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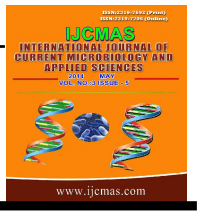

Original Research Article

Influence of Transgenic *Bt* Crop Root Exudates on Rhizospheric Soil Microflora

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A B S T R A C T

In soil root exudates govern which organisms reside in the rhizosphere . Therefore any change to the quality of crop residues and rhizosphere inputs could modify the dynamics of the composition and activity of organisms in soil .Insect resistant *Bt* crops have the potential to change the microbial dynamics, biodiversity and essential ecosystem functions in soil, because they usually produce insecticidal 'cry' proteins through all parts in plants. Bacteria and Yeast populations were increased in rhizospheric soils and *Actinomycete* and fungal populations were reduced. Similarly, in case of beneficial microorganisms *Rhizobium*, *Azospirillum* and Phosphate solubilizers were reduced in Bt cotton soils compared to Non Bt cotton soils but, *Azatobacter* populations were increased three times in Bt cotton soils compared to non Bt cotton soils. However, *Cellulomonas* populations were unaffected.

Keywords

Bt cotton, *cry* protein, *Cellulomonas, Rhizobium, Azospirillum, Azatobacter*

Introduction

Soil microbial diversity is an important index of agricultural productivity (Nimisha Sharma et al,.2010; Kent and Triplett, 2002; Isik, 2008). Both plant and the soil types influences the microbial diversity. Rhizosphere microbial diversity usually influenced by both plant and soil types. Plants and microorganisms interact with each other and co evaluate together. Their balance is important for sustainable biotechnology especially genetic engineering have revolutionized crop improvement and increased the availability of valuable new traits. The adoption of genetically modified crops has dramatically increased in the last 11 years (Gupta and yeates,1997). Insect resistant Bt crops have the potential to change the microbial dynamics, biodiversity and essential ecosystem functions in soil

because they produce insecticidal 'cry' proteins through all parts of the plant. Soil microorganisms are involved in numerous important processes like decomposition of organic matter, nutrient mineralization, Regulation of plant pathogens, decomposition of agricultural chemicals and improvement of soil structure (Bruinsma *et al*., 2003; Bondgett *et al*., 1999). It is widely acknowledged that root exudates govern which organisms should reside in the rhizosphere. Therefore, any change in the quality and quantity of root exudates could potentially modify the biodiversity of soil microbiota and may cause changes in both harmful and beneficial microorganisms. The present study discusses on the effects of Bt crops on soil microbial diversity (Rui, 2005; Schenick, 1976).

Materials and Methods

Sample collection

The soil samples from rhizospheric area (up to 20cm from the plant to a dimensions of 15cm height X 7cm diameter) of transgenic Bt cotton plant and non transgenic Bt cotton plant were selected . Five such samples were collected from each field and pooled together into a single sterilized polythene bag . The samples were immediately taken into laboratory and subjected to microbial analysis.

Plating and enumeration of viable Microbial populations

One gram of each soil sample was weighed and suspended in 100ml of sterile water separately. The above samples were serially diluted with sterile water up to 10^6 dilutions by transferring 1ml from the 100ml sample to test tubes containing 9ml of sterile water. The viable number of bacterial counts from Rhizospheric samples of *Bt* and non *Bt* were enumerated using pour plate method. The population of soil bacteria was estimated on soil extract agar (Bernt and Rovira, 1955). Similarly soil fungi was estimated on Martin Rose Bengal Streptomycin sulphate agar medium (Martin, 1950). For counting of Actinomycete population Kusters Agar medium (Balagurunathan and Subramanyan,1992) and for yeast population counting Yeast extract Agar medium (Windle Taylor, 1958) were employed. 1ml aliquots from the appropriate dilutions were pipetted out into sterile petridishes. A 20ml of respected medium was aseptically poured into petriplates and the petriplates were rotated in clock wise and anti clock wise direction and allowed to solidify. For each dilution three replicates were maintained. The inoculated plates were incubated at 37^0C for 24 to 72hr. The microbial colonies were enumerated using colony counter after incubation period. The petri dishes which contained countable colonies were selected for enumeration and expressed as number of colony forming units per gram or ml (CFU/ gram or ml) of the sample analysed.

Estimation Nitrogen fixing Microbial Population

Beneficial microorganisms such as *Rhizobium, Azatobacter* and *Azospirillum* were enumerate using specific media. For enumeration of Rhizobial populations Yeast Extract Mannitol Agar (YEMA) medium (Vincent, 1970) was employed. White, Gummy colonies on the YEMA medium were enumerated. They were confirmed by Gram staining method of Huckers modification . *Azatobacterial*

populations were counted on Ashby's medium .Yellow, gummy colonies were selected and enumerated later confirmed by Grams staining. Rojo Congo medium was employed for enumeration of *Azospirillum*.

Estimation of phosphate solubilizers

Phosphate solubilizers were isolated and enumerated on Pickovskaya's agar medium(Pikovskaya,1948). On Pikovskaya medium phosphate solubulizers formed a clear zone around the developed colonies.

Estimation of organic matter degrading bacteria

Organic matter degrading bacteria were isolated on Hans medium (Glucose-5g/l; $(NH_4)_2SO_4$- 3gm/l; K_2HPO_4 -1.5g/l; $NaH_2PO_4.H_2O$ -0.5g/l; MgCl2-0.1g/l;MgCl2.$2H_2O$-0.1g/l; Thiamine Hcl - $5x10^{-3}$g/l) and were enumerated.

Results and Discussion

Micro organisms are the dominant organisms, both interms of biomass and activity in soil and they are involved in numerous important processes, including decomposition of organic matter, nutrient mineralization, regulation of plant pathogens and decomposition of organic matter, nutrient mineralization, regulation of plant pathogens and decomposition of agricultural chemicals and improvement of soil structure (Breinsma *et al*, 2003). The total heterotrophic aerobic bacterial, fungal, actinomycetes and yeast populations enumerated was present in Table.1. In Bt rhizospheric soils among the four tested microbial populations viz., Bacteria, fungi, actinomycetes and Yeasts, Yeast populations ($48X10^4$) were high followed by bacterial populations. In contrast, non Bt cotton soils exhibited more bacteria (41×10^4), and actinomycetes (18×10^3). The fungal and yeast populations were high in Bt rhizospheric soils. Similarly, significant differences were also observed in the composition of the microbiota in Australian soils associated with residues of Bt cotton and non Bt cotton.

The per cent distribution of microbial populations in Bt and Non Bt cotton soils were presented in Fig-1a &b. Beneficial soil microbiota was also assessed in Bt and non Bt rhizospheric soils. These include symbiotic (*Rhizobium*) and Asymbiotic Nitrogen Fixing Bacteria (*Azospirillum* and *Azatobacter*) phosphate solubilizing bacteria and celllulase degrading *Cellulomonas*. In Bt Rhizoospheric spoils Rhizobial, Azospirillum and phosphate solubilizing bacterial populations were reduced, compared to non Bt Rhizospheric soils. *Azatobacter* population were increased almost four times compared to non Bt soils. However, *Cellulomonas* populations were un affected in both the soils (Table-2). The beneficial bacterial populations in Bt rhizospheric soils composed of *Rhizobium* (83.1 %) *Azospirillum* (0.17 %) *Azotobacter* (3.32 %) Phosphate solubilizing bacteria (4.15%) and cellulose degrading bacteria (3.3 %).

The bacterial genera of the non Bt Rhizospheric samples composed of 92.2 % *Rhizobium*, 0.23% of *Azospirillum*, 1.67 % *Azatobacter,* 3.6 % of phosphate solubilizing bacteria and 2.3% cellulose degrading bacteria. Soil microorganisms play an important role in soil process that determines plant productivity. Soil ecosystem is not only the reservoir pool of exotic genes and their expression products of Bt transgenic

Table.1 Aerobic, Heterotrophic Microbial population density of Rhizosphere soils

S. No	Sample	Microbial population Density (Cfu / gm soil)			
		Bacteria	Fungi	Actinomycetes	Yeasts
1.	Bt Rhizosphere soil	$27(\pm3)\times10^4$	29×10^4	27×10^2	48×10^4
2.	Non Transgenic Rhizosphere soil	41×10^4	18×10^4	32×10^2	18×10^4

Table.2 Beneficial Microbial population Density in Rhizosphere soils of Bt and Non Bt cotton

S. No	Types of organisms	Bt Rhizosphere (Cfu/gm soil)	Non Bt Rhizosphere (Cfu/gm soil)
1	Symbiotic Nitrogen fixing bacteria	7×10^6	11×10^6
2	Asymbiotic nitrogen fixing bacteria *Azospirillum* *Azatobacter*	 15×10^3 78×10^4	 28×10^3 20×10^4
3	Phosphate Solubilizing bacteria	35×10^4	43×10^4
4	Cellulase degrading bacteria	28×10^4	27×10^4

Figure.1A Distribution of Microbial populations in Bt cotton soils

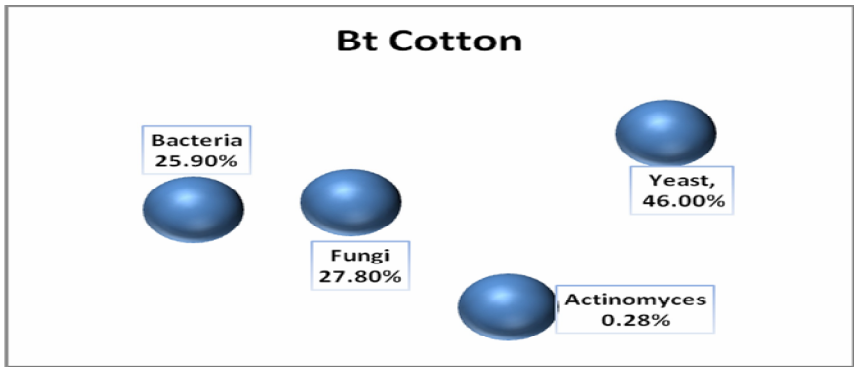


Figure.1B Distribution of Microbial populations in Non Bt cotton soils

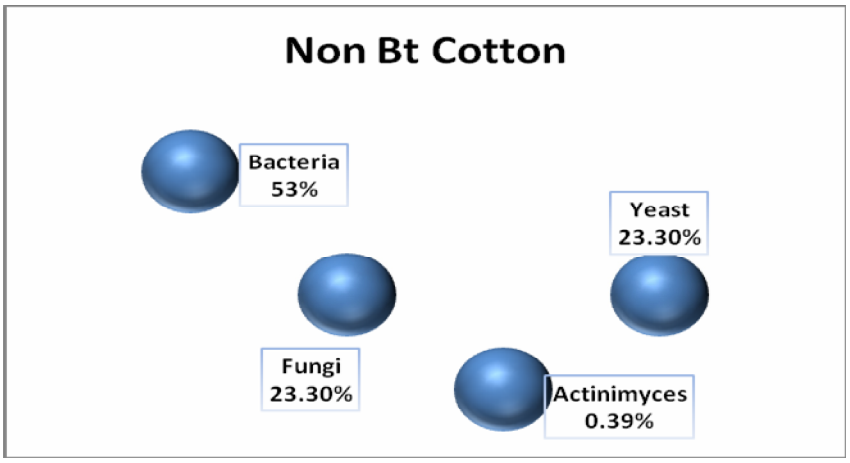


Fig.2A Distribution of beneficial microbial population in Bt Cotton soils

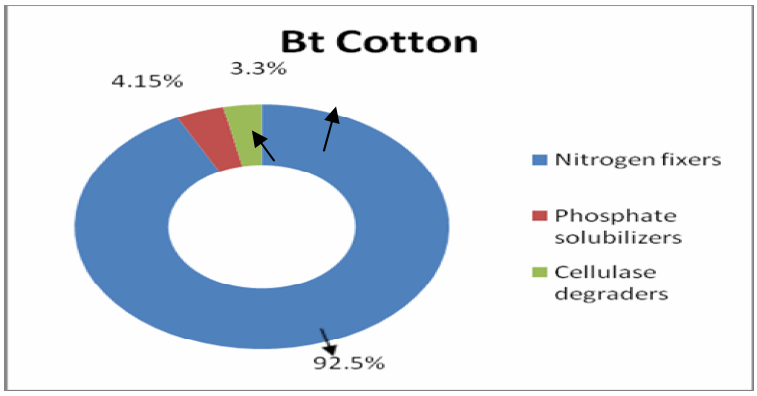
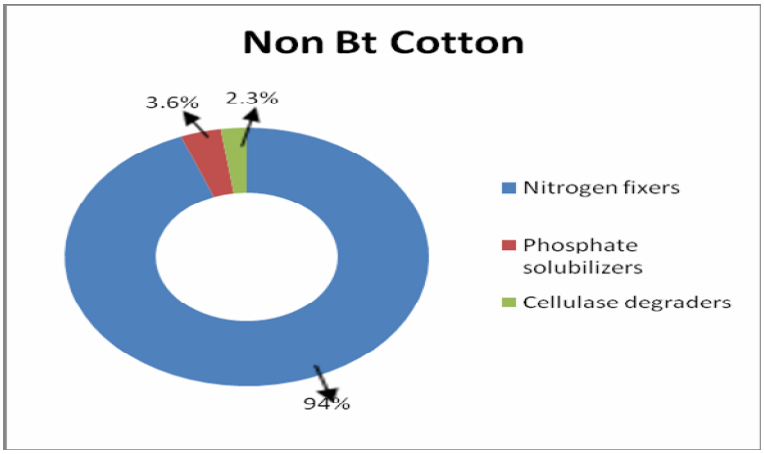


Fig.2B Distribution of beneficial microbial population in Non Bt Cotton soils



crops but also the centre of biosphere and terminal habitat of microorganisms. Usually the Bt gene expressing products of transgenic Bt crops and Bt toxins could be introduced into soil through root exudation or decomposition of the crop residues (Saxena and Stotzky, 2000). Once in the soil the toxin could be observed or bound on clay particles, humic compounds or organic mineral complexes and then be protected against degradation by soil microorganisms (Rui *et al*, 2005,) and the soil biochemical properties (Sun *et al*, 2007). Due to the expression of Bt insecticidal protein, unintentional modifications of agronomic traits changed for Bt transgenic crops compared with non Bt counterparts. Bt transgenic crops can affect soil ecosystem through two pathways, *i.e.*, biomass incorporation and root exudates. In our study some microbial populations are affected and some are unaffected. Shen *et al* (2006) showed that the richness of the microbial communities in rhizosphere soil did not differ between Bt and the non Bt cotton. Even the functional diversity of Microbial communities was not different in rhizosphere soils between Bt and non Bt cotton.

In contrast in our study, some soil specific microbial populations were affected by Bt cotton and some specific microbial populations were unaffected. A decrease in specific microbial population could lead

to a decrease in decomposition process after the level and composition of soil organic matter and have secondary effects on the survival of plant pathogens. Similarly loss of particular trophic groups of meso fauna could cause a loss of specific pathways within nutrient cycling process. Thus affecting important bio-geochemical pathways. Hence, this study strongly recommends the necessity for monitoring the Bt cotton cultivation to ensure the safety of the terrestrial biodiversity and the ecosystem at large.

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