms *viz*., *Helicoverpa armigera* (Hubner), the green bollworm; *Eariasvittella* (Fabricius), the spotted bollworm; *Eariasinsulana* (Biosdual), the spiny bollworm and *Pectinophoragossypiella* (Saunders), the pink bollworm infested cotton (Bhamare and Wadnerkar, 2018) and inflicted 30-80 per cent yield losses (Kranthi *et al*., 2009). Even after the introduction of bollworm-resistant *Bt* cotton, which now covers more than 95 per cent of area under cotton, losses caused by insect-pests have been estimated at a whopping 30 per cent (Dhaliwal *et al*., 2010 and 2015). The 87.43 per cent avoidable yield losses were estimated due to bollworm complex in *Bt* cotton (Jadhav *et al*., 2019). Worldwide more than US $ 5 billion per annum accounted for managing the bollworm outbreak and its losses (Crop Life, 2017).

In India so far six *Bt* cotton events have been approved for commercial cultivation, however currently more than 95 per cent of the cotton area is covered by only Monsanto's two-gene (Cry 1Ac + Cry 2Ab) *Bt* event called Mon-15985 (Kranthi, 2016). Under this background, in view of the availability of various transgenic cotton hybrids for farmers and the variability in the performance of the Cry 1Ac and Cry 2Ab toxins among the plant parts and different growth stages of crops, the present investigation was planned to study the survival and development of bollworms on different *Bt* cotton hybrids. In India insect resistant (*Bt*) technology in cotton hybrids delivered broad based benefits by saving losses caused by bollworm and boosted cotton yield to 500 kg lint per hectare (ISAAA, 2017). However, recently many studies exhibited bollworm survive and develop on elite *Bt* cotton hybrids (Mahalakshmi and Prasad, 2013, Naik *et al*., 2012, Soujanya *et al*. 2010, Naik *et al*., 2014, Kranthi, 2016, Shera and Arora, 2016a,Likhitha and Bhamare, 2018a&b and; Rupnar, 2018). Moreover, severe damage to bolls by *P. gossypiella* and yield-losses

Table-1 : Growth indices of *E. vittella* reared on different plant part of *Bt* cotton hybrids.

Treatments	Growth Index		
	Squares (90 days old crop)	Bolls (120 days old crop)	Bolls (150 days old crop)
AJEET-155 BG-II	3.89	3.88	6.85
BHAKTI NCS-245 BG-II	4.14	4.27	7.84
JAY BG-II	3.55	3.37	6.30
YUVA 7215-2 BG-II	3.64	3.90	5.00
Dr. BRENT MRC-7347 BG-II	3.33	3.65	5.63
RCH-2 BG-II	3.37	4.17	7.17
NHH-44 Non-Bt	10.63	10.86	11.90

Table-2 : Survival indices of *E. vittella* reared on different plant part of different *Bt* cotton hybrids.

Treatments	Survival Index		
	Squares (90 days old crop)	Bolls (120 days old crop)	Bolls (150 days old crop)
AJEET-155 BG-II	0.53	0.53	0.70
BHAKTI NCS-245 BG-II	0.56	0.60	0.76
JAY BG-II	0.48	0.46	0.68
YUVA 7215-2 BG-II	0.47	0.50	0.63
Dr. BRENT MRC-7347 BG-II	0.44	0.47	0.64
RCH-2 BG-II	0.45	0.56	0.72
NHH-44 non-Bt	1.00	1.00	1.00

were observed in *Bt*-cotton in many regions of Gujarat and some parts of AP, Telangana and Maharashtra during 2014. High *P. gossypiella* larval recovery on *Bt*-II in conjunction with high LC_{50} values for Cry1Ac and Cry2Ab in major cotton-growing districts of central and southern India provided evidence of field-evolved resistance in *P. gossypiella* to *Bt*-I and *Bt*-II cotton (Naik *et al*., 2018).

Materials and Methods

The popular *Bt* cotton hybrids having different crop durations (early, medium and late) were selected and cultivated on the Research Farm of Department of Agricultural Entomology and the laboratory investigations on survival and development of Spotted bollworm *E. vittella* on field collected *Bt* cotton plant structures or parts at pre-determined intervals were conducted at the Post Graduate Laboratory, Department of Agricultural Entomology, College of Agriculture, Latur (Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra)-India during 2018. The experiment was conducted with different larval instars by feeding them on squares and bolls of cotton hybrids.

The initial culture of bollworms *viz*., *Eariasvittella* Fabricius were developed by collecting large number of larvae from the cotton field.The collected larvae were reared individually in round plastic vials by feeding them on natural diet (flowers, squares and bolls of non-*Bt* cotton) every day till pupation. Pupae were transferred 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. The freshly emerged adults were released into standard oviposition cage covered with black muslin cloth. 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 stored in transparent plastic boxes. After hatching, neonate larvae were transferred separately into plastic vials to avoid cannibalism.

The experiment was carried out in completely randomized design (CRD) with three replications using ten larvae per replication with different larval instars by feeding them on squares and bolls of six BG-II cotton hybrids (AJEET-155, BHAKTI NCS-245, JAY, YUVA 7215-2, RCH-2, Dr. BRENT MRC-7347) and one non-*Bt* cotton hybrid (NHH-44)as a control. The cotton plant parts or structures *viz.,* squares and bolls of different cotton hybrids were collected randomly in labelled plastic bags at pre-determined interval of 90, 120 and 150 days old crop and 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 respective *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 larval mortality of *E. vittella* in all the instars was investigated individually by exposing them to different cotton plant structures *viz*., squares and bolls of different cotton hybrids at 90 and; 120 and 150 days old crop, respectively. The observation on weight of the surviving

larvae was recorded after 24, 48 and 72 h of exposure and the weight of pupae was also recorded from each treatment. In addition, other parameters *viz*., per cent pupation and adult emergence from the surviving larvae were observed. The growth and survival index were calculated for each bollworm on different treatments using the formulae given by Vennila *et al*. (2006).

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

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

The data in respect of survival and development of bollworms on *Bt* cotton hybrids of different events was statistically analyzed by standard 'analysis of variance'. The null hypothesis was tested by 'F' test of significance at 5 per cent level (Gomez and Gomez, 1984).

Results and Discussion

Effect of different plant parts of different *Bt* cotton hybrids on larval mortality of *E. vittella* : All the *Bt* cotton hybrids under investigation revealed superior results over NHH-44 non-*Bt* cotton hybrid. The mortality of *E. vittella* larvae was evidenced during first, second and third instars only, when fed on squares of different *Bt* cotton hybrids at 90 days old crop. The larvae of *E. vittella* fed on squares of Dr. BRENT MRC-7347 BG-II noticed maximum mortality. The per cent larval mortality varied with the instars. At 120 days old crop evidenced that the first, second and third instar larvae of *E. vittella* fed on bolls of JAY BG-II registered maximum mortality. The larval mortality of *E. vittella* was less on green bolls compared to squares. The mortality of *E. vittella* larvae was noticed during first and second instars only, when fed on bolls of different *Bt* cotton hybrids at 150 days old crop. The larvae of *E. vittella* fed on bolls of YUVA 7215-2 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.

The results on the per cent mortality of different larval instars of *E. vittella* fed on squares and bolls of different*Bt* cotton hybrids at pre-determined intervals are in harmony with the results Rupnar (2018) who revealed that the mortality of early larval instars of *E. vittella* fed on squares and bolls of *Bt* cotton hybrids of different event was higher than the later instars. Among the *Bt* cotton hybrids of different events, NCS-207 BG-II registered maximum larval mortality over rest of *Bt* cotton hybrids. Analogously, Likhitha and Bhamare (2018b) revealed that the mortality of early larval instars of *E. vittella* fed on squares and bolls of *Bt* cotton hybrids of different event was higher compared to the later instars. Hallad *et al*. (2014) indicated that the mortality of *E. vittella* was significant across genotypes or events at different days of interval. Tulasi 4 BG-II showed 100.0 per cent mortality followed by Tulasi 4 BG-I (99.4 per cent) and Nathbaba (99.3 per cent), the morality was decreased to 87.7 and 65.7 per cent in Tulasi 4 BG-II and Nathbaba at 135 DAS, respectively.

Effect of different plant parts of different *Bt* cotton hybrids on larval weight of *E. vittella* : The data on mean weight of *E. vittella* larval instars that survived beyond 24, 48 and 72 h after exposure on squares of different *Bt* cotton hybrids at 90 days old crop evidenced that minimum larval weight was recorded on all the *Bt* cotton hybrids under investigation compared to NHH-44 non-*Bt* cotton hybrid**.** The Dr. BRENT MRC-7347 BG-II hybrid was found less palatable to *E. vittella* as larval weight was very less compared to other *Bt* and NHH-44 non-*Bt* cotton hybrids. At 120 days old crop evidenced that minimum larval weight was recorded on all the *Bt* cotton hybrids under investigation compared to NHH-44 non-*Bt* cotton hybrid. The JAY BG-II cotton hybrid was revealed less palatable to *E. vittella* as larval weight was very less compared to other *Bt* and NHH-44 non-*Bt* cotton hybrid. At 150 days old crop evidenced that minimum larval weight was recorded on all the *Bt* cotton hybrids under investigation compared to NHH-44 non-*Bt* cotton hybrid. The YUVA 7215-2 BG-II hybrid was evidenced less palatable to *E. vittella* as larval weight was very less compared to other *Bt* and NHH-44 non-*Bt* cotton hybrid.

The results on effect of squares and bolls of different *Bt* cotton hybrids on larval weight of *E. vittella* are in close agreement with the findings of Rupnar (2018) who revealed that early instar larvae of *E. vittella* fed on squares and bolls of different *Bt* events hybrids evidenced diminution in larval weight. Among the *Bt* cotton hybrids of different events, NCS-207 BG-II found significantly superior over rest of *Bt* cotton hybrids. Analogously, Likhitha and Bhamare (2018b) reported that early instar larvae of *E. vittella* feeding on squares and bolls of *Bt* cotton hybrids of different events exhibited decrease in larval weight.More or less similar findings are reported by Naik *et al.* (2014) who observed that exposure of different instar larvae to plant parts of *Bt* event hybrids exhibited adverse effects on the growth and development of *P. gossypiella* with decline in larval weight. Analogously, Naik *et al*. (2012) revealed that exposure of later instar larvae of *H. armigera* to plant parts of *Bt* cotton hybrids of different events exhibited adverse effects on the larval weight. Soujanya *et al*. (2010) exhibited that the exposure of different instar larvae to plant parts of stacked *Bt* and *Bt* cotton cultivars exhibited adverse effects on the growth and development of *P. gossypiella* with decrease in larval weight.

Effect of different plant parts of different *Bt* cotton hybrids on pupation of *E. vittella* : All the *Bt* cotton hybrids under investigation showed better results over NHH-44 non-*Bt* cotton hybrid. The first, second and third instar larvae of *E. vittella* fed on squares of Dr. BRENT MRC-7347 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 I, II and III instar larvae fed on squares of different *Bt* cotton hybrids. All the *Bt* cotton hybrids under investigation demonstrated superior results over NHH-44 non-*Bt* cotton hybrid. The first, second and third instar larvae of *E. vittella* fed on bolls of JAY BG-II registered lowest pupation. The gradual increase in per cent pupation of *E. vittella* was recorded in I, II and III instar larvae fed on bolls of different *Bt* cotton hybrids. All the *Bt* cotton hybrids under investigation proved effective over NHH-44 non-*Bt*cotton hybrid. The first and second instar larvae of *E. vittella* fed on bolls of YUVA 7215-2 BG-II registered lowest pupation. The gradual increase in per cent pupation of *E. vittella* was recorded when I and II instar larvae fed on bolls of different *Bt* cotton hybrids.

The results on the per cent pupation of surviving larvae of different instars of *E. vittella* fed on squares and bolls of different *Bt* cotton hybrids at pre-determined intervals are comparable with the findings of Rupnar (2018) who revealed that early instar larvae of *E. vittella* fed on squares and bolls of *Bt*cotton hybrids of different events recorded significantly lowest pupation with malformed pupae compared to non-*Bt* cotton hybrid. Among the *Bt* cotton hybrids of different events, NCS-207 BG-II was found superior over rest of *Bt* cotton hybrids. Similarly, Likhitha and Bhamare (2018b) revealed that feeding of early instar larvae of *E. vittella* on squares and bolls of *Bt* cotton hybrids of different events recorded lowest pupation with malformed pupae. More or less similar results were documented by 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. Naik *et al*. (2012) revealed that exposure of later instar larvae to plant parts of *Bt* event hybrids observed least pupation with formation of small pupae having less weight.

Effect of different plant parts of different *Bt* cotton hybrids on pupal weight of *E. vittella* : The data on mean pupal weight of *E. vittella* reared on squares of different *Bt* cotton hybrids at 90 days old crop evidenced that minimum pupal weight was observed on all the *Bt* cotton hybrids under investigation compared to NHH-44 non-*Bt* cotton hybrid. At 120 days old crop evidenced that minimum pupal weight was recorded on all the *Bt* cotton hybrids under investigation compared to NHH-44 non-*Bt* cotton hybrid. At 150 days old crop evidenced that minimum pupal weight was recorded on all the *Bt* cotton hybrids under investigation compared to NHH-44 non-*Bt* cotton hybrid.

The results on mean pupal weight of *E. vittella* reared on squares and bolls different of *Bt* cotton hybrids at pre-determined interval are in accordance with the findings of Rupnar (2018) who revealed that early instar larvae of *E. vittella* fed on squares and bolls of *Bt* cotton hybrids of different events recorded significantly lowest pupal weight with malformed pupae compared to non-*Bt* cotton hybrid. Among the *Bt* cotton hybrids of different events, NCS-207 BG-II was found superior over rest of *Bt* cotton hybrids. Alike, Likhitha and Bhamare (2018b) noticed that rearing of early instar larvae of *E. vittella* on squares and bolls of *Bt* cotton hybrids of different events produced malformed pupae with lowest pupal weight. Naik *et al*. (2012) revealed that exposure of different instar larvae to plant parts of *Bt* event hybrids produced small pupae with lesser weight. Cry toxin levels were changed significantly as the season progressed (Akin *et al*., 2004).

Effect of different plant part of different *Bt*cotton hybrids on adult emergence of *E. vittella* : All the *Bt* cotton hybrids expressed superior results over NHH-44 non-*Bt* cotton hybrid. The first, second and third instar larvae of *E. vittella* fed on squares of Dr. BRENT MRC-7347 BG-II registered lowest adult emergence at 90 days old crop. The per cent adult emergence varied with the instars. The gradual increase in per cent adult emergence of *E. vittella* was recorded in I, II and III instar larvae fed on squares of different *Bt* cotton hybrids. All the *Bt* cotton hybrids under investigation expressed superior results over NHH-44 non-*Bt* cotton hybrid. The first, second and third instar larvae of *E. vittella* fed on bolls of JAY BG-II registered lowest adult emergence at 120 days old crop. The gradual increase in per cent adult emergence of *E. vittella* was recorded in I, II and III instar larvae fed on bolls of different *Bt* cotton hybrids. All the *Bt* cotton hybrids under investigation expressed superior results over NHH-44 non-*Bt* cotton hybrid. The first and second instar larvae of *E. vittella* fed on bolls of YUVA BG-II registered lowest adult emergence at 150 days old crop. The gradual increase in per cent adult emergence of *E. vittella* was recorded in I and II instar larvae fed on bolls of different *Bt* cotton hybrids.

The results on adult emergence of *E. vittella* fed on squares and bolls of different *Bt*cotton hybrids at pre-determined intervals are overlapped with findings of

Rupnar (2018) who evidenced that early instar larvae fed on squares and bolls of *Bt* cotton hybrids documented minimum adult emergence of *E. vittella*. Similarly, Likhitha and Bhamare (2018b) indicated that rearing of early instar larvae of *E. vittella* on squares and bolls of *Bt* cotton hybrids of different events recorded lowest adult emergence.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. 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 different *Bt* cotton hybrids : The growth and survival indices for *E. vittella* were very low when reared on squares and bolls on different *Bt* cotton hybrids compared to NHH-44 non-*Bt* cotton hybrid. Both growth index and survival index values were high for the larvae of *E. vittella* reared on bolls compared to squares. The low growth and survival indices can be attributed to longer developmental period, low per cent pupation and less adult emergence on *Bt* cotton hybrids. The results on growth and survival indices of different larval instars of *E. vittella* fed on squares and bolls of different *Bt* cotton hybrids at pre-determined interval are in concurrence with the findings of Rupnar (2018) who stated that the growth and survival index values for *E. vittella* were low for the larvae fed on squares compared to those fed on bolls of *Bt* cotton hybrids of different events. Similarly, Likhitha and Bhamare (2018b) claimed that the growth and survival indices for *E. vittella* were low for the larvae reared on squares compared to those reared on bolls of *Bt* cotton hybrids of different events.

Conclusion

From the present investigation it can be concluded that the mortality of early larval instars of *E. vittella* fed on squares and bolls of different *Bt* cotton hybrids was higher than the later larval instars. Exposure of later instar larvae to different plant parts of different *Bt* cotton hybrids also showed adverse effects on the growth and development such as reduced larval weights, prolonged larval developmental period, reduced pupation, formation of small pupae with less weight, reduction in adult emergence with low growth and survival indices for *E. vittella*. The growth and survival index values were also low for the larvae reared on squares compared to those reared on bolls of different *Bt* cotton hybrids.

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