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Accumulation and Persistence of Cry1Ac δ-Endotoxin in Rhizospheric Soils Under Continuous Bt Cotton (Gossypium hirsutum) Cultivation: A Field Study from Khammam District, Telangana, India

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Abstract

The large-scale and continuous cultivation of Bacillus thuringiensis (Bt) cotton expressing the Cry1Ac δ-endotoxin raises significant concerns about toxin accumulation in agricultural soils. This study quantified Cry1Ac δ-endotoxin levels in rhizospheric soils of Bt and non-Bt (NBt) cotton fields cultivated continuously for 10–12 years in Khammam District, Telangana, India, across six growth stages (0–150 days after sowing). Using a quantitative ELISA-based assay (Envirologix Quanti Plate Kit), Bt toxin was undetected in NBt soils, while Bt rhizospheric soil showed a progressive accumulation from 41.18 ppb at the seedling stage to a peak of 174.22 ppb at post-harvest (150 days). Concurrent physicochemical analysis demonstrated significantly reduced available nitrogen (22.5 to 15.4 ppm), phosphorus (11.5 to 7.2 ppm), potassium (18.3 to 16.4 ppm) and organic carbon (0.7 to 0.6%) in Bt soils compared to NBt soils across all growth stages. Microbial colony-forming unit (CFU) counts for bacteria (26 to 9 $\times 10^6$ CFU/g), fungi (14 to 7 $\times 10^4$ CFU/g), and actinomycetes (12 to 6 $\times 10^4$ CFU/g) were significantly lower in Bt rhizospheric soils. These findings indicate cumulative toxin persistence and concurrent deterioration of soil physicochemical and biological quality under long-term Bt cotton monoculture. This study calls for mandatory soil quality monitoring frameworks for GM crop cultivation zones in subtropical India.

Keywords: *Bt cotton; Cry1Ac δ-endotoxin; rhizospheric soil; ELISA quantification; soil microbial community; Khammam; Telangana; transgenic crops; soil quality; toxin persistence*

1. Introduction

Bt cotton, genetically engineered to express the Cry1Ac δ-endotoxin from Bacillus thuringiensis, was commercially approved in India in 2002 and has since become the dominant cotton variety, covering approximately 93% of India's 11.7 million hectares of cotton cultivation area [1]. The Khammam District of Telangana State represents one of the most intensive Bt cotton cultivation zones, with over 95% adoption and continuous mono-cropping spanning 10–12 consecutive years. While Bt cotton has demonstrably reduced bollworm pressure and insecticide usage in initial adoption phases, concerns about the environmental fate of the Cry1Ac protein — particularly in soil ecosystems — have intensified globally [2,3].

Bt toxin enters the soil ecosystem through two principal pathways: (i) root exudates from living plants throughout the growing season [4], and (ii) decomposing plant biomass incorporated post-harvest [5]. Once introduced, the Cry1Ac protein can bind rapidly and tightly to soil clay particles, humic acids, and organo-mineral complexes, thereby gaining protection from microbial degradation [6]. This binding mechanism allows the toxin to retain insecticidal and potentially ecotoxicological activity for extended periods. Studies under temperate conditions have reported toxin persistence exceeding 234 days [7]. However, systematic field-based quantification under subtropical Indian conditions — with

distinctly different soil types, temperatures, and microbial communities — remains critically underrepresented in the scientific literature [8].

The rhizosphere, defined as the zone of soil directly influenced by root activity, harbours microbial populations 10–100 times denser than bulk soil and mediates essential biogeochemical processes including nitrogen cycling, phosphorus solubilization, and organic matter decomposition [9]. Disruption of rhizospheric microbial communities by persistent agrochemicals or transgenic proteins could therefore have cascading consequences for soil fertility and long-term agricultural productivity [10]. In the context of India's rapidly expanding Bt cotton cultivation, a comprehensive, temporally resolved field assessment of Cry1Ac accumulation and its concurrent effects on soil quality parameters is urgently needed.

This study addresses this gap by: (i) quantifying Cry1Ac δ-endotoxin across six crop growth stages in Bt and NBt rhizospheric soils from three field sites in Khammam District; (ii) characterizing changes in physicochemical soil parameters including macronutrients and micronutrients; and (iii) enumerating microbial populations (bacteria, fungi, actinomycetes) to assess biological soil health across the cotton cropping cycle.

2. Materials and Methods

2.1 Study Area and Soil Sampling

Three villages in Khammam District, Telangana State, India — Pandithapuram (17°14'N, 80°09'E), Ragunathapalem (17°08'N, 80°22'E), and Kanapuram (17°19'N, 80°15'E) — were selected as field sites (Figure 1). These sites had been under continuous Bt cotton (var. Tulasi, Bollgard I expressing Cry1Ac) monoculture for 10–12 years at the time of sampling. Adjacent NBt cotton fields served as controls. Soils were silty loam (sand 30%, silt 70%, clay 20–23%) with a bulk density of 1.42 g/cm³. All fields received 10 t/ha farmyard manure and recommended NPK fertilizers prior to sowing, and fungicide applications (copper oxychloride/carbendazim/mancozeb) at 2–3 times during the crop cycle. Rainfall during the study year averaged 1,200 mm (June–September 2012).

Rhizospheric soil samples were collected at six growth stages: Stage 0 (0 days — initial seedling), Stage 1 (30 days — one-month-old plant), Stage 2 (60 days — budding), Stage 3 (90 days — boll formation), Stage 4 (120 days — harvest), and Stage 5 (150 days — post-harvest). At each sampling, 10 core samples (1–10 inches depth, 3-inch bore) were collected from randomly selected Bt cotton plants along three transects per plot, then composited into a representative sample. Root-adhering soil was separated by vigorous shaking, passed through a 2 mm sieve, and stored at 4°C for subsequent analysis.

2.2 Cry1Ac δ-Endotoxin Quantification by ELISA

Cry1Ac δ-endotoxin was quantified using the Envirologix Quanti Plate Kit (Envirologix Inc., Portland, USA) with Cry1Ac-specific polyclonal antibodies. For each sample, 0.5 g of soil was extracted with 1.5 ml extraction buffer in 2 ml Eppendorf tubes, vortexed for 5 min, incubated 24 h at room temperature, and centrifuged at 16,000 rpm at 15°C. The supernatant (100 µl per well) was applied to ELISA plates. Standards at 40 and 200 ppb Cry1Ac were included. Colour development was read at OD_{450} nm (Envirologix plate reader). NBt soil spiked with Bt cotton seed powder served as a positive extraction control. All samples were run in triplicate.

2.3 Physicochemical Analysis of Soils

Soil pH was measured in a 1:5 (w/v) suspension using an ELICO digital pH meter with calomel glass electrode. Organic carbon was determined by the Walkley-Black wet oxidation method [11]. Mineralizable nitrogen was estimated by the alkaline $KMnO_4$ method [12]. Available phosphorus was measured by Olsen's method, and potassium by neutral ammonium acetate extraction. Micronutrients (Zn, S) were extracted by DTPA and analysed by Atomic Absorption Spectrophotometry (AAS). Boron was determined by hot-water extraction [13]. All analyses were performed in triplicate.

2.4 Microbial Enumeration

Total viable counts of bacteria, fungi, and actinomycetes were determined by the serial dilution plate method. One gram of representative soil was suspended in 100 ml sterile distilled water; 1 ml aliquots from 10^{-6}, 10^{-4}, and 10^{-5} dilutions were plated on Nutrient Agar, Starch-Casein Agar, and Rose Bengal Agar, respectively. Incubation conditions were: bacteria 37°C/24–48 h; actinomycetes 28°C/7–14 days; fungi 28°C/5–7 days. CFU per gram soil was calculated as: CFU/g = (average colony count × dilution factor) / sample weight. All estimations were performed in triplicate.

2.5 Statistical Analysis

Data are presented as mean ± standard deviation of triplicate measurements. Differences between Bt and NBt soils at each growth stage and across growth stages were analysed by two-way ANOVA followed by Duncan's Multiple Range Test (DMRT). Statistical significance was set at $p < 0.05$. All analyses were performed using SPSS v.21.

3. Results

3.1 Cry1Ac δ-Endotoxin Levels in Rhizospheric Soils

Cry1Ac δ-endotoxin was not detected in any NBt soil sample at any growth stage. In Bt rhizospheric soils, toxin was first detected at the seedling stage (41.18 ppb) and accumulated progressively throughout the crop cycle, reaching a maximum of 174.22 ppb at post-harvest (Table 1, Figure 1). A statistically significant difference ($p < 0.001$) was observed between Bt and NBt soils at all growth stages. The pattern of accumulation — accelerating from budding (Stage 2) through boll formation (Stage 3) — coincides with peak root growth and exudate production, consistent with root-derived toxin being the dominant input pathway during active crop growth.

Table 1. Cry1Ac δ-Endotoxin Levels (ppb) in Bt and NBt Rhizospheric Soils Across Growth Stages

Growth Stage	Days After Sowing	Bt Soil (ppb)	NBt Soil (ppb)	p-value
Seedling (Stage 0)	0	41.18 ± 3.2	ND	< 0.001
One-month plant (Stage 1)	30	68.45 ± 5.1	ND	< 0.001
Budding (Stage 2)	60	98.72 ± 7.8	ND	< 0.001
Boll formation (Stage 3)	90	131.56 ± 9.4	ND	< 0.001
Harvest (Stage 4)	120	156.33 ± 11.2	ND	< 0.001
Post-harvest (Stage 5)	150	174.22 ± 12.6	ND	< 0.001

ND = Not Detected; values are mean ± SD (n=3); p-values from two-way ANOVA

3.2 Physicochemical Properties of Rhizospheric Soils

Both Bt and NBt soils had similar pH (~7.7 and 7.6 respectively) and were classified as mildly alkaline. However, systematic differences in nutrient availability were observed across all growth stages. Available nitrogen declined significantly in Bt soils (from 22.5 at Stage 0 to 15.4 ppm at Stage 4), while NBt soils maintained relatively stable nitrogen levels (24.5–23.0 ppm). Similar declining trends were noted for phosphorus, potassium, zinc, and boron in Bt soils (Table 2). Organic carbon was consistently lower in Bt soils (0.65% mean) versus NBt soils (0.75% mean).

Table 2. Chemical Characteristics of Bt and NBt Rhizospheric Soils (Macro- and Micronutrients, ppm)

Stage	Soil	N (ppm)	P (ppm)	K (ppm)	Zn (ppm)	B (ppm)	OC (%)
Seedling	Bt	22.5	11.5	18.3	0.60	1.50	0.70
Seedling	NBt	24.5	12.5	18.9	0.90	1.70	0.80

Stage	Soil	N (ppm)	P (ppm)	K (ppm)	Zn (ppm)	B (ppm)	OC (%)
Budding	Bt	19.5	9.2	17.1	0.50	1.38	0.64
Budding	NBt	24.2	12.3	18.7	0.84	1.60	0.78
Boll Formation	Bt	16.2	8.5	17.0	0.45	1.35	0.62
Boll Formation	NBt	24.0	12.0	18.5	0.82	1.58	0.74
Harvest	Bt	15.7	7.4	16.6	0.42	1.34	0.61
Harvest	NBt	23.5	11.4	18.2	0.80	1.54	0.72

All values are mean of triplicate measurements; OC = Organic Carbon

3.3 Microbial Population Dynamics

Total culturable microbial populations were significantly lower in Bt rhizospheric soils compared to NBt soils across all growth stages (Table 3). Bacterial CFU declined from 26 × 10^6/g at Stage 0 to 9 × 10^6/g by Stage 4 in Bt soils, compared to relatively stable counts (30–28 × 10^6/g) in NBt soils. Fungal populations decreased from 14 × 10^4/g to 7 × 10^4/g in Bt soils (NBt: 18–16 × 10^4/g). Actinomycetes showed a similar declining trend in Bt soils (12–6 × 10^4/g) versus NBt soils (15–13 × 10^4/g). All differences were statistically significant ($p < 0.05$ by two-way ANOVA with DMRT).

Table 3. Microbial Population (CFU/g Soil) in Bt and NBt Rhizospheric Soils

Growth Stage	Bacteria Bt (×10^6)	Bacteria NBt (×10^6)	Fungi Bt (×10^4)	Fungi NBt (×10^4)	Actino. Bt (×10^4)	Actino. NBt (×10^4)
Seedling (0 d)	26	30	14	18	12	15
One-month (30 d)	22	29	12	17	10	14
Budding (60 d)	18	28	10	17	9	14
Boll Formation (90 d)	14	29	9	16	7	13
Harvest (120 d)	9	28	7	16	6	13
Post-harvest (150 d)	11	29	8	17	7	14

Values represent mean CFU/g dry soil (n=3 composite samples per village per stage)

4. Discussion

The progressive accumulation of Cry1Ac δ-endotoxin in Bt rhizospheric soils — from 41.18 ppb at seedling stage to 174.22 ppb post-harvest — represents one of the first temporally resolved, field-based toxin quantification studies from subtropical India. The pattern mirrors observations from temperate studies: Saxena and Stotzky (2002) demonstrated that Cry toxin released from Bt corn root exudates remained larvicidally active for at least 180 days after binding to soil clay particles [4]. Tapp and Stotzky (1995, 1998) showed insecticidal activity persisting beyond 234 days in soil-bound fractions [7]. Our finding of maximum toxin at post-harvest (150 days) is consistent with the hypothesis that plant biomass incorporation during crop termination delivers a concentrated toxin pulse into the soil environment.

The concurrent decline in available nitrogen (by ~32% across the crop cycle in Bt soils) is particularly significant given nitrogen's central role in crop productivity. The macronutrient depletion pattern in Bt soils — nitrogen > phosphorus > potassium — suggests that microbially-mediated nitrogen transformations (specifically nitrification and mineralization) may be disproportionately affected. This is supported by the progressive decline in bacterial populations, many of which

are implicated in nitrogen cycling. The significantly lower organic carbon in Bt soils (mean 0.65% vs. 0.75% in NBt) further indicates reduced substrate availability for heterotrophic microbial activity.

Our microbial enumeration data show 65% reduction in bacterial CFU and 50% reduction in fungal and actinomycete populations at peak depletion in Bt soils compared to NBt. While some researchers have reported transient or non-significant differences between Bt and NBt crop microbial communities [15], these contrasting reports often involve short-term studies (one or two seasons) under temperate conditions. The 10–12 year continuous monoculture at our study sites likely represents a cumulative ecological stress scenario, where successive toxin additions have progressively exceeded a threshold tolerable to sensitive microbial populations [16].

The absence of Cry1Ac in NBt soils throughout the study confirms that the toxin detected in Bt soils is exclusively transgene-derived and not a background soil contaminant. This finding underscores the importance of maintaining paired non-GM controls in long-term environmental monitoring programs. Soil pH (~7.7) at our sites falls within the range reported to promote maximum Cry protein adsorption to clay particles (pH 5–7), potentially explaining the high toxin persistence observed [6]. Subtropical temperatures may additionally slow microbial degradation of soil-bound toxin, as has been reported for other soil-bound proteins in tropical environments.

From a regulatory and agricultural management perspective, these data suggest that mandatory Cry toxin monitoring — using validated ELISA protocols — should be incorporated into Indian GM crop environmental surveillance frameworks. Crop rotation, green manuring, and reduced chemical fertilizer inputs may help restore soil microbial community structure in long-term Bt cotton cultivation zones.

5. Conclusion

Continuous cultivation of Bt cotton for 10–12 years in Khammam District resulted in significant and progressive accumulation of Cry1Ac δ-endotoxin (41.18–174.22 ppb) in rhizospheric soils, unmatched by NBt control soils. Concurrent deterioration of soil physicochemical quality (30–37% reduction in available nitrogen and phosphorus) and substantial declines in total bacterial, fungal, and actinomycete populations were documented. These findings collectively indicate that long-term, large-scale Bt cotton monoculture poses measurable risks to soil ecosystem health in subtropical India, and warrant systematic policy-driven monitoring and sustainable soil management interventions.

References

[1] James, C. (2022). ISAAA Brief 55: Global Status of Commercialized Biotech/GM Crops. ISAAA, Ithaca, NY.

[2] Icoz, I., & Stotzky, G. (2008). Fate and effects of insect-resistant Bt crops in soil ecosystems. Soil Biology and Biochemistry, 40(3), 559–586.

[3] Saxena, D., & Stotzky, G. (2000). Insecticidal toxin from Bacillus thuringiensis is released from roots of transgenic Bt corn in vitro and in situ. FEMS Microbiology Ecology, 33(1), 35–39.

[4] Saxena, D., & Stotzky, G. (2002). Bt toxin released from root exudates and biomass of Bt corn has no apparent effect on earthworms, nematodes, protozoa, bacteria, and fungi. Soil Biology and Biochemistry, 34(1), 133–137.

[5] Zwahlen, C., Hilbeck, A., Gugerli, P., & Nentwig, W. (2003). Degradation of the Cry1Ab protein within transgenic Bacillus thuringiensis corn tissue in the field. Molecular Ecology, 12(3), 765–775.

[6] Tapp, H., & Stotzky, G. (1998). Persistence of the insecticidal toxin from Bacillus thuringiensis subsp. kurstaki in soil. Soil Biology and Biochemistry, 30(4), 471–476.

[7] Tapp, H., & Stotzky, G. (1995). Insecticidal activity of the toxins from Bacillus thuringiensis subspecies kurstaki and tenebrionis adsorbed and bound on pure and soil clays. Applied and Environmental Microbiology, 61(5), 1786–1790.

[8] Sarkar, B., Patra, A. K., Purakayastha, T. J., & Megharaj, M. (2009). Assessment of biological and biochemical indicators in soil under transgenic Bt and non-Bt cotton crop in a sub-tropical environment. Environmental Monitoring and Assessment, 156(1), 595–604.

[9] Lynch, J. M., & Whipps, J. M. (1990). Substrate flow in the rhizosphere. Plant and Soil, 129, 1–10.

[10] Morrissey, J. P., Dow, J. M., Mark, G. L., & O'Gara, F. (2004). Are microbes at the root of a solution to world food production? EMBO Reports, 5(10), 922–926.

[11] Walkley, A., & Black, I. A. (1934). An examination of the Degtjareff method for determining soil organic matter. Soil Science, 37(1), 29–38.

[12] Subbiah, B. V., & Asija, G. L. (1956). A rapid procedure for the estimation of available nitrogen in soils. Current Science, 25, 259–260.

[13] Berger, K. C., & Truog, E. (1939). Boron deficiency as revealed by plant and soil tests. Journal of the American Society of Agronomy, 31(4), 307–319.

[14] Donegan, K. K., Palm, C. J., Fieland, V. J., Porteous, L. A., Ganio, L. M., Schaller, D. L., & Seidler, R. J. (1995). Changes in levels, species and DNA fingerprints of soil microorganisms associated with cotton expressing the Bacillus thuringiensis var. kurstaki endotoxin. Applied Soil Ecology, 2(2), 111–124.

[15] Saxena, D., & Stotzky, G. (2001). Bacillus thuringiensis (Bt) toxin released from root exudates and biomass of Bt corn has no apparent effect on earthworms, nematodes, protozoa, bacteria, and fungi in soil. Soil Biology and Biochemistry, 33(9), 1225–1230.

[16] Dunfield, K. E., & Germida, J. J. (2004). Impact of genetically modified crops on soil- and plant-associated microbial communities. Journal of Environmental Quality, 33(3), 806–815.

[17] Brusetti, L., Francia, P., Bertolini, C., Pagliuca, A., Borin, S., Sorlini, C., & Daffonchio, D. (2004). Bacterial communities associated with transgenic Bt 176 maize and its non-transgenic counterpart. Plant and Soil, 266(1), 11–21.

[18] Stotzky, G. (2002). Release, persistence, and biological activity in soil of insecticidal proteins from Bacillus thuringiensis. In Genetically Engineered Organisms: Assessing Environmental and Human Health Effects. CRC Press, Boca Raton, FL.

[19] Palm, C. J., Donegan, K., Harris, D., & Seidler, R. J. (1994). Quantification in soil of Bacillus thuringiensis var. kurstaki delta-endotoxin from transgenic plants. Molecular Ecology, 3(2), 145–151.

[20] Velmourougane, K., & Sahu, A. (2013). Impact of Bt (Cry1Ac) and non-Bt cotton on soil biological attributes. Plant, Soil and Environment, 59(11), 498–503.