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Effect of *Bt* cotton on survival and development of tobacco caterpillar, *Spodoptera litura* (Fabricius) on different events of *Bt* hybrids

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Transgenic Bt Cotton hybrids expressing cry toxins with different events are available in India for cultivation offering resistance to bollworms. In the present investigation the impact of Bt Cotton hybrids derived from different events were studied in the laboratory on the growth and development of tobacco caterpillar, *Spodoptera litura*. The larval mortality when fed on leaves and squares of RCH 2 BG-II featuring Cry 1 AC + Cry 2 Ab was highest compared to other Bt Cotton hybrids with corresponding events. The susceptibility level of *S. litura* to RCH2 BG-II varied instar wise and the late instars had very little effect on the survival and developmental bioLogyof the insect. The development of *S. litura* was drastically affected as indicated by lowest growth and survival indices. The cry protein content was estimated from different plant parts of different events of transgenic *Bt* cotton hybrids at 60, 90, 120 and 150 DAS during the seasons.

Key words: Spodoptera litura, Bt cotton, survival, expansion, cry 1 AC + Cry 2 Ab.

INTRODUCTION

Toxins from a soil bacterium, *Bacillus thuringiensis* are widely employed in control of many insect pests from decades. Earlier, sprays containing *Bt* toxins was used to control agricultural pests. Since, 1996, plants engineered to produce these toxins showed great potential in pest management by reducing reliance on insecticides. However, continuous exposure to these toxins imposes a selection pressure on insect populations favouring evolution of resistance to *Bt* toxins expressed in transgenic plants (Tabashnik, 1994; Shelton et al., 2002). Recently, field evolved resistance to *Bt* toxins present in plants has been reported in several species of major pests (Tabashnik et al., 2008; Bagla, 2010).The released transgene events against insect pests of cotton stand at

five. *Bt* cotton harboring Cry1Ac has come very popular occupying an area of 66 lakh hectare was intended to manage the bollworms that had become the major cause for yield losses, in cotton, in the country. On the other hand, the two gene event (Cry1Ac+ Cry2Ab) released in 2005 for commercial cultivation occupies a reasonable area in the country.

This genotype was expected to combat *Helicoverpa armigera* as well as *Earias vittella* (Fab.) and it was also promoted as a resistance management tool in other countries like India, Australia and Europe. The technology is expected to provide cotton growers with significant ecological and economic advantage. Nath Seeds Private Limited introduced the Cry 1Ac obtained

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from China into its popular genotypes while JK Hybrid Seeds Private Ltd has introduced the indigenous Cry 1Ac developed by Bose institute into its cultivated genotypes. Today the Indian farmer is faced with the choice of at least 280 genotypes harboring one of the four released transgene events against insect pests with at least 6 more in the pipeline.

Among the major insect pests, bollworms which cause damage mainly to the economic parts of the crop are the dreaded pests influencing the economic well being of the cotton growers. The causes for repeated pest outbreaks and control failures may be due to pest resistance to most common and repeatedly used classes of insecticides and dwindling of natural enemies due to broad spectrum insecticides (Kranthi et al., 1997), warranting to search for alternate measures that are cost effective, selective and eco-friendly for the management of these noxious pests. Unhindered use of agrochemicals not only causes harm to the environment but also contribute to the development of resistance and resurgence in insect pests. At this juncture, advances made in the genetic engineering lead to the development of transgenic Bt cotton, which promise sustainable cotton production.

Transgenic *Bt* cotton with Cry 1Ac gene is not effective against *S. litura* (Soujanya et al., 2009). Hence, the addition of Cry 2Ab to *Bt* cotton provides increased efficacy against bollworms (Soujanya et al., 2009). The stacked *Bt* cotton technology has used the same soil bacterium as in the case of *Bt* cotton, but it has two genes working at the same time which delays the development of resistance to the *Bt* toxin as the insects can develop resistance to one gene faster than two genes working in the same genotype. Due to the different in structure and insecticidal mechanism to Cry2A genes are promising for management of resistance in insects (Kumar et al., 2004; Sivasupramaniam et al., 2003).

The expression of Cry protein in different plant parts at a given point of time and different growth stages is not uniform. Stacked Bt cotton and Bt cotton cultivars control the bollworms upto 110 days only and thereafter the toxin expression level decreases as the plant age advances (Chinna et al., 2013; Kranthi et al., 2005; Adamczyk et al., 2001). Laboratory studies have shown that the addition of second Bt protein (Cry2Ab) to Bt cotton may reduce the survival of bollworms relative to Bt cotton with only one protein (Stewart et al., 2001). Bt gene does not allow the development of bollworm population because of the inherent toxicity of the Bt cotton against bollworms (Kranthi et al., 2000). This leads to minimum usage of insecticides and is considered as one of the best tools of Integrated Pest Management against bollworm complex. It has been proved eco-friendly in the management of bollworm complex (Hamilton et al., 2002; Romeis et al., 2006). Hence, it fits very well in Integrated Pest Management programme of bollworm complex. Introduction of cotton transgenic expressing Cry1Ac

toxins under the influence of constitutive promoter is to render pest populations resistant in a short period of time. Selection for resistance to Cry1Ac significantly reduced the fitness of *O. nubilalis*. Resistant insects exhibited reduced pupal weight and increased developmental time compared with susceptible and F_1 larvae derived from reciprocal crosses of resistant and susceptible parents. In addition, resistant insects exhibited a higher proportion of unsuccessful matings and lower fertility than the susceptible strain (Andre et al., 2010).

MATERIALS AND METHODS

Laboratory bioassays for S. litura on Bt cotton hybrid was conducted at Regional Agricultural Research Station, Lam for two consecutive season during Kharif, 2007 to 2008 and Kharif, 2008 to 2009. The experiment was conducted with all the larval instars that is, from I to V instars by feeding on leaves and squares of different Bt hybrids belonging to four Bt events viz. RCH 2 Bt (Cry1Ac), JK Durga Bt (Modified Cry 1Ac), Nath baba Bt (Fusion Cry 1Ac) and RCH 2 BGII (Cry 1Ac + 2 Ab), their corresponding non Bt hybrids as control. The seeds of RCH 2 Bt, RCH 2 BGII and RCH 2 non Bt hybrids obtained from M/S Rasi seeds, Coimbatore, Tamil Nadu, while JK Durga Bt and JK Durga non Bt seeds was obtained from J.K. Agri Genetics Ltd. Hyderabad and Nath baba Bt and Nath baba non Bt Seeds from Nath Seeds, Aurangabad, Maharashtra. The field collected larvae were reared on the natural diet upto pupation and the adults released from the pupae were kept for oviposition in cages. The eggs laid on castor leaves. Subsequent instars were maintained on natural diet and bioassay was conducted at ten larvae for instars for each replication in each event of Bt cotton was used. The mortality of all the five instars of S. litura larvae were studied individually by exposing to leaves and squares of RCH 2 BGII, RCH 2 Bt, JK Durga Bt and Nath baba Bt hybrids and their corresponding non Bt hybrids with three replication at 70 to 80 days old crop.

The plant parts were replaced daily with fresh *Bt* plant parts to avoid death or growth reduction due to tissue drying or nutritional deterioration. 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.*, percent pupation and adults emergence were observed from the larvae. The growth index and survival index were calculated for *H. armigera* and *S. litura* population on different treatments using the formulae given by Vennila et al. (2006).

Growth index: Per cent pupation / Larval developmental period (days),

Survival index: Number of moth's emerged / total number of neonates used.

The data collected for the two years were pooled and analyzed (ANOVA) to compare the survival and development of American boll worm on *Bt* cotton.

RESULTS AND DISCUSSION

The survival and development of *S. litura* larvae was observed instar wise on tender leaves and squares of RCH 2 *Bt* (Mon 531 event), JK Cry1Ac event, Nath baba *Bt* (GMF event), RCH 2 BGII (Mon 15985 event), RCH 2 non *Bt*, JK Durga non *Bt* and Nath baba non *Bt* cotton

| Tracting and | | | Leaves | | | | | Squ |
|---------------------|-----------------------------|-----------------------------|----------------------------|----------------------------|-------------|----------------------------|----------------------------|-----|
| Treatment | l instar | ll instar | III instar | IV instar | V instar | l instar | ll instar | I |
| RCH 2 Bt | 11.66 (19.88) ^b | 10.00 (18.04) ^{bc} | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 10.00 (18.04) ^b | 5.00 (10.45) ^b | 0 |
| RCH 2 non Bt | 11.66 (19.88) ^b | 6.66 (14.75) ^c | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 10.00 (18.04) ^b | 6.66 (14.75) ^b | 0 |
| JK Durga <i>Bt</i> | 10.00 (18.43) ^b | 10.00 (18.04) ^{bc} | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 11.66 (19.88) ^b | 6.66 (14.75) ^b | 0 |
| JK Durga non Bt | 13.33 (21.14) ^b | 13.33 (21.33) ^b | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 13.33 (21.33) ^b | 8.33 (16.20) ^b | 0 |
| Nath baba <i>Bt</i> | 11.66 (19.88) ^b | 10.00 (18.04) ^{bc} | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 13.33 (21.33) ^b | 5.00 (10.45) ^b | 0 |
| Nath baba non Bt | 10.00 (18.43) ^b | 6.66 (14.75) ^c | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 11.66(19.88) ^b | 8.33 (16.20) ^b | 0 |
| RCH 2 BGII | 100.00 (90.00) ^a | 83.33 (65.95) ^a | 50.00 (45.95) ^a | 20.00 (27.71) ^a | 0.00 (0.00) | 71.66(57.85) ^a | 43.33 (41.16) ^a | 33. |
| F-TEST | Sig | Sig | Sig | Sig | | Sig | Sig | |
| SEm± | 1.97 | 1.95 | 0.03 | 0.04 | | 2.49 | 3.50 | |
| CD(P=0.05) | 5.98 | 5.92 | 1.09 | 1.31 | | 7.5 | 10.63 | |

Table 1. Percent mortality of larval instars of S.litura on test hybrids.

Figures in parentheses are angular transformed values, Numbers followed by same superscript are not statistically different.

hybrids at 70 to 80 days old crop because of the peck activity of *S. litura* were caused maximum damage on leaves and green bolls during the periods that will predict actually effect to Bt events. Different parameters *viz.*, larval mortality, weight of larvae and pupae and adult emergence from the surviving larvae were studied and presented here under.

The larval mortality of *S. litura* was maximum on RCH 2 BGII compared to other test hybrids. Mortality of first instar larvae was 100.00% on RCH 2 BGII and gradually decreased as the age of the larvae increased. In second instar the mortality was 83.33%. In third and fourth instars the mortality was 50.00 and 20.00%, respectively. The late age worms (fifth instar) were resistant to all the test hybrids and survival was cent percent. The mortality of *S. litura* larvae was observed only during first and second instars stages on all the other test hybrids. The third, fourth and fifth instars survived on leaves without any mortality. The first instar larval mortality on RCH 2 *Bt*, RCH

2 non Bt, JK Durga Bt, JK Durga non Bt, Nath baba Bt and Nath baba non Bt was 11.66, 11.66, 10.00, 13.33, 11.66 and 10.00% respectively. There was no significant difference between the treatments. Similarly in second instar also the mortality varied from 6.66 to 10.00%. RCH 2 BGII with stacked genes for S. litura resistance was the best hybrid with the maximum mortality of S. litura larvae (Table 1). Similar observations were made by Donglin et al. (2006) on the effect of S. litura on stacked Bt cotton and non Bt cotton hybrids and Henneberry et al. (2001) on the effects transgenic cotton on cabbage looper, tobacco bud worm and beet army worm. Guifen et al. (2006) also observed decreasing survival rate of S. litura on NuCOTN 33B (Fused Cry1Ab/Ac).

The mortality of larvae on squares of RCH 2 BGII hybrid was seen only up to third instar and decreased gradually with increase in age. The maximum mortality of 71.66% was recorded in first instar larvae. In second and third instar, the mortality was only 43.33 and 33.33% respectively on RCH 2 BGI hybrids the larva to 13.33%, whe to 8.33% only. squares was nil cotton hybrids t percent mortalit instar larvae of evident from the was significantly

The minimum when fed on lea BGII hybrid wit was significantly (Table 2 and Fig second instar la RCH 2 BGII h leaves. The ma was recorded on *Bt* (18.66 mg), J non *Bt* (18.66 m and RCH 2 no

| Treatment | Mean w | eight (mg/lar instar after | va) of II | Weight | Mean | weight (mg/la III instar aftei | rva) of | Weight | Mean we IV |
|------------------------|----------------------|-------------------------------|----------------------|----------------------|----------|-----------------------------------|---------------------|----------------------|---------------------|
| | 24 h | 48 h | 72 h | gain (%) | 24 h | 48 h | 72 h | gain (%) | 24 h |
| | 18.66 | 22.06 | 28.00 | 42.86 | 33.66 | 35.73 | 44.40 | 27.92 | 52.40 |
| RCH 2 <i>Bt</i> | (4.37) ⁰ | (4.75) | (5.53) | (40.90) ⁰ | (5.84) | (6.01) ⁰ | (6.70) | (31.88) ⁰ | (7.27) |
| | 17.73 | 23.80 | 29.60 | 45.94 | 35.00 | 37.00 | 49.06 | 34.77 | 54.43 |
| RCH 2 non Bt | (4.27) ⁰ | (4.92) ^C | (5.48) ⁰ | (42.66) ⁰ | (5.95) | (6.12) ^u | (7.04) | (36.12) ^u | (7.41) ^u |
| | 18.66 | 22.00 | 27.80 | 42.44 | 33.93 | 36.20 | 45.80 | 30.13 | 53.93 |
| JK Durga <i>Bt</i> | (4.37) | (4.74) | (5.31) | (40.64) | (5.86) | (6.05) | (6.80) | (33.28) | (7.37)00 |
| | 17.93 | 23.5 <u>3</u> | 28.93 | 44.70 | 35.20 | 37.06 | 48.20 | 33.67 | 53.26 |
| JK Durga non <i>Bt</i> | (4.29) | (4.90) | (5.42) | (41.95) | (5.97) | (6.12) ^u | (6.97) | (35.46) | (7.33) |
| | 18.80 | 22.13 | 28.13 | 43.12 | 34.06 | 36.53 | 47.00 | 31.91 | 54.73 |
| Nath baba Bt | (4.39) | (4.76) | (5.34) | (41.04) | (5.87) | (6.08) | (6.89) ^u | (34.38)00 | (7.43) ^u |
| | 18.66 | 23.80 | 29.46 | 45.68 | 34.86 | 36.93 | 47.73 | 33.00 | 53.93 |
| Nath baba non Bt | (4.37) | (4.92) | (5.47) | (42.52) | (5.94) ° | (6.11) ^ଘ | (6.94) ^u | (35.05) | (7.37)00 |
| | 16.33 | 17.40 | 18.00 | 11.11 | 32.73 | 33.60 | 35.86 | 10.76 | 51.20 |
| RCH-2 BGII | (4.10) ^{°°} | (4.23) ັ | (4.30) ^{°°} | (19.42)ິ | (5.76) ິ | (5.83) ິ | (6.03) ີ | (19.07) ິ | (7.19) ິ |
| F-TEST | Sig | Sig | Sig | Sig | Sig | Sig | Sig | Sig | Sig |
| SEm± | 0.04 | 0.03 | 0.06 | 0.78 | 0.01 | 0.01 | 0.02 | 1.20 | 0.02 |
| CD(P=0.05) | 0.13 | 0.09 | 0.18 | 2.38 | 0.03 | 0.04 | 0.08 | 2.56 | 0.06 |
| Initial weight | 16.00 | | | | 32.00 | | | | 50.00 |

Table 2. Effect of test hybrid leaves on S.litura larval weight.

Figures in parentheses are square root transformed values, numbers followed by same superscript are not statistically different.

exposure to leaves. All these hybrids are statistically on par and significantly inferior over the RCH 2 BGII hybrid. The percent weight gain was lowest (11.11%) in RCH 2 BGII hybrid. The maximum weight gain (45.94 %) at 72 h after exposure was recorded in RCH 2 non *Bt* hybrid followed by Nath baba non *Bt* (45.68%), JK Durga non *Bt* (44.70%), Nath baba *Bt* (43.12%), RCH 2 *Bt* (42.86%) and JK Durga *Bt* (42.44%). All these are statistically on par with each other.

The mean larval weight of third instar larvae fed on leaves was minimum in RCH 2 BGII hybrid at 24, 48 and 72 h after feeding compared to other test hybrids. Only 0.73 mg weight increase was recorded at 24 h after feeding on RCH 2 BGII hybrid where as 3.2 mg weight increase was noticed in JK Durga non *Bt* hybrid followed by RCH 2 non *Bt*, Nath baba non *Bt*, Nath baba *Bt*, JK Durga *Bt* and RCH 2 *Bt* after 24 h of feeding on leaves. The percent weight gain after 72 h feeding was maximum in RCH 2 non *Bt* (34.77) followed by JK Durga non *Bt*, Nath baba non *Bt*, Nath baba *Bt*, JK Durga *Bt* and RCH 2 *Bt* hybrids and all are statistically on par. The percent weight gain during third instar was lowest (10.76%) in RCH 2 BGII and it is statistically superior over other hybrids. The mean larval weight of fourth instar larvae after feeding for 24 h was maximum in Nath baba *Bt* (54.73 mg) and minimum in RCH 2 BGII hybrid (51.20 mg). After three days of feeding the perc in RCH 2 BGII The maximum Durga non *Bt* (non *Bt* (28.63% baba *Bt* (28.08 RCH 2 *Bt* (2 statistically on p RCH 2 BGII h lower larval and index of *S.litura* present studies of Parker et a (2004) and Souj fed on RCH 2









RCH 2 Bt RCH 2 non Bt JK Durga Bt JK Durga non Bt Nath baba Bt Nath baba non Bt RCH-2 BG-II III INSTAR



IV INSTAR

Figure 1. Effect of test hybrid leaves on S.litura larval weight (mg).

| Treatments | Mean we II | eight (mg/la instar aftei | rva) of | Weight | Mean | weight (mç III instar a | g/larva) of fter | Weight | Меа | n weig IV in |
|-------------------------|----------------------|------------------------------|----------------------|-----------------------|----------------------|----------------------------|-----------------------|----------------------|----------------------|------------------|
| | 24 h | 48 h | 72 h | gain (%) | 24 h | 48 h | 72 h | gain (%) | 24 h | 48 |
| RCH 2 Bt | 18.20 | 22.66 | 31.66 | 49.46 | 38.26 | 44.66 | 51.66 | 30.31 | 59.40 | 64.4 |
| | (4.32) ^b | (4.81) ^b | (5.67) ^b | (44.69) ^b | (6.22) ^b | (6.72) ^b | (7.22) ^b | (33.40) ^b | (7.73) ^c | (8.05 |
| RCH 2 non Bt | 21.53 | 24.73 | 33.33 | 52.00 | 39.40 | 46.66 | 53.13 | 32.24 | 59.20 | 65. ⁻ |
| | (4.69) ^d | (5.02) ^d | (5.81) ^{bc} | (46.14) ^{bc} | (6.31) ^d | (6.86) ^{cd} | (7.32) ^{bcd} | (34.58) ^b | (7.72) ^c | (8.10 |
| JK Durga <i>Bt</i> | 18.73 | 23.13 | 34.26 | 53.29 | 39.73 | 46.66 | 52.53 | 31.46 | 57.93 | 65.8 |
| | (4.38) ^b | (4.86) ^{bc} | (5.89) ^{bc} | (47.79) ^c | (6.34) ^d | (6.86) ^{cd} | (7.28) ^{bc} | (34.11) ^b | (7.64) ^b | (8.14 |
| JK Durga non <i>Bt</i> | 21.00 | 24.93 | 34.80 | 54.00 | 38.86 | 46.93 | 52.46 | 31.38 | 58.80 | 66.3 |
| | (4.63) ^{cd} | (5.04) ^d | (5.94) ^c | (46.31) ^{bc} | (6.27) ^{bc} | (6.88) ^d | (7.27) ^{bc} | (34.06) ^b | (7.70) ^{bc} | (8.17 |
| Nath baba <i>Bt</i> | 18.93 | 23.86 | 33.73 | 52.56 | 38.66 | 46.06 | 54.26 | 33.65 | 58.80 | 66. ⁻ |
| | (4.40) ^b | (4.93) ^c | (5.85) ^{bc} | (46.46) ^{bc} | (6.25) ^b | (6.82) ^c | (7.40) ^α | (35.44) ^b | (7.70) ^{bc} | (8.16 |
| Nath baba non <i>Bt</i> | 20.26 | 24.86 | 35.26 | 54.62 | 39.53 | 46.93 | 53.33 | 32.50 | 59.26 | 66.9 |
| | (4.55) ^c | (5.03) ^d | (5.98) ^c | (47.65) ^c | (6.32) ^{cd} | (6.88) ^d | (7.33) ^{cd} | (34.74) ^b | (7.73) ^c | (8.2 |
| RCH 2 BGII | 16.73 | 18.20 | 20.00 | 20.00 | 36.86 | 37.11 | 39.46 | 8.76 | 56.33 | 63.3 |
| | (4.15) ^a | (4.32) ^a | (4.52) ^a | (26.54) ^a | (6.11) ^a | (6.18) ^a | (6.32) ^a | (17.15) ^a | (7.53) ^a | (7.98 |
| F-TEST | Sig | Sig | Sig | Sig | Sig | Sig | Sig | Sig | Sig | Sig |
| SEm± | 0.02 | 0.02 | 0.04 | 0.73 | 0.02 | 0.01 | 0.03 | 0.80 | 0.02 | 0.0 |
| CD(P=0.05) | 0.08 | 0.07 | 0.22 | 2.21 | 0.08 | 0.05 | 0.10 | 2.43 | 0.07 | 0.0 |
| Initial weight | 16.00 | | | | 36.00 | | | | 55.00 | - |

Table 3. Effect of test hybrid squares on S. litura larval weight.

Figures in parentheses are square root transformed values, numbers followed by same superscript are not statistically different.

less weight gain in all the three instars *viz.*, second, third and fourth instars tested. The RCH 2 BGII hybrid was superior over the other hybrids tested in having less preference for feeding by *S. litura* (Table 3 and Figure 2). The maximum mean weight of 21.53 mg of the second instar larvae of *S. litura* when fed on squares of was recorded on RCH 2 non *Bt* at 24 h after feeding which is statistically on par with JK Durga non *Bt* (21.00 mg). The mean weight gain was lowest (0.73 mg) on RCH 2 BGII and the maximum weight gain was

on RCH 2 non *Bt* (5.53 mg). The minimum mean larval weight gain was recorded on RCH 2 BGII hybrid after 72 h feeding on squares (4.0 mg). The maximum larval weight gain was recorded on Nath baba non *Bt* at 48 h (8.86 mg) and 72 h (19.26 mg) of feeding. Nath baba non *Bt* was statistically on par with JK Durga non *Bt*, JK Durga *Bt*, Nath baba *Bt* and RCH 2 non *Bt* hybrids at 72 h after feeding. The percent weight gain in second instar larvae was minimum on RCH 2 BGII (20.00%) and maximum on Nath baba non *Bt*

(54.62%). The III instar larvae JK Durga *Bt* aft initial mean wei Durga *Bt* (39.73 followed by Nat non *Bt* (39.40 mg) at 24 h af RCH 2 BGII hy *litura* larvae. In 48 and 72 h afte









RCH 2 Bt RCH 2 non Bt JK Durga Bt JK Durga non Bt Nath baba Bt Nath baba non Bt RCH 2 BG-II

III INSTAR



IV INSTAR

Figure 2. Effect of test hybrid squares on S.litura larval weight (mg).

| Trootmonto | | | Leaves | | | | | Squares |
|------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|-----------------------|----------------------|
| | l instar | ll instar | III instar | IV instar | V instar | l instar | ll instar | III instar |
| | 117.52 | 136.53 | 138.46 | 140.00 | 135.93 | 132.33 | 132.80 | 132.73 |
| RCH 2 Bt | (10.15) | (11.70) | (11.78) | (11.85) ⁰ | (11.68) | (11.52) | (11.54) | (11.54) |
| | 137.66 | 134.13 | 137.46 | 138.33 | 138.13 | 133.26 | 132.40 | 135.53 |
| RCH 2 non Bt | (11.75) ^u | (11.60) | (11.74) | (11.78) ⁰ | (11.77) | (11.56) | (11.52) | (11.66)ັ |
| | 135.73 | 137.86 | 138.00 | 138.66 | 134.53 | 133.06 | 131.06 | 132.20 |
| JK Durga <i>Bt</i> | (11.67) [°] | (11.76) [°] | (11.76) ⁰ | (11.79) ⁰ | (11.62) | (11.55) | (11.47) | (11.56) ⁰ |
| | 139.13 | 138.60 | 138.10 | 139.33 | 136.40 | 134.46 | 134.73 | 134.93 |
| JK Durga non <i>Bt</i> | (11.81)ັ | (11.79) ^ບ | (11.77) ⁰ | (11.82) | (11.70) | (11.61) | (11.62) ^{cu} | (11.63) ^u |
| | 135.46 | 136.46 | 137.46 | 137.96 | 138.06 | 132.13 | 132.46 | 133.80 |
| Nath baba Bt | (11.66) ^ບ | (11.70) | (11.74) | (11.76) | (11.77) | (11.51) ⁰ | (11.53) ^D | (11.58) ⁰ |
| Nath baba non | 138.20 | 139.20 | 139.00 | 137.06 | 137.00 | 134.80 | 134.86 | 134.26 |
| Bt | (11.77) ^u | (11.81) ⁶ | (11.81) ⁰ | (11.72) ^D | (11.72) | (11.63) | (11.63) ^u | (11.60) [°] |
| | 0.00 | 104.00 | 113.00 | 120.80 | 125.66 | 101.26 | 109.33 | 114.13 |
| RCH 2 BGII | (0.70) ^a | (10.22) ^a | (10.65) ^a | (11.01) ^a | (11.23) ^a | (10.08) ^a | (10.48) ^a | (10.70) ^a |
| F-TEST | Sig | Sig |
| SEm± | 0.08 | 0.04 | 0.07 | 0.07 | 0.03 | 0.03 | 0.02 | 0.01 |
| CD(P=0.05) | 0.02 | 0.13 | 0.22 | 0.23 | 0.09 | 0.10 | 0.08 | 0.04 |

Table 4. Mean weight (mg) of pupae from surviving larvae of S. litura on test hybrids.

Figures in parentheses are angular transformed values, numbers followed by same superscript are not statistically different.

0.86, 1.11 and 3.46 mg, respectively. The overall weight gain during third instar was only 8.76%. The mean larval weight of third instar gradually increased from 39.73 mg at 24 h after feeding to 46.66 mg in 48 h after feeding and 52.53 mg at 72 h after feeding on JK Durga Bt hybrid. The third instar larvae attained maximum weight of 54.26 mg, when it was fed on Nath baba Bt, and it is statistically on par with Nath baba non Bt (53.33 mg) and RCH 2 non Bt (53.13 mg) at 72 h. The percent weight gain of third instar due to feeding on squares of cotton hybrids was in the range of 30.31 and 33.65 mg. All these test hybrids are statistically on par with each other except RCH 2 BGII hybrid. The fourth instar S. litura larvae preferred squares of RCH 2 Bt hybrid in the initial

stages and reached its maximum weight of 59.40 mg after 24 h of feeding. RCH 2 Bt hybrid was statistically on par with RCH 2 non Bt (59.20 mg), Nath baba non Bt (59.26 mg), Nath baba Bt (58.80 mg) and JK Durga non Bt (58.80 mg) in the preference levels. The least preferred host during fourth instar was RCH 2 BGII hybrid with only 56.33 mg mean weight at 24 h after feeding. The mean initial weight during the fourth instar was 55.00 mg. In all the test hybrids the gradual increase in mean larval weight was recorded. The minimum weight gain (11.26 mg that is, 17.00%) was recorded on RCH 2 BGII hybrid at 72 h after feeding. A maximum of 24.00 mg weight gain (30.38%) was recorded in JK Durga non Bt followed by Nath baba non Bt (29.74%), JK Durga

Bt (29.42%) and these hybrids a other. The perc S. litura on lea cotton hybrids. resistant to S. percent pupatio hybrid was low c it is statistically The first instar la hybrid died co some larvae of s 2 BGII survived and pupated (T second instar l There was 50.00

| Treatments | | | Leaves | | | | | Square |
|-------------------------|-------------------------------|-------------------------------|--------------------------------|--------------------------------|-------------------|-------------------------------|-------------------------------|-----------------|
| Treatments | l instar | II instar | III instar | IV instar | V instar | l instar | ll instar | III inst |
| | 88.33 | 90.00 | 100.00 | 100.00 | 100.00 | 90.00 | 95.00 | 100.0 |
| RCH 2 Bt | (70.11) ^b 88.33 | (71.56) ^b 93.33 | (90.00) ^b 100.00 | (90.00) ^b 100.00 | (90.00) 100.00 | (71.56) ^b 90.00 | (79.54) ^b 93.33 | (90.00 100.0 |
| RCH 2 non Bt | (70.11) ⁰ | (77.71) ⁰ | (90.00) ⁰ | (90.00) ⁰ | (90.00) | (71.56) ⁰ | (77.71) ^D | (90.00 |
| JK Durga <i>Bt</i> | 90.00 (71.56) ^b | 90.00 (71.56) ^b | 100.00 (90.00) ^b | 100.00 (90.00) ^b | 100.00 (90.00) | 88.33 (70.11) ^b | 93.33 (77.71) ^b | 100.0 (90.00 |
| JK Durga non <i>Bt</i> | 86.67 (68.85) ^b | 86.67 (68.85) ^b | 100.00 (90.00) ^b | 100.00 (90.00) ^b | 100.00 (90.00) | 86.67 (68.85) ^b | 91.66 (73.40) ^b | 100.0 (90.00 |
| Nath baba Bt | 88.83 (70.11) ^b | 90.00 (71.56) ^b | 100.00 (90.00) ^b | 100.00 (90.00) ^b | 100.00 (90.00) | 86.67 (68.85) ^b | 95.00 (79.54) ^b | 100.0 (90.00 |
| Nath baba non <i>Bt</i> | 90.00 (71.56) ^b | 93.33 (77.71) ^b | 100.00 (90.00) ^b | 100.00 (90.00) ^b | 100.00 (90.00) | 88.33 (70.11) ^b | 91.66 (73.40) ^b | 100.0 (90.00 |
| RCH 2 BGII | 0.00 (0.00) ^a | 16.67 (23.85) ^a | 50.00 (45.00) ^a | 80.00 (63.54) ^a | 100.00 (90.00) | 28.34 (34.23) ^a | 56.67 (48.84) ^a | 66.67 (54.78 |
| F-TEST | Sig | Sig | Sig | Sig | NS | Sig | Sig | Sig |
| SEm± | 1.40 | 3.60 | 0.62 | 0.78 | | 1.69 | 4.56 | 0.75 |
| CD(P=0.05) | 4.23 | 11.00 | 1.90 | 2.38 | | 5.11 | 13.83 | 2.30 |

Table 5. Percent pupation of surviving larvae of S. litura on test hybrids.

Figures in parentheses are angular transformed values, Numbers followed by same superscript are not statistically different.

in fourth instar larvae when fed on RCH 2 BGII hybrid and this is significantly superior over other hybrids. The survival first and second instar larvae of *S. litura* fed on leaves of test hybrids except RCH 2 BG -II hybrid was more till the end of the larval stage. Hence the per cent pupation in these hybrids was more compared to RCH 2 BGII hybrid. There was no significant difference between the percent pupation in other test hybrids. All are statistically on par with each other. In third, fourth and fifth instars larvae, there was no mortality; hence all the larvae pupated at the end. In RCH 2 BGII hybrid also 100.00% pupation was recorded in fifth instar larvae.

The percent pupation of larvae when fed on squares of RCH 2 BGII hybrid was less in first, second and third instars compared to other test hybrids and it is statistically superior over other hybrids. The first instar larvae when fed on RCH 2 BGII hybrid squares resulted in lowest percent pupation (28.34). The pupation percent in other test hybrids varied between 86.67 and 90.00 and all are statistically on par. In second instar, the pupation percent was 56.67 in RCH 2 BGII and > 90.00% in other hybrids. The percent pupation of third instar larvae fed on squares of RCH 2 BGII

was 66.67% and in third, fourth mean pupal wei larvae were rea mg) and it is sig hybrids. Nath ba 2 non *Bt* (137.66 each other. In pupation of first of second, third on leaves of R other test hybrid 140.00 mg was r

| Tracting and a | | | Leaves | | | | | Squar |
|------------------------|----------------------|----------------------|----------------------|----------------------|----------|----------------------|----------------------|---------|
| Treatments | l instar | ll instar | III instar | IV instar | V instar | l instar | ll instar | III ins |
| | 88.33 | 90.00 | 100.00 | 100.00 | 100.00 | 90.00 | 95.00 | 100.0 |
| RCH 2 Bt | (70.11) ⁰ | (71.56) ^D | (90.00) ^D | (90.00) ⁰ | (90.00) | (71.56) ^D | (79.54) ⁰ | (90.0 |
| | 88.33 | 93.33 | 100.00 | 100.00 | 100.00 | 90.00 | 93.33 | 100.0 |
| RCH 2 non Bt | (70.11) ⁰ | (77.71) ^D | (90.00) ^D | (90.00) ⁰ | (90.00) | (71.56) ^D | (77.71) ^D | (90.0 |
| | 90.00 | 90.00 | 100.00 | 100.00 | 100.00 | 88.33 | 93.33 | 100.0 |
| JK Durga <i>Bt</i> | (71.56) ^D | (71.56) ⁰ | (90.00) ^D | (90.00) ^D | (90.00) | (70.11) ⁰ | (77.71) ^D | (90.0 |
| | 86.66 | 86.66 | 100.00 | 100.00 | 100.00 | 86.66 | 91.66 | 100.0 |
| JK Durga non <i>Bt</i> | (68.85) ^D | (68.85) ^D | (90.00) ^D | (90.00) ⁰ | (90.00) | (68.85) ^D | (73.40) ^D | (90.0 |
| | 88.33 | 90.00 | 100.00 | 100.00 | 100.00 | 86.66 | 95.00 | 100.0 |
| Nath baba Bt | (70.11) ^D | (71.56) ^D | (90.00) ^D | (90.00) ⁰ | (90.00) | (68.85) ^D | (79.54) ^D | (90.0 |
| | 90.00 | 93.33 | 100.00 | 100.00 | 100.00 | 88.33 | 91.66 | 100.0 |
| Nath baba non Bt | (71.56) ^D | (77.71) ^D | (90.00) ^D | (90.00) ⁰ | (90.00) | (70.11) ^D | (73.40) ^D | (90.0 |
| | 0.00 | 3.33 | 20.00 | 36.66 | 100.00 | 6.66 | 16.66 | 23.3 |
| RCH 2 BGII | (0.00) ^a | (6.14) ^a | (26.07) ^a | (37.22) ^a | (90.00) | (12.29) ^a | (23.85) ^a | (28.7 |
| F-TEST | Sig | Sig | Sig | Sig | | Sig | Sig | Sig |
| SEm± | 1.40 | 4.15 | 1.61 | 0.75 | | 2.48 | 4.61 | 0.83 |
| CD(P=0.05) | 4.23 | 12.59 | 4.90 | 2.30 | | 8.63 | 14.01 | 2.54 |

Table 6. Percent adult emergence of S.litura on test hybrids.

Figures in parentheses are angular transformed values, Numbers followed by same superscript are not statistically different.

were fed on leaves of RCH 2 *Bt* hybrid and it is statistically at par with other hybrids except RCH 2 BGII (Table 5). The fifth instar larvae fed on leaves of RCH 2 non *Bt* recorded 138.13 mg of pupal weight followed by Nath baba *Bt* (138.06 mg), Nath baba non *Bt* (137.00 mg), JK Durga non *Bt* (136.40 mg) and RCH 2 *Bt* (135.93 mg) hybrids and all are statistically on par with each other. RCH 2 BGII hybrid was the least preferred host with only 125.66 mg of mean pupal weight, when fifth instar larvae were reared on this hybrid. The different instars fed on squares exhibited similar trend in pupal weight as those fed on

leaves. The larvae of *S. litura* fed on RCH 2 BGII hybrid recorded least pupal weight compared to other hybrids. The first instar larvae fed on Nath baba non *Bt* squares recorded the maximum pupal weight (134.8 mg) and is on par with JK Durga non *Bt* (134.46 mg), RCH 2 non *Bt* (133.26 mg) and JK Durga *Bt* (133.56 mg). The lowest mean pupal weight of 101.26 mg was recorded in RCH 2 BGII hybrid. The mean pupal weight was increased gradually with increase in age of larvae on RCH 2 BGII hybrid. The maximum pupal weight (136.06 mg) was recorded on JK Durga non *Bt* hybrid and it was statistically on par with RCH 2 *Bt* (136 mg), Nath baba (135.00 mg). R (121.13 mg) pup were reared on The percent second instar la was more than par with each 100.00% when reared on the te fifth instar th emergence in al

| Testhala | Survival indices | | | | | | | |
|-------------------------|------------------|---------|--------|---------|--|--|--|--|
| Test hybrids | Leaves | Squares | Leaves | Squares | | | | |
| RCH 2Bt | 11.11 | 10.71 | 1.00 | 1.00 | | | | |
| RCH 2 non Bt | 11.54 | 10.00 | 1.00 | 1.00 | | | | |
| JK Durga <i>Bt</i> | 10.00 | 10.00 | 1.00 | 1.00 | | | | |
| JK Durga non Bt | 11.11 | 10.54 | 1.00 | 1.00 | | | | |
| Nath baba Bt | 10.00 | 10.71 | 1.00 | 1.00 | | | | |
| Nath baba non <i>Bt</i> | 11.54 | 11.11 | 1.00 | 1.00 | | | | |
| RCH2 BG II | 1.85 | 2.78 | 0.20 | 0.23 | | | | |

Table 7. Growth and survival indices for S. litura on test hybrids.

The adult emergence was very poor when first, second and third instars were fed on squares of RCH 2 BGII hybrid. There was only 6.66, 16.66 and 23.33% adult emergence in first, second instar and third instar, when larvae were reared on RCH 2 BGII hybrid. This hybrid is least preferred by the *S. litura*. The other test hybrids recorded > 86.00% adult emergence when first and second instar larvae were reared on these hybrids. Cent percent adult emergence was observed when third, fourth and fifth instars reared on the test hybrids.

The growth index values for S. litura larvae reared on leaves were 1.85 on RCH 2 BGII hybrid as against 11.11 on RCH 2 Bt, 10.00 on JK Durga Bt and 10.00 on Nath baba Bt, 11.54, 11.11 and 11.54 on their corresponding non Bt versions while on squares the growth index values were 2.78 on RCH 2 BGII hybrid as against 10.71 on RCH 2 Bt, 10.00 on JK Durga Bt and 10.71 Nath baba Bt followed by 10.00 on RCH 2 non Bt, 10.54 on JK Durga non Bt and 11.11 Nath baba non Bt hybrids. The survival index values for S. litura larvae reared on leaves were 0.20 on RCH 2 BGII hybrid as against 1.00 on RCH 2 Bt, JK Durga Bt, Nath baba Bt, RCH 2 non Bt, JK Durga non Bt and Nath baba non Bt hybrids, while on squares the survival index values were 0.23 on RCH 2 BGII hybrid as against 1.00 on RCH 2 Bt, JK Durga Bt, Nath baba Bt, RCH 2 non Bt, JK Durga non Bt and Nath baba non Bt hybrids. The data indicated that the growth and survival index were low when reared on leaves compared to those reared on squares in all the events of *Bt* cotton tested. However the growth and survival indices were low when the larvae were fed with RCH 2 BGII hybrid compared to other Bt and non Bt hybrid (Table 7).

REFERENCES

- Adamczyk JJ, Hardee DD, Armes LC, Sumer DV (2001). Correlating differences in larval survival and development of bollworm (Lepidoptera: Noctuidae) and fall armyworm (Lepidoptera: Noctuidae) to differential expression of Cry1Ac delta endotoxin in various plant parts among commercial cultivars of transgenic *Bacillus thuringiensis* cotton. J. Econ. Entomol. 94(1):284-290.
- Andre CLB, Terence SA, Sek YT, Blair SD (2010). Fitness costs of Cry1Ab resistance in a field-derived strain of Ostrinia

nubilalis (lepidoptera: crambidae). J. Econ. Entomol. 103(4):1386-1393.

- Bagla P (2010). Hardy cotton-munching pests are latest blow to GM crops. Science 327:1439.
- Chinna BNV, Prasad NVVSD, Krishna MSR, Ramachndra-rao G (2013). Spatial and Temporal expression of Bt toxins on commercial Bt cotton hybrids. J. Cotton Res. Dev. 27(1):80-84.
- Donglin H, Qin LH, Xia JS (2006). Effects of Zhongmiansuo 45 and Zhongmiansuo 41 on experimental population of *Spodoptera litura*. Acta Phytophylacica Sinica 33(1):1-5.
- Guifen Z, Hao W F, Xue LW, Ying GT (2006). Early instar response to plant delivered *Bt* toxin in a herbivore *Spodoptera litura* and predator *Propylaea japonica*. Crop Prot. 25(6):527-533.
- Hamilton K A, Goodman RE, Fuchs RL (2002). Safety assessment of insect protected cotton. In J. A. Thomas and R. L. Fuchs (eds.): Biotechnology and Safety Assessment. Academic press, London, U.K. pp. 435-465.
- Henneberry TJ, Jech JF, Torre T (2001). Effects of transgenic cotton on cabbage looper, tobacco budworm and beet armyworm larval mortality and development and foliage consumption in the laboratory. Southwest. Entomol. 26:325-338.
- Kranthi KR, Armes NJ, Nagarjun RGV, Raj S, Sundaramurthy VT (1997). Seasonal dynamics of metabolic mechanisms mediating pyrethriod resistance in *Helicoverpa armigera* in Central India. Pestic. Sci. 50:91-98.
- Kranthi KR, Kranthi S, Ali S, Banerjee SK (2000). Resistance to Cry 1Ac deltaendotoxin of *Bacillus thuringiensis* in a laboratory selected strain of *Helicoverpa armigera*. Curr. Sci. 78:1001-1004.
- Kranthi KR, Naidu S, Dhawad CS, Tatwawadi A, Mate K, Patil E, Bharose AA, Behere GT, Wadaskar RM, Kranthi S (2005).Temporal and intraplant variability of Cry 1Ac expression in *Bt* cotton and its influence on the survival of the cotton bollworm, (*Helicoverpa armigera* Hubner) (Noctuidae: Lepidoptera). Curr. Sci. 88:796-800.
- Kumar UV, Sangeetha P, Bahrathi M (2004). Analysis of Cry2A proteins encoded by genes from indigenous isolates of Bacillus thuringiensis for toxicity against *Helicoverpa armigera*. Curr. Sci. 86:566-570.
- Parker CD, Mascarenhas VJ, Lutrell RG, Knighten K (2000). Survival rates of tobacco budworm (Lepidoptera: Noctuidae) larvae exposed to transgenic cottons expressing insecticidal protein of *Bacillus thuringiensis* Berliner. J. Entomol. Sci. 35(2):105-117.
- Romeis J, Meissle M, Bigler F (2006). Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. Nat. Biotechnol. 24(1):63-71.
- Shelton AM, Zhao JZ, Roush RT (2002). Economic, ecological food safety, and social consequences of the deployment of Bt transgenic plants. Ann. Rev. Entomol. 47:845-881.
- Sivasupramaniam S, Ruschke LG, Osborn JA, Oppenhuizen ME, Mullins JW, Watson KG, Mueller G (2003). BollgardII Improvements in efficacy and spectrum against lepidopteran pests of cotton. Proceedings of the 2003 Beltwide Cotton Conferences National cotton council of America Nashville TN 38182 U.S.A.
- Soujanya PL (2008). Effect of *Bt* toxions (Cry 1Ac and Cry 1Ac + 2 Ab) on the development and management of bollworm complex with

special references to (*Pectinophora gossypiella* Saunders) and (*Spodoptera litura* Fabricius) on cotton. Ph.D. Thesis Acharya N.G. Ranga Agricultural University, Rajendranagar, Hyderabad.

- Soujanya PL, Rao PA, Prasad NVVSD (2009). Expression of *Bacillus thringiensis* insect control protein Cry1Ac over time in different bplant parts of Bt cotton hybrids. J. Ecobiol. 25(1)29-34.
- Stewart SD, Adamczyk JJ, Knighten KS, Davis FM (2001). Impact of *Bt* cottons expressing one or two insecticidal proteins of *Bacillus thuringiensis* on growth and survival of Noctuid Iarvae. J. Econ. Entomol. 94(3):752-760.
- Tabashnik (1994). Evolution of resistance to *Bacillus thuringiensis*. Ann. Rev. Entomol. 39:47-79.
- Tabashnik BE, Gassmann AJ, Crowder DW, Carriere Y (2008). Insect resistance to Bt crops: Evidence versus theory. Nat. Biotechnol. 26:199-202.
- Usha S, Regupathy A (2004). Measuring the efficacy of *Bt* cotton by using field bioassay of (*Helicoverpa armigera* Hubner). Proceedings of National Symposium on strategies for sustainable cotton production-A global vision 23-25, November, 2004, UAS, Dharwad, pp. 132-135.
- Vennila S, Panchbhai PR, Biradar VK (2006). Growth and survival of *Helicoverpa armigera* (Hubner) and *Spodoptera litura* (Fab.) on transgenic *Bt* cotton. J. Cott. Res. Dev. 20(1):131-133.