

Effect of Bt Cotton on Enzymes Activity and Microorganisms in Rhizosphere

Usha Mina (Corresponding author)

Division of Environmental Sciences, Indian Agricultural Research Institute, New Delhi-110012, India

Tel: 112-584-1866 ext 4798 E-mail: usha_env@iari.res.in

Anita Chaudhary

Division of Environmental Sciences, Indian Agricultural Research Institute, New Delhi-110012, India

Tel: 112-584-1866 ext 4805 E-mail: anita_envr@yahoo.com

Anju Kamra

Division of Nematology, Indian Agricultural Research Institute, New Delhi-110012, India

Tel: 112-584-2721 E-mail: anjukamra@yahoo.com

Research support was provided by Department of Biotechnology (DBT), New Delhi, India to Division of Environmental Sciences, IARI, India.

Abstract

For three consecutive years *Bacillus thuringiensis* (Bt) transgenic cotton (var. Mech 162) and its isogenic non Bt counterpart were assessed for the risks of transgenic crop on the soil ecosystem under Indian subtropical conditions. To observe effect of Bt cotton on soil biochemical properties, activities of dehydrogenase, alkaline phosphatase, nitrate reductase and urease soil enzymes were assayed at its different growth stages i.e., seedling, vegetative, flowering, bolling and harvesting stages. To observe effect of Bt cotton on soil microorganisms, number of nematodes, collembola and ants representing micro, meso and macrofauna, respectively were observed in Bt and non- Bt cotton plants rhizosphere at different growth stages.

Results showed no significant difference ($P < 0.05$) in alkaline phosphatase, nitrate reductase and urease activity between Bt and non- Bt cotton rhizosphere during crop growth period. However, dehydrogenase activity was significantly high ($P < 0.05$) in the Bt cotton rhizosphere as compared to non Bt cotton rhizosphere through out the observation period. At most of the growth stages numbers of micro, meso and macro fauna were more in Bt cotton rhizosphere as compared to non Bt cotton rhizosphere. The temporal and spatial variations observed in number of nematodes, collembola and ants between Bt and non Bt cotton plants rhizosphere were significant. The present study shows that the Mech 162 variety of Bt cotton was not posing any risk to soil microorganisms and soil biochemical properties.

Keywords: *Bacillus thuringiensis*, Transgenic, Cotton, Enzymes, Soil, Microorganisms, Mesofauna, Microfauna

1. Introduction

In India nine million hectares (m ha) area was under cotton cultivation and it is one fourth of the global area under cotton cultivation (35 m ha). *Bacillus thuringiensis* (Bt) cotton was introduced in India in 2002. Following its success, during the last 7 years (2002-2008), the area under Bt cotton has increased by 7.6 m ha from 0.029 m ha (James, 2008).

Although there are diverse benefits of Bt cotton, public concern also exist because both *in vitro* and *in vivo* studies on Bt cotton showed that Bt toxin produced in leaves, stems and roots of Bt cotton plants is introduced in soil. Bt-toxin from Bt cotton plants introduced into the soil through two pathways, i.e., biomass incorporation and root exudates (Saxena and Stotzky 2001; Mina *et al.*, 2008; Liu 2009). Bt toxin released in soil get adsorbed or bound on clay particles, humic components, or organic mineral complexes and then be protected against degradation by soil microorganisms (Tapp *et al.*, 1995). Although Bt toxin also found naturally in many soils, but continuous growing of Bt crops on same location enhance its existing levels to a certain concentration that might affect the composition and activity of soil microbial communities (Donegan and Seidler 1999; Stotzky

2004; Wei *et al.*, 2006; Griffiths *et al.*, 2007; Rui *et al.*, 2005) and the soil biochemical properties (Rui *et al.*, 2005; Fang *et al.*, 2007; Sun *et al.*, 2007; Sarkar *et al.*, 2009).

Several experiments were conducted to assess risk of Bt cotton on flora and fauna in diverse agroecosystems (Zhang *et al.*, 2000; Li *et al.*, 2002; Liu *et al.*, 2002; Men *et al.*, 2003; Bai *et al.*, 2003). Some studies indicate that Bt cotton has no negative effects on soil flora and fauna and may even have beneficial effects (Saxena and Stotzky 2001; Sarkar *et al.*, 2009), while some have reported adverse effects (Cui and Xia 2000; Tan *et al.*, 2002). However, similar experimental studies on risk assessment of Bt cotton with respect to soil ecosystem in India are very limited. Climatically India is a subtropical country, thus as compared to temperate and sub temperate countries, biological and biochemical response of Indian soil to increasing acreage of Bt cotton may vary. Therefore, we have studied the effects of Bt cotton (var. Mech 162, approved by Genetic Engineering Approval Committee for commercial cultivation in North India) on activity of soil enzymes and microorganisms at Indian Agriculture Research Institute farms, New Delhi, India. An evaluation of the ecological risks of Bt cotton was made on the basis of changes in enzymes activity and number of nematodes, collembola and ants as compared to non Bt cotton in the respective rhizospheres.

2. Materials and methods

2.1 Field preparation and cultural practices

Field experiments were conducted at the research farm of the Indian Agricultural Research Institute, New Delhi, India. The soil of the experimental site was slightly alkaline with silty clay loamy texture and has low organic matter content. Experiments were conducted for three consecutive years (2004, 2005 and 2006) in a large plot (450 m²) where Bt and non Bt cotton had ever planted. This plot was subdivided into 24 subplots (3.75m x 5m). Treated seeds of non Bt cotton (Mech 162-Bt) and Bt cotton (Mech 162+Bt), procured from MAHYCO (Mahyco seed limited, Mumbai) were sown in plots at the rate of 15 kg/ha. Each subplot was sown with a different cotton line, under irrigated conditions. Distance between row to row and plant to plant was 60 cm and 45 cm respectively. To ensure uniform germination and better stand, line sowing with seed drill was carried out. Seeds were sown uniformly at a depth of 4-5 cm. A 6 m buffer zone was also established surrounding the main plot. Recommended dose of NPK fertilizer (660 g N as Urea, 250 g P as SSP, 350 g K as MOP) were applied in the field. Recommended dose of phosphorus and Potash was applied at time of sowing, whereas nitrogen was given in split dose, half at the time of thinning and other half at flowering stage. First irrigation to cotton was given one week after sowing and subsequent irrigations at an interval of two weeks. Cotton crop at maturity was harvested in the first week of December in all the three years. Soil samples of experimental field for physicochemical characterization (Table 1) were collected from the top layer (0–20 cm). The soil was air-dried at room temperature, passed through a 2 mm sieve, homogenized by mixing three times with a shovel and stored at room temperature before use.

2.2 Soil Sampling

Soil samples from the rhizosphere of Bt cotton and non Bt cotton plants were collected at regular interval (30 days interval, coinciding with different growth stage of cotton) since the date of sowings till harvesting in all the three years. First Sampling was done before seed sowing from prepared field, second sampling 30 Days After Sowing (DAS) (at seedling stage, growth stage1), third sampling 60 DAS (at vegetative stage, growth stage 2), fourth sampling 90 DAS (at Flowering stage growth stage3), fifth sampling 120 DAS (at Boll formation stage, growth stage 4) and last sixth sampling 150 DAS (at mature stage, growth stage 5). Ten rhizosphere samples (0-15cm) were taken along two transects across each plot and were mixed to make a representative sample for analysis. Collected rhizospheric soil samples were processed by air drying, grinding and sieving (passed through a 1 mm sieve) and analysed for all enzymes activities except dehydrogenase and nitrate reductase. For observing dehydrogenase as well as nitrate reductase activity, the soil samples were kept moist.

2.3 Dehydrogenase activity

Dehydrogenase activity was analyzed as described by Min *et al.* (2001). Five grams of soil was incubated for 12 h at 37°C in 5 ml of a TTC solution (5 g TTC in 0.2M Tris-HCl buffer, pH 7.4). Two drops of concentrated sulfuric acid were added immediately after the incubation to end the reaction. The sample was then blended with 5 ml of toluene and shaken for 30 min at 250 rpm, followed by centrifuging at 4500g for 5 min to extract TPF. The optical density of the red colour extract supernatant was measured at 492 nm using UV-Vis spectrophotometer (UV-1201, Shimadzu Corp, Japan). Soil dehydrogenase activity was expressed as $\mu\text{g TPF g}^{-1} 12 \text{ h}^{-1}$

2.4 Alkaline phosphatase activity

Alkaline phosphatase activity was measured spectrophotometrically as described by Tabatabai and Bremner (1969). 1 g soil was placed in a 50 ml erlenmeyer flask and treated with 0.25 ml of toluene and 4 ml MUB buffer (P^H 11) and 1 ml of p- nitrophenolphosphate solution made in same buffer. After that the flask contents were mixed and incubated for 1 h at 37°C. After 1 h of incubation 1 ml of $CaCl_2$ (0.5 M) and 4 ml of NaOH (0.5 M) were added to the flask. The colored soil suspension was filtered through Watmann no. 2 filter paper and absorbance of filtrate was measured at 400 nm. The phosphatase activity was expressed as μg p-nitrophenol $g^{-1} h^{-1}$

2.5 Urease activity

Urease enzyme activity in soil sample was estimated according to “determination of urea remaining” methodology (Tabatabai, 1994). This methodology estimate urea hydrolysis in soils on account of urease enzyme activity. For this 5 g of soil mixed with 5 ml of urea solution (10 mg urea/ml) in a 50-ml erlenmeyer flask, and incubated for 5 h at 37°C. After 5 h, 50 ml 2M KCl-PMA was added to flasks and kept for 1h shaking. After 1 h shaking soil suspension was filtered under suction through whatmann no 42 filter paper. Out of filtrate 2 ml of aliquot was taken and mixed with 10 ml 2M KCl-PMA and 30 ml colouring reagent (25 ml 2.5% DAM + 10 ml of 0.25% TSC in 500 ml acid reagent). This mixture was first kept in water bath for 30 minutes and than kept in ice coldwater for 15 minutes for colour development. Absorbance of coloured end product was measured at 527nm. Activity of Urease enzyme in soil was expressed as μg urea N hydrolysed $g^{-1} h^{-1}$

2.6 Nitrate reductase activity

Nitrate reductase activity was determined using methodology of Abdellmagid and Tabatabai (1987). For this 5 gm field-moist soil was mixed with 4 ml of 0.19 mM 2,4 dinitrophenol, 1 ml of (25 mM) substrate solution (KNO_3) and 5 ml distilled water into screw cap test tubes. These tubes were incubated at 37°C for 24 h and the control was frozen immediately at -20°C. After incubation 10 ml of 4M KCL solution was added in the samples and control and filtered immediately. 5 ml of the filtrate was used for color development with 3 ml of 0.19 M ammonium chloride buffer and 2 ml of color reagent. Absorbance of coloured end product was measured at 520nm. Activity of nitrate reductase enzyme in soil was expressed as μg NO_2^- $Ng^{-1} h^{-1}$

2.7 Nematodes

Nematodes were extracted from Bt and non Bt cotton plants rhizosphere (250 gm) by the Baermann technique (Van Gundy 1982) in water. Nematodes samples from Bt and non Bt cotton plants in water suspension were uniformly placed over tissue paper supported by screen in a petriplates, which were filled with water. After placing the sample, petriplates were covered with respective cap and incubated at $24 \pm 2^\circ C$ for 36 h. To estimate the total number of nematodes, the content of the petriplates were transferred to a flat bottom partitioned counting dish (36 squares), and the number of nematodes were counted in 10 random squares using stereoscopic dissecting microscope (Nikon, 10- 100 X zoom). Total nematodes in the sample were calculated by multiplying the average count in 10 squares by 36.

2.8 Collembola and Ants

Collembola and ants were extracted from soil cores (5 cm dia x 20 cm deep; collected from Bt and non Bt cotton plants rhizosphere) using Tullgreen funnel ("Berlese funnels"). Reagent grade ethylene glycol was used to capture the Collembola and ants (moving out from the soil core as the soil heated up from bulbs light energy) in 275 ml plastic cups. Collected collembola and ants were counted using stereoscopic dissecting microscope (Nikon, 10 - 100 X zoom)

2.9 Experimental design and statistics

This study was designed as a randomized complete block. The block treatment was the month of sampling and the two cotton lines were the main source of variation. The interaction of month and cotton lines was used for testing significance of the sources of variation. These treatments were tested with analysis of variance (ANOVAs) using SPSS software.

3. Results and discussion

Seven ANOVAs were calculated to test significant differences among the variables due to Bt cotton. ANOVA results for the dehydrogenase enzyme is given in Table 2. Cotton line was a significant source of variation in one of seven ANOVA's. Year block term was non significant in all seven ANOVA's, whereas month by cotton variety interaction source of variation being non significant for three variables. Therefore the interaction term was used to test the variation on account of cotton variety.

On average dehydrogenase, alkaline phosphatase, nitrate reductase and urease enzymes activity was high in Bt cotton rhizosphere as compared to non Bt rhizosphere (Table 3). Except dehydrogenase enzyme, differences in activity of alkaline phosphatase, nitrate reductase and urease enzymes between Bt and non Bt plants rhizosphere were statistically non significant. Over the crop growth period (of 150 days) the activity of Phosphatase, nitrate reductase and urease enzymes activity was high in the rhizosphere at early vegetative and flowering stage, whereas activity of dehydrogenase enzyme was high at boll formation stage (Figure 1). Activity of all the four enzymes declined at crop harvesting stage (Figure 1). On comparing enzymes activity for the interaction of crop variety (Bt and non Bt trait) over a monthly period, activity of dehydrogenase and urease enzymes were non significant and activity of Phosphates and nitrate reductase enzymes were significant (Table 3).

Significant differences ($P < 0.05$) were observed in the number of nematodes, collembola and ants between the Bt and non Bt cotton rhizosphere (Table 4). Number of nematodes, collembola and ants were more in Bt plants rhizosphere as compared to non Bt plants rhizosphere. Number of nematodes, collembola and ants were highest in Bt and non Bt cotton rhizosphere at flowering stage.

The biochemical properties of soil have often been proposed as early and sensitive indicators of soil ecosystem health (Oliveira and Pampulha, 2006). Activities of soil enzymes indicate the direction and strength of all kinds of biochemical processes in soil and act as key biological indicator of soil. Soil enzymes play an essential role in energy transfer, environmental quality, organic matter decomposition, nutrient cycling and crop productivity (Tabatabai, 1969; Kumar *et al.*, 1992). Measurement of enzymes activity in combination with count of number of key microorganisms provides sensitive information of the changes occurring in soil (Brooke 1995). The results are consistent with an experiment conducted with Cry 1A Bt cotton (Sukang-103) and non Bt cotton (Sumian-12) (Shen *et al.*, 2006). Their study demonstrated enhanced phosphatase, nitrate reductase and urease activity in the rhizosphere of Bt cotton, but most of the time differences in enzymes activity between Bt and non-Bt cotton rhizosphere were non significant. Similarly, Sun *et al.*, (2007) also observed stimulated activities of urease, phosphatase, invertase and cellulase enzymes by the addition of Bt cotton tissues in soil. In present study variation in dehydrogenase enzyme activity between Bt and non-Bt cotton rhizosphere was significant ($P < 0.05$) and activity of dehydrogenase enzyme was high in Bt cotton rhizosphere. This observation of the present study is consistent with the observation of Wu *et al.*, (2004) observed enhanced dehydrogenase activity in soil incubated with Bt transgenic rice straw (during 7-14 days of incubation, later on it declined) compared to soil without straw. Results of the present study with respect to dehydrogenase enzyme were contrary to research performed by Liu *et al.* (2008) and Oliveira *et al.* (2008). They observed no significant differences in dehydrogenase activity in Bt maize and Bt rice rhizosphere. The enhancement in activity of dehydrogenase in Bt cotton rhizosphere may be due to altered composition of its root exudates. Root exudates have a profound qualitative and quantitative effect on the rhizospheric microorganisms (Schenck, 1976).

In the present study temporal variation was observed in number of nematodes, collembola and ants in Bt and non Bt plants rhizosphere. Temporal variation in number of nematodes, collembola and ants is a complicating factor in impact assessment of Bt transgenic cotton, because the impact of the genetic modification may be transient and minor compared to the variation caused by soil condition and growth stage of crop (Rasche *et al.*, 2006). Number of nematodes, collembola and ants in Bt plants rhizosphere were significantly more than non Bt plants rhizosphere. This observation of the present study is contrary to Saxena and Stotzky (2001) observation. They observed that transgenic corn crops had no apparent effect on nematodes. However these results should be considered as preliminary, as nematodes, collembola and ants are mobile and slight change in their niche environment may affect their mobility. In general, we speculate that effects of Bt cotton on the number of nematodes, collembola and ants in rhizosphere might mainly depend on the extent of change in root exudates (quality and/or quantity) that may related to variety differences. More detailed studies on the composition and diversity of these groups of microorganisms using different molecular and biochemical techniques are necessary.

In conclusion, based on three year field experiments it was found that Mech 162 variety of Bt cotton has no adverse effect on soil biochemical and microbial indicators. The differences caused by growing Bt cotton were not as large as those resulting from seasonal changes indicating that effect of Bt cotton on soil ecology was within normal variation expected in conventional agriculture.

References

- Abdellmagid, H.M., & Tabatabai, M.A. (1987). Nitrate reductase activity of soils. *Soil Biology and Biochemistry*, 19, 412-427.
- Bai, L., Zhang, L., Chen, X., & Feng, H. (2003). Composition and diversity of the weed community in transgenic Bt cotton (four Bollgard strains) fields. *Acta Phytoecol Sin*, 27, 610-616.

- Brookes, P.C. (1995). The use of microbial parameters in monitoring soil pollution by heavy metal. *Biology and Fertility of Soils*, 19, 269-279.
- Cui, J., & Xia, J. (2000). Effects of transgenic Bt cotton R93-6 on the insect community. *Acta Entomologica Sinica*, 4, 343-351.
- Donegan, K.K., & Seidler, R.J. (1999). Effects of transgenic plants on soil and plant microorganisms. In: SG Pandalai (ed) *Recent research development in microbiology*, 3(II). Research Signpost, Trivandrum, India. pp 415-424.
- Fang, M., Motavalli, P.P., Kremer, R.J., & Nelson, K.A. (2007). Assessing changes in soil microbial communities and carbon mineralization in Bt and non-Bt corn residue-amended soils. *Applied Soil Ecology*, 37, 150-160.
- Griffiths, B.S., Birch, A.N.E., Caul, S., Thompson, J., Heckmann, L.H., Krogh, P.H., & Cortet, J. (2007). The role of laboratory, glasshouse and field scale experiments in understanding the interactions between genetically modified crops and soil ecosystems: A review of the ECOGEN project. *Pedobiology*, 51(3), 251-260.
- Hu, Hai-Yan., Liu, Xiao-Xia., Zhao, Zhang-Wu., Sun, Jian-Guang., Zhang, Qing-Wen., Liu, Xing-Zhong and Yu, Yong. (2009). Effects of repeated cultivation of transgenic Bt cotton on functional bacterial populations in rhizosphere soil *World Journal of Microbiology and Biotechnology*, 25(3), 357-366.
- James, C. (2008). Global Status of Commercialized Biotech/GM Crops. *ISAAA Briefs*. ISAAA, Ithaca, New York
- Kumar, J.D., Sharma, G.D., & Mishra, R.R. (1992). Soil microbial population numbers and enzyme activities in relation to altitude and forest degradation. *Soil Biology and Biochemistry*, 24, 761-767.
- Li, J., Liu, Q., Jin, X., Liu, J., & Liu, Y. (2002). The niches of major pests and natural enemies between Bt cotton and common cotton. *Entomology Journal of East China*, 11, 51-54.
- Liu, Wenke. (2009). Effects of Bt transgenic crops on soil ecosystems: a review of ten year research in China. *Frontiers in Agriculture of China*, 3(2), 190-198.
- Liu, W., Wan, F., & Guo, J. (2002). Structure and seasonal dynamics of arthropods in a transgenic Bt cotton field. *Acta Ecologia Sinica*, 22, 729-735.
- Liu, Wei., Lu H.H., Wu, W., Wei, Q.K., Chen, Y.X., & Thies, J.E. (2008). Transgenic Bt rice does not affect enzyme activities and microbial composition in the rhizosphere during crop development. *Soil Biology and Biochemistry*, 40, 475-486.
- Men, X., Ge, F., Yin, X., & Cai, D. (2003). Diversities of arthropod community in transgenic Bt cotton and nontransgenic cotton agroecosystems. *Chinese Journal of Ecology*, 22, 26-29.
- Min, H., Ye, Y.F., Chen, Z.Y., Wu, W.X., & Du, Y.F. (2001). Effects of butachlor on microbial populations and enzyme activities in paddy soil. *Journal of Environmental Science and Health*, B36, 581-595.
- Mina, U., Khan, Shakeel. A., Chaudhary, Anita., Choudhary, R., & Aggarwal, P.K. (2008). An Approach For Impact Assessment Of Transgenic Plants On Soil Ecosystem. *Applied Ecology and Environmental Research*, 6(3), 1-19.
- Oliveira, A., & Pampulha, M.E. (2006). Effects of long term heavy metal contamination on soil microbial characteristics. *Journal of Bioscience and Bioenergy*, 102(3), 157-161
- Oliveira, A., Pampulha, M.E. & Bennet, J.P. (2008). A two year field study with transgenic *Bacillus Thuringiensis* maize: Effects on soil microorganisms. *Science of Total Environment*, 405, 351-357.
- Rui, Y.K., Yi, Guo-Xang., Zhao, J., Wang, B.M., Li, Z.H., Zhai, Z.X., & He, Z.P. (2005). Changes of Bt toxin in the rhizosphere of transgenic Bt cotton and its influence on soil functional bacteria. *World Journal of Microbiology and Biotechnology*, 21, 1279-1284.
- Rasche, F., Hodl, V., & Poll, C. (2006). Rhizospheric bacteria affected by transgenic potatoes with antibacterial activities compared with the effects of soil, wild type potatoes, vegetation stage and pathogen exposure. *FEMS Microbial Ecology*, 56, 219-235.
- 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, 595-604.

- Saxena, D., & Stotzky, G. (2001). Bacillus thuringiensis (Bt) toxin released from root exudates and biomass of Bt corn had no apparent effect on earthworm, nematodes, protozoa, bacteria and fungi in soil. *Soil Biology and Biochemistry*, 33, 1225-1230.
- Schenck, N.G. (1976). *Microorganisms and Root Development and Function*. Madison, FL: Soil and Crop Science Society.
- Shen, R.F., Cai, H., & Gong, W. H. (2006). Transgenic Bt cotton has no apparent effect on enzymatic activities or functional diversity of microbial communities in rhizosphere soil. *Plant Soil*, 285, 149-159.
- Stotzky, G. (2004). Persistence and biological activity in soil of the insecticidal proteins from Bacillus thuringiensis, especially from transgenic plants. *Plant Soil*, 266, 77-89
- Sun, C.X., Chen, L.J., Wu, Z.J., & Zhou, L.K. (2007). Soil persistence of Bacillus thuringiensis(Bt) toxin from transgenic Bt cotton tissues and its effect on soil enzymes activities. *Biology and Fertility of Soil*, 43, 617-620.
- Tabatabai, M.A., & Bremner, J.M. (1969). Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry*, 1, 301-307.
- Tabatabai, M.A. (1994). Soil enzymes. In: Weaver RW, Angel JS, Bottomley PS. (Eds). *Methods of soil analysis. Part 2. Microbial and biochemical properties*. Soil Science Society of America, Madison, pp 775-833.
- Tan, S., Chen, X., & Li, D. (2002). Progress in the studies on Helicoverpa spp Resistance to transgenic Bt cotton and its management strategy. *Acta Entomologica Sinica*, 45, 138-144.
- Tapp, L, Calamai, L., & Stotzky, G. (1995). Insecticidal activity of the toxins from Bacillus thuringiensis subspecies kurstaki and tenebrionis adsorbed and bound on pure and soil clays. *Applied Environmental Microbiology*, 61, 1786-1790.
- Van Gundy, S.D. (1982). Nematodes. In: Page AL, Miller RH, Keeney DR. (Eds). *Methods of Soil Analysis. Part 2. American Society of Agronomy*, Madison, WI, pp. 1124-128.
- Wei, X.D., Zou, H.L., Chu, L.M., Liao, B., Ye, C.M., & Lan, C.Y. (2006). Field released transgenic papaya affects microbial communities and enzyme activities in soil. *Plant and Soil*, 285 (1-2), 347-358.
- Wu, W.X., Ye, Q.F., Min, H., Duan, X.J., & Jin, W.M. (2004). Bt transgenic rice straw effects the culturable microbiota and dehydrogenase and phosphatase activities in a flooded paddy soil. *Soil Biology and Biochemistry*, 36, 289-295.
- Zhang, Q., Li, S., Chen, Z., Fu, H., & Yang, Y. (2000). Study of the population dynamics changes of the beneficial and injurious living beings on the field of Bt cotton. *Journal of Hebei Normal University. Natural Science Edition*, 24, 4521-523.

Table 1. Physico-chemical properties of soil at experimental site

Properties	Values
Sand (%)	46
Silt (%)	33
Clay (%)	21
Textural class	Silty clay loam
Bulk density (g cm ⁻³)	1.38
Hydraulic conductivity (cm d ⁻¹)	3.53
Percolation rate (cm d ⁻¹)	2.85
pH	8.1
Electrical conductivity (dS m ⁻¹)	0.48
CEC (cmol (p ⁺) kg ⁻¹)	7.30
Organic carbon (%)	0.45
Total N (ppm)	667
Available nitrogen (kg ha ⁻¹)	232
Available phosphorous (kg ha ⁻¹)	37
Available potassium (kg ha ⁻¹)	291

Table 2. Example of ANOVA for dehydrogenase enzyme

Source of variation	Degree of freedom	Sum of squares	Mean squares	F value	Probability
Year	2	0.208	0.104	0.287	0.751
Month	5	29.595	5.91	16.32	0.000
Cotton variety	1	4.28	4.28	11.83	0.001
Year X Cotton variety	2	1.49	0.746	2.059	0.132
Month x cotton variety	5	2.63	0.527	1.453	0.211
Error	115	41.69	0.363		
Total	144	654.2			

Significant source of variation (P<0.05) are in bold font

Table 3. Enzymes activity in Bt and non Bt cotton rhizosphere during crop growth period

Month	Dehydrogenase ($\mu\text{g TPF g}^{-1}\text{h}^{-1}$)	Phosphatase ($\mu\text{g TPF g}^{-1}\text{h}^{-1}$)	Nitrate reductase ($\mu\text{g p-nitrophenol g}^{-1}\text{h}^{-1}$)	Urease ($\mu\text{g urea N hydrolysed g}^{-1}\text{ soil h}^{-1}$)
1	19.1	165.1	0.5	154.7
2	20.1	180.9	0.8	154.6
3	22.1	170.4	0.5	154.2
4	22.5	165.8	0.3	144.4
5	22.4	166.9	0.2	144.7
6	21.1	146.5	0.3	143.7
CD (P < 0.05)	1.79	10.5	0.03	1.20
Treatment				
Bt plant rhizosphere(a)	21.1	166.2	0.5	149.9
Non Bt plant rhizosphere (b)	18.1	165.6	0.4	149.4
CD(P < 0.05)	1.19	ns	ns	ns
Month * Treatment				
1*a	18.3	165.8	0.5	154.5
1*b	19.8	164.4	0.6	154.9
2*a	19.2	174.2	0.9	154.7
2*b	20.8	187.1	0.8	154.6
3*a	21.9	179.7	0.5	153.2
3*b	22.3	161.1	0.4	155.4
4*a	22.1	174.7	0.3	144.8
4*b	22.8	156.9	0.2	144.1
5*a	21.9	168.2	0.2	145.6
5*b	22.8	165.6	0.1	143.1
6*a	20.7	135.1	0.4	145.6
6*b	21.3	158.0	0.2	141.1
CD(P < 0.05)	ns	14.8	ns	1.7

Table 4. Number of nematodes, collembola, ants in Bt and non Bt cotton rhizosphere

	Nematodes Numbers 250 g⁻¹ soil	Collembola Numbers 250 g⁻¹ soil	Ants Numbers 250 g⁻¹ soil
Month			
1	661.5	1.0	3.5
2	724.4	2.4	1.3
3	1065.4	4.1	2.8
4	1212.9	9.6	6.3
5	942.8	8.3	4.6
6	681.7	4.6	2.0
CD (P < 0.05)	26.99	1.33	1.12
Treatment			
Bt rhizosphere(a)	808.2	5.4	3.9
Non Bt rhizosphere (b)	787.1	4.6	2.9
CD(P < 0.05)	15.58	0.77	0.69
Month * Treatment			
1*a	674.5	1.0	3.8
1*b	648.5	1.0	3.3
2*a	735.0	2.8	1.0
2*b	713.7	2.0	1.5
3*a	1078.7	5.0	2.8
3*b	1052.0	3.3	2.8
4*a	1220.7	9.0	6.0
4*b	1205.0	10.3	6.5
5*a	946.0	9.0	2.3
5*b	939.7	7.5	7.0
6*a	671.7	5.5	1.5
6*b	691.7	3.8	2.5
CD(P < 0.05)	ns	ns	ns

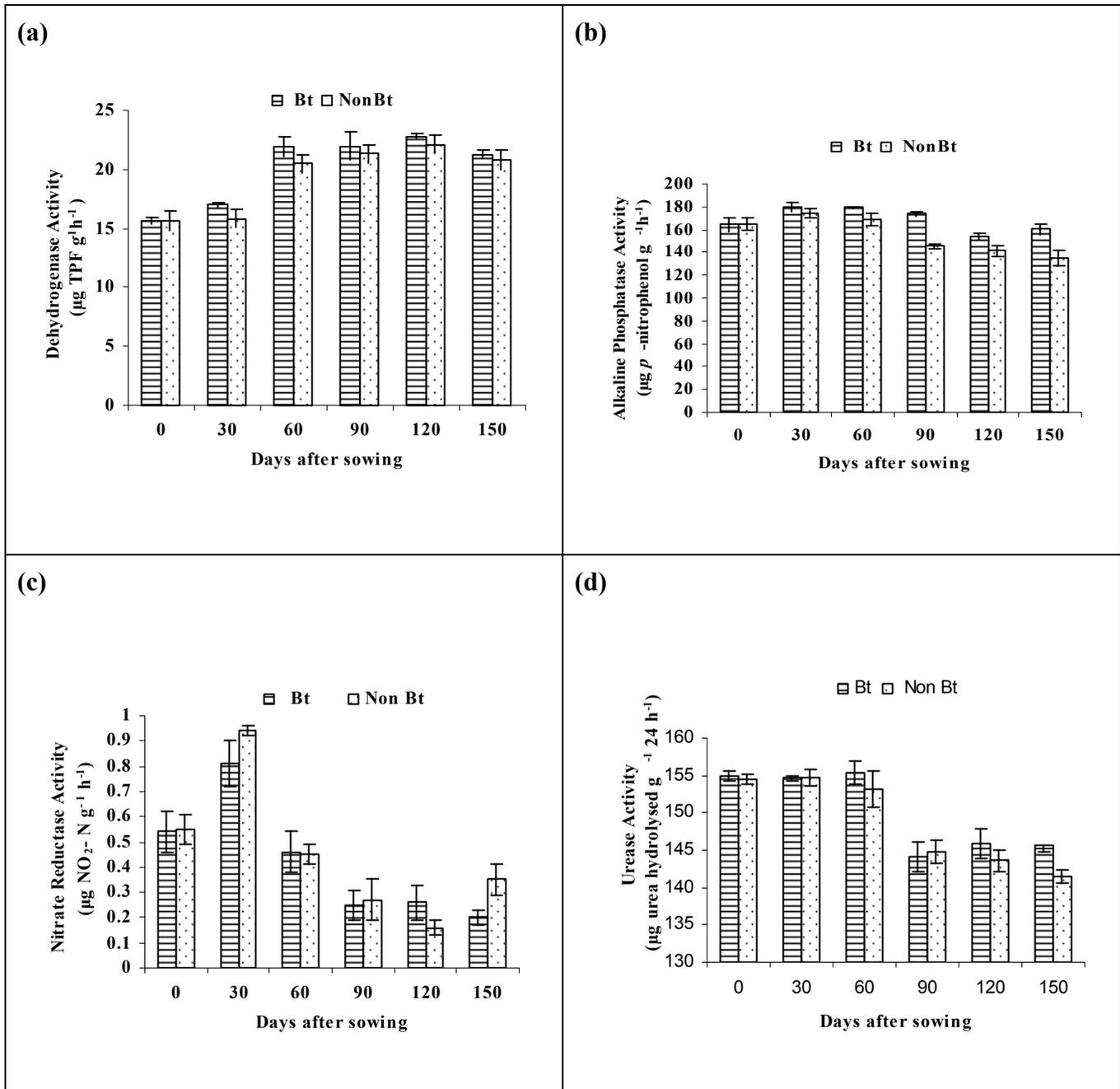


Figure 1. Variations in activity of (a) Dehydrogenase (b) Alkaline phosphatase (c) Nitrate reductase and (d) Urease enzymes at different growth stages in Bt and Non Bt cotton rhizosphere