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Laboratory Evaluation of transgenic *Bt* cotton and non *Bt* cotton plant parts against third instar larvae of *Spodoptera litura* (Fab.) (Noctuidae: Lepidoptera)

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ABSTRACT

The lab performance of certain released *Bt* cotton hybrids for their effect on *Spodoptera litura* were evaluated in comparison with non *Bt* cotton plant parts. *Bt* cotton hybrids *viz.*, *Bt* bunny, six Bollgard II (cry 1 Ac + cry 2 Ab genes) hybrids e.g. RCH 2 *Bt* , RCH 515 *Bt* , RCH 596 *Bt*, RCH 530 *Bt*, RCH 134 *Bt* and RCH 533 *Bt* and two non *Bt* cotton *viz.*, Non *Bt* bunny and RCH 2 non *Bt* cotton against cotton leaf worm, *S. litura* third instar larva. Four plant parts *viz.*, young green bolls, top fully opened young leaves, middle leaves and squares were tested. Among the *Bt* cotton hybrids and non *Bt* cotton evaluated, RCH 2 *Bt* top fully opened young leaves showed highest per cent mortality followed by squares, middle leaves and young green bolls observed at 168 hours after the treatments (HAT) followed by top fully opened young leaves, squares, middle leaves and young green bolls of RCH 515 *Bt* which recorded 46.67, 38.34, 37.67 and 30.00 per cent mortality, respectively after 168 HAT when compared to squares, green bolls, top fully opened leaves and middle leaves of RCH 2 non *Bt* cotton which recorded 4.83, 4.17, 3.33 and 0.00 per cent mortality respectively after 168 HAT.

Key words: Transgenic *Bt* cotton, Toxicity, *Spodoptera litura*, crop pest

INTRODUCTION

The most common biological pesticides used include Cry proteins from *Bacillus thuringiensis* Berliner (*Bt*), which is a soil bacterium. It produces crystalline inclusions containing these proteins during sporulation. This protein binds to the larval gut lining and damages it inducing an antifeeding behavior in the larvae and finally death. Transgenic cotton which produces *B. thuringiensis* toxins have been shown effective against many lepidopteran pests in the field and laboratory (Benedict *et al.*, 1996; Gore *et al.*, 2001). *Bt* transgenic cotton acreages have increased rapidly in recent years around the world (James, 2004) because *Bt* toxins are environmentally safe and reduce the use of broad spectrum insecticides (Lambert and Peferoen, 1992). However, growing *Bt* cotton successively raises the potential for resistance adaptation of the target insects to the toxins (McGaughey and Whalon, 1992). Insect resistant Indian transgenic *Bt* cotton produced insecticidal crystal protein from the soil bacterium *B. thuringiensis* and released for commercial use during 1996 in USA. In India, bollgard *Bt* gene of Monsanto was introduced in to the Indian cotton hybrids developed by MAHYCO (Maharashtra hybrid seed company limited Mumbai).

Bt cotton is cultivated in 6.2 million hectares, 35 per cent was irrigated and 65 per cent rainfed land. In India, among the states Maharashtra topped in the area under *Bt* cotton (2.8 million hectares) followed by Andhra Pradesh, Gujarat and Tamil Nadu (Anonymous, 2008). *Bt* cotton had increased the yield by 64 per cent and pesticide usages was reduced 25 per cent. The Indian Market Research Bureau International (IMRBI) survey indicates that bollgard crop of 2005 -2006 helped Indian farmers to earn an additional income of Rs. 2,100 crores. The net profit increase for bollgard farmers is Rs. 6,727 per acre, over conventional cotton farmers (Anonymous, 2007). Leaf worm, *Spodoptera litura* Fab. (Noctuidae: Lepidoptera) is one of the most destructive pests of cotton which feeds on foliage and some times bolls, is a secondary pest of cotton (Allen *et al.*, 2000). It has been found that *S. litura* has a greater potential to survive in the presence of *Bt* toxins when compared to other bollworms. In the present study, four different plant parts of *Bt* and non *Bt* cotton hybrids were tested for their effect on third instar larvae of *S. litura*.

MATERIALS AND METHODS

Mass culturing of *S. litura*

The field collected egg masses of *S. litura* were used to initiate the mass culturing under laboratory conditions. The egg masses were kept in the egg cage. After

emergence, first instar larvae were transferred to the castor leaves. The newly emerged larvae when settled on the leaves, the leaves were taken and kept in the conical flask containing water. Five day old larvae were transferred to plastic buckets with castor leaves kept in conical flask containing water at the rate of 25 larvae/bucket. The leaves were changed and the faecal pellets removed from the container every 24h. The grown up larvae were allowed to pupate in soil. Moths was collected on emergence and released in oviposition cage for egg laying. The required larvae for the different treatments were taken from the culture.

Host plants

Six Bollgard II (cry 1 Ac + cry 2 Ab genes) *Bt* cotton and two non *Bt* cotton hybrids were obtained from Rasi Seeds (P) Ltd, Auttur, Salem (District), Tamil Nadu. Seeds were grown in mud pots in greenhouse with one plant in each pot. Thirty five days after sowing, the plants had approximately 15 nodes. The green bolls, top fully expanded young leaves, squares and middle leaves were used for the experiments.

Mortality of third instar larvae of *S. litura*

Plant parts of *viz.,* green young bolls, top young fully opened leaves, squares and middle leaves of *Bt* and non *Bt* cotton were used to feed the third instar larvae of *S. litura*. Bioassay was conducted. Plant parts of cotton were placed on moistened filter paper kept in petri dish and 30 third instar larvae were released in to each petri dish. To avoid drying of plant parts, the filter paper was moistened at regular intervals. The leaves, bolls and squares were changed at 24 hr interval and the fecal pellets and dead larvae were removed from the petri dish every 24hr. The experiment was conducted under laboratory condition (28 ± 1^0C and 80 ± 5% RH). Mortality was recorded at 24 hr intervals for seven days. Three replications were maintained for each treatment.

The methods of Gomez and Gomez (1984) were followed in scrutinizing the data from various experiments. Square root and angular transformations were adopted for the data in numbers and percentage, respectively (Abbott, 1925). Means in simple CRD analysis were separated by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

The laboratory evaluation of certain released *Bt* hybrids in comparison with their non *Bt* versions revealed that there was wide variation among the different hybrids on mortality of *S. litura* (Tables 1 to 4). Seven *Bt* cotton hybrids (plant parts *viz.,* green bolls, top fully opened young leaves, squares and middle leaves) were tested for their toxicity in comparison with non *Bt* cotton hybrids. Data on per cent mortality of *S. litura* after 24, 48, 72, 96, 120, 144 and 168 hours after treatment (HAT) were recorded. The results on toxicity of green young bolls of *Bt* and non *Bt* cotton hybrids (Table 1) revealed that significant differences in the mortality level of third instar larvae on green young bolls could be observed from the bioassay results. In all seven hybrids no mortality was observed up to 72 hours after treatments (HAT). Maximum mortality was observed in green young bolls of RCH 515 *Bt* followed by RCH 2 *Bt* which was on par with RCH 596 *Bt*, RCH 533 *Bt*, RCH 530 *Bt*, RCH 134 *Bt* and *Bt* bunny, no morality was observed in green young bolls of non *Bt* cotton hybrids at 72 HAT. At 120 HAT, highest mortality was noticed in RCH 2 *Bt* (25.34 %) which had on par with RCH 515 *Bt* followed by RCH 530 *Bt* , RCH 134 *Bt* and *Bt* bunny (23.50%). Other hybrids namely RCH 596 *Bt* and RCH 533 *Bt* registered of 22.50 per cent mortality, respectively. At 144 HAT, highest mortality of 27.33 per cent were observed in RCH 2 *Bt* which was followed by RCH 515 *Bt*, RCH 530 *Bt* 24.50 per cent which had on par with (RCH 530 *Bt*, RCH 134 *Bt*, *Bt* bunny, RCH 596 *Bt* and RCH 533 *Bt*) Similar results was observed in *Spodoptera exigua* (Stapel *et al.*, 1998), *Helicoverpa armigera* (Hubner) (Zhang *et al.*, 2004; Men *et al.*, 2005) and *H. zea* (Gore *et al.*, 2001) in which larvae avoid food treated with *Bt* insecticides or transgenic plants. At 168 HAT, the highest mortality was recorded in RCH 2 *Bt*, this was followed by RCH 533 *Bt* other hybrids were on par RCH 530 *Bt* (33.34%), RCH 134 *Bt* , RCH 596 *Bt* and RCH 515 *Bt* (30.00%). In case of non *Bt* cottons up to 144 HAT, no per cent mortality was registered. The lowest mortality was recorded in non *Bt* cotton viz., RCH 2 non *Bt* and RCH 2 non *Bt* the hybrids RCH 2 *Bt*, RCH 515 *Bt* showed highest mortality at 7 days after the treatment. Zhang *et al*. (2004) found that *H. armigera* neonates showed lower consumption and higher mortality on transgenic cotton leaves compared to non transgenic cotton leaves.

The results on toxicity of top younger fully opened leaves of *Bt* and non *Bt* cotton hybrids (Table 2) revealed that the all seven hybrids and two non *Bt* showed no mortality up to 72 HAT. At 96 HAT, hybrid RCH 515 *Bt* and RCH 2 *Bt* showed 21.66 and 20.83 per cent mortality respectively, which was followed by RCH 596 *Bt* other three hybrids *viz.*, RCH 530 *Bt*, RCH 533 *Bt*, RCH 134 *Bt* and *Bt* bunny which were at on par in their efficacy. In case of non *Bt* cottons, no mortality was observed at 144HAT. The hybrid, RCH 515 *Bt* was found effective next to RCH 2 *Bt* which were on par with RCH 530 *Bt*, RCH 596 *Bt*, RCH 134 *Bt* and RCH 533 *Bt* at 120 hours after treatments. The mortality

Table 1. Per cent mortality of third instar larva of *S. litura* on different transgenic *Bt* and non *Bt* cotton.

Treatments (Green young bolls)	Per cent mortality (Mean of three replications)				
	96 HAT	120 HAT	144 HAT	168 HAT	Mean
Bt bunny	18.00^{b}	23.50^{ab}	24.34^{b}	27.33^{b}	13.31
Non *Bt* bunny	0.00^{c}	0.00^{c}	0.00^{c}	3.33^{c}	0.47
RCH 2 *Bt* BG II	20.00^{b}	25.34^{a}	27.33^{a}	34.00^{a}	15.23
RCH 2 non *Bt*	0.00^{c}	0.00^{c}	0.00^{c}	4.17^{c}	0.59
RCH 515 *Bt* BG II	22.33^{a}	24.34^{a}	25.00^{ab}	30.00^{ab}	14.52
RCH 596 *Bt* BG II	20.00^{b}	22.50^{b}	23.50^{b}	32.33^{ab}	14.00
RCH 530 *Bt* BG II	19.00^{b}	23.50^{ab}	24.50^{b}	33.34^{ab}	14.33
RCH 134 *Bt* BG II	18.00^{b}	23.50^{ab}	24.50^{b}	32.33^{ab}	14.04
RCH 533 *Bt* BG II	20.00^{b}	22.50^{b}	23.50^{b}	33.33^{ab}	14.19

HAT- Hours after treatments, Values in parentheses are *arc sine* transformed values, BG II (Bollgard II, cry 1 Ac + cry 2 Ab genes), In a column means followed by a common letter are not significantly different by DMRT (P=0.05); mean of 30 larvae per replication

of *S. litura* recorded at 144 HAT, RCH 515 *Bt* showed highest per cent mortality (36.66 %), followed by RCH 2 *Bt*, RCH 530 *Bt* while other two hybrids *viz.*, RCH 533 *Bt* and RCH 134 *Bt* were on par. Benedict *et al.*, (1992) had observed the feeding deterrent effect of *Bt* cotton leaves against *Heliothis virsecens.* At 168 hours after treatment the per cent mortality recorded was higher in RCH 2 *Bt*, whereas other six hybrids *viz.*, RCH 515 *Bt*, RCH 596 *Bt*, RCH 530 *Bt*, RCH 533 *Bt* , RCH 134 *Bt* and *Bt* bunny were on par, while in non *Bt* bunny and RCH 2 non *Bt* lowest per cent mortality was registered. Li *et al.* (2006) found that the percentage of feeding damage by *Trichoplusia ni* larvae on *Bt* cotton was only 15.67%, while 84.47% damage was recorded in non *Bt* cotton.

The toxicity level of squares of *Bt* and non *Bt* cotton hybrids (Table 3) indicated that all hybrids showed no mortality up to 72 HAT. Maximum mortality was found in RCH 2 *Bt* followed by *Bt* bunny which have on par with RCH 530 *Bt*, RCH 134 *Bt* , and RCH 515 *Bt* which were equal toxicity with RCH 596 *Bt* and RCH 533 *Bt* at 96 HAT. At 120 HAT different *Bt* cotton hybrids showed the same trend. Highest mortality was noticed in the hybrid RCH 2 *Bt* which was on par with RCH 515 *Bt* followed by RCH 134 *Bt* where as other four hybrids namely RCH 533 *Bt* , RCH 530 *Bt*, RCH 596 *Bt* and *Bt* bunny were sowed equal efficacy. The results reported by Murugan *et al.* (2003) observed that first instar larvae of *H. armigera* when reared on the squares of transgenic cultivars showed highest mortality at 72 hours. At 144 HAT, the hybrid, RCH 2 *Bt* showed highest mortality which was on par with RCH 134 *Bt* followed by RCH 515 *Bt* other two hybrids *viz.*, RCH 530 *Bt* and RCH 533 *Bt* which were on par and *Bt* bunny. At 168 HAT, per cent mortality was higher in, RCH 2 *Bt* which was on par with RCH 515 *Bt*,

Table 2. Per cent mortality of third instar larvae of *S. litura* on different transgenic *Bt* and non *Bt* cotton.

Treatments (Top younger fully opened leaves)	Per cent mortality (Mean of three replications)				
	96 HAT	120 HAT	144 HAT	168hr	MEAN
Bt bunny	14.16^{b}	19.16^{c}	25.00^{c}	36.67^{a}	13.57
Non *Bt* bunny	0.00^{c}	0.00^{d}	0.00^{d}	3.33^{b}	0.48
RCH 2 *Bt* BG II	20.83^{a}	29.16^{b}	34.16^{ab}	43.33^{a}	18.21
RCH 2 non *Bt*	0.00^{c}	0.00^{d}	0.00^{d}	3.33^{b}	0.48
RCH 515 *Bt* BG II	21.66^{a}	34.16^{a}	36.66^{a}	46.67^{a}	19.88
RCH 596 *Bt* BG II	15.83^{b}	28.33^{b}	28.33^{bc}	38.33^{a}	15.83
RCH 530 *Bt* BG II	15.83^{b}	29.16^{b}	30.00^{abc}	38.33^{a}	16.19
RCH 134 *Bt* BG II	13.33^{b}	28.33^{b}	28.33^{bc}	36.67^{a}	15.24
RCH 533 *Bt* BG II	14.16^{b}	26.66^{b}	29.16^{bc}	38.33^{a}	15.47

HAT- Hours after treatments, Values in parentheses are *arc sine* transformed values, BG II (Bollgard II, cry 1 Ac + cry 2 Ab genes) In a column means followed by a common letter are not significantly different by DMRT (P=0.05); mean of 30 larvae per replication

Table 3. Per cent mortality of third instar larvae of *S. litura* on different transgenic *Bt* and non *Bt* cotton.

Treatments (Squares)	Per cent mortality (Mean of three replications)				
	96 HAT	120 HAT	144 HAT	168 HAT	Mean
Bt bunny	20.83^{b}	21.33^{b}	25.83^{c}	33.67^{c}	14.52
Non *Bt* bunny	0.00^{d}	0.00^{c}	0.00^{d}	0.00^{e}	0.00
RCH 2 *Bt* BG II	25.00^{a}	26.83^{a}	30.00^{a}	39.33^{a}	17.30
RCH 2 non *Bt*	0.00^{d}	0.00^{c}	0.00^{d}	4.83^{d}	0.69
RCH 515 *Bt* BG II	18.33^{bc}	25.83^{a}	28.33^{ab}	38.34^{a}	15.83
RCH 596 *Bt* BG II	18.33^{bc}	22.33^{b}	27.33^{b}	36.66^{b}	14.95
RCH 530 *Bt* BG II	19.00^{b}	22.33^{b}	28.33^{ab}	37.01^{ab}	15.23
RCH 134 *Bt* BG II	19.00^{b}	23.50^{b}	29.33^{a}	36.66^{b}	15.49
RCH 533 *Bt* BG II	18.33^{bc}	23.50^{b}	28.33^{ab}	33.68^{c}	14.83

HAT- Hours after treatments, Values in parentheses are *arc sine* transformed values, BG II (Bollgard II, cry 1 Ac + cry 2 Ab genes), In a column means followed by a common letter are not significantly different by DMRT (P=0.05); mean of 30 larvae per replication

Table 4. Per cent mortality of third instar larvae of *S. litura* on different transgenic *Bt* and non *Bt* cotton.

Treatments (Middle leaves)	Per cent mortality (Mean of three replications)				
	96 HAT	120 HAT	144 HAT	168 HAT	Mean
Bt bunny	16.66^{bc}	21.66^{c}	28.33^{a}	30.00^{b}	13.80
Non *Bt* bunny	0.00^{d}	0.00^{d}	0.00^{b}	0.00^{d}	0.00
RCH 2 *Bt* BG II	21.66^{ab}	23.33^{b}	35.00^{a}	36.67^{a}	16.66
RCH 2 non *Bt*	0.00^{d}	0.00^{d}	0.00^{b}	0.00^{d}	0.00
RCH 515 *Bt* BG II	26.66^{a}	33.33^{a}	36.67^{a}	37.67^{a}	19.19
RCH 596 *Bt* BG II	18.33^{bc}	26.66^{b}	31.67^{a}	30.00^{b}	15.23
RCH 530 *Bt* BG II	16.66^{bc}	26.66^{b}	28.33^{a}	29.33^{bc}	14.42
RCH 134 *Bt* BG II	15.83^{c}	26.66^{b}	30.00^{a}	31.00^{b}	14.78
RCH 533 *Bt* BG II	17.50^{bc}	26.66^{b}	31.67^{a}	33.67^{b}	15.64

HAT- Hours after treatments, Values in parentheses are *arc sine* transformed values , BG II (Bollgard II, cry 1 Ac + cry 2 Ab genes), In a column means followed by a common letter are not significantly different by DMRT (P=0.05); mean of 30 larvae per replication

next best hybrid of RCH 530 *Bt* which registered 37.01 per cent mortality of *S. litura* followed by RCH 596 *Bt* which have same toxicity with RCH 134 *Bt* , RCH 533 *Bt* which have similar efficacy with *Bt* bunny. The non *Bt* bunny which showed through out the period of study no mortality up to 168 HAT where as RCH 2 non *Bt* which also registered no mortality up to 144 HAT at 168 HAT, only 4.83 per cent showed the least per cent mortality was observed. Prasad and Rao (2008) reported that the *Bt* hybrids were found highly effective against *H. armigera* with very low larval population of pink bollworm.

The results on toxicity of middle leaves of *Bt* and non *Bt* cotton hybrids (Table 4) showed no mortality at 72 HAT. Maximum mortality was noticed in hybrid RCH 515 *Bt* followed by RCH 2 *Bt*, RCH 596 *Bt* while other three hybrids (RCH 533 *Bt*, RCH 530 *Bt* and *Bt* bunny) and RCH 134 at 96 hours after treatment. Highest mortality was noticed in the hybrid RCH 515 *Bt* (33.33 %) followed by RCH 596 *Bt* which was equal toxicity with RCH 530 *Bt*, RCH 134 *Bt* and RCH 533 *Bt* and *Bt* bunny registered at 120 hours after treatment. These findings are in conformity with the reports of Murgan *et al* (2003) who reported that after 120 hours of feeding on the *Bt* cultivars *viz.,* MECH 12 *Bt*, MECH 16 *Bt*, and MECH 184 *Bt*, 92.80, 66.70 and 51.70 per cent mortality respectively, was observed in the early instar larvae of *H. armigera*. The hybrid, RCH 2 *Bt*, RCH 515 *Bt*, RCH 533 *Bt*, RCH 596 *Bt*, RCH 134 *Bt* and *Bt* bunny recorded 38.36.67, 35.00, 31.67, 31.67, 30.00 and 28.33 per cent mortality at 144 HAT. At 168 hours after treatment, the per cent mortality was higher in RCH 2 *Bt*, which was on par with RCH 515 *Bt* followed by RCH 533 *Bt* while other three hybrids *viz.,* RCH 134 *Bt*, RCH 596 *Bt* and *Bt* bunny were found to be on par. Bagade *et al*. (2005) reported that transgenic *Bt* cotton was found effective against three bollworms (*H. armigera*, *Earias spp.* and *P.gossypiella*) as well as Luttrell *et al.* (1998) reported more tolerance in *S. frugiperda* against Cry 1 Ac than other bollworms. In case of RCH 2 non *Bt* and non *Bt* bunny were registered no mortality thorough out period of study.

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