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Transgenic *Bt* and non transgenic cotton effects on larval mortality and development of *Helicoverpa armigera* (Hubner) and *Spodoptera litura* (Fabricius)

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ABSTRACT: The effect of various tissues (leaf, square and green bolls) of *Bt* and non *Bt* cotton cultivar on larval mortality and development of *Helicoverpa armigera* (Hubner) and *Spodoptera litura* (Fabricius) was studied in Department of Entomology, Punjab Agricultural University, Ludhiana. Different *H. armigera* larval instars *i.e.* 2nd, 3rd and 4th of when fed with different plant parts like leaves, squares and bolls of transgenic *Bt* cotton hybrid resulted in 100 per cent mortality. Similarly, 2nd ,3rd and 4th larval instars of *S. litura* when fed on different plant parts (leaves, squares and green bolls) of transgenic *Bt* cotton cultivar resulted in 100 per cent mortality up to 12 days of treatment except when 2nd, 3rd and 4th instar fed on squares in all the treatments. The pupation took place after 18 days of treatment when 2nd instar fed with squares of *Bt* cotton cultivars. However, pupation was only 3.33 per cent when 3rd instar larvae of *S. litura* fed on squares after 14 days of treatment but these pupae were deformed. No adult emergence took place in any treatment whereas in case of non *Bt* cotton, 100 per cent adult emergence took place.

Key words : Bollgard II, development, *Helicoverpa armigera,* mortality, *Spodoptera litura*

Cotton production in India is severely constrained due to the damage inflicted by insect pests, particularly lepidopterans. The most serious of these pests is the american bollworm, *Helicoverpa armigera* (Hubner). Other significant lepidopteran pests of cotton in India include pink bollworm, *Pectinophora gossypiella* (Saunders), spotted bollworm, *Earias vitella* (Fabricius); spiny bollworm, *Earias insulana* (Boisd) and tobacco caterpillar, *Spodoptera litura* (Fabricius.). Toxins from a soil bacterium, *Bacillus thuringiensis* (*Bt*) are widely used for control of many insect pests from decades. Transgenic cotton, expressing the ä-endotoxin gene from the bacterium *B. thuringiensis* is a convincing answer to manage cotton bollworms (Kumar and Grewal, 2016). Although BG I cotton expressing Cry1Ac is providing good control of bollworms but its replacement with BG II expressing dual genes Cry 1Ac and Cry 2Ab proteins has provided increased efficacy against bollworm complex and enhanced spectrum of activity against beet armyworm, *Spodoptera exigua* (Hubner) and tobacco caterpillar, *S. litura* which have been predicted to be major pests under changing scenario (Rao *et al.,* 2015). The neonates of *H. armigera* and *S. litura* are highly susceptible to these toxicants as life begins with egg stage. However, under field condition the later larval instars of *S. litura* were found attacking the leaves and squares of Bollgard II and that may be due to decline in resistance or poor expression of genes in cotton plants. Survival of larvae up to late instars would led to enhanced damage and acquisition of resistance. The dreaded pests *H. armigera* and *S. litura* have many alternate cultivated hosts like pigeonpea, castor, groundnut etc. which are presently non *Bt*

cultivars. Due to plant protection in these crops, migration of different instars to *Bt* cotton could not be ruled out. The efficacy of *Bt* toxins expressed in cotton plants may not be sufficient to contain grown up larvae. Migration of *S. litura* to cotton at later stage of crop growth is more prone to such problem. There is scanty information regarding instar wise efficacy on different *Bt* cotton target tissues expressing different level of cry toxins. Therefore, present investigation was undertaken to analyze the larval mortality and development variation in respect of different instars to understand pattern of resistance, in different *Bt* cotton target tissues expressing different level of cry toxins.

MATERIALS AND METHODS

Bt cotton, MRC 7017 expressing dual genes of cry1Ac and cry2Ab proteins of Mansanto Mahyco India Pvt Ltd and non *Bt* cotton cultivar LH 2076 was grown at Entomological Research Farm, Punjab Agricultural University, Ludhiana. The bioassay studies were carried out at 110 to 125 DAS (day after sowing) for *H. armigera* and *S. litura* using 2nd, 3rd and 4th instar larvae from the cultures maintained in IPM Laboratory of Department of Entomology, PAU, Ludhiana. Different instars of *H. armigera* and *S. litura* were fed with different plant parts like leaf, squares and bolls of *Bt* and non *Bt* cotton. Each treatment comprised of 20 larvae each of different instars of *H. armigera* and *S. litura* with three replications. The treatments comprised of second instar larvae fed with terminal leaf (youngest leaf from apical or side branches), second instar larvae fed with pre candle and candle size squares, third instar larvae fed with terminal leaf, third instar larvae fed with pre candle/ candle size squares, third instar fed with green bolls, 4th instar larvae fed with pre candle size squares, 4th instar larvae fed with green bolls, all the treatments (1 to7) with non *Bt* tissues in a separate set and equal number of larvae/ treatment. The observations on the per cent mortality of different larval instars recorded at different time intervals. The observations on development of larvae, per cent pupation and adult emergence of *H. armigera* and *S. litura* were also recorded.

RESULTS AND DISCUSSION

Effect of BG II cotton on different instars of *H. armigera* : No mortality of larvae of *H. armigera* was observed after one day of treatments. However, after three days of treatments, significantly higher mortality was recorded in second instar larvae when fed on squares (100%), third instar larvae fed on leaves (100%) and fourth instar larvae fed with green bolls (91.66) in comparison to all other treatments (Fig 4). It was observed that feeding of leaf discs from cry1Ac/Cry2Ab cotton resulted in mortality of second instars of *S. frugiperda* ranging from 69 to 93 per cent depending on plant age.

After 5 days of treatments, 100 per cent mortality was recorded when 3rd and 4th instar larvae of *H. armigera* fed with squares. However. Significantly higher mortality (100%) in neonates of *H. armigera* when fed on *Bt* cotton leaves than those fed on *Bt* flower bolls (93%). After 7 days of treatments, maximum per cent mortality of larvae of *H. armigera* was observed in 2nd instar larvae fed on leaves (100%). After 10 days of treatments maximum mortality was observed in 4th instar fed with green bolls (100%). After 12 days of treatments 100 per cent mortality was recorded in all the treatment. No larvae of *H. armigera* fed on leaves, squares and bolls entered the pupal stage.

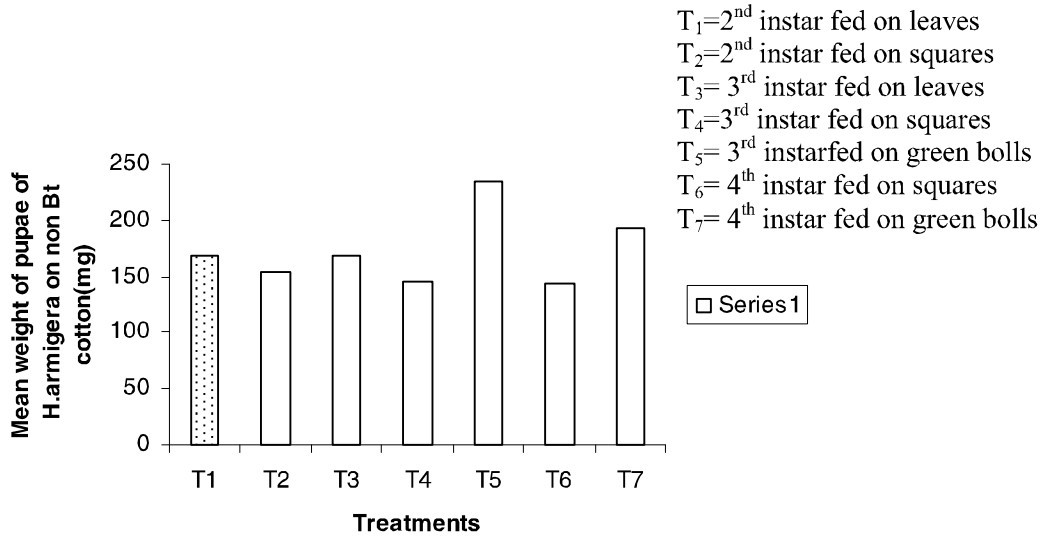

Fig. 1. Pupal weight of different intars of *H. armigera* on non *Bt* cotton cultivar when fed with leaf, squares and bolls

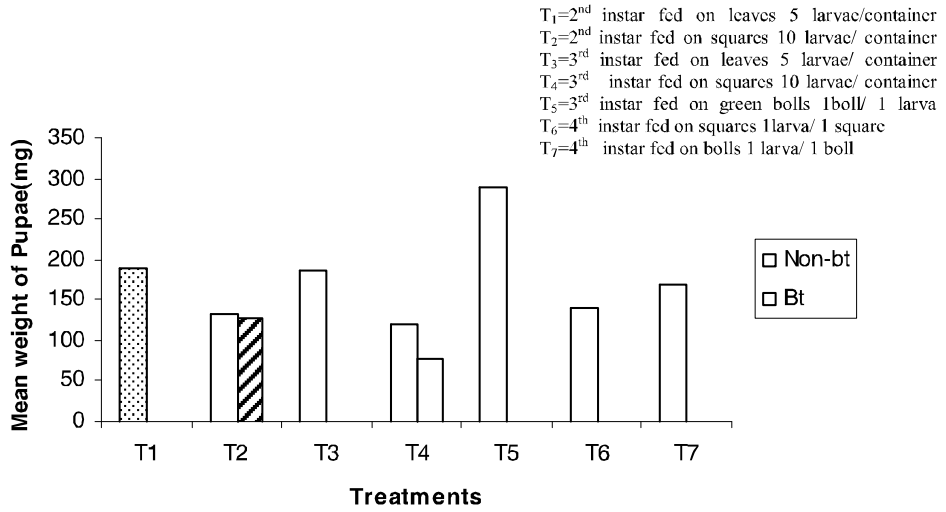

Fig. 2. Pupal weight of different intars of *S. litura* on *Bt* and non *Bt* cotton cultivar when fed with leaf, squares and bolls

The cotton data presented in Table 1 showed that when different instar of *H. armigera* fed on non *Bt* leaves, squares and bolls, all the larvae were able to complete their development. In all the cases 100 per cent pupation (Fig 1) and adult emergence took place. The results are in conformity with the earlier findings of Quyang *et al.,* (2011) who found that larvae of *H. armigera* fed on *Bt* cotton had a decreased pupation rate and fewer emerged as adult in comparison with larvae fed on non *Bt* cotton under field and laboratory conditions.

Effect of *Bt* cotton on different instar of *S. litura* : No mortality of larvae of *S. litura* was observed after one day of treatments. However, after three days of treatments, significantly higher mortality was recorded in

Table 1. Effect of non *Bt* LH 2076 cotton on the per cent pupation and adult emergence of *H. armigera* at different interval

Treatment	Per cent pupation of *H. armigera* days after treatment (DAT)								Per cent adult emergence
	10	12	14	16	18	20	22	24	
T1 (2nd instar fed on leaves)	0.00(0.00)	0.00(0.00)	20.00(26.55)	40.00 (39.21)	60.00 (50.74)	70.00 (56.76)	90.00(71.53)	100.00	100.00
T2 (2nd instar fed on squares)	0.00(0.00)	0.00(0.00)	20.00(26.55)	50.00(44.98)	70.00(56.76)	80.00(63.40)	100.00(89.96)	100.00	100.00
T3 (3rd instar fed on leaves)	0.00(0.00)	30.00(33.19)	80.00(63.40)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00	100.00
T4 (3rd instar fed on squares)	0.00(0.00)	50.00(44.98)	80.00(63.40)	100.00(89.96)	100.00(89.96)	100.00 (89.96)	100.00(89.96)	100.00	100.00
T5 (3rd instar fed on green bolls)	30.00(33.19)	70.00(56.96)	100.00 (89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00	100.00
T6 (4th instar fed on squares)	20.00(26.55)	60.00(50.74)	100.00 (89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00	100.00
T7 (4th instar fed on green bolls)	20.00(26.55)	70.00(56.76)	100.00 (89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00	100.00
LSD (p=0.05)	(0.014)	(0.000001)	(0.06)	(0.08)	(0.08)	(0.08)	(0.06)	NS	NS

Mean of three replications; Figures in the parentheses indicate arc sine transformation

Table 2. Effect of *Bt* cotton hybrid MRC7017 on the per cent mortality of *S. litura* at different time interval

Treatment	Per cent mortality of *S. litura* (DAT)								
	1	3	5	7	10	12	14	16	18
T1 (2nd instar fed on mature leaves)	0.00	66.66	93.33(81.11)	100.00	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00
T2 (2nd instar fed on squares)	0.00	56.66	70.00(61.89)	70.00	80.00(68.04)	80.00(68.04)	83.33(70.04)	83.33(70.04)	86.66
T3 (3rd instar fed on mature leaves)	0.00	33.33	86.66(72.26)	93.33	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00
T4 (3rd instar fed on squares)	0.00	23.33	46.66(43.05)	76.66	76.66(61.19)	86.66(68.82)	86.66(68.82)	86.66(68.82)	96.66
T5 (3rd instar fed on green bolls)	0.00	50.00	50.00(44.98)	66.66	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00
T6 (4th instar fed on squares)	0.00	0.00	0.000(0.000)	33.33	33.33(29.98)	33.33(29.98)	100.00(89.96)	100.00(89.96)	100.00
T7 (4th instar fed on green bolls)	0.00	33.33	100.00(89.96)	100.00	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00
LSD (p=0.05)	NS	NS	(37.68)	NS	(36.93)	(36.97)	(12.03)	(12.03)	NS

Mean of three replications; Figures in the parentheses indicate arc sine transformation

Plate 1. Pupa formation on *Bt* and non *Bt* cotton

second instar larvae of *S. litura* when fed on leaves (66.66) and squares (56.66) in comparison to all other treatments. However, the data was statistically non significant. After 5 days of treatments, 100 per cent mortality was recorded when 4th instar larvae of *S. litura* fed on green bolls followed by 2nd instar larvae fed on mature leaves(93.33). Similarly, according to Naik *et al.,* (2013) larval mortality when fed on leaves and squares of RCH2 BGII featuring cry1Ac+cry2Ab was highest compared to other *Bt* cotton hybrids. After 7 days of treatments, 100 per cent mortality of larvae of *S. litura* was recorded when 2nd instar fed on mature leaves followed by 3rd instar larvae fed on mature leaves (93.33) (Table 2). According to Kumar and Grewal, 2015 the neonates, 1st and 2nd instar larvae of *S. litura* when fed on leaves of transgenic *Bt* cotton resulted in 100 per cent mortality as compared to non *Bt* cotton (0.00). However, 2nd instar larvae fed on squares of *Bt* cotton resulted in 100 oer cent mortality in most of *Bt* cotton cultivars, MRC7017, BCHH 6488, BCHH 6588 and Ankur 3028 except NCS 855 (82.16) and non *Bt* (6.63). After 12 days of treatments, maximum mortality was observed in 3rd instar fed with squares (86.66). After 14 days of treatments, maximum mortality was observed in 4th instar fed with squares (100). After 18 days of treatment, maximum mortality was observed in 3rd instar fed with squares (96.66).

Effect of *Bt* cotton on Pupation and adult emergence of *S. litura* : After 7 days of treatment, no pupation took place in any treatment. After 12 days of treatment, only 3.33 per cent pupation took place in T_2 (2nd instar fed on squares). After 14 days of treatment, 6.66 per cent pupation took place in T_2 and 3.33 per cent pupation took place in T_4 (3rd instar fed on squares). After 18 days of treatment, 13.33 per cent pupation took place in T_2 (2nd instar fed on squares). However, no adult emergence took place in any treatment (Fig. 3).

Pupal weight fed on leaves and squares : A marked difference was found in pupal weight of larvae fed on *Bt* and non *Bt* cotton (Fig 1). Pupal weight was higher on non *Bt* cotton (189mg) (2nd instar fed on leaves), 133.2mg (2nd instar fed on squares),185.9 mg (3rd instar fed on leaves), 121.3 mg (3rd instar fed on squares), 288.2mg (3rd instar fed on green bolls), 141.1mg (4th instar fed on squares),168.5 mg (4th instar fed on bolls) as compared to *Bt* cotton 127.6mg (2nd instar fed

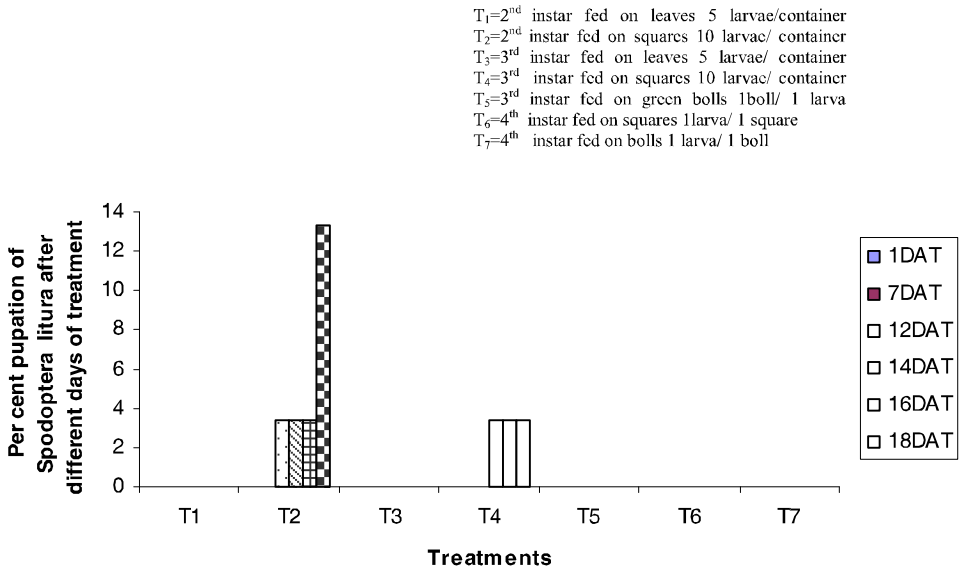

Fig. 3. Percent pupation of *Spodoptera litura* on *Bt* cotton after different days of treatment

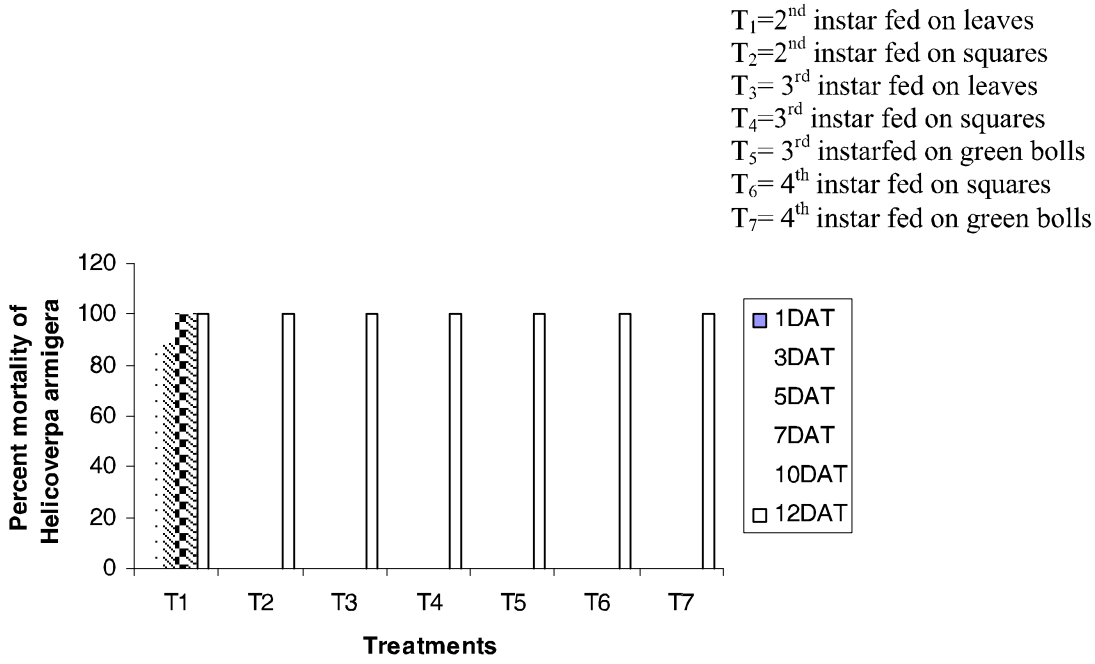

Fig. 4. Effect of *Bt* cotton hybrid MRC 7017 on the per cent mortality of *H. armigera* at different time interval

on squares) and 77.9mg (3rd instar fed on squares) (Fig. 2) (Plate 1). Arshad and Suhail (2011) also recorded a marked difference in pupal weight of larvae fed on *Bt* and non *Bt* cotton cultivars.

The data presented in Table 3 showed that when different instars of *S. litura* fed on non *Bt* leaves, squares and bolls, all the larvae were able to complete their development. In all the cases, 100 per cent pupation and adult emergence took place. The results are in conformity with Govindan *et al.,* (2010) who

Table 3. Effect of non *Bt* cotton LH 2076 on the per cent pupation and adult emergence of *S. litura* at different interval

Treatment	Per cent pupation *S. litura* (days after treatment) (DAT)								Per cent adult
	10	12	14	16	18	20	22	24	emergence
T1 (2nd instar fed on leaves)	0.00(0.00)	0.00(0.00)	20.00(26.55)	40.00 (39.21)	60.00(50.74)	70.00 (56.76)	90.00(71.53)	100.00	100.00
T2 (2nd instar fed on squares)	0.00(0.00)	0.00(0.00)	20.00(26.55)	50.00(44.98)	70.00(56.76)	80.00(63.40)	100.00(89.96)	100.00	100.00
T3 (3rd instar fed on leaves)	0.00(0.00)	30.00(33.19)	80.00(63.40)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00	100.00
T4 (3rd instar fed on squares)	0.00(0.00)	50.00(44.98)	80.00(63.40)	100.00(89.96)	100.00 (89.96)	100.00 (89.96)	100.00(89.96)	100.00	100.00
T5 (3rd instar fed on green bolls)	30.00(33.19)	70.00(56.76)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00	100.00
T6 (4th instar fed on squares)	20.00(26.55)	60.00(50.74)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00	100.00
T7 (4th instar fed on green bolls)	20.00(26.55)	70.00(56.76)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00	100.00
LSD (p=0.05)	(0.01)	(0.000001)	(0.06)	(0.08)	(0.08)	(0.08)	(0.06)	NS	NS

Mean of three replications; Figures in the parentheses indicate arc sine transformation

tested *Bt* cotton hybrids *viz*. *Bt* bunny, six bollgard II (cry1Ac+cry2Ab genes) hybrids *viz.*, RCH2 *Bt*, RCH 596 *Bt*, RCH 134 *Bt* and RCH 533 *Bt* and two non *Bt* cotton cultivars against third instar larvae of *S. litura*.

From all this it was concluded that cry protein content was significantly high in leaves from top canopy leaves followed by green bolls in all *Bt* events. Square bracts and square buds had significantly lower protein expression in all the *Bt* events. The present findings are in accordance with Badiger *et al.,* (2013) who reported higher expression of cry1Ac in leaf canopy(3.55-0.42 and3.66-0.47µg/g fresh weight) in comparison to that in fruiting parts(1.85-0.14 and 1.83 to 0.15).

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