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# Comparative survival and development of spotted bollworm, *Earias vittella* (Fabricius) on *Bt* and isogenic non *Bt* cotton genotypes under field cage conditions

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**ABSTRACT**: Survival and development of *Earias vittella* (Fabricius) was studied on four *Bt* cotton hybrids, one each from four different events, *viz.*, MRC 6301 *Bt* (*cry1Ac* gene), JKCH 1947 *Bt* (modified *cry1Ac* gene), NCEH 6R *Bt* (fusion *cry1Ac*/*cry1Ab* gene) and MRC 7017 BG II (*cry1Ac* and *cry2Ab* genes) along with their isogenic non-*Bt* genotypes at different crop ages under field cage conditions at the Cotton Research Farm, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Four plants per genotype were tagged and covered with portable screen cages in the field. Fifty neonates from the stock culture were placed gently using soft camel hair brush and were evenly distributed on each tagged plant with in cages each time on 90, 120 and 150 days old crop. The observations were recorded on larval survival, larval period, larval growth index, pupal survival, pupal period and survival index. All *Bt* cotton hybrids showed zero survival index indicating strong in-built resistance against *E. vittella* as compared to the isogenic non-*Bt* genotypes under field conditions. None of the neonates could manage to complete the larval period and reach pupal stage at any of the selected crop ages (90, 120 and 150 days old crop) signifying that toxin content was still sufficient to cause 100 per cent mortality of neonates even when released at later crop stages. Conversely, larval survival varied from 56.5 to 63.0 per cent on non-*Bt* genotypes. Among non-*Bt* genotypes, the survival index was higher on JKCH 1947 followed by NCEH 6R, MRC 7017 and MRC 6301.

**Key words:** *Bt* cotton, spotted bollworm, *Earias vittella,* survival and development

As many as 1326 species of insects have been recorded on cotton crop right from sowing to maturity in different cotton growing areas of the world and 162 species have been reported on the cotton crop in India, of which 24 species have attained pest status (Arora *et al.*, 2011). Among these, the bollworm complex comprising of American bollworm [*Helicoverpa armigera* (Hübner)], spotted bollworm [*Earias vittella* (Fabricius)], spiny bollworm [*E. insulana* (Boisduval)] and pink bollworm [*Pectinophora gossypiella* (Saunders)] are the key pests in Punjab (Dhawan *et al.*, 2012). The genus *Earias* (superfamily Noctuoidea; family Nolidae; subfamily Eariadinae) is widely distributed in the Old World and Australasia, and some species are pests in many of the cotton growing countries of Africa and Asia. The species attacking cotton include *E. vittella* and *E. insulana* in India, *E. biplaga* Walker in Africa, *E. huegeliana* Gaede in Australia and *E. cupreoviridis* Walker in China. The larvae cause damage by boring into growing shoots, buds, flowers and bolls. As soon as the terminal shoot of young cotton is bored, the growing tip loses its turgidity and droops. The larvae can cause excessive shedding of fruiting bodies and the circular holes produced by spotted and spiny bollworms in the fruiting bodies remain filled with excreta. The yield losses caused by *E. vittella* and *E. insulana*, and estimated by chemical control method, were 44 per cent in *G. hirsutum* and 48.2 per cent in *G. arboreum* (Shera, 2009) varieties.

Transgenic *Bt* cotton expressing genes coding for soil inhabiting spore forming bacterium, *Bacillus thuringiensis* Berliner (*Bt*) toxins offers the state of the art and yet simple to use technology for bollworm management. So

far, six transgene events, *viz.* MON 531 (*cry1Ac*), Event 1 (modified *cry1Ac*), GFM event (fusion *cry1Ab*/*cry1Ac*), BNLA 601 (*cry1Ac*), MLS 9124 (*cry1C* gene), MON 15985 (*cry1Ac* and *cry2Ab*) have been approved by Genetic Engineering Approval Committee (GEAC) in India (Choudhary and Gaur, 2011). Whilst substantial information has been generated on the susceptibility of American bollworm, *H. armigera* to *Bt* cotton in India (Kranthi *et al.*, 2009; Basavaraja *et al.*, 2011; Shera *et al.*, 2012), quantitative data are sparse on the relative efficacy of different transgenic *Bt* cotton against *E. vittella*. Most of the earlier studies on toxicity of *Bt* cotton hybrids to *E. vittella* have tracked the fate of larvae under laboratory conditions at the most up to seven days. The present studies were, therefore, conducted to know survival and development of *E. vittella* on *Bt* cotton hybrids along with the respective isogenic non-*Bt* genotypes at different crop ages under field cage conditions.

## MATERIALS AND METHODS

The study was conducted on four *Bt* cotton genotypes from private sector (Table 1), one each belonging to different events approved by GEAC in India in comparison to the respective isogenic non *Bt* genotypes under field cage conditions at the Cotton Research Farm, Department of Plant Breeding and Genetics, Punjab Agricultural University (PAU), Ludhiana.

The culture of *E. vittella* was maintained from the field collected larvae in Plant Growth Chamber at 27 ± 2$^0$C temperature and 70 ± 5 per cent relative humidity. The larvae were collected from cotton and okra grown fields and were reared in glass jars (10 x 15 cm) covered with muslin cloth on okra, *Abelmoschus esculentus* (L.) fruits, the most preferred natural food. Food was changed daily till the onset of pupation. The pupae were separated (B& and @&) on the basis of well developed knob like structure at the antero-dorsal end of male cocoon and placed in jars having moist sponge at bottom covered with filter paper. The freshly emerged male and female moths were paired and released into glass jars (15 x 20 cm) lined with muslin cloth for oviposition. A cotton-swab dipped in 10 per cent honey solution was hung from top of muslin cloth covering the mouth of the jar which provided food for the adults. The female moth laid eggs on the lined muslin cloth which was removed daily and replaced with new one to facilitate further oviposition. The oviposited muslin cloth was examined twice a day and neonates hatching from eggs were used for the present study.

The selected cotton genotypes were sown on 15$^{th}$ May 2011. The row to row spacing was 67.5 cm and plant to plant spacing was 75 cm. All agronomic practices were applied as per Punjab Agricultural University recommendations (Anonymous, 2011) except that no plant protection measures were used against bollworms throughout the cropping season. Four plants per genotype were tagged and covered with portable screen cages (1.5 m x 1.5 m x 1.5 m) in the field when crop was fifteen days old to exclude the natural population of bollworms and prevent the entry of any natural enemies. Fifty neonates from the stock culture were placed gently using soft camel hair brush and were evenly distributed on each tagged plant with in cages each time at 90, 120 and 150 days old crop. The fruiting bodies fallen on the ground were inspected daily two times in the morning and evening hours for the damage and presence of the larvae. The recovered larvae on these fallen fruiting bodies were again placed on the respective plant. Single plant of each genotype was considered as one replication. The observations were recorded on larval survival, larval period, pupal survival, pupal period. The larval growth index and survival index were worked out for each genotype.

Means, standard deviations (SD) and standard errors (SE) of different parameters were calculated using window MS excel functions. The

significance of differences were tested by F-tests, while the significance of differences between treatment means were compared using least significant difference at 5 per cent probability level. The data were transformed using appropriate transformations wherever necessary and subjected to statistical analysis (ANOVA) using CRD in CPCS1 programme.

## RESULTS AND DISCUSSION

**a) Ninety days old crop:** The data pertaining to survival and development parameters of E. *vittella* neonates released on 90 days old crop are presented in Table 2. The results showed cent per cent larval mortality as none of the larvae survived and completed their larval period to reach pupal stage in any of the *Bt* cotton hybrids. However, in isogenic non-*Bt* cotton genotypes, larval survival varied from 58.25 to 63 per cent. Among the latter, lowest survival was recorded in MRC 6301 (58.25 %) and it was at par with MRC 7017 (59.75 %) and NCEH 6R (60.75 %). The larval survival was significantly higher in JKCH 1947 (63%) which was *at par* with NCEH 6R. However, the mean larval period (in days) was significantly greater in MRC 6301 non *Bt* (10.06 ± 0.10) and it was *at par* with MRC 7017 non *Bt* (10.04 ± 0.10) and NCEH 6R non *Bt* (9.93 ± 0.10), whereas, the larval duration was lowest in JKCH 1947 non *Bt* (9.70 ± 0.15). The growth index was less (5.68) in MRC 6301 non-*Bt* followed by 5.95 in MRC 7017 non *Bt*, 6.11 in NCEH 6R non-*Bt* and 6.49 in JKCH 1947 non *Bt*.

The results on pupal survival and duration of *E. vittella* following release on 90 days old crop revealed that survival (adult emergence) varied from 75.25 to 76.25 per cent among different non *Bt* genotypes with non significant differences. Likewise, there was no significant variation with respect to pupal duration which varied from 7.84 ± 0.17 to 8.06 ± 0.11 days in different non *Bt* genotypes. The survival index was 0.0 in all *Bt* hybrids as compared to 0.44, 0.45, 0.46 and 0.48 in non *Bt* genotypes namely MRC 6301, MRC 7017, NCEH 6R and JKCH 1947, respectively (Table 2).

**b) One hundred and twenty days old crop:** On 120 days old crop also (Table 3), none of the neonates completed it larval duration and developed to pupa as cent per cent mortality was observed in all *Bt* cotton hybrids. Nevertheless, larval survival varied from 57.75 to 62.50 per cent in their isogenic non *Bt* genotypes. Among different non *Bt* hybrids, the lowest survival was recorded in MRC 6301 (57.75%) and it was *at par* with MRC 7017 (58.50%) and NCEH 6R (60%). Significantly higher larval survival was recorded in JKCH 1947 (62.50%). Conversely, the mean

**Table 1.** Details of *Bt* cotton along with the respective isogenic non-*Bt* genotypes

| Genotype | Event | Insect resistance gene(s) | Source |
|---|---|---|---|
| MRC 6301 *Bt* | Mon 531 | *cry1Ac* | Maharashtra Hybrid Seed Company, Jalna-431203 (Maharashtra) |
| MRC 6301 Non-*Bt* | - | - | |
| JKCH 1947 *Bt* | Event 1 | Modified *cry1Ac* | JK Agri Genetics Ltd., Hyderabad - 500016 (Andhra Pradesh) |
| JKCH 1947 Non-*Bt* | - | - | |
| NCEH 6R *Bt* | GFM event | Fusion *cry1Ac/cry1Ab* | Nath Bio Genes (I) Ltd., Aurangabad-431005 (Maharashtra) |
| NCEH 6R Non-*Bt* | - | - | |
| MRC 7017 BG II | Mon 15985 | *cry1Ac* + *cry2Ab* | Maharashtra Hybrid Seed Company, Jalna-431203 (Maharashtra) |
| MRC 7017 Non-*Bt* | - | - | |

**Table 2.** Comparative survival and duration of *E. vittella* larvae and pupae on *Bt* cotton and their isogenic non *Bt* genotypes at 90 days of crop age under field cage conditions

| Genotypes | Larval survival (%) | Larval duration (days) | | Growth index | Pupal Survival (%) | Pupal duration (days) | | Survival index |
|---|---|---|---|---|---|---|---|---|
| | | Range | Mean ± SE | | | Range | Mean ± SE | |
| MRC 6301 *Bt* | 0.00(4.05) | - | - | 0.00 | - | - | - | 0.00 |
| MRC 6301 N *Bt* | 58.25(49.73) | 9 - 11 | 10.06 ± 0.10 | 5.68 | 75.25 | 7 - 9 | 8.06 ± 0.11 | 0.44 |
| JKCH 1947 *Bt* | 0.00(4.05) | - | - | 0.00 | - | - | - | 0.00 |
| JKCH 1947 N *Bt* | 63.00(52.52) | 8 - 10 | 9.70 ± 0.15 | 6.49 | 76.25 | 7- 8 | 7.84 ± 0.13 | 0.48 |
| NCEH 6R *Bt* | 0.00(4.05) | - | - | 0.00 | - | - | - | 0.00 |
| NCEH 6R N *Bt* | 60.75(51.19) | 9 - 10 | 9.93 ± 0.10 | 6.11 | 75.88 | 7 - 8 | 7.95 ± 0.14 | 0.46 |
| MRC 7017 BG II | 0.00(4.05) | - | - | 0.00 | - | - | - | 0.00 |
| MRC 7017 N *Bt* | 59.75(50.61) | 9 - 11 | 10.04 ± 0.10 | 5.95 | 75.75 | 7 - 9 | 8.02 ± 0.15 | 0.45 |
| SEm± | 0.54 | | 0.08 | | 0.60 | | 0.16 | - |
| CD (p=0.05) | (1.57) | | 0.25 | | NS | | NS | |

Figures in parentheses are corresponding arcsine transformed values

larval period (in days) was significantly higher in MRC 6301 non *Bt* (10.34 ± 0.10) which was *at par* with MRC 7017 non *Bt* (10.27 ± 0.10) and NCEH 6R non *Bt* (10.13 ± 0.11). At the same time, larval duration was significantly less in JKCH 1947 non *Bt* (10.00 ± 0.11). The growth index was less in MRC 6301 non *Bt* (5.59), followed by MRC 7017 non *Bt* (5.70), NCEH 6R non *Bt* (5.92) and it was comparatively more in JKCH 1947 non *Bt* (6.25).

Similar trend was observed with respect to pupal survival and duration at 120 days old crop as that recorded on 90 days old crop (Table 3). The pupal survival varied from 72.10 to 73.10 per cent among all non *Bt* genotypes with non significant differences. Likewise, pupal duration in all non *Bt* genotypes was also non significant and it varied from 10.14 ± 0.18 to 10.36 ± 0.14 days on MRC 6301 non *Bt* and JKCH 1947 non *Bt*, respectively. The survival index on non *Bt* genotypes, *viz.*, MRC 6301, MRC 7017, NCEH 6R and JKCH 1947 was 0.42, 0.42, 0.43 and 0.46, respectively, as compared to 0.0 in all *Bt* cotton hybrids.

**c) One hundred and fifty days old crop:**

A perusal of Table 4 also revealed zero per cent survival as none of the larvae could manage to survive and reached pupal stage in any of *Bt*

**Table 3.** Comparative survival and duration of *E. vittella* larvae and pupae on *Bt* cotton and their isogenic non-*Bt* genotypes at 120 days of crop age under field cage conditions

| Genotypes | Larval survival (%) | Larval duration (days) | | Growth index | Pupal Survival (%) | Pupal duration (days) | | Survival index |
|---|---|---|---|---|---|---|---|---|
| | | Range | Mean ± SE | | | Range | Mean ± SE | |
| MRC 6301 *Bt* | 0.00(4.05) | - | - | 0.00 | - | - | - | 0.00 |
| MRC 6301 N *Bt* | 57.75(49.44) | 9 - 12 | 10.34 ± 0.10 | 5.59 | 72.10 | 9 - 11 | 10.36 ± 0.14 | 0.42 |
| JKCH 1947 *Bt* | 0.00(4.05) | - | - | 0.00 | - | - | - | 0.00 |
| JKCH 1947 N *Bt* | 62.50(52.22) | 9 - 11 | 10.00 ± 0.11 | 6.25 | 73.10 | 8 - 11 | 10.14 ± 0.18 | 0.46 |
| NCEH 6R *Bt* | 0.00(4.05) | - | - | 0.00 | - | - | - | 0.00 |
| NCEH 6R N *Bt* | 60.00(50.75) | 9 - 11 | 10.13 ± 0.11 | 5.92 | 72.38 | 9 - 11 | 10.23 ± 0.12 | 0.43 |
| MRC 7017 BG II | 0.00(4.05) | - | - | 0.00 | - | - | - | 0.00 |
| MRC 7017 N *Bt* | 58.50(49.88) | 9 - 11 | 10.27 ± 0.10 | 5.70 | 72.13 | 9 - 11 | 10.36 ± 0.13 | 0.42 |
| SEm± | 0.41 | | 0.07 | | 0.32 | | 0.16 | - |
| CD (p=0.05) | (1.20) | | 0.22 | | NS | | NS | |

Figures in parentheses are corresponding arcsine transformed values

hybrids on 150 days old crop. However, it is pertinent to mention that one out of 50 larvae survived for 7 days on green bolls of JKCH 1947 *Bt*. In comparison, larval survival varied from 56.50 to 61 per cent on isogenic non *Bt* genotypes. Among the latter, the larval survival was lowest in MRC 6301 (56.50%) and it was *at par* with MRC 7017 (57.00 %) and NCEH 6R (57.25%). Significantly higher survival was observed in JKCH 1947 non *Bt* (61%). On the contrary, larvae took significantly more days to reach pupal stage in MRC 6301 non *Bt* (12.40 ± 0.12) and it was *at par* with MRC 7017 non *Bt* (12.33 ± 0.12) and NCEH 6R non *Bt* (12.16 ± 0.13). However, the larval period was significantly less in JKCH 1947 non *Bt* (11.91 ± 0.15) which was *at par* with NCEH 6R non *Bt*. The growth index comparatively less in MRC 6301 non *Bt* (4.56) which was followed by MRC 7017 non *Bt*, NCEH non *Bt* and JKCH 1947 non *Bt* in which 4.62, 4.71 and 5.12 larval growth index was observed, respectively.

On 150 days old crop also, no variation with respect to pupal survival among different non *Bt* genotypes was observed (Table 4). It varied from 67.40 (MRC 6301 non *Bt*) to 69.00 (JKCH 1947 non *Bt*) per cent, respectively. The pupal duration (in days) among non *Bt* genotypes also showed non-significant differences and it ranged from 17.29 ± 0.17 and 17.61 ± 0.15 on JKCH 1947 non *Bt* and MRC 6301 non *Bt*, respectively. The survival index was 0.0 in all the *Bt* hybrids whereas it was 0.38, 0.39, 0.39 and 0.42 in non *Bt* genotypes, *i.e.* MRC 6301, MRC 7017, NCEH 6R and JKCH 1947, respectively.

The present results thus showed zero survival index in all *Bt* cotton hybrids belonging to four different events, *i.e.* MRC 6304 *Bt* (*cry1Ac* gene), MRC 7017 BG II (*cry1Ac* and *cry2Ab* genes), NCEH 6R *Bt* (fusion *cry1Ab*/*cry1Ac* gene) and JKCH 1947 *Bt* (modified *cry1Ac* gene). It indicated that all the selected *Bt* cotton hybrids had strong in-built resistance under field conditions as they were highly effective against *E. vittella* as compared to their isogenic non *Bt* genotypes. Most of the earlier studies on toxicity of *Bt* cotton hybrids to *E. vittella* have tracked the fate of larvae under laboratory conditions at the most up to seven days. No one has reported, whether surviving larvae reached up to pupal or adult stage. Kumar and Indrapriyadarshani (2010) reported that on squares, RCH 2 *Bt* recorded higher neonate mortality (96%) of *E. vittella* followed by MECH 162 *Bt* (93.33%) and MECH 184 *Bt* (89.33%) as compared to 40.00, 58.67 and 46.67 per cent mortality in the respective non *Bt* genotypes at 65 DAS. At 110 DAS, mortality in

**Table 4.** Comparative survival and duration of *E. vittella* larvae and pupae on *Bt* cotton and their isogenic non *Bt* genotypes at 150 days of crop age under field cage conditions

| Genotypes | Larval survival (%) | Larval duration (days) | | Growth index | Pupal Survival (%) | Pupal duration (days) | | Survival index |
|---|---|---|---|---|---|---|---|---|
| | | Range | Mean ± SE | | | Range | Mean ± SE | |
| MRC 6301 *Bt* | 0.00(4.05) | - | - | 0.00 | - | - | - | 0.00 |
| MRC 6301 N *Bt* | 56.50(48.72) | 11 - 14 | 12.40 ± 0.12 | 4.56 | 67.40 | 16 - 19 | 17.61 ± 0.15 | 0.38 |
| JKCH 1947 *Bt* | 0.00(4.05) | - | - | 0.00 | - | - | - | 0.00 |
| JKCH 1947 N *Bt* | 61.00(51.34) | 10 - 13 | 11.91 ± 0.15 | 5.12 | 69.00 | 15 - 18 | 17.29 ± 0.17 | 0.42 |
| NCEH 6R *Bt* | 0.00(4.05) | - | - | 0.00 | - | - | - | 0.00 |
| NCEH 6R N *Bt* | 57.25(49.15) | 11 - 13 | 12.16 ± 0.13 | 4.71 | 68.33 | 15 - 18 | 17.31 ± 0.13 | 0.39 |
| MRC 7017 BG II | 0.00(4.05) | - | - | 0.00 | - | - | - | 0.00 |
| MRC 7017 N *Bt* | 57.00(49.01) | 11 - 14 | 12.33 ± 0.12 | 4.62 | 67.81 | 16 - 18 | 17.56 ± 0.15 | 0.39 |
| SEm± | 0.49 | | 0.09 | | 0.76 | | 0.15 | |
| CD (p=0.05) | (1.43) | | 0.28 | | NS | | NS | |

Figures in parentheses are corresponding arcsine transformed values

MECH 184 *Bt* (41.33%) and MECH 162 *Bt* (44.0%) was *at par* with their isogenic non *Bt* genotypes recording 38.66 and 41.33 per cent mortality, respectively. However, RCH 2 *Bt* showed a significantly high mortality (92%) as compared to the respective non *Bt* (34.67%) genotype and other *Bt* hybrids. Somashekara *et al*. (2011) compared efficacy of RCH 2 *Bt* and Bunny *Bt* with the respective BG II genotypes against *E. vittella* subjected to bioassay for five days on squares and reported that both BG II hybrids recorded cent per cent mortality while it was 98.50 and 99.05 per cent on RCH 2 *Bt* and Bunny *Bt*, respectively, at 60 DAS. It decreased progressively in all *Bt* hybrids at 90 DAS and at 120 DAS, the mortality in RCH 2 BG II and Bunny *Bt* 2 was 95.44 and 94.48 per cent as compared to 76.67 and 74.38 per cent in their BG I genotypes, respectively. In Egypt, Dahi (2012) has reported zero per cent infestation in bolls of the genetically modified *G. barbadense Bt* varieties (Giza 80, Giza 90 and Giza 89) when they were artificially infested by *E. insulana* neonate larvae in laboratory conditions as compared to 76 - 100 per cent infestation in the respective isogenic non *Bt* varieties.

The expression of Cry toxin in *Bt* cotton has been reported to vary among plant structures and also decline with age of the crop (Manjunatha *et al.,* 2009, Sagar *et al.*, 2011). The temporal and spatial variability of *Bt* protein expression might allow susceptible larvae to survive and complete their development at certain times and/or in certain plant structures which provide a sub-lethal toxin dose. But the present study showed that none of the neonates could manage to develop and reach pupal stage when they were released on plants of 90, 120 and 150 days old crop under field cage conditions indicating that toxin content was still sufficient to cause 100 per cent mortality even when released at later crop stages.

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