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Effect of *Bt* cotton hybrids on larval mortality and development of *Helicoverpa armigera* (Hubner) and *Spodoptera litura* (Fabricius)

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ABSTRACT: Different larval instars (1st, 2nd, 3rd and 4th) of *H. armigera* when fed on the different plant parts like leaves and squares of transgenic *Bt* cotton hybrids suffered in 100 per cent mortality. However, last instar larvae of *H. armigera* fed to the different plant parts, led to 80-100 per cent larval mortality. No larva survived in case of MRC 7031. However, pupation took place in case of MRC 7017 and MRC 6301 but pupae were deformed. Only 1-2 adults were formed but they were not able to survive for more than 3 days and all of them were males. Similarly, different larval instars (1st, 2nd, 3rd and 4th) of *S. litura* when fed on the different plant parts like leaves and squares of transgenic *Bt* cotton hybrids recorded in 100 per cent mortality of larvae upto 7 days of the treatment. Last instar of *S. litura* when fed to the different plant parts mortality of larvae ranged from 80-100 per cent. No larva survived in case of MRC 7031 and Ankur 3028 when fed on the leaves. The last instar larvae of *S. litura* were able to feed on the leaves of MRC 7017, MRC 7031. They were also able to feed on squares of MRC 7031 and Ankur 3028. The pupation took place in case of MRC 7017 when fed on squares and bolls, but the pupae were deformed. However, adults were formed in case of Ankur 3028 when fed on bolls. All the adults so obtained were males and they were not able to survive for more than 2 days. The results clearly represent decreased sensitivity in grown up stages of larvae in comparison to early instars.

Key words: *Bt* cotton, 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.). Resistance of cotton bollworms to insecticides is a major concern because of extensive reliance on these materials for control (Men *et al.*,, 2005). Transgenic cotton, expressing the ä-endotoxin gene from the bacterium *Baccillus thuringiensis* (*Bt*) is a convincing answer to manage cotton bollworms (Naranjo, 2005). Although BG I cotton expressing Cry1Ac is effective against bollworms but its replacement with BG II expressing dual genes Cry 1Ac and Cry 2Ab proteins has provided increased efficacy against bollworm complex and tobacco caterpillar, *S. litura* which have been predicted to be major pests in emerging scenario. The neonates are highly susceptible to these toxicants and life begins with egg stage. However, the decline in resistance or poor expression might have lead to survival of insects till adult stage. Survival of larvae upto 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* (LH 2076) cultivars. Due to plant protection in these crops migration of different instars to *Bt* cotton could not be ruled out. Migration to cotton at later stage of crop growth is more prone to such problem. As such there are no studies to compare instar wise efficacy of

cry toxins using discrete generations. Therefore, present investigation was undertaken to analyze the mortality variation in respect of different instars to understand pattern of resistance, in different *Bt* cotton transgenic events expressing different cry toxins.

Bt cotton hybrids were grown in the field under unprotected condition at Entomology Research Farm, Punjab Agricultural University, Ludhiana. Four *Bt* cotton cultivars MRC 7031, MRC 7017, MRC 6301, Ankur 3028 and non *Bt* (LH 2076) were selected for the bioassay. The bioassay studies were carried out at 110 to 125 DAS (day after sowing) for *H. armigera* and *S. litura* using 1st, 2nd, 3rd ,4th and 5th instar larvae from the cultures maintained in IPM laboratory. Different plant parts like leaf, squares and bolls were fed to the different instars of *H. armigera* and *S. litura*. Each treatment comprised of 10 larvae each of different instars of *H. armigera* and *S. litura* with three replications. The observations on the per cent mortality of different larval instars recorded after 3, 5, 7 and 8 days. The observations on development of larvae, per cent pupation and adult emergence of *H. armigera* and *S. litura* were also recorded.

Effect of *Bt* cotton on different instar of *H. armigera*: The mortality of *H. armigera* was assessed using 1st, 2nd, 3rd, 4th and last larval instar. The data presented in Table 1 revealed 100 per cent mortality of first instar larva of *H. armigera* when fed on leaves of *Bt* cotton MRC 7031 and MRC 7017 followed by MRC 6301 (86.66%) after 5 days. The first instar larva of *H. armigera* when fed on *Bt* cotton squares, per cent mortality was significantly higher in all cotton hybrids being *at par* with each other. However, no larval mortality was observed in non *Bt* LH 2076 (control). After 7 days, 100 per cent mortality was recorded in all cotton hybrids. However, Muhammad *et al.*, (2009) recorded significantly higher mortality (100%) in neonates of *H. armigera* when fed on *Bt* cotton leaves than those fed on *Bt* flower bolls (93%). Similarly, significantly higher mortality (Table 1 and 2) was observed in 2nd and 3rd instar larvae of *H. armigera* fed on leaves and squares of MRC 7031, MRC 7017 and MRC 6301 after 7 days.

Cent per cent mortality was observed in 4th instar larvae after 5 days when fed on leaves and squares of MRC 7031, MRC 7017 and MRC 6301 (Table 2). The data presented in Table 3 revealed that after 5 days significantly lower mortality in last instar larvae fed on MRC 7017 leaves (56.66%), squares (36.66%) and bolls (16.66%) was recorded. No significant difference was observed among three cotton hybrids except non *Bt* (LH 2076). However, after 7 days significantly higher mortality was observed in last larval instar fed with leaves of MRC 7031 (100.00%), MRC 7017 (100.00%) followed by bolls of MRC 7031 (76.66%). Similarly, after 8 days, significantly higher mortality was observed in larvae fed with leaves and bolls of MRC 7031, leaves of MRC 7017 (100.00%) followed by leaves of MRC 6301 (86.66%). No larval mortality was recorded in control. However, few studies available are in close agreement with the present study (Govindan *et al.,* 2010, Arshad *et al.,* 2009 and Bird and Akhurst, 2007).

The data presented in Table 4 revealed that no pupation was recorded in larvae fed with leaves of MRC 7031 and MRC 7017. However, 10 per cent pupation was recorded in MRC 7031 as compared to non *Bt* (100). Similarly, 16.66 and 10 per cent pupation was observed in MRC 7017 and MRC 6301 when fed with squares as compared to non *Bt* LH 2076 (100%). No pupation was observed in larvae fed with bolls of MRC 7031 as compared to 16.66 per cent in MRC 7017 and MRC 6301 followed by non *Bt* LH 2076 (100%). 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. The

Table 1 Effect of *Bt* cotton (leaf and squares) on first and second instar of *Helicoverpa armigera* (Hubner)

Cotton cultivar	First instar						Second instar					
	Per cent mortality (days after treatment)						Per cent mortality (days after treatment)					
	3 day		5 day		7 day		3 day		5 day		7 day	
	Leaf	Square	Leaf	Square	Leaf	Square	Leaf	Square	Leaf	Square	Leaf	Square
MRC7031	86.66	76.66	100.00	100.00	100.00	100.00	86.66	86.66	100.00	100.00	100.00	100.00
	(68.82)	(61.19)	(89.96)	(89.96)	(89.96)	(89.96)	(68.82)	(68.82)	(89.96)	(89.96)	(89.96)	(89.96)
MRC7017	86.66	76.66	100.00	100.00	100.00	100.00	76.66	100.00	100.00	100.00	100.00	100.00
	(68.82)	(61.19)	(89.96)	(89.96)	(89.96)	(89.96)	(61.19)	(89.96)	(89.96)	(89.96)	(89.96)	(89.96)
MRC6301	76.66	66.66	86.66	100.00	100.00	100.00	76.66	100.00	86.66	100.00	100.00	100.00
	(61.19)	(54.76)	(68.82)	(89.96)	(89.96)	(89.96)	(61.19)	(89.96)	(68.82)	(89.96)	(89.96)	(89.96)
Non *Bt* (LH 2076)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CD(p=0.05)	(7.21)	(6.06)	(4.41)	(0.06)	(0.06)	0.06	(6.75)	(4.41)	(4.41)	(0.06)	(0.06)	(0.06)

Table 2 Effect of *Bt* cotton (leaf and squares) on third and fourth instar of *Helicoverpa armigera* (Hubner)

Cotton cultivar	Third instar						Foruth instar					
	Per cent mortality (days after treatment)						Per cent mortality (days after treatment)					
	3 day		5 day		7 day		3 day		5 day		7 day	
	Leaf	Square	Leaf	Square	Leaf	Square	Leaf	Square	Leaf	Square	Leaf	Square
	86.66	66.66	100.00	100.00	100.00	100.00	100.00	86.66	100.00	100.00	100.00	100.00
MRC7031	(68.82)	(54.76)	(89.96)	(89.96)	(89.96)	(89.96)	(89.96)	(68.82)	(89.96)	(89.96)	(89.96)	(89.96)
	76.66	66.66	86.66	76.66	100.00	100.00	100.00	66.66	100.00	100.00	100.00	100.00
MRC7017	(61.19)	(54.76)	(68.82)	(61.19)	(89.96)	(89.96)	(89.96)	(54.76)	(89.96)	(89.96)	(89.96)	(89.96)
	56.66	36.66	100.00	100.00	100.00	100.00	86.66	86.66	100.00	100.00	100.00	100.00
MRC6301	(48.82)	(37.20)	(89.96)	(89.96)	(89.96)	(89.96)	(68.82)	(68.82)	(89.96)	(89.96)	(89.96)	(89.96)
Non *B t*(LH 2076)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CD(p=0.05)	(6.51)	(5.66)	(4.41)	(3.61)	(0.06)	(0.06)	(4.41)	(7.05)	(0.06)	(0.06)	(0.06)	(0.06)

Table 3. Effect on *Bt* cotton (leaf, square and boll) on last instar of *Helicoverpa armigera* (Hubner) and *Spodoptera litura* (Fabricius)

Cotton cultivar	Per cent mortality (Days after treatment)											
	3 day			5 day			7 day			8 day		
	Leaf	Square	Boll	Leaf	Square	Boll	Leaf	Square	Boll	Leaf	Square	Boll
						Helicoverpa armigera						
MRC 7031	36.66	16.66	0.00	56.66	26.66	36.66	100.00	76.66	76.66	100.00	86.66	100.00
	(37.20)	(23.84)	0.00	(48.82)	(30.98)	(37.20)	(89.96)	(61.19)	(61.19)	(89.96)	(68.82)	(89.96)
MRC 7017	56.66	36.66	16.66	56.66	36.66	36.66	100.00	56.66	66.66	100.00	76.66	76.66
	(48.82)	(37.80)	(23.84)	(48.82)	(37.20)	(37.20)	(89.96)	(48.82)	(54.76)	(89.96)	(61.19)	(61.19)
MRC 6301	36.66	0.00	0.00	56.66	36.66	36.66	66.66	56.66	46.66	86.66	86.66	76.66
	(37.20)	0.00	0.00	(48.82)	(37.20)	(37.20)	(54.76)	(48.82)	(43.05)	(68.82)	(68.82)	(61.19)
Non *Bt* (LH 2076)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	(0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00)	0.00
CD(p=0.05)	(5.58)	(5.49)	(4.41)	(5.42)	(5.86)	(5.66)	(3.27)	(5.71)	(5.79)	(4.41)	(7.21)	(5.10)
						Spodoptera litura						
MRC 7031	66.66	0.00	0.00	66.66	70.00	0.00	76.66	70.00	26.66	100.00	70.00	66.66
	(54.76)	0.00	0.00	(54.76)	(56.76)	0.00	(61.19)	(56.76)	(30.98)	(89.96)	(56.76)	(54.76)
MRC 7017	0.00	0.00	16.66	36.66	66.66	16.66	36.66	66.66	76.66	76.66	100.00	76.66
	0.00	0.00	(23.84)	(37.20)	(54.76)	(23.84)	(37.20)	(54.76)	(61.19)	(61.19)	(89.96)	(61.19)
Ankur 3028	0.00	36.66	26.66	100.00	36.66	36.66	100.00	43.66	36.66	100.00	66.66	66.66
	0.00	(37.20)	(30.98)	(89.96)	(37.20)	(37.20)	(89.96)	(43.05)	(37.20)	(89.96)	(54.76)	(54.76)
Non *Bt* (LH 2076)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CD(p=0.05)	(3.27)	(3.27)	(5.70)	(4.62)	(4.62)	(5.49)	(4.87)	(4.53)	(6.06)	(3.61)	(3.27)	(5.86)

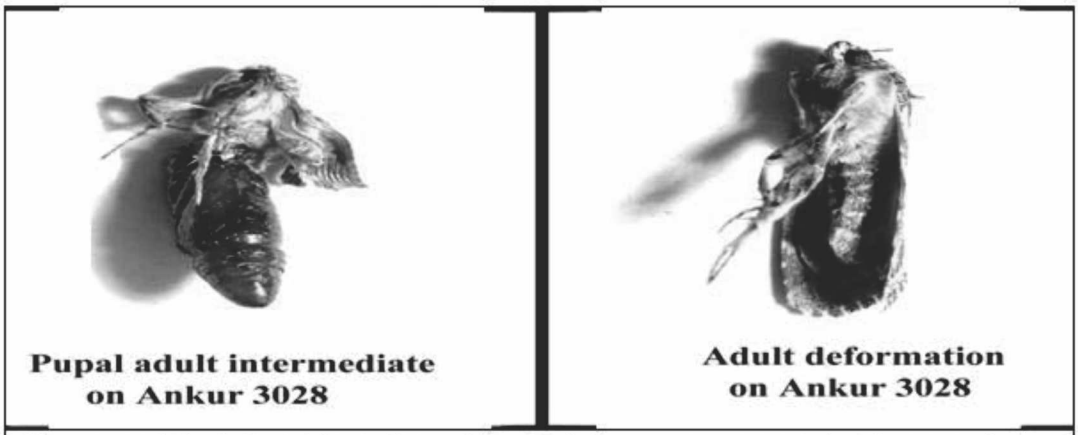

Plate 1 Adult deformities of *Spodoptera litura* on Bt cotton (Ankur 3028)

data presented in Table 4 revealed that no adult emergence was observed in any *Bt* cotton hybrid when fed on leaves. However, 10 per cent adult emergence was observed in MRC 7017 (squares and bolls), 16.66 per cent in MRC 6301 (bolls) as compared to 100 per cent on non *Bt* (LH 2076). Only 1-2 adults were formed but they were not able to survive for more than 3 days and all of them were males.

Effect of *Bt* cotton on different instars of *S. litura*: The mortality of *S. litura* was assessed using 1st, 2nd, 3rd, 4th and last instar larva. The data presented in Table 5 revealed that the first instar larva of *S. litura* when fed on *Bt* cotton leaves resulted in 100 per cent mortality in MRC 7031 followed by Ankur 3028 (86.66%). However, on 3rd day 100 per cent mortality was observed in MRC 7017 squares followed by Ankur 3028 squares (86.66%) and MRC 7031 (squares). On 7th day 100 per cent mortality was recorded in all cotton hybrids being *at par* with each other. No larvel mortality was observed in control. Data in Table 5 and 6 showed that 2nd and 3rd instar larvae fed with leaves and squares of MRC 7031, MRC 7017 and Ankur 3028 showed significantly

Table 4 Effect of *Bt* cotton (leaf, square and boll) on pupation and adult emergence of *Helicoverpa armigera* (Hubner) and *Spodoptera litura* (Fabricius)

Cotton cultivar	Per cent pupation Plant parts			Per cent adult emergence Plant parts		
	Leaf	Square	Boll	Leaf	Square	Boll
			Helicoverpa armigera			
MRC 7031	0. 00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
MRC 7017	0.00(0.00)	16.66(23.84)	16.66(23.84)	0.00(0.00)	10.00(18.42)	10.00(18.42)
MRC 6301	10.00(18.42)	10.00(18.42)	16.66(23.84)	0.00(0.00)	0.00(0.00)	16.66(23.84)
Non *Bt*(LH 2076)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)
CD(p=0.05)	(0.041)	(4.41)	(6.24)	(0.029)	(0.041)	(4.41)
			Spodoptera litura			
MRC 7031	0.00(0.00)	26.66 (30.98)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
MRC 7017	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
Ankur 3028	0.00(0.00)	26.66(30.98)	26.66(30.98)	0.00(0.00)	0.00(0.00)	16.66(23.84)
Non *Bt*(LH 2076)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)
CD(p=0.05)	(0.029)	(5.10)	(3.61)	(0.029)	(0.029)	(4.41)

Table 5. Effect of *Bt* cotton (leaf and squares) on first and second instar of *Spodoptera litura* (Fabricius)

Cotton cultivar	First instar						Second instar					
	Per cent mortality (days after treatment)						Per cent mortality (days after treatment)					
	3 day		5 day		7 day		3 day		5 day		7 day	
	Leaf	Square	Leaf	Square	Leaf	Square	Leaf	Square	Leaf	Square	Leaf	Square
MRC7031	100.00	66.66	100.00	100.00	100.00	100.00	56.66	36.66	76.66	76.66	100.00	100.00
	(89.96)	(54.76)	(89.96)	(89.96)	(89.96)	(89.96)	(48.82)	(37.20)	(61.19)	(61.19)	(89.96)	(89.96)
MRC7017	50.00	100.00	76.66	100.00	100.00	100.00	43.33	26.66	76.66	73.33	100.00	100.00
	(44.98)	(89.96)	(61.19)	(89.96)	(89.96)	(89.96)	(41.13)	(30.98)	(61.19)	(58.98)	(89.96)	(89.96)
Ankur 3028	86.66	76.66	86.66	86.66	100.00	100.00	26.66	20.00	46.66	100.00	100.00	100.00
	(68.82)	(61.19)	(68.82)	(68.82)	(89.96)	(89.96)	(30.98)	(26.05)	(43.05)	(89.96)	(89.96)	(89.96)
Non *Bt*(LH 2076)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CD(p=0.05)	(7.00)	(4.87)	(5.70)	(4.41)	(0.06)	(0.06)	(5.71)	(8.50)	(5.99)	(5.10)	(0.06)	(0.06)

Table 6. Effect of *Bt* cotton (leaf and squares) on third and fourth instar of *Spodoptera litura* (Fabricius)

Cotton cultivar	Third instar						Fourth instar							
	Per cent mortality (Days after treatment)						Per cent mortality (Days after treatment)							
	3 day		5 day		7 day		3 day		5 day		7 day		8 day	
	Leaf	Square	Leaf	Square	Leaf	Square	Leaf	Square	Leaf	Square	Leaf	Square	Leaf	Square
MRC7031	0.00	0.00	76.66	86.66	100.00	100.00	0.00	0.00	86.66	36.66	100.00	86.66	100.00	100.00
	0.00	0.00	(61.19)	(68.82)	(89.96)	(89.96)	0.00	0.00	(68.82)	(37.20)	(89.96)	(68.82)	(89.96)	(89.96)
MRC7017	9.66	0.00	86.66	100.00	100.00	100.00	0.00	0.00	100.00	56.66	100.00	66.66	100.00	100.00
	(18.10)	0.00	(68.82)	(89.96)	(89.96)	(89.96)	0.00	0.00	(89.96)	(48.82)	(89.96)	(54.76)	(89.96)	(89.96)
Ankur 3028	0.00	0.00	76.66	66.66	100.00	100.00	0.00	0.00	83.33	16.66	100.00	86.66	100.00	100.00
	0.00	0.00	(61.19)	(54.76)	(89.96)	(89.96)	0.00	0.00	(66.11)	(23.84)	(89.96)	(68.82)	(89.96)	(89.96)
Non *Bt*(LH 2076)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CD(p=0.05)	(0.53)	0.00	(6.75)	(5.49)	(0.06)	(0.06)	0.00	0.00	(6.24)	(6.32)	(0.06)	(7.05)	(0.06)	(0.06)

higher mortality on 7th day being *at par* with each other. The data presented in Table 6 revealed that no mortality was observed in 4th instar larvae fed with leaves and squares of cotton hybrid within 3 days of feeding. However, on 5th day 100 per cent mortality was recorded in MRC 7017 leaves followed by MRC 7031 and Ankur 3028. Peng *et al.,* (2008) also studied the effects of two transgenic cotton lines (G1560 and GK19) carrying a Cry1A gene against *S. litura*. They found no significant difference in larval population in conventional and *Bt* cotton fields.

On 7th day 100 per cent mortality was observed in all cotton hybrids being at par with each other. The results are in accordance with Govindan *et al.,* (2010) who 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 against third instar larvae of *S. litura*. Last instar larvae fed with leaves squares and bolls of *Bt* cotton hybrids (Table 3) showed 66.66 per cent mortality on 3rd day as observed in MRC 7031 (leaves) followed by 36.66 per cent (squares) in Ankur 3028. On 4th day 100 per cent mortality was observed in Ankur 3028 (leaves). On 8th day, 100 per cent mortality was observed in MRC 7031, Ankur 3028 (leaves) and MRC 7017 (squares). The data presented in Table 4 revealed that no pupation was observed in larvae fed with leaves of MRC 7031, MRC 7017 and Ankur 3028. However, 26.66 per cent pupation was observed in MRC 7031 (squares) and Ankur 3028 (squares and bolls) as compared to non *Bt* LH 2076 (100%). Similar, results were obtained by Hallad *et al.,* (2011) who found that mortality in late instars (3rd and 4th) was less compared to second instars. The pupae obtained were deformed when fed on squares of MRC 7031 on leaves of MRC 7017 and when fed on square and bolls of Ankur 3028. Adults emerged only in case of MRC 7017 and Ankur 3028 when fed on bolls. All the adults so obtained were males and were not able to survive for more than 2 days (Plate 1). There was a marked difference in larval development period between *Bt* cotton (27.75 days) and on non *Bt* cotton (16.68 days) flower bolls. Pupal weight was significantly higher for larvae fed on non *Bt* cotton (LH 2076) compared with *Bt* cotton plant parts (leaves and flower bolls). The data presented in Table 4 also revealed that no adult emergence was observed in any hybrid (leaves). However, 16.66 per cent adult emergence (Plate 1) was observed in Ankur 3028 (bolls) as compared to 100 per cent emergence in non *Bt* (LH 2076). According to Liu *et al.,* (2005) there was significant growth inhibition of *H. armigera* larvae when they were fed on a diet containing *Bt* transgenic cotton powder.

Our results indicated that there is decreased sensitivity in the grown up larvae of *H. armigera* and *S. litura* as compared to early instar. The decreased sensitivity in late instar may be due to increased physiological resistance to Cry toxins, or reduced binding sites in mid gut epithelium or may be due to deceased feeding demand. Survival of late instar larvae of these insects especially at mid stages of crop growth would lead to accumulation of resistance alleles in the population. However, the issues need to be addressed critically and it calls for still further investigations.

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