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DECOMPOSITION OF BT COTTON AND NON BT COTTON RESIDUES UNDER VARIED SOIL TYPES

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ABSTRACT

Use of the insecticidal cry proteins from the bacterium, *Bacillus thuringiensis* (Bt) in cotton has raised a number of concerns, including the ecological impact on soil ecosystems.Greenhouse study was conducted during the 2011 wet season (March to August) at the Institute of Agricultural Sciences of Banaras Hindu University. It was carried out on three different soil orders that includedentisol, inceptisol and alfisol. Bt cotton (var.NCS-138) and its non-transgenic isoline (var.NCS-138) were grown until maturity. A no croppot was maintained for all the three soil orders. The highest rate of decomposition was found in alluvial soil compared to black and red soils in 50 days after incorporation (DAI). Thereafter the rate of decomposition was slowed downby100 DAI and the constant rate of decomposition was found in 150 DAI. The rate of decomposition was higher in non Bt than Bt crop residues.

Keywords: Bt cotton, soil types, decomposition

INTRODUCTION

Many excellent accounts of the transgenic crops with low pesticide input in agro-ecosystems have been documented (**Beura and Rakshit,2011; Brookes and Barfoot, 2006; Ferry *et al*.,2006**). Genetically engineered cotton (*Gossypium* spp.) expressing crystalline proteins (e.g., Cry1Ab, Cry1Ac, and Cry13A) for control of lepidopteran and coleopteran pests and encoded by genes derived from soil bacterium Bt are widely adopted in India(**Qaimand Zilberman,2003; Flores *et al*., 2005**). Bt cotton accounted for 90 % of the cotton acreage in 2010-11 in India(**Ramasundaram and Vennila, 2013**).

A large-scale cultivationof transgenic Bt-plants may result in long-lasting negative impact on the environment(**Vadakattu and Gupta,2008**). First, the cultivation of these plants leads to accumulate of Bt-toxin in soil (**Sun*et al*.,2005; Sun*et al*.,2007**). Second, the decomposition of transgenic plants takes significantly longer time compared to that of isogenic lines(**Saxena *et al*., 1999**).. Third, the transfer of delta-endotoxin-encoding genes to the genome of agricultural crops affects simultaneously several entirely different traits of genetically modified plants, thus exerting pleotropic effects(**Saxena and Stotzky, 2001**). Further the increased concerns of the impacts of Bt transgenic plants on soil ecosystems with reference to soil microorganism species, population, and biodiversity (**Angel, 1994; Jepson *et al*., 1994**)add gravity to this issue.These prompted action for a well defined risk assessment study of Bt cotton under diverse agroecological set up. (**Wolfenbarger and Phifer, 2000; Bruinsma *et al*., 2003**).

Bt toxin acts selectively. The toxin produced by *Bacillus thuringein*sis adsorbed and bound rapidly (in <30 minutes, the shortest time studied) on clay size fraction of soil, on humic acids, aluminium hydroxyl-polymer. The binding of the toxins on these surface-active particles reduce their availability to microbes, which is probably responsible for the persistence of the toxin in soil. However, ithas also been shown that these effects are dependent on nature of soil fieldsite, seasonal variation, and method of analysis used to assessthe community(**Beura and Rakshit, 2013;Blackwoodet al., 2004**).

Although numerous laboratory and field studies have showed no unexpected ecological risk at the insect community-level above-ground, few studies have addressed the possible impact of cry protein released from living or decaying roots of Bt maize (*Zea mays*)on soil microbial communities under varied soil types. Here the test hypothesis is that coleopteran-active Bt maize does not affect non target ecological processes, such as decomposition or the function of the associated saprophytic microbial community. The present work was designed to determine whetherresidue from commonly grown Bt and non Bt cotton differ in rate of decomposition under varied soil types.

MATERIAL AND METHODS

Experimental site

Agreenhouse study was conducted at the Institute of Agricultural Sciences, Banaras Hindu University (BHU), Varanasi, Uttar Pradesh, India during (March to August) in 2011. Crop residues of Bt cotton (var.NCS-138) and its non transgenic isoline were collected from the harvest of previous pot experiment. Plant samples were dried in sunlight for 10-15 days. Thereafter plant samples were kept in an oven at 50^0C to attain constant weight. Cotton plants separated into leaves and stems, grinded and passed through 2 mm sieve.Crop residues were kept in small bags and were incorporated in the soils of the present pot experiment up to five months and soil samples were obtained periodically. The small bags are made up of two pieces of square nylon netting material (127mm× 127mm) with mesh size 0.79mm were sown together on three side with heavy duty nylon thread.A no crop residues pot was maintained with three replications for all the three soil types. The experimental design was a factorial experiment under completely randomized design with three replications.The treatments were assigned to the experimental pots randomly using a table of random numbers.The cultivated soils of three orders viz; entisol, inceptisol, and alfisol were collected from the previous pot experiment conducted in 2010 in which Bt cotton and non Bt cotton was grown for impact assessment study in a net house of Soil Science and Agricultural Chemistry Department, BHU(**Beura and Rakshit,2011**).

Analysis of Soil

Three different soils were analysed for different physicochemical parameters using standard procedure (**Jackson, 1967**) (Tab 1).Bulk density of soil varied from 1.34-1.53 Mg m^{-3} .The experimental soils were sandy loam to silty clay loam in texture. Among the three soils, black and alluvial soils were slightly alkaline and red soil was acidic in reaction. All the soils hadlow organic matter content., EC varied from0.31-0.64 dSm^{-1} , CEC from 18.25 to 31.85 Cmol $(p+)kg^{-1}$,available N from 173 to 240 Kg ha^{-1}, P from 8 to 20 Kg ha^{-1}, and K from 109 to 244 Kg ha^{-1}.

Table 1 Physico-chemical properties of initial experimental soil

Parameter	Values								
	Red soil			Black soil			Alluvial		
Physical	Bt crop	Non Bt crop	No crop	Bt crop	Non Bt crop	No crop	Bt crop	Non Bt crop	No crop
Bulk density(Mg m^{-3})	1.34	1.33	1.29	1.52	1.53	1.50	1.43	1.45	1.39
Particle density(Mg m^{-3})	2.50	2.51	2.49	2.65	2.63	2.61	2.56	2.57	2.56
Water holding capacity (%)	39.4	40.1	38.9	45.40	46.3	44.9	41.6	40.8	39.9
Sand (%)	46.0	46.0	46.0	11.7	11.7	11.7	48.78	48.78	48.78
Silt (%)	32.8	32.8	32.8	52.7	52.7	52.7	30.48	30.48	30.48
Clay (%)	21.5	21.5	21.5	35.6	35.6	35.6	20.44	20.44	20.44
Soil texture	Silty clay loam	Silty clay loam	Silty clay loam	Clayey	Clayey	Clayey	Sandy loam	Sandy loam	Sandy loam
Electro-chemical and Chemical properties									
pH_w (1 : 2.5)	6.3	6.1	6.4	7.5	7.3	7.4	7.2	7.0	7.2
Electrical conductivity (dSm^{-1})	0.32	0.34	0.31	0.61	0.64	0.61	0.43	0.45	0.44
CEC{Cmol(p+)kg^{-1}}	18.2	19.2	18.0	31.85	32.12	30.92	19.55	20.00	28.98
Organic carbon (%)	0.34	0.39	0.31	0.40	0.42	0.39	0.38	0.40	0.37
AvailableN (kg ha^{-1})	176	178	173	238	240	236	232	239	229
Available P (kg ha^{-1})	9	10	8	14	15	13	18	20	17
Available K(kg ha^{-1})	110	119	109	238	244	235	232	237	230

The decomposition data for each plant part and hybrid were fitted using non-linear regression to the exponential decay model (**Olsen,1963**).

Ln(Xt/Xo)=-kt

Where X_0 is the weight of litter at time 0 ,X_t is the weight of litter at time t , t is the incubation period in month,k is the decomposition constant ($month^{-1}$).
Approximately 4g of air dried plant sample was placed in smallbags. The smallbags were weighted aloneand again withthe plant material. The bags were closed at the top by folding the top 1.3 cm of the bag over and stapled four times. The smallbags were buried to a depth of 10 cm in to the pot on 15March, 2011.Data obtained from all the observation were statistically analysed and least significant 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

A rate constant is an extremely useful quantitative characteristic of a chemical or physical process. Rate constant (k) indicates the rate of decomposition of crop residues and it followed the first order kinetics. The rate constant was significantly higher in non Bt cotton crop residues than Bt cotton crop residue treatments due to difference in total C, total N, biomass fractions (soluble, hemicelluloses, cellulose, lignin) and carbon nitrogen ratio, between Bt and non Bt crop residues (Tab 2). There were differences in C:N ratios between initial Bt and non-Bt cotton residues; however, these differences did not result in differences in their rates of decomposition or mass of C remaining over time. These data suggest that the Bt and non-Bt cotton hybrids used in this study should not cause differences in carbon sequestration when their residues decompose under similar environmental conditions**. Saxena *et al*. (1999) and Flores et al.(2005)**found that Bt hybrids decomposed slower than non Bt isoline when assessed using carbon di-oxide evolution techniques in laboratory studies. **Saxena and Stotzky (2001)** found that lignin content of the Bt hybrids was 33-97% higher than for the non Bt hybrids. In another study by **Flores *et al*. (2005)**, a mixture of Bt maize leaves and stems had a 96% higher lignin content than near- isogenic non Bt hybrid. Initially the rate of decomposition of Bt protein was very fast within few hours, afterward it binds with clay particles and becomes resistant to be decomposed by microorganisms. The rate of decomposition was fast by 50 days after incorporation (DAI), thereafter it was slowed down and the rate of decomposition was almost constant in the next two observations (100 DAI and 150 DAI)for both Bt and non BT crop residues(Fig.1).
The rate of decomposition is influenced by many factors. Because decomposition is a biological process carried out primarily by bacteria and fungi, its speed will be affected by temperature and soil moisture. Generally decomposition increases exponentially with temperature. In the initial stages (0 to 3 months) of residues breakdown, small soluble carbon molecules, like starches and amino acids, are lost first leaving behind the more recalcitrant molecules like lignin (**Tarkalson et al., 2008**). Decomposition during this first phase is rapid because these molecules are easy to break down and are energy rich. The second stage of decomposition - the breakdown of lignin - is much slower because lignin consists of very large and complex molecules.

Table 2 Rate constant (10^{-2}per month) of residues at different days after incorporation (DAI)

DAI	Cultivar (C)	Soil types(S)			Mean
		S1	S2	S3	
50	Non-Bt (V_1)	6	18	24	18
	Bt (V_2)	5	8	5	14
	Mean	5.5	13	14.5	
	LSD(0.01) C =0.12,S =0.12,C×S =0.20				
	SEm± C =0.04,S=0.04,C×S=0.07				
100	Non-Bt (V_1)	4	5	6	7.5
	Bt (V_2)	2	3	4	3.5
	Mean	3	4	5	
	LSD(0.01) C =0.01,S=0.01,C×S=0.01				
	SEm± C=0.003, S=0.003, C×S=0.004				
150	Non-Bt (V_1)	4	4	4	6
	Bt (V_2)	2	2	4	4
	Mean	3	3	4	
	LSD(0.01) C =0.01, S=0.01,C×S=0.01				
	SEm± C= 0.003,S=0.003,C×S=0.004				

Legend:S_1=Red soil, S_2= Black soil, S_3=Alluvial soil, V_1=Non-Bt cultivar, V_2= Bt cultivar

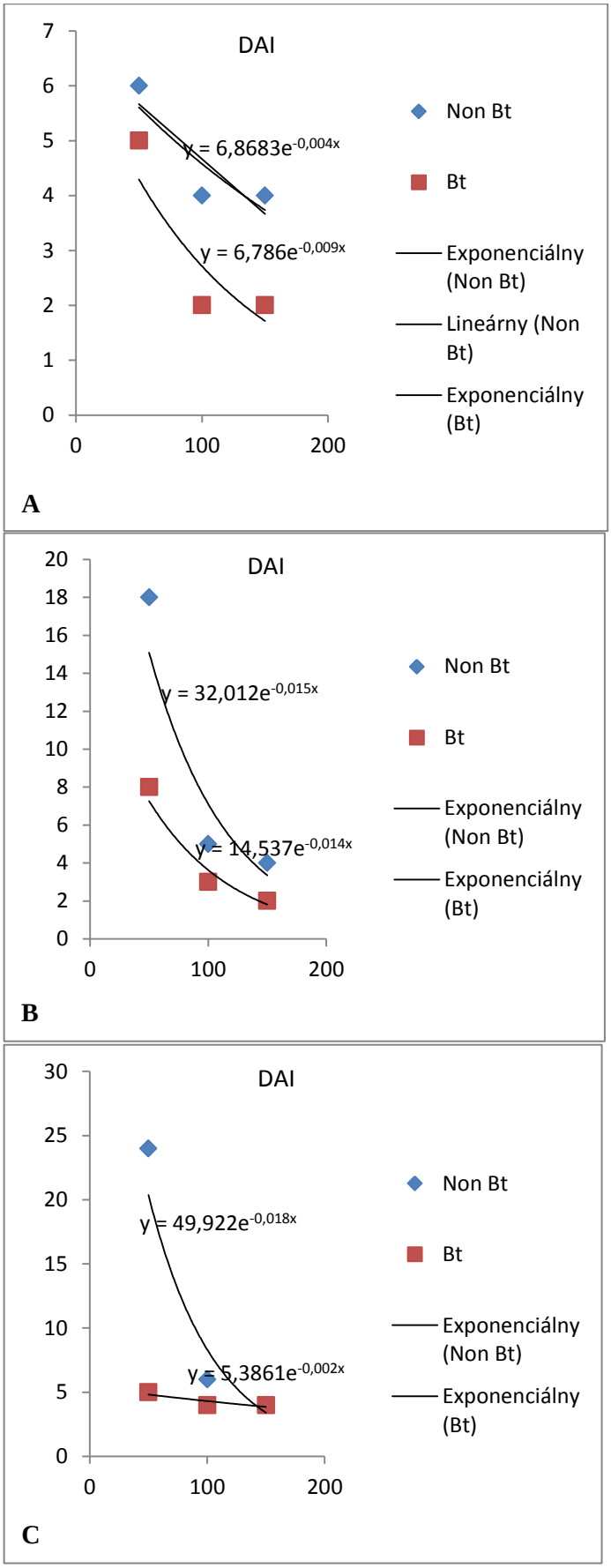

Figure 1 Rate of decomposition of Bt and non Bt crop residues under varied soil types (A – Red soil, B – black soil, C – Alluvial soil).

Koskella and Stotzky (1997) stated that differences in pH could influence the conformation of the Cry proteins. The C and N termini in the peptide bonds of the Cry proteins could be altered. This leads to changes in the relative susceptibility to cleavage by proteases. By increasing the pH of the soil, the Cry proteins can be more easily cleaved by proteases. The biodegradation would therefore be enhanced when soil pH is higher. **Tapp and Stotzky (1998)** observed in their experiments that pH of the soil was the only physicochemical characteristics that influenced the retention of the Cry proteins in soil The microbial culture was as well affected by the pH. Microbes showed the highest efficiency at pH 7 in soils. In the present experiment a lower degradation of the Cry proteins could therefore be explained by a low pH. Soils with low pH (red soil) showed no decrease in insecticidal activity, whereas soils with high pH (alluvial and black soil) showed a significant decrease in insecticidal activity. The potential hazard from Cry proteins in soil to non target organisms is increased at low pH as the microbes show less activity and only low amounts of toxins are degraded. The soil pH is therefore an important parameter for Cry protein degradation in soil environments.Among the three different soils,the rate of decomposition was the lowest in red soil followed by black and alluvial soil due to a higher rate of absorption of Bt protein.

CONCLUSION

The rate of decomposition was higher in non Bt than Bt crop residues.Generally, Bt cotton residue with high lignin content and higher C:N ratio decomposed at a lower rate when incorporated in the soil. Distinctive variations in the rate constant were evident amongst the soils selected for study. The highest rate of decomposition was found in alluvial soil compared to black and red soils in 50 DAI. Thereafter rate of decomposition was slow in 100 DAI and the constant rate of decomposition was found in 150 DAI. As Bt crop residues were to decompose more slowly, changes could also occur in carbon cycling and nutrient availability.

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