A STUDY ON QUANTITY OF Bt TOXIN IN Bt AND NBt COTTON RHIZOSPHERE

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Abstract

Genetically engineered plants and their residues may pose direct or indirect impacts on different ecosystem functions. The aim of this experiment was to determine the amount of Bt toxin (Cry 1 Ac δ endotoxin) present in the rhizosphere of Bt cotton during the crop period at regular intervals. Bt toxin was estimated from the rhizospheric soils of near isogenic Non Bt cotton crop for as a control. Cry 1 Ac δ endotoxin was estimated using Envirologix Quanti Kit plate (ELISA) method. The soil samples were collected in which Bt cotton was cultivating for more than ten years as monocrop, similarly Non Bt cotton fields where never cultivated transgenic crop were selected for this study. The quantity of the Cry 1Ac toxin levels in the rhizosphere of Bt cotton was estimated at different crop stages and it was 41.13 ppb at pre cultivation stage which is higher than that of Non Bt rhizosphere soil i.e.15.3 ppb. The toxin concentration increased gradually during different crop stages i.e 69.32 ppb, 95.24 ppb, 103.35 ppb at 30, 60,90 days crop stages respectively. It was decreased to 92.37 ppb at harvest stage and shown higher levels (173.24 ppb) at postharvest stage. But in case of Non Bt rhizosphere it is almost same at all the stages of crop. The results suggests that there is a significant difference between the Bt and Non Bt soils with respect to quantity of Cry toxin and there is also significant difference between different crop stages of Bt cotton with respect to Non Bt cotton.

Keywords: Bt toxin, ELISA, δ endotoxin, ppb, rhizosphere, monocrop

INTRODUCTION:

Bt cotton varieties produce toxin in each part of the plant. Bt toxin from leaves and other above ground plant parts may enter soil only after defoliation and cotton harvest, but roots with toxin are in constant contact with the soil systems. Bt toxin levels in fine roots were found to be as high as that in younger leaves. Root hairs and sloughed epidermal cells contribute a significant amount of root material in the rhizosphere of actively growing plants. Hence, the levels of Bt toxin entering the soil system also significantly higher (Gupta *et al.*, 2004). Throughout their entire life cycle transgenic Bt cotton plant synthesizes active Cry protein. This active toxin does not require a high pH and specific proteases for activation as in case of protoxin. This characteristic feature has impact on soil organisms (Singh *et al.*, 2012; Stotzky 2004; Tapp *et al*., 1994). A small portion of Cry toxin can be inactivated or removed by insect larvae, degradation and mineralization by microorganisms or by sun light. The excess amount of toxin accumulates in bound form on soil particles (Martina and Jeanne, 2008).

There are several studies showing that the endotoxin released into the soil by decomposition process adsorbed by the colloidal particles and persists in its active form for a long time and may pose impact on the soil microbial diversity and their metabolic functions. Martina and Jeanne, 2008; Tarafdar *et al*., 2012). Another study by Tapp and Stotzky (1998), reveal that the toxin remained active in soil against insect larvae for more than 230 days. The present study focuses on the estimation of bt toxin (Cry 1 Ac δ endotoxin) from the rhizosphere of Bt cotton and non Bt cotton at different stages of crop.

MATERIALS AND METHODS:

Soil sampling

Three villages were selected for field study which was located in Khammam district, Telangana State, India. The selected sites are being used to cultivate Bt cotton continuously for more than ten years consecutively without an alternate crop. Rhizospheric soils were collected from 60 days crop of Bt Cotton. Rhizospheric soil samples were taken from five fields from each village. Five transects across each plot were chosen. The soil samples were collected at different points (five points) from each transects to get 125 soil samples from one village. Like this from all the three villages separate 125 Bt cotton rhizospheric soils were collected.

All the 125 samples from each village were mixed to get one representative soil sample. After removal of plant debris, the samples were sieved using 2mm mesh size sieve and air dried (Amith *et al.*, 2013). Then they were labeled and transported to the laboratory in polyethylene bags and

stored at 4^0C, and were further used for the quantification of Cry 1 Ac δ endotoxin.

Collection of rhizospheric soil at different stages of Bt and NBt cotton crop

Bt and Non Bt soil samples were collected at different stages of Bt and Non Bt cotton crop from the same experimental sites at six intervals, prior to the plantation to post- harvesting stage. First sampling was done before sowing the seed i.e pre cultivation stage (0 Days), second sampling at one month old plant i.e, growth stage-1 (30 Days), third sampling at budding stage i.e growth stage- 2 (60 Days), fourth sampling at boll formation i.e growth stage- 3 (90 Days), fifth sampling at harvest i.e growth stage- 4 (120 Days) and last sixth sampling at post-harvest stage (150 days).

The rhizospheric soil samples were collected by shaking the roots vigorously to separate the loosely bound bulk soil. The soil samples at pre-vegetation and post- harvest stage were collected from 0-15 cm depth using a 5 cm diameter soil corer (Barea *et al.,* 2005).

Quantification of Cry1 Ac δ endotoxin from the Soil samples:

For extraction of Cry1Ac endotoxin from rhizosphere soil samples, the following protocol was fallowed.

Soil was room dried and sieved by using 2mm sieve and 0.5 gram of soil was mixed with 1.5 ml of extraction buffer, using 2ml capacity of Eppendorf tubes in triplicates. The soil samples were then homogenized by shaking in vortex shaker for 5 minutes. Suspension was then incubated for 24 hrs at room temperature and after that they were centrifuged at 16000 rpm at 15^0C and supernatant was collected for ELISA test (Namita *et al.,* 2009).

A set of Cry 1 Ac protein standards at four different concentrations namely 1ppm, 0.2 ppm, 0.04 ppm,0 ppm were used to get the calibration. 100 µl of each sample extract was added into the wells of ELISA plate. The contents of the plate were mixed thoroughly by moving the plate in a rapid circular motion on the bench top for 20-30 seconds. The plate was then covered by a strip of parafilm to prevent the evaporation and incubated for 15 minutes at ambient temperature in the orbital plate shaker at 200 rpm. Then 100 µl of Cry 1Ab enzyme conjugate was added to each well. The contents were further thoroughly mixed in the similar way and the plate was incubated for 3 hours. After incubation, the plate was flooded with wash buffer thrice. After washing the plate it was inverted and soaked on a paper towel. Then 100 µl of substrate was added to each well under dark condition to avoid the light reaction with substrate. The contents of the plate were thoroughly mixed again and it was wrapped with a parafilm to avoid evaporation. After this the plate was incubated for half an hour in the orbital

plate shaker at 200 rpm. Finally 100 µl of stop solution was added to stop the reaction. The intensity of developed color was measured at 450 nm by ELISA reader within 30 minutes of addition of stop solution. The interpretations of results were tabulated and analyzed using Repeated Measures ANOVA.

RESULTS AND DISCUSSION:

Saxena *et al.,* (2010) reported Bt toxin content in rhizospheric soils of corn and estimated the amount using same kit and studied about the persistence and effect of the toxin. Sims and Ream (1997) calculated that approximately 486 g/ acre (1174 g/ ha) or 1.6 μg/g of soil of Bt protein would be added to soil from a mature transgenic cotton crop.

In the present study Cry 1Ac δ endotoxin was estimated from all the representative soil samples of Bt and Non Bt cotton Rhizosphere. In the Bt rhizospheric soils the amount of Cry 1Ac δ endotoxin levels had increased gradually through per-cultivation to post- harvest stage. The quantity of Bt toxin at 0 Days stage was 41.13 ppb and increased gradually up to103.35 ppb at 90 Days crop level. This can be attributed to increase in the root secretions in the rhizosphere up to flowering and budding stage of Bt cotton crop. At 120 Days growth stage the toxin quantity was dropped to 92.37 ppb and this may be due to decrease in the root secretions as the plant attains harvest stage. At post- harvest stage the quantity was high (173.24 ppb) among all other soil samples, as amendments of plant debris containing Bt toxin occurs to the soil (fig.1).

In Non Bt rhizospheric environment the range of toxin was 13.2 to 15.4 ppb. Low levels of the toxin at different stages of growth was very less as the Non Bt cannot produce Cry 1Ac δ endotoxin in its tissues and tissue secretions. The low levels of detected toxin may be due to indigenous microorganisms which produce toxin in the soil. Mean and Standard Deviations of quantity of Cry 1 Ac endotoxin have been summarized for different days for Bt rhizosphere and NBt rhizosphere separately (Table 1).

Further, Repeated Measures ANOVA has been conducted for days and soils simultaneously (Table 2 and 3). It can be concluded that there is significant (p value at < 0.001) impact of days on quantity of Cry 1 Ac endotoxin and there is also interaction effect of days & soils on quantity of Cry 1 Ac δ endotoxin (Table 2).

There is significant (1% level with p value 0.000) difference between Bt rhizosphere and over the observed period (150 days) with respect to quantity of Cry 1 Ac δ endotoxin in which Bt sample showed more Cry 1 Ac endotoxin than NBt soil sample (Table 3).

CONCLUSION:

The quantity of the Cry 1Ac toxin levels in the rhizosphere of Bt cotton was estimated at different crop stages and it was higher than that of Non Bt rhizosphere soil, in all the soil samples. The toxin concentration increased gradually during different crop stages i.e from pre cultivation to flowering stage, and it was decreased at harvest stage and again shown higher levels at post-harvest stage. But in case of Non Bt rhizosphere it is almost same at all the stages of crop. The high level of toxin was because of root exudates and root sloughed off material which was continuously added during the plant growth and the toxin which already existing in that environment (Mina *et al.,* 2008; Saxena *et al.,* 1999; Tapp *et al.,* 1994; Tapp and Stotzky, 998). This was evident by a large difference between the quantity of toxin at pre cultivation stages of both Bt and Non Bt cotton soils. The difference was almost three folds higher in Bt than the Non Bt soil.

The adsorption, persistence and fate of the toxin which was added continuously in to the rhizospheric environment should be studied in detail. In case of Bt rhizosphere, microbes are immediate receivers of root exudates or root material and they were in continuous contact with the toxin, in addition to the accumulated toxin present in the soil (Tarafdar *et al.,* 2012). Any change in the dynamics of rhizosphere may effect the microbial interactions there by plant microbe relations.

List of tables:

Table 1: Mean and Standard deviation of Quantity of Cry 1Ac δ endotoxin from Rhizosphere soil at different stages of Bt and NBt cotton crop

Days	Mean and Standard Deviation of Cry 1 Ac δ Endotoxin (ppb)	
	Bt	NBt
0 days	41.14 ± 0.01	15.14 ± 0.01
30 days	69.33 ± 0.01	14.3 ± 0.1
60 days	94.58 ± 1.15	14.4 ± 0.1
90 days	103.34 ± 0.01	13.6 ± 0.1
120 days	92.38 ± 0.02	13.2 ± 0.1
150 days	173.25 ± 0.02	15.3 ± 0.1

Table 2: Tests of With-in Subjects Effects

Source	Sum of Squares	df	Mean Square	F-value	p-value
Days	14,731.425	5	2,946.285	25764.99**	0.000
DaysVs Organisms	14,550.855	5	2,910.171	25449.18**	0.000
Error(days)	2.287	20	0.114		

** Significant at 1 % level

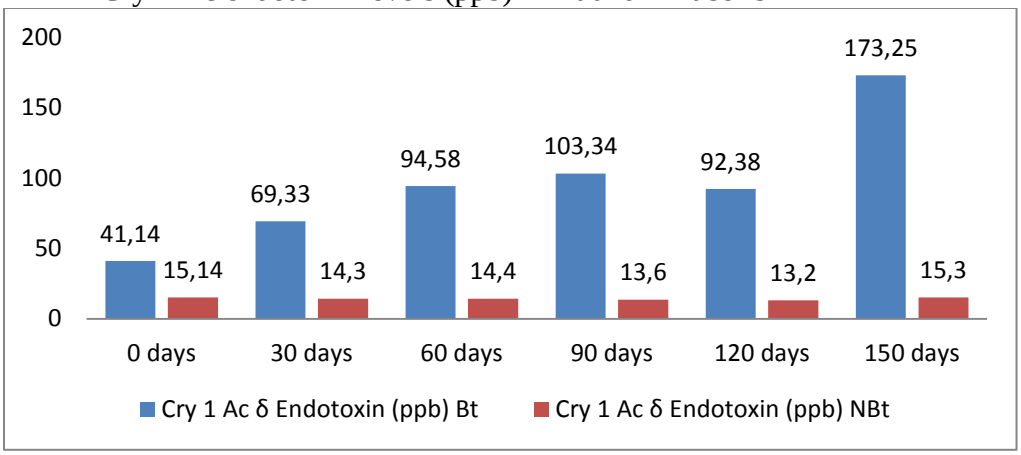
Table 3: Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F-value	p-value
Intercept	108,890.100	1	108,890.100	986571.60**	0.000
Organisms	59,559.589	1	59,559.589	539624.80**	0.000
Error	0.441	4	0.110		

** Significant at p value less than 0.001.

List of Figures:

Cry 1 Ac endotoxin levels (ppb) in Bt and NBt soils



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