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Effect of drip fertigation on endotoxin expression in intra *hirsutum* Bt Cotton

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Abstract
Endotoxin expression in leaf of cotton genotype First Class BG-II was studied, sown under different irrigation and fertigation levels in medium deep black soil at Agricultural Research Station Dharwad, Karnataka during *kharif* season of 2015-16. Results revealed that expression of *Cry 1Ac* and *Cry 2Ab* protein content in leaf was non-significant at 60, 90, 120 and 150 DAS (*Cry 1Ac* 2.70, 1.77, 1.29 and 0.83µg g^{-1}fresh weight and *Cry 2Ab* 47.66, 30.66, 25.14 and 15.14 µg g^{-1}fresh weight, respectively) in different irrigation levels, expect at 45 DAS. Fertigation with 100 per cent recommended (RD) N & K in six equal splits recorded significantly higher protein content in leaves at 45, 60, 90 and 120 DAS (*Cry 1Ac* 3.33, 2.84, 2.21 and 1.33µg g^{-1}fresh weight and *Cry 2Ab* 62.22, 49.32, 32.32 and 27.32) and was non-significant at 150 DAS. *Cry 1Ac* and *Cry 2Ab* protein content (3.24 and 59.33µg g^{-1}fresh weight) were significantly higher at 45 DAS in C_1 (Drip irrigation at 1.0 Etc + 100% RD N & K in 4 splits through soil (25% each as basal and at 30, 60 & 90 DAS) and no significant difference was observed for *Cry 1Ac* and *Cry 2Ab* protein content in C_2 (Furrow Irrigation at 0.8 IW/CPE ratio +100% RD N & K in 4 splits through soil (25% each as basal and at 30, 60 & 90 DAS).

Keywords: Bt cotton, *Cry* protein, endotoxin, irrigation, fertigation

Introduction
Cotton is a major commercial and industrial crop known as "White gold" and "King of fiber crops". In India the productivity is lower because of pest attack, which requires intensive use of pesticides. Cotton cultivation had become uneconomical in many parts of the country due to high cost of pesticides, lower yields and insects attaining resistance to the insecticides. Bt cotton was introduced in India during 2002. The Bt cotton contains a foreign gene obtained from bacillus thuringiensis. This bacterial gene, introduced genetically into the cotton seeds, This Bt cotton offers good resistance to bollworms. The yields of Bt cotton are also found to be higher. Possible alteration of the levels of endotoxin in plant parts with manipulation of nutrient management practices could be a sound practice to enhance the production, so also it helps for further extension of endotoxin content at later stages of crop cycle when the natural level in the plant decline (Adamczyk *et al*, 2000) [1].

Water becomes a scarce resource due to increased demand for industrial, agricultural and domestic purposes, besides increased cost on fertilizer necessitated for development and adoption of alternative agro techniques which help in effective and efficient utilization of these inputs. The use of micro irrigation techniques and fertigation is only way to manage these resources efficiently. Drip irrigation is a type of micro-irrigation system that has the potential to save water and nutrients by allowing water drop by drop slowly to the roots of plants, either from above the soil surface or buried below the surface where water runoff is less. Nutrients are second most important limiting factor of crop production after water. Improved plant K status also promoted the uptake of other macro and micronutrients, indicating an improved capacity of the root system. The goal is to optimize water and input usage. The present study was aimed to assess the cry protein expression in Bt cotton hybrid as influenced by irrigation and fertigation yield under medium deep black soils.

Materials and Methods
A field experiment was conducted during 2015-16 during *kharif* at Agricultural Research Station Dharwad, Karnataka to estimate the endotoxin expression in *intra hirsutum* Bt cotton hybrid influenced by different levels of irrigation and fertigation. The soil of the site was medium deep black with neutral pH (7.3), available N, P_2O_5, K_2O were 285, 35 and 525 kg

ha^{-1} with 0.56 per cent organic carbon. The experiment was laid out in factorial randomized block design with three replications. Main plots consisted of three irrigation regimes *viz*., I_1: 1.0 Etc, I_2 : 0.8 Etc and I_3 :0.6 Etc and sub plot consisted of three fertigation levels i.e., F_1: 100 per cent RDN & K (150:75 kg N & K ha^{-1}), F_2: 75 per cent RDN & K (112.5: 56.25 kg N & K ha^{-1}) and F_3: 50 per cent RDN & K (75:37.5 kg N & K ha^{-1}), with two control plots C_1: Drip irrigation at 1.0 Etc + 100 per cent RDF in 4 splits through soil (25 per cent each as basal and at 30, 60 & 90 DAS) and C_2: Furrow Irrigation at 0.8 IW/CPE ratio +100 per cent RDF in 4 splits through soil (25 per cent each as basal and at 30, 60 & 90 DAS). Irrigation was given at three days interval based on Etc level and fertigation of N and K was given in six equal splits at 15 days interval. Entire dose of (100%) P_2O_5 was applied as basal at the time of sowing to all the treatments.

Leaf samples were collected at 45, 60, 90, 120 and 150 DAS for quantification of *Cry1Ac* as well as *Cry2Ab* using Quan-T ELISA plate kits from Desi-Gen, Maharashtra. The samples *viz.*, third leaf from the top attached to main stem were collected in ice box and carried to laboratory About 15-20 mg of sample was collected for further analysis. Weighed samples were taken in microfuge by adding standard extraction buffer 500 ml (furnished in the kit) and then macerated for 10 minutes. Macerated samples were subjected for centrifugation at 3000 rpm for 30 seconds, after 10 min time gap same sample was subjected for second round centrifugation at 8000 rpm for 30 sec. Then the supernatant was extracted and stored at 4°C. Before loading the sample extract to plate the positive and negative standards were diluted with standard buffer (both provided in kit). Before loading, ELISA plate was washed 2-3 times with standard buffer with multi-channel pipette. Then standards were loaded to the plate (three each). Later samples stored at 4°C were diluted 1:1000 with standard buffer and loaded to plate (250 ml each sample). Then the sample in the plate was washed with conjugate buffer and the plate was incubated at room temperature for 30 min for color development. Then the plate was subjected to ELISA reader. Based on absorption values the quantification of *Cry* protein was assessed with help of sigma plot version 8.01 programme.

The data collected from the experiment were subjected to statistical analysis as described by Gomez and Gomez (1984) [5].

Results and Discussion

The *Cry 1Ac* and *Cry2Ab* protein content in leaves was non-significant at all the growth stages due to irrigation levels but differed significantly at 45 DAS. However irrigation at 1.0 Etc recorded higher *Cry 1Ac* and *Cry2Ab* protein content in leafs over 0.8 and 0.6 Etc irrigation levels at 60, 90, 120 and 150 DAS (2.70, 1.77, 1.29 and 0.83µg g^{-1}fresh weight, and *Cry2Ab*, 47.66, 30.66, 25.14 and 15.14 µg g^{-1}fresh weight, respectively). Hallikeri *et al.* (2009) [6] at Dharwad observed that higher *Cry* protein expression in Bt-cotton from 90 to 150 days after sowing (DAS) with irrigation of 0.8 IW/CPE as compared to other irrigation regimes. Effect of moisture regimes on pest incidence was less and nor significant at early stages (60 and 90 DAS). However, pest incidence was significantly affected with moisture regimes at 120 DAS, owing to the decreased level of *Cry* protein and increased effect of soil moisture.

Fertigation with 100 per cent RD N & K (150:75 NK kg ha^{-1}) recorded significantly higher *Cry 1Ac* protein content at 45, 60, 90 and 120 DAS (3.33, 2.84, 2.21 and 1.33 µg g^{-1}fresh weight) and *Cry 2Ab* protein content (62.22, 49.32, 32.32 and 27.32 µg g^{-1}fresh weight). However it was on par with 75 per cent RD N & K at 45, 60 and 120 DAS, and there was non-significant difference at 150 DAS. Ghongane *et al* (2009) [2] reported that *Cry* protein content marginally increased with increase in fertilizer levels and it declined with the advancement of the crop growth. Interaction effect of different irrigation and fertigation levels was non-significant. However highest protein content was recorded in I_1F_1 (drip irrigation at 1.0 Etc with 100% RD N & K fertigation) over all other treatment combinations. Police Patil (2007) [8] and Basavanneppa (2012) [3] also noticed increased *Cry* protein concentration with application of higher nutrient levels. In present study higher *Cry* protein concentration was noticed in initial crop growth stages (45 DAS) and declining trend was observed in subsequent growth stages. The findings were in agreement with the reports of Pettigrew *et al.* (2000) [7] and Adamczyk and summer (2001) [2]. They reported a decline in leaf *Cry* protein concentration in aged cotton plants. At particular stage of the crop cry protein concentration changes with change in the nutrient levels. Studies of Sun *et al.* (2002) [9] and Wan *et al.* (2005) [10] also confirmed the fact that expression pattern vary within the season with higher concentration at the beginning and least at the end.

Significantly highest *Cry 1Ac* and *Cry 2Ab* protein content was recorded at 45 DAS in Drip irrigation at 1.0 Etc with 100% RD N & K in 4 splits as soil application (C_1) (3.24 and 59.33 µg g^{-1}fresh weight, respectively) and at later stages there was no significant difference was observed for *Cry 1Ac* and *Cry 2Ab* protein expression over Furrow Irrigation at 0.8 IW/CPE ratio with 100% RD N & K in 4 as soil application (C_2).

Conclusion

The study indicated that fertigation and split application of N & K had significant effecton *Cry1Ac* and *Cry 2 Ab* concentration in cotton leaves. Drip at 1.0 Etc & fertigation with 100% RD N & K (150:75 NK kg ha^{-1}) applied in six equal splits at 15 days interval found optimum higher Cry 1 Ac and *Cry 2 Ab* protein content in cotton leaves.

Table 1: Cry 1 Ac protein content in Bt cotton leaves as influenced by irrigation and fertigation levels at different growth stages

Treatment	*Cry 1 Ac* protein content µg g^{-1} fresh leaf weight				
	45 DAS	60 DAS	90 DAS	120 DAS	150 DAS
Irrigation levels (I)					
I_1: 1.0 Etc	3.09	2.70	1.77	1.29	0.83
I_2 : 0.8 Etc	2.96	2.51	1.68	1.19	0.78
I_3 : 0.6 Etc	2.69	2.24	1.61	1.01	0.60
S. Em±	0.11	0.14	0.15	0.08	0.11
CD at 5%	0.32	NS	NS	NS	NS
Fertigation levels (F)					
F_1 : 100% RD N & K	3.33	2.84	2.21	1.33	0.82

F_2 : 75% RD N & K	3.08	2.46	1.51	1.16	0.76
F_3 : 50% RD N & K	2.33	2.15	1.35	1.01	0.63
S. Em±	0.11	0.14	0.15	0.08	0.11
CD at 5%	0.32	0.43	0.46	0.23	NS
Interactions (I x F)					
I_1F_1	3.41	3.12	2.31	1.55	0.94
I_1F_2	3.33	2.65	1.47	1.23	0.84
I_1F_3	2.52	2.34	1.54	1.09	0.72
I_2F_1	3.36	2.86	2.11	1.33	0.89
I_2F_2	3.12	2.55	1.73	1.22	0.84
I_2F_3	2.41	2.13	1.21	1.03	0.62
I_3F_1	3.23	2.55	2.20	1.12	0.62
I_3F_2	2.78	2.17	1.32	1.04	0.62
I_3F_3	2.06	1.99	1.30	0.89	0.56
S. Em±	0.18	0.25	0.27	0.13	0.18
CD at 5%	NS	NS	NS	NS	NS
Comparision with controls					
C_1	3.24	2.37	2.08	1.11	0.78
C_2	2.18	1.93	1.27	1.03	0.59
S. Em±	0.17	0.23	0.36	0.14	0.17
CD at 5%	0.50	0.67	NS	NS	NS

Irrigation Levels **Fertigation levels (F)**
I1: 1.0 Etc F1: 100% RD N & K (150: 75: 75 kg ha-1)
I2: 0.8 Etc F2: 75% RD N & K (112.5: 75: 56.25 kg ha-1)
I3: 0.6 Etc F3: 50% RD N & K (75: 75: 37.5 kg ha-1)

Controls
C1: Drip irrigation at 1.0 Etc + 100% RD N & K in 4 splits through soil (25% each as basal and at 30, 60 & 90 DAS).

C2: Furrow Irrigation at 0.8 IW/CPE ratio +100% RD N & K in 4 splits through soil (25% each as basal and at 30, 60 & 90 DAS).

Table 2: *Cry 2 Ab* protein content of Bt cotton as influenced by irrigation and fertigation levels at different growth stages

Treatment	*Cry 2 Ab* protein content µg g^{-1} fresh leaf weight				
	45 DAS	60 DAS	90 DAS	120 DAS	150 DAS
Irrigation levels (I)					
I_1: 1.0 Etc	61.26	47.66	30.66	25.14	15.14
I_2 : 0.8 Etc	60.73	47.02	30.02	24.45	14.45
I_3 : 0.6 Etc	58.10	46.26	29.26	23.15	13.15
S. Em±	0.87	0.65	0.65	1.18	1.18
CD at 5%	2.62	NS	NS	NS	NS
Fertigation levels (F)					
F_1 : 100% RD N & K	62.22	49.32	32.32	27.32	17.32
F_2 : 75% RD N & K	60.76	47.19	30.19	23.96	13.96
F_3 : 50% RD N & K	57.11	44.43	27.43	21.45	11.45
S. Em±	0.87	0.65	0.65	1.18	1.18
CD at 5%	2.62	1.96	1.96	3.52	NS
Interactions (I x F)					
I_1F_1	63.23	50.43	33.43	28.28	18.28
I_1F_2	62.06	47.52	30.52	25.28	15.28
I_1F_3	58.49	45.01	28.01	21.84	11.84
I_2F_1	63.09	49.22	32.22	27.99	17.99
I_2F_2	61.82	47.52	30.52	23.81	13.81
I_2F_3	57.29	44.33	27.33	21.55	11.55
I_3F_1	60.33	48.32	31.32	25.70	15.70
I_3F_2	58.41	46.53	29.53	22.78	12.78
I_3F_3	55.56	43.93	26.93	20.96	10.96
S. Em±	1.51	1.13	1.13	2.04	2.04
CD at 5%	NS	NS	NS	NS	NS
Comparision with controls					
C_1	59.33	46.30	29.30	24.68	14.68
C_2	51.52	45.24	28.24	20.67	10.67
S. Em±	1.63	1.07	1.07	1.88	1.88
CD at 5%	4.80	3.14	3.14	NS	NS

Irrigation Levels **Fertigation levels (F)**
I1: 1.0 Etc F1: 100% RD N & K (150: 75: 75 kg ha-1)
I2: 0.8 Etc F2: 75% RD N & K (112.5: 75: 56.25 kg ha-1)
I3: 0.6 Etc F3: 50% RD N & K (75: 75: 37.5 kg ha-1)

Controls

C1: Drip irrigation at 1.0 Etc + 100% RD N & K in 4 splits through soil (25% each as basal and at 30, 60 & 90 DAS).

C2: Furrow Irrigation at 0.8 IW/CPE ratio +100% RD N & K in 4 splits through soil (25% each as basal and at 30, 60 & 90 DAS).

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