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SOIL SCIENCE

Decomposition of Bt Cotton Residues affecting Soil microbial activity under varied Soils

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Abstract

The effect of transgenic *Bacillus thuringiensis* (*Bt*) cotton residues on soil microbiological activity was investigated. Greenhouse study was carried out during the 2011 wet season (March to August) at Institute of Agricultural Sciences, Banaras Hindu University. It was experimented on three different soil orders that included entisol, inceptisol and alfisol. *Bt* cotton (var.NCS-138) and its non-transgenic isoline (var.NCS-138) were grown until maturity along with one control treatment. Microbial population count, Dehydrogenage activity and Microbial Biomass Carbon (MBC) were estimated following standard protocols. The decomposition of cotton crop residues resulted increased micro-flora populations and microbial biomass carbon (MBC). When residue was retained, non- *Bt* cotton showed higher populations of micro-flora as well as MBC that of *Bt*-cotton. Results from the study revealed that a significant reduction (7.5%) of the dehydrogenase activity was there in case of *Bt*-cotton. The interaction effect between soil type and varieties was found to be non significant for the soil micro-flora populations for different sampling stages throughout the incorporation period.These results suggest that *Bt*-transgenic cotton tissues have no apparent effect on soil microbial activity.

Highlights

- *Bt*-transgenic cotton tissues have no apparent effect on soil microbial activity.

Keywords: *Bt*-cotton, soil types, soil micro-flora

Soil ecosystem is not only the reservoir pool of exotic genes and their expression products of *Bt* transgenic crops but also the centre of biosphere and terminal habitat of microorganism. However, the large-scale commercial release of *Bt* crops is of public concern because of the potential threat to natural and agricultural ecosystems (Hails 2000, Stotzky 2000, 2004, Hu 2009, Velmourougane 2013). More and more attention has been paid worldwide to analyse the impact of the *Bt* transgenic crops on soil ecosystem in recent years. Usually, the *Bt* genes-expressing products into soil through root exudation or decomposition of the crop residues (Palm *et al.* 1996; Sims and Holden 1996; Saxena *et al.* 1999; Saxena and Stotzky 2000). Then the toxin could be adsorbed or bound on clay particles, humic components, or organic mineral complexes followed by degradation through soil microorganisms. Likewise it could be deposited to a certain concentration that might affect the composition and activity of soil microbial communities (Tapp and Stotzky 1995; Crechio and Stotzkty 2001; Rui *et al.* 2005) and the soil biochemical

properties (Rui *et al.* 2005; Sun *et al.* 2007;Kumari *et al.* 2014; Beura and Rakshit,2013). In addition there are many modifications in crop-soil ecosystem being raised after replacement of conventional crops by Bt transgenic crops, such as *Plant Physiology*ogical characteristics, biomass quantity and composition, degree of dependence on pesticides, fertilization structure etc. In soil ecosystems, therefore, *Bt* transgenic products expose potential toxicity to sensitive microorganism, which affect the transformation and cycle of carbon and nutrients. The changes in microbial communities associated with transgenic crop residues are relatively variable and transient in comparison with some other well-accepted agricultural practices. Since minor alterations in the diversity of the microbial community, such as the removal or appearance of specific functional groups of bacteria such as plant-growth-promoting rhizobacteria, phytopathogenic organisms, or key organisms responsible for nutrient cycling processes, could affect soil health and ecosystem functioning, the impact that plant residue may have on the dynamics of soil microbial populations and in turn health, and ecosystem sustainability, requires further study. Future work needs to address long-term effects of transgenic crops in rotation, while keeping in mind that these effects should not only be compared with a non-transgenic counterpart, but also to other acceptable changes in the agro ecosystem, such as growing a novel non-transgenic plant or utilizing a new agronomic practice. Keeping in view the above points in mind, the investigation has been carried out to determine if microbial activity differ in *Bt* and non *Bt* cotton residue amendments under varied soil type.

Materials and Methods

Experimental site

The present investigation was carried out in a net house on three different soil types at Institute of Agricultural sciences, B.H.U. during March to August in 2011. *Bt* cotton crop residues (cv.NCS-138) and its non transgenic isoline (cv.NCS138) plant residues were incorporated in the soils (Figure 1) up to five month and soil sample were obtained periodically. A control treatment was also maintained with three replications for all the three soil types. The experimental design was a factorial experiment under completely randomized block design with three replications. The cultivated soils of three order viz; Entisol, Inceptisol, and alfisol were collected from previous pot experiment in which *Bt* cotton and non *Bt* cotton was grown for impact assessment study in net house of Soil Science and Agricultural chemistry, BHU.

Figure 1: (a) Experimental view in net house (b) square litter bags of nylon netting material (127 mm · 127 mm) with a mesh size of 0.79 mm filled up with air dried plant sample

Analysis of Soil

The preliminary physico-chemical characterization was done for the initial soil samples. Among the three soils, two were slightly alkaline and one was acidic in reaction. All the soils have low organic matter content, bulk density of soil varied from 1.38-1.51 Mg m^{-3}, EC from 0.32-0.61dsm^{-1} ,CEC from 18.25 to 31.85 Cmol (p+) kg^{-1}, available N from 167 to 238 kg ha^{-1}, P from 8 to 18 kg ha^{-1} and K from 110 to 165 kg ha^{-1} respectively.

Microbial Population

For enumerating the microbial population of soil, composite soil, samples were made by pooling together the samples of soil from the entire plastic cup to each. Total bacteria, fungi and actinomycetes population were estimated by following the serial dilution and plating techniques as described by

Schmidt and Caldwell (1967). Dehydrogenase activity and Microbial Biomass Carbon were also determined following the standard protocol by Casida *et al.* 1964 and Wu *et al.* 1990 respectively.

Data obtained from all the observation were statistically analysed applying the factorial CRD. The least square difference (LSD) values were calculated to test the significance of treatment difference and LSD values were evaluated at 1% level of significance.

Results and Discussion

Bacterial population depends upon the decomposition of crop residues. Bacterial population was at its higher for the case of faster decomposition. Rate of decomposition of crop residues was higher during initial phase after incorporation for which it was maximum at 50 days after incorporation. Thereafter the bacteria population has been decreased gradually as the rate of decomposition slowed down.

Table 1. Bacterial population (cfu x 10^6 g^{-1} soil) in soil at different day after incorporation.

DAI	Cultivar (C)	Soil types(S)				Mean
		S_1	S_2	S_3		
50	Non-Bt (V_1)	41.33	45.00	48.67		45.00
	Bt (V_2)	37.00	42.00	44.00		41.00
	No crop (V_3)	30.33	36.33	39.00		35.22
	Mean	36.22	41.11	43.89		
	LSD(0.01) C = 5.95, S=5.95,CxS=10.31 SEm± C= 1.46,S=1.46,CxS=2.53					
100	Non-Bt (V_1)	28.67	39.67	43.33		37.22
	Bt (V_2)	26.67	32.00	36.67		31.78
	No crop (V_3)	24.67	30.67	28.67		27.98
	Mean	26.64	34.11	36.22		
	LSD(0.01) C =4.74S=4.74,CxS=8.22 SEm± C=1.17, S=1.17, CxS=2.02					
150	Non-Bt (V_1)	17.33	20.00	21.67		19.66
	Bt (V_2)	15.67	19.33	20.67		18.53
	No crop (V_3)	12.33	15.33	16.00		14.66
	Mean	15.33	18.22	19.44		
	LSD(0.01) C = 5.95, S=5.95,CxS=10.31 SEm± C= 1.46,S=1.46,CxS=2.53					

(S_1 = Red soil, S_2 = Black soil, S_3 = Alluvial soil, V_1 = Non-Bt cultivar, V_2 = Bt cultivar, V_3 = no crop, DAI = Days after incorporation

From Table 1, it has been observed that soil under non *Bt* cotton residues produced significantly higher bacterial population than *Bt* cotton crop residues. Results in the study are in accordance with that by Saxena *et al.* (2001), Sun *et al.* (2007).

Results from Table 2 predicted that faster rate of decomposition increased the fungi population. It was observed that Fungi population was maximum at 50 days after incorporation. Thereafter rate of decomposition was slowed down. It has been observed that soil under non *Bt* cotton residues produced significantly higher fungi population than *Bt* cotton crop residues. Results in the study were in accordance with that of Saxena *et al.* (2001). Among the three different types of soil, red soil produced highest fungal population followed by black soil and alluvial soil. This might be for the acidic nature and

Table 2. Fungi population (cfux10^4 g^{-1} soil) in soil at different day after incorporation.

DAI	Cultivar (C)	Soil types (S)			Mean
		S_1	S_2	S_3	
50	Non-Bt (V_1)	45.33	36.33	38.33	39.99
	Bt (V_2)	42.33	35.33	33.33	36.99
	No crop (V_3)	40.67	32.67	27.33	33.55
	Mean	42.77	34.77	32.99	
	LSD(0.01) C = 4.47, S=4.47,CxS=7.74 SEm± C= 1.10, S=1.10,CxS=1.90				
100	Non-Bt (V_1)	32.67	26.67	27.33	28.89
	Bt (V_2)	28.67	22.67	24.00	25.11
	No crop (V_3)	25.67	18.00	19.33	21.00
	Mean	29.00	22.44	23.55	
	LSD(0.01) C =4.24, S=4.24,CxS=7.35 SEm± C=1.04, S=1.04, CxS=1.81				
150	Non-Bt (V_1)	24.00	21.67	13.67	19.78
	Bt (V_2)	23.00	20.67	12.33	18.66
	No crop (V_3)	18.67	15.33	11.00	17.74
	Mean	21.89	19.22	12.33	
	LSD(0.01) C = 3.48, S=3.48,CxS=6.03 SEm± C= 0.86,S=0.86,CxS=1.48				

($S_{1=}$Red soil, $S_{2=}$ Black soil, $S_{3=}$Alluvial soil, $V_{1=}$Non-Bt cultivar, $V_{2=}$ Bt cultivar,V_3=no crop, DAI=Days after incorporation)

abundance of coarse clay in red soil. The interaction effect between soil types and varieties was found to be non-significant in various sampling stages throughout the incorporation period.

Table: 3 Actinomycetes population (cfux10^5g^{-1}) in soil at different day after incorporation.

DAI	Cultivar (C)	Soil types(S)			Mean
		S_1	S_2	S_3	
50	Non-Bt (V_1)	37.33	41.00	48.00	42.11
	Bt (V_2)	34.33	39.67	45.33	39.77
	No crop (V_3)	29.67	34.67	42.00	35.44
	Mean	33.77	38.44	45.11	
	LSD(0.01) C = 4.31, S=4.31,CxS=7.47 SEm± C= 1.06,S=1.06,CxS=1.84				
100	Non-Bt (V_1)	26.33	33.00	41.33	33.54
	Bt (V_2)	25.00	31.00	36.67	30.89
	No crop (V_3)	22.00	28.00	33.00	27.67
	Mean	24.44	30.66	37.00	
	LSD(0.01) C =3.56,S=3.56,CxS=6.17 SEm± C=0.87, S=0.87, CxS=1.52				
150	Non-Bt (V_1)	17.33	23.67	29.67	23.55
	Bt (V_2)	15.00	21.00	27.00	21.00
	No crop (V_3)	13.33	20.00	24.00	19.11
	Mean	15.22	21.55	26.89	
	LSD(0.01) C = 4.53, S=4.53,CxS=7.85 SEm± C= 1.11,S=1.11,CxS=1.97				

($S_{1=}$Red soil, $S_{2=}$ Black soil, $S_{3=}$Alluvial soil, $V_{1=}$Non-Bt cultivar, $V_{2=}$ Bt cultivar,V_3=no crop, DAI=Days after incorporation)

Data from Table 3 resulted that actinomycetes population was higher for faster rate of decomposition. It was found maximum at 50 days after incorporation and decreased as the duration prolonged. It has also been observed that soil under non *Bt* cotton residues produced significantly higher actinomycetes population than *Bt* cotton crop residues. Results in the study are in agreement with Saxena *et al.* (2001). The interaction effect between soil type and varieties was found to be non-significant during different sampling stages throughout the incorporation period.

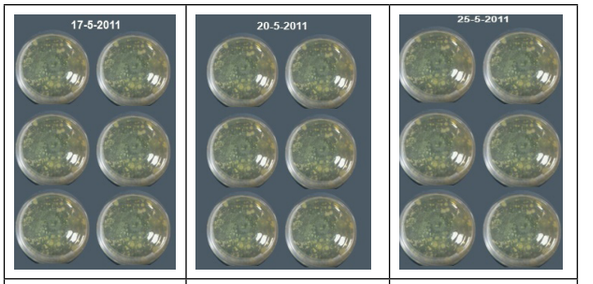

Figure 2. Microbial population in soil at 50 days after incorporation.

Significant reduction (7.5%) in the dehydrogenase activity was observed in the residues of *Bt* cotton incorporation over non-*Bt* isoline. The lower dehydrogenase activity in residues of *Bt* cotton is in conformity with the result of Beura and Rakshit (2011), Masto *et al.* 2006 and Wu *et al.* (2004). It might have been partly because of unfavourable conditions in the soil under *Bt* cotton crop residues or because of a negative effect of *Bt*- toxins on certain microbial groups, which might have retarded metabolic activities in the soil. The interaction effect between soil type and *Bt* cotton was found to be non-significant for different sampling stages throughout the incorporation period.

Microbial biomass carbon was at higher site, where degradation of cotton residues was fast. (Table 5). Degradation of non-*Bt* cotton was significantly higher than *Bt* cotton during all the growth stages. Maximum degradation of *Bt* protein took place at 30 days after incubation. This result is in agreement with by Li *et al.* (2005) who reported that *Bt* protein in cotton leaves degraded rapidly in the first several days, then got slowed down, and then entered a relative stable stage, in which Cry1Ac protein content was kept at a concentration of about 50 ngg^{-1}. This might be for the higher temperature and lower humidity in the first few days. In natural environment, the insecticidal protein in *Bt* cotton degraded much rapidly in the initial period, reaching 85% for the first month, slowed down during winter season, then degraded quickly in the next spring until it became undetectable in late April. At the

same time, MBC was significantly higher in black and alluvial soil as compared to that of red soil at 50 and 100 days interval whereas it was highest for alluvial soil than red soils. MBC declined with time for all the soil studied. Cotton type had no significant effect on C dynamics.

Table 4. Dehydrogenase activity ug TPF g soil^{-1}day^{-1} in soil at different day after incorporation.

DAS	Cultivar (C)	Soil types (S)			
		S_1	S_2	S_3	Mean
50	No crop (V_3)	39.15	42.23	48.41	43.26
	Bt (V_2)	35.52	40.60	44.34	40.15
	Non-Bt (V_1)	31.56	37.38	37.55	38.30
	Mean	35.41	40.07	43.43	
	LSD(0.01) C = 4.14, S=4.14,CxS=7.18 SEm± C= 1.02,S=1.02,CxS=1.76				
100	Non-Bt (V_1)	34.09	40.60	44.08	39.59
	Bt (V_2)	32.08	36.37	40.34	36.26
	No crop (V_3)	28.04	33.64	34.55	33.77
	Mean	31.40	36.98	39.65	
	LSD(0.01) C =5.96,S=5..96,CxS=10.32 SEm± C=1.46, S=1.46, CxS=2.53				
150	Non-Bt (V_1)	30.74	34.81	39.40	34.98
	Bt (V_2)	26.05	30.27	35.37	30.56
	No crop (V_3)	23.89	28.28	31.67	27.94
	Mean	26.89	31.12	35.48	
	LSD(0.01) C = 5.20, S=5.20,CxS=9.01 SEm± C= 1.28,S=1.28,CxS=2.21				

($S_{1=}$Red soil, $S_{2=}$ Black soil, $S_{3=}$Alluvial soil, $V_{1=}$Non-Bt cultivar, $V_{2=}$ Bt cultivar, V3=no crop, DAI=Days after incorporation)

Correlation of microbial population with enzyme activity and microbial biomass carbon

The relationships of soil microbial population, dehydrogenase enzyme activity and microbial biomass carbon illustrated in Table 3. This positive correlation suggests that *Bt* cotton residue incorporation has not changed the microbial activity significantly in soil.

Table 5. Soil microbial biomass carbon in soil at different day after incorporation.

DAS	Cultivar (C)	Soil types(S)				Mean
		S_1	S_2	S_3		
50	Non-Bt (V_1)	300.92	315.04	232.32	282.67	
	Bt (V_2)	292.49	300.25	205.93	266.22	
	No crop (V_3)	284.94	287.50	199.00	257.14	
	Mean	292.78	300.93	212.41		
	LSD(0.01) C = 6.11, S=6.11,CxS=10.58 SEm± C= 1.50,S=1.50,CxS=2.60					
100	Non-Bt (V_1)	207.11	224.60	232.04	217.63	
	Bt (V_2)	195.46	216.19	205.93	207.58	
	No crop (V_3)	193.63	208.11	199.09	200.27	
	Mean	198.73	216.30	212.20		
	LSD(0.01) C =6.08 ,S =6.08,CxS =10.54 SEm± C =1.49,S=1.49,CxS=2.59					
150	Non-Bt (V_1)	156.27	165.34	182.15	167.92	
	Bt (V_2)	150.64	163.34	169.48	156.46	
	No crop (V_3)	146.60	155.41	160.56	154.19	
	Mean	151.17	161.36	170.73		
	LSD(0.01) C = 5.54, S=5.54,CxS=9.59 SEm± C= 1.36,S=1.36,CxS=2.36					

($S_{1=}$Red soil, $S_{2=}$ Black soil, $S_{3=}$Alluvial soil, $V_{1=}$Non-Bt cultivar, $V_{2=}$ Bt cultivar, V3=no crop, DAI=Days after incorporation)

Table 6. Correlation matrix between important microbiological parameters

	Bacterial population	Fungi population	Actinomycetes population	Dehydrogenase activity	Microbial Biomass Carbon
Bacterial population	1	0.94**	0.98**	0.95**	0.95**
Fungi population	0.94**	1	0.97**	0.91**	0.98**
Actinomycetes population	0.99**	0.97**	1	0.95**	0.98**
Dehydrogenase activity	0.95**	0.92**	0.95**	1	0.91**
Microbial Biomass Carbon	0.96**	0.98**	0.98**	0.91**	1

** significant p=0.01

Conclusion

The decomposition of cotton crop residues results in increased micro-flora populations. When residue was retained, non- *Bt* cotton showed higher populations of micro-flora compared with *Bt*- cotton. The interaction effect between soil type and varieties was found to be non significant for the soil micro-flora populations in different soil sampling throughout the incorporation period.

References

Beura K and Rakshit A 2011. Effect of Bt cotton on nutrient dynamics under varied soil type. *Italian Journal of Agronomy* **6**(4):25-28.

Beura K and Rakshit A 2013. Bt cotton influencing enzymatic activities under varied soils. *Open Journal of Ecology* **3**: 505-509.

Casida LE, Klein DA Jr. and Santero T 1964. Soil dehydrogenase activity. *Soil Science* **98**: 371-376.

Crecchio and Stotzky 2001. Biodegradation and insecticidal activity of the toxin from Bacillus thuringiensis subsp. kurstaki bound on complexes of montmorillonite-humic acids-Al hydroxypolymers, *Soil Biology and Biochemistry* **33**: 573–581.

Hails RS 2000. Genetically modified plants – the debate continues. *Trends in Ecology and Evolution* **15**: 14–18.

Hu HY, Liu XX, Zhao ZW, Sun JG, Zhang QW, Liu XZ and Yu Y 2009. Effects of repeated cultivation of transgenic Bt cotton on functional bacterial populations in rhizosphere soil. *World Journal of Microbiology and Biotechnology* **25**: 357–366.

Jenkinson DS and Powelson DS 1976. The effects of biological treatments on metabolism in soil. V.A. method for measuring soil biomass. *Soil Biology Biochemistry* **8**:209-213.

Klein DA, Loh TC and Goulding RL 1971. Arapid procedure to evaluate dehydrogenase activity of soils low in organic matter. *Soil Biology Biochemistry* **3**: 385-387.

Kumari S, Rakshit A, Beura K 2014. Decomposition of bt cotton and non bt cotton residues under varied soil types. *The Journal of Microbiology, Biotechnology and Food Sciences* **3**(5): 360-363.

Li YH, Zhang YJ, Wu KM, Yuan GH, Guo YY 2005. Degradation dynamics of Cry1Ac insecticidal protein in leaves of Bt cotton under different environments, *Scientia Agricultura Sinica* **38**(4): 714–718.

Masto RE, Chhonkar PK, Singh D, Patra AK 2006. Changes in soil biological and biochemical characteristics in a long-term field trial on a sub-tropical inceptisol, *Soil Biology and Biochemistry* **38**:1577–1582.

Palm CJ, Schaller DL, Donegan KK, Seidler RJ 1996. Persistence in soil of transgenic plant produced Bacillus thuringiensis var. kurstaki δ-endotoxin, *Canadian Journal Microbiology* **42**:1258–1262.

Rui YK 2005. Dynamics of Bt toxin and plant hormones in rhizosphere system of transgenic insecticidal cotton (Gossy posium L.), *Letters in Biotechnology* **16**(5): 515–517.

Saxena D and Stotzky G 2001. Bt corn has a higher lignin content than non-Bt corn. *American Journal of Botany* **88**: 1704–1706.

Saxena D, Flores S and Stotzky G 1999. Insecticidal toxin in root exudates from Bt corn. *Nature* **402**: 480.

Saxena D and Stotzky G 2001. Insecticidal toxin from Bacillus thuringiensis is released from roots of transgenic Bt corn in vitro and in situ FEMS *Microbiological Ecology* **33**: 35–39.

Sims SR, Holden LR 1996. Insect bioassay for determining soil degradation of Bacillus thuringiensis var. kurstaki CryIA (b) protein in corn tissues, *Environ Entomology* **25**: 659–664.

Stotzky G 2000. Persistence and biological activity in soil of insecticidal proteins from Bacillus thuringiensis and of bacterial DNA bound on clays and humic acids. *Journal of Environmental Quality* **29**: 691–705.

Sun CX, Chen LJ, Wu ZJ, Zhou LK 2007. Soil persistence of Bacillus thuringiensis (Bt) toxin from transgenic Bt cotton tissues and its effect on soil enzyme activities, *Biology and Fertility of Soils* **43**: 617–620.

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 Environmental Microbiology* **61**(5): 1786–1790.

Velmourougane K, Sahu A 2013. Impact of transgenic cottons expressing cry1Ac on soil biological attributes. *Plant Soil and Environment* **59**: 108–114.

Wu LC, Li XF, Ye QF, Wang HY 2004. Expression and root exudation of Cry1Ab toxin protein in cry1Ab transgenic rice and its residue in rhizosphere soil. *Environmental Science* **25**(5): 116–121.

Wu J, Jörgensen RG, Pommerening B, Chaussod R, Brookes PC 1990. Measurement of soil microbial biomass C by fumigation-extraction–an automated procedure. *Soil Biology and Biochemistry* **22**: 1167–1169