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Leaf δ- endotoxin content and performance of *Bt* cotton (*Gossypium hirsutum*) genotypes as influenced by fertility levels for target yields*

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Bacillus thuringensis (*Bt*) cotton (*Gossypium hirsutum*) is rapidly dominating cotton-growing regions of the world. The introduction of commercial cotton producing the insecticidal protein is improving grower's profitability, reducing environmental pollution from synthetic insecticides, and increasing workers safety. However, poor performances of transgenic cultivars during boll period, and variable performances between different regions are the constraints in growing *Bt* cotton. The loss of efficacy is associated with a reduction of insecticidal Cry 1 Ac δ - endotoxin protein. Kranthi *et al*. (2005) reported that the Cry 1 Ac expression was found to be variable among hybrids and also in different parts. Further, Pettigrew and Adamczyk (2006) also found variation in plant δ-endotoxin content due to nitrogen fertilization. Hence, a study was envisaged to quantify δ - endotoxin as influenced by graded fertilization for target yields in *Bt* genotypes and its impact on crop performance at college of Agriculture, Dharwad, Karnataka during 2006–7 under rainfed conditions.

The experiment was conducted on black clayey soil with 7.5 *p*H, low in available nitrogen (256 kg/ha), medium in available phosphorus (20.6 kg/ha) and high in available potassium (340 kg/ha) contents. A total of 476.5 mm of rainfall was received in 55 rainy days during the crop period. Four cotton hybrids, viz. MRC 6322 (intra hirsutum Cry 1 Ac BG I hybrid), MRC 6918 (intra hirsutum Cry 1 Ac BG I hybrid), MRC 7351 (interspecific H X B Cry 1 Ac + Cry 2Ab BG II hybrid) and MRC 7201 (interspecific H X B Cry 1 Ac + Cry 2Ab BG II hybrid) were supplied with three fertilizer levels for different yield targets, viz. F_1 – 145:39:99 NPK kg/ha (for a yield target of 2.0 tonnes/ha), F_2 – 181:49:124 NPK kg/ha (2.5 tonnes/ha) and F_3 – 217:59:148 NPK kg/ha (3.0 tonnes/ha). Nutrient levels were worked for target yield based on uptake value of 4.45 - N, 0.83-P_2O_5 and 7.47 K_20 kg/ha per 100 kg of seed cotton (Das *et al*., 1991) and soil nutrient status. The crop was sown on 29 June 2006 at spacing of 90 cm × 60 cm. Totally there were 12 treatment combinations tested using randomized complete block design with three replications. Periodic observations on δ- endotoxin content in leaf and number of bad opened bolls besides total dry matter, nitrogen uptake and seed cotton yield were made.

Quantitative estimation of Cry 1 Ac protein in bollgard hybrids was done using commercially available Quant-T ELISA 96-well plate kits. About 5 mg of lyophilized tissue samples were taken in a 1.5 ml microfuge tube, by the addition of 500 μl of ice-cold sample extraction buffer and then macerated at 300 rpm using motor driven pestle for 30 sec., later it was chilled on ice for 10 min. and again macerated for 30 sec. Finally, the sample was subjected to centrifugation at 8 000 rpm for 15 min. Then the supernatant was extracted and stored at 4°C. before loading the sample extracts to plate the positive and negative standards were diluted with standard buffer. After loading plates were incubated at 37°C for 1.5 hr in a humid environment, later the samples were discarded and washed with wash buffer and conjugate buffer twice allowing the plate to stand five minutes with wash buffer in the wells in between washes. The plate was incubated at room temperature for 30 min. for colour development. Then, the plate was subjected to ELISA reader. Based on absorbance values of the plate at 405 nm the quantification of Cry protein was done with the help of sigma plot version 8.01 programme.

In the present investigation δ-endotoxin content decreased with advancement in age (Table 1) which was in

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Table 1 δ-endotoxin concentration (µg/g fresh weight of leaves) and number of good opened bolls in *Bt* cotton genotypes as influenced by nutrient management for targeted yields

Treatment	δ-endotoxin at 75 DAS				δ-endotoxin at 105 DAS				δ-endotoxin at 120 DAS				No. of good bolls/plant			
Bt hybrid	Fertilizer for target yield				Fertilizer for target yield				Fertilizer for target yield				Fertilizer for target yield			
	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean
MRC 6322	1.63^{e}	1.89^{cd}	1.99^{abc}	1.84^{b}	0.78^{a}	0.81^{a}	1.04^{a}	0.88^{a}	0.20^{a}	0.49^{a}	0.46^{a}	0.39^{a}	63.27^{ab}	68.53^{a}	71.27^{a}	67.69^{a}
MRC 6918	1.42^{f}	1.73^{de}	1.83^{cde}	1.66^{c}	0.72^{a}	0.87^{a}	0.92^{a}	0.83^{a}	0.20^{a}	0.49^{a}	0.45^{a}	0.39^{a}	51.00^{bcd}	49.27^{cd}	62.20^{abc}	53.49^{b}
MRC 7351	1.73^{de}	1.93^{bcd}	2.13^{ab}	1.93^{ab}	0.82^{a}	0.89^{a}	1.01^{a}	0.91^{a}	0.18^{a}	0.31^{a}	0.57^{a}	0.32^{a}	52.13^{bcd}	55.73^{bcd}	54.13^{bcd}	54.00^{b}
MRC 7201	1.71^{de}	1.94^{bcd}	2.19^{a}	1.97^{a}	0.85^{a}	0.97^{a}	1.07^{a}	0.96^{a}	0.36^{a}	0.37^{a}	0.44^{a}	0.37^{a}	43.67^{d}	55.40^{bcd}	59.67^{abc}	52.91^{b}
Mean	1.62^{c}	1.80^{b}	2.04^{a}		0.79^{a}	0.89^{a}	1.01^{a}		0.24^{a}	0.41^{a}	0.48^{a}	0.40^{a}	52.52^{b}	57.23^{ab}	61.32^{a}	
		SE ±	LSD (P=0.05)			SE ±	LSD (P=0.05)			SE ±	LSD (P=0.05)			SE ±	LSD (P=0.05)	
Hybrid (G)		0.02	0.12			0.06	NS			0.06	NS			1.26	6.42	
Fertlizer (F)		0.02	0.10			0.05	NS			0.05	NS			1.09	5.55	
G × F		0.04	0.20			0.09	NS			0.11	NS			2.18	11.10	

Means with same alphabet do not differ significantly, F_1, 145:39:99 NPK kg/ha (2.0 tonnes/ha); F_2, 181:49:124 NPK kg/ha (2.5 tonnes/ha); F_3, 217:59:148 NPK kg/ha (3.0 tonnes/ha); DAS, days after sowing; NS, not significant

line with observation made by Kranthi *et al.* (2005a). Further, genotypic variation in ä-endotoxin content also confirm the findings of Kranthi *et al.* (2005a). In fact, the δ-endotoxin Cry 1 Ac protein concentration in different cotton hybrids differed significantly at 75 days after sowing (DAS) and thereafter the differences were not significant. Significantly higher δ-endotoxin content (1.97 µg/g fresh weight (fw)) was recorded with MRC 7201. The other Bollgard II cotton, MRC 7351, was at par with it. Bollgard I cotton hybrids recorded lower δ-endotoxin contents and the lowest endotoxin content was observed in MRC 6918. The delta endotoxin concentration also differed significantly due to different levels of fertilizer application for targeted yield at 75 DAS. Increase in fertilizer level increased the δ-endotoxin concentration, and significantly higher δ-endotoxin concentration was recorded with high level of fertilize ($F_{3,}$ 2.04 µg/g fw), followed by F_2 (medium level) and F_1(low level), the latter being the lowest. These observations corroborate well with Pettigrew and Adamczyk (2006), and Bruns and Abel (2003) who attributed increase in δ-endotoxin content to increased nitrogen usage. Differences among fertilizer levels were not significant at later stages. Interaction effects were also significant at 75 days only, increase in fertilizer level targeted for higher yield recorded higher δ-endotoxin content particularly in Bollgard II, and MRC 7201 recorded the highest ä-endotoxin content with F_3 level and MRC 7351 at same level of fertilizer was on par with it. MRC 6918 recorded the lowest ä-endotoxin content particularly at F_1 level, among all the treatment combinations.

Further, number of bad opened bolls/plant varied significantly among cotton hybrids except at last picking (Table 2). At first picking, MRC 6918 recorded the maximum number of bad opened bolls, and the trend remained same throughout. While MRC 6322 recorded the least number of infested bolls, and other Bollgard II hybrids (cvs. MRC 7351 and MRC 7201) were at par with it. Interestingly, different fertilizer levels did not result in any significant variation in number of bad opened bolls. However, varieties responded differently in presence of varied levels of fertilizer during the initial three pickings. During the first two pickings almost all hybrids except MRC 6918 recorded lower number of bad opened bolls with moderate (F_2) to low (F_1) fertilizer level while MRC 6918 recorded increased number of bad opened bolls with increasing fertilizer levels in spite of total number of bolls being lowest with this hybrid in comparison to other hybrids at all the fertilizer levels (Table 1). During third picking, again number of bad opened bolls was lower with F_1 level. Here, despite higher toxin content, number of infested bolls was more with increased fertilizer level. This was probably due to availability of more bolls for the insect and relatively lower concentration of toxin in the leaf (dilution effect due to larger biomass production; Table 3). Among all the hybrids and fertilizer levels, MRC 6322 recorded higher number of

Table 2 Number of bad opened bolls/plant at different pickings in *Bt* cotton genotypes as influenced by nutrient management for targeted yields

Treatment	I picking				II picking				III picking				IV picking			
Bt hybrid	Fertilizer for target yield				Fertilizer for target yield				Fertilizer for target yield				Fertilizer for target yield			
	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean
MRC 6322	3.27^{b}	2.60^{a}	2.60^{b}	2.82^{b}	2.47^{c}	2.47^{c}	2.40^{c}	4.47^{c}	5.40^{b}	4.47^{bc}	4.27^{bc}	4.71^{c}	4.60^{b}	5.27^{a}	5.73^{a}	5.20^{a}
MRC 6918	8.87^{a}	8.33^{a}	9.47^{a}	8.89^{a}	6.07^{ab}	6.07^{ab}	6.13^{ab}	7.87^{a}	6.93^{abc}	8.00^{ab}	9.34^{a}	8.09^{a}	6.67^{a}	6.00^{a}	6.53^{a}	6.40^{a}
MRC 7351	3.27^{b}	3.93^{b}	3.33^{b}	3.51^{b}	3.20^{c}	3.20^{c}	3.00^{c}	3.13c	5.00^{bc}	5.67^{bc}	6.00a^{bc}	5.59^{b}	5.93^{a}	5.60^{a}	5.60^{a}	5.71^{a}
MRC 7201	3.93^{b}	2.80^{b}	3.40^{b}	3.38^{b}	2.27^{c}	2.27^{c}	3.00^{c}	4.20^{bc}	4.47^{bc}	6.00^{abc}	5.13^{bc}	5.20^{b}	5.97^{a}	4.47^{a}	6.80^{a}	5.74^{a}
Mean	4.83^{a}	4.43^{a}	4.70^{a}		3.50^{a}	3.50^{a}	3.65^{a}		5.22^{a}	6.27^{a}	6.19^{a}		5.79^{a}	5.33^{a}	6.17^{a}	
	SE ±		LSD (P=0.05)		SE ±		LSD (P=0.05)		SE ±		LSD (P=0.05)		SE ±		LSD (P=0.05)	
Hybrid (G)	0.27		1.39		0.23		1.18		0.35		1.81		0.60		NS	
Fertlizer (F)	0.23		NS		0.20		NS		0.30		NS		0.52		NS	
G × F	0.47		2.41		0.40		2.05		0.61		3.14		1.04		NS	

Means with same alphabet do not differ significantly, F_1, 145:39:99 NPK kg/ha (2.0 tonnes/ha); F_2, 181:49:124 NPK kg/ha (2.5 tonnes/ha); F_3, 217:59:148 NPK kg/ha (3.0 tonnes/ha); DAS, days after sowing; NS, not significant

Table 3 Total dry matter production (g/plant), nitrogen uptake (kg/ha) and seed cotton yield (kg/ha) in *Bt* cotton genotypes as influenced by nutrient management for target yields

Treatment	TDM at I picking				N uptake (kg/ha)				Seed cotton yield (kg/ha)			
Bt hybrid	Fertilizer for target yield				Fertilizer for target yield				Fertilizer for target yield			
	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean
MRC 6322	399.33^{c}	434.05^{b}	456.75^{a}	430.04^{a}	133^{abc}	136^{abc}	155^{a}	141^{a}	3062^{ab}	3067^{ab}	3730^{a}	3286^{a}
MRC 6918	389.08^{c}	421.92^{b}	435.25^{b}	415.42^{b}	119^{c}	117^{c}	126^{bc}	121^{b}	2472^{b}	2494^{b}	2769^{b}	2578^{b}
MRC 7351	394.63^{c}	430.05^{b}	438.75^{b}	421.15^{ab}	133^{abc}	146^{ab}	140^{abc}	140^{a}	2857^{b}	3006^{ab}	3290^{ab}	3051^{a}
MRC 7201	390.01^{c}	425.70^{b}	431.77^{b}	415.83^{b}	121^{bc}	135^{abc}	146^{ab}	134^{a}	2561^{b}	2997^{ab}	3086^{ab}	2881^{ab}
Mean	393.26^{c}	427.93^{b}	440.63^{a}		126^{b}	134^{ab}	142^{a}		2738^{b}	2891^{ab}	3219^{a}	
	SE ±		LSD (P=0.05)		SE ±		LSD (P=0.05)		SE ±		LSD (P=0.05)	
Hybrid (G)	1.81		9.24		1.45		12		80		408	
Fertlizer (F)	1.57		8.00		1.25		11		69		353	
G × F	3.15		16.01		2.51		21		139		406	

Means with same alphabet do not differ significantly, F_1, 145:39:99 NPK kg/ha (2.0 tonnes/ha); F_2, 181:49:124 NPK kg/ha (2.5 tonnes/ha); F_3, 217:59:148 NPK kg/ha (3.0 tonnes/ha); DAS, days after sowing; NS, not significant good opened bolls which increased with increase in fertilizer levels followed by Bollgard II hybrids (Table 1).

MRC 6322 recorded the maximum seed cotton yield at any given level of fertilizer (Table 3). The yields with F_3 (3.7 t/ha) exceeded the target (3.0 tonnes/ha). The yield levels of MRC 7351 and MRC 7201 were comparable with MRC 6322. However, the numerical differences with F_3 level were the maximum (440 and 644 kg seed cotton yield difference between MRC 6322 and MRC 7351 and MRC 6322 and MRC 7201, respectively). While, MRC 6918 recorded significantly lower seed cotton yield at F_3 (2 760 kg/ha) and the desired target could not be achieved with this hybrid with F_3 though other lesser targets were achieved with respective fertilizer levels.

SUMMARY

Two Bollgard I (cvs.MRC 6322 and MRC 6918) and two Bollgard II (cvs.MRC 7351 and MRC 7201) hybrids were supplied with three fertilizer levels (145:39:99, 181:49:124 and 217:59:148 NPK kg/ha, respectively[1]) targeted for 2.0, 2.5 and 3.0 tonnes/ha of seed cotton yield. δ-Endotoxin content was higher in Bollgard II hybrids and MRC 6322 was comparable and the toxin content increased with increase in fertilizer level. However, the number of bad opened bolls differed among genotypes and it followed the total number of bolls rather than δ- endotoxin content. Thus, the study brings two important points. First one is that in the selection of Bollgard I or Bollgard II hybrids, the intensity of population of pest in question matters most. And, secondly targeted yields set in the study were easily achieved and often exceeded. Hence, it is possible to achieve target yield of 3.0 tonnes/ha using desired level of fertilizer in most of the *Bt* hybrids, and this point needs emphasis as cotton is a commercial crop and for farmer monetary returns matter most.

REFERENCES

Bruns H A and Abel C A. 2003. Nitrogen fertility effects on δ-endotoxin and nitrogen concentrations of maize during early growth. *Agronomy Journal* **95**: 207–11.

Das S K, Sharma K L, Neelam Sahan and Bhaskar Rao U M. 1991. Nutrient balance and sustainable agriculture in Southern Plateau and Hills Region of India. *Fertilizer News* **36**: 43–9.

Kranthi K R, Naidu S, Dhawad C S, Tatwawadi A, Mate K, Patil E, Bharose A A, Behere G T, Wadaskar R M and Kranthi S, 2005. Temporal and intra-plant variability of Cry 1 Ac expression in *Bt* cotton and its influence on the survival of the cotton bollworm, *Helicoverpa armigera* (Hubner) (Noctuidae: Lepidoptera). *Current Science* **89**: 291–8.

Pettigrew W T and Adamczyk J J. 2006. Nitrogen fertility and planting date effects on lint yield and Cry Ac endotoxin production. *Agronomy Journal* **98**: 691–7.