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RESEARCH ARTICLE

SOIL BIOLOGICAL INDICATORS AS A KEY TO ASSESS SOIL HEALTH UNDER TRANSGENIC COTTON GROWING RHIZOSPHERIC SOILS OF PERAMBALUR DISTRICT

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ABSTRACT

To assess the soil health under transgenic Bt cotton grown soils (RCH-2 Bt, Bunny Bt and NHH 44 Bt) expressing cry1Ac gene were evaluated for their effects on soil microbial population, Microbial Biomass carbon (MBC), Microbial Biomass Nitrogen (MBN), soil respiration, and Dehydrogenase (DHA) activity to ascertain for rhizosphere soil quality under field conditions. The soil biological indicators viz.,microbial population, DHA, soil respiration, MBC and MBN were higher in Bt cotton grown soils when compared to non Bt cotton grown soils indicating no adverse effects of Bt toxin on rhizosphere soil microbial activity. These results suggest that cultivation of Bt cotton expressing cry1Ac gene may not pose ecological or environmental risk.

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INTRODUCTION

Identification of biological indicators of soil quality is reported as critically important by several authors (Doran and Parkin, 1994; Abawi and Widmer, 2000) because soil quality is strongly influenced by microbiological mediated processes such as nutrient cycling, nutrient capacity and aggregate stability. Soil health is defined as the continued capacity of soil to function as a vital living system, by recognizing that it contains biological elements that are key to ecosystem function within land-use boundaries (Karlen *et al.,* 2001). Genetically modified cotton genotypes incorporating a crystal (Cry) toxin producing cry1Ac gene derived from Bacillus thuringiensis (Bt), were introduced in India for commercial cultivation in the year 2002 (Morse *et al*. 2005). The transgenic crop, now popularly called Bt cotton, represents about 90% of cotton cultivated area in India. In India no comprehensive field study is available on the effects of growing transgenic cotton on soil biology. In general, few or no toxic effects of Cry proteins on woodlice, collembolans, mites, earthworms, nematodes, protozoa, and the activity of various enzymes in soil have been reported.

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Although some effects, ranging from no effect to minor and significant effects, of Bt plants on microbial communities in soil have been reported, using both culturing and molecular techniques. They were mostly the result of differences in geography, temperature, plant variety, and soil type and, in general, were transient and not related to the presence of the Cry proteins. Bt cotton (events MON531, MON757, and MON1076) expresses the Cry1Ac protein at about 1.56, 12.6, and 12.2 mg g^{-1} of fresh leaf tissue, respectively, and at about 0.86, 9.9, and 12.7 mg g^{-1} of fresh seed tissue, respectively (AGBIOS, 2005). Thus, the Bt-toxin has the potential to enter the soil system throughout the Bt-cotton-growing season, through root release and root turnover processes (Motavalli *et al*. 2004). While Bt occurs naturally in soil, growth of transgenic Bt-crop causes a large increase in the amount of Cry endotoxin present in agricultural systems, e.g. roughly 0.25 g ha^{-1} produced naturally (calculated from approximately 1000 Bacillus thuringiensis spores g^{-1} soil (Blackwood and Buyer 2004). Thus the transgenic plants, either through the products of introduced genes and modified rhizosphere chemistry or through altered crop residue quality, have the potential to significantly change the essential ecosystem functions such as nutrient mineralization, carbon turnover and plant growth (Callaghan *et al*. 2005). With this preamble, the investigation was taken

- To assess the safety of Bt cotton expressing *cry1Ac* on soil microbial communities / microbial respiration.
- To assess the effect of the Bt cotton plants expressing the *cry1C* gene on the soil Microbial Biomass carbon (MBC), Microbial Bio mass Nitrogen (MBN) and Dehydrogenase activity

MATERIALS AND METHODS

The rhizosphere soil samples from Bt and non Bt cotton growing areas of different taluks of Perambalur district were collected where Bt cotton had been planted at least for the previous 3-5 consecutive years, which was compared with the adjoining fields where non-Bt cotton was growing during that period. In order to collect rhizosphere soil samples, Bt cotton plants were vigorously shaken by hand for 10 min, paying attention to the roots integrity. The actual limit for shaking and thus for sampling for this soil fraction was considered as reached when roots non-adhering soil particles were completely removed. Continue shaking for another 10 min in one litre of a sterile 0.9 % NaCl solution to remove the adhering rhizosphere soil (Angle *et al*., 1996). The rhizosphere soil samples were collected 10 days before harvest and transported to lab in polythene bags and stored in 5º C for biological analysis. The rhizosphere soil samples were subjected for soil biological properties viz., Microbial Biomass carbon, Microbial Biomass Nitrogen, soil respiration, Dehydrogenase activity and soil microbial population to ascertain for soil quality.

Soil Biological analyses

a. **Soil respiration** was measured as the CO_2 evolved from moist soil by alkali trap method.
b. **Dehydrogenase activity** was determined by following the method of Casida etal.,1964, by the colorimetric measurement of reduction of 2,3,5 triphenyl tetrazolium chloride (TTC)
c. **Microbial Biomass Carbon (MBC)and Microbial Bio mass Nitrogen (MBN)** were measured by using chloroform-fumigation extraction method as reported by Vance etal.,1987,
   MBC = 2.64 EC (where EC is the difference between organic C extracted by K_2SO_4 in fumigated soil and organic C extracted by K_2SO_4 in non fumigated soil.
d. **Soil Microbial population analysis** was done by following serial dilution method**.**

RESULTS AND DISCUSSION

Impact of Bt cotton on soil microbial population (Table.1)

The bacterial, fungal and actinomycetes population was significantly higher in Bt cotton grown soil compared with non-Bt soil. The increase in microbial population indicates no adverse effects of growing Bt cotton on soil microbial activity. The differences in the microbial population between Bt and non-Bt cotton may be attributed to variations in root exudates quantity, composition and root characteristics bring about by the genetic makeup of the cotton rather than expression of cry gene.

Perambalur District Map

These results were well supported by Yan *et al*. 2007 who have shown that the qualitative and quantitative differences in root exudation of Bt cotton could strongly influence the structure of microbial communities in the rhizosphere. Higher microbial populations in transgenic cotton grown soil were also reported by several workers (Shen *et al*. 2006, Kapur *et al*. 2010). Hu *et al*. (2009) that transgenic Bt cotton was not found to affect the rhizosphere functional bacterial population.

Table.1 Effect of Bt and non Bt cotton on soil microbial population in Perambalur district (Mean values of ten villages in each taluks)

SI.No	Taluks	General microflora in Bt cotton grown soils (CFU /g)			General microflora in non Bt cotton grown soils (CFU /g)		
		Bacteria x 10^6	Fungi x 10^3	Actinomycetes x 10^3	Bacteria x 10^6	Fungi x 10^3	Actinomycetes x 10^3
1.	Veppanthattai	42	15.0	4.8	29	14.7	3.8
2.	Perambalur	58	14.3	4.0	33	13.8	2.8
3.	Alathur	30	14.8	5.2	25	12.0	2.9
4.	Veppur	35	16.5	5.7	25	14.3	3.1
	Range values	30-58	14.3-16.5	4.0-5.7	25-33	12.0-14.7	2.8-3.8
	SD	8.034	1.491	0.56	4.877	1.913	0.814

Table.2 Effect of Bt and non Bt cotton on soil microbial respiration and Dehydrogenase activity in soils of Perambalur district (Mean values of ten villages in each taluks)

S.No.	Taluks	Bt cotton grown soils		Non Bt cotton grown soils	
		DHA (µg TPF/ g / h	Soil respiration µg of CO_2/ g / h	DHA (µg TPF/ g / h	Soil respiration µg of CO_2/ g / h
1.	Veppanthattai	0.2137	224	0.071	164
2.	Perambalur	0.2281	264	0.068	181
3.	Alathur	0.1983	308	0.075	202
4.	Veppur	0.1739	286	0.079	201
	Range values	0.174 -0.228	224-308	0.068-0.079	168-202
	SD	0.024	26.464	0.006	16.494

Table.3 Effect of Bt and non Bt cotton on soil Microbial Bio mass Carbon (MBC) and Microbial Bio mass Nitrogen (MBN) in soils of Perambalur district (Mean values of ten villages in each taluks)

S.No.	Taluks	Bt cotton grown soils		Non Bt cotton grown soils	
		MBC (µg /g)	MBN (%)	MBC (µg /g)	MBN (%)
1.	Veppanthattai	191	1.481	170	0.0813
2.	Perambalur	185	0.784	165	0.0732
3.	Alathur	175	0.427	162	0.0835
4.	Veppur	181	0.691	169	0.0918
	Range values	175-191	0.43-1.48	162-170	0.073-0.092
	SD	4.671	0.310	3.273	0.007

Impact of Bt cotton on soil DHA level and soil respiration (Table.2)

Soil enzymes were suggested as one of the potential biological indicators of soil quality because of their relationship to soil biology, ease of measurement, and rapid response to changes in soil management. Soil DHA reflects the total range of oxidative activity of soil microflora and, consequently it may be a good indicator of microbiological activity in the soil. Bt cotton grown soil showed significantly higher DHA as compared to non-Bt cotton grown soil. The DHA level in Bt cotton grown soil ranged from 0.174 -0.228 µg TPF/g/ h and in non Bt soil it was between 0.068-0.079 µg TPF / g/ h. The higher DHA in Bt cotton grown soil is mainly attributed to the higher microbial activity stimulated by higher root density in Bt cotton compare with non-Bt cotton. DHA is considered as an indicator of the oxidative metabolism in soils and thus of the microbiological activity (Garcia *et al*. 1997) because it is linked to viable cells. Studies by Furczak and Joniec (2007) showed that stimulation of DHA was accompanied by an increase in the number of the microbial groups and improvement in other living conditions. The increased soil respiration rate with Bt cotton soils in all the taluks of Perambalur district was in the range of 224 -308 µg of CO_2/ g / h when compared to non Bt soil in our study is the outcome of higher root volume in Bt cotton compare to non-Bt cotton that have stimulated the microbial growth and activity by enhanced resource availability as reported by Velmourougane and Sahu, 2013. Among the different taluks, Perambalur taluk registered highest DHA level of 0.2281 µg TPF/g/ h, whereas Alathur taluk registered highest soil respiration rate of 308 µg of CO_2/ g / h in Bt cotton grown soils.

Impact of Bt cotton on soil MBC and soil MBN (Table.3)

Microbial population and biomass is a small but very dynamic and essential component of nutrient cycling in soil. Soil under Bt cotton hybrids had higher MBC range values of 175 to191 µg/g and MBN values of 0.43 -1.48 % compared with the non Bt MBC values (162 -170 µg/g) and MBN values 0.073 to 0.092 %. The increased MBC and MBN values in Bt cotton grown soil is attributed to higher root volume compared with non-Bt cotton. Possibly readily metabolisable carbon and nutrient availability at Bt cotton rhizosphere and differences in root exudates are perhaps the most influential factors contributing to increased microbial colonization and

subsequent higher MBC and MBN values in soils under Bt cotton. Earlier, Sarkar *et al*. (2009) reported a significant correlation between root volume of Bt cotton and soil MBC and MBN that supported the findings of Jagadish *et al* (2012) that soil MBC and MBN increased with root growth and rooting density of the crop.

Conclusion

The adoption of genetically modified (GM) crops has increased dramatically in the last 15years. However, the introduction of GM plants into agricultural ecosystems has raised a number of questions, including the ecological impact of these plants on soil ecosystems. The soil biological indicators like microbial population, DHA, soil respiration, MBC and MBN were higher in Bt cotton grown soils when compared to non Bt soils. In conclusion, this study has demonstrated that cultivation of transgenic Bt cotton expressing cry1Ac gene had no adverse effects on soil biological activities such as soil respiration, dehydrogenase, microbial biomass carbon, microbial bio mass nitrogen and soil microbial population. Based on the overall observations, growing Bt cotton was found to have a positive impact on soil biological activities. Temporal variations observed between Bt and non-Bt cotton were attributable to differences in genetic makeup of the cotton hybrids rather than gene expression. Our results suggest that cultivation of Bt cotton expressing cry1Ac gene may not pose ecological or environmental risk.

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