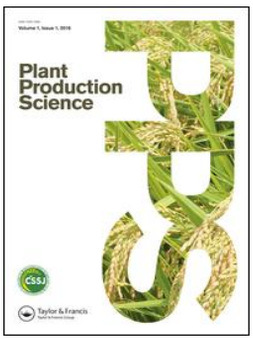

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Impact of *Bt*-cotton on soil microbiological and biochemical attributes

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ABSTRACT

Transgenic *Bt*-cotton produces *Bt*-toxins (*Cry* proteins) which may accumulate and persist in soil due to their binding ability on soil components. In the present study, the potential impacts of *Bt*- and non-*Bt* genotypes of cotton on soil microbial activity, substrate use efficiency, viable microbial population counts, and nutrient dynamics were studied. Two transgenic *Bt*-cotton genotypes (CIM-602 CIM-599) expressing *cry1 Ac* gene and two non-*Bt* cotton genotypes (CIM-573 and CIM-591) were used to evaluate their impact on biological and chemical properties of soil across the four locations in Punjab. Field trials were conducted at four locations (Central Cotton Research Institute-Multan, Naseer Pur, Kot Lal Shah, and Cotton Research Station-Bahawalpur) of different agro-ecological zones of Punjab. Rhizosphere soil samples were collected by following standard procedure from these selected locations. Results reveled that *Bt*-cotton had no adverse effect on microbial population (viable counts) and enzymatic activity of rhizosphere soil. Bacterial population was more in *Bt*-cotton rhizosphere than that of non-*Bt* cotton rhizosphere at all locations. Phosphatase, dehydrogenase, and oxidative metabolism of rhizosphere soil were more in *Bt*-cotton genotypes compared with non-*Bt* cotton genotypes. Cation exchange capacity, total nitrogen, extractable phosphorous, extractable potassium, active carbon, Fe and Zn contents were higher in rhizosphere of *Bt*-cotton genotypes compared with non-*Bt* cotton genotypes. It can be concluded from present study that the cultivation of *Bt*-cotton expressing *cry1 Ac* had apparently no negative effect on metabolic, microbiological activities, and nutrient dynamics of soils. Further work is needed to investigate the potential impacts of *Bt*-cotton on ecology of soil-dwelling insects and invertebrates before its recommendation for extensive cultivation.

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Bt-cotton; *Bt*-toxin; ecology; phosphatase; dehydrogenase; rhizosphere

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Introduction

Cotton (*Gossypium hirsutum* L.) is an important fiber crop bearing different biotic and abiotic stresses. Severe attack of sucking and chewing pests on crop leads to intensive use of pesticides (Benedict & Altman, 2001; James, 2002) that ultimately cause several health and environmental issues. Different strategies are being adopted to reduce the heavy reliance on pesticides, among them development of transgenic crops executes as the promising technology for this. Due to promising effect of transgenic technology on pest suppression, the cultivated area under *Bt* cotton in Pakistan has been increased to more than 90% in last few years (Sabir et al., 2011). Instead of benefits offered by transgenic plants, the cultivation of *Bt* corn engineered with 176 events may also have sublethal effects on bio-diversity and non-target organisms (Zangerl et al., 2001). The soil micro-organisms are tightly related to status of the soil ecosystem, and considered as sensitive indicators reflecting the changes in rhizosphere (Hartmann et al., 2014). Root exudates composition depends on cultivar, plant species, and physiological status of the plant (Saxena et al., 2002). The diverse microbial communities sustain their growth in close proximity to the plant roots using decomposed organic matter and root exudates. Plant genetic transformation can alter rhizosphere chemistry (Gasson, 2000; Kowalchuk et al., 2003; Lynch et al., 2004) that can also cause distinct changes in root exudates and root structural properties (Velmourougane & Sahu, 2013).

Commercial cultivation of *Bt*-cotton and their remains after harvest may lead to addition and persistence of Cry proteins in rhizosphere (Stotzky, 2004). Karuri et al. (2013) reported that *Cry1Ac* protein from *Bt*-cotton was present in soil up to 30 days from the first detection at 150DAS (days after sowing). *Bt*-toxins remain protected from decomposition by soil micro-organism when adsorbed on clay particles, humic components, and organic mineral complexes (Tapp et al., 1995). The *Cry* proteins are produced in plants by expression of *Cry* gene persisting in the soil (Muchaonyerwa et al., 2004) and *Cry1Ac* protein in *Bt* cotton has been shown to remain in soil up to 140 days

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(Palm et al., 1996). The variability in degradation times for *Cry* protein could be caused by different types of crop plants, type of *Cry* proteins, and ecological factors (temperature, nutrients, soil pH, types, and amount of clay minerals and organic matter in soil) (Stotzky, 2004).

The exudates of *Bt*-cotton influence the rhizospheric microflora and enzymatic activities occurring in rhizosphere (Singh et al., 2013). These changes may be transient depending upon soil type, crop stage, as well as environmental conditions (Velmourougane & Sahu, 2013). In Pakistan, previously no study has been conducted with primary objective to investigate the effects of growing *Bt*-cotton on soil microbiological and chemical attributes. Possible risks, if any, attached with growing *Bt*-cotton demand a comprehensive study to find out the microbiological or chemical changes in rhizosphere of *Bt*-cotton. Keeping in view the above facts that genetic makeup and varietal difference may contribute to modify the rhizosphere microbial community and different biochemical transformations, the present study was conducted to evaluate the potential role of two local *Bt*-cotton genotypes and counterpart (two non-*Bt* cotton genotypes) on selected microbiological and chemical attributes at different locations of Punjab, Pakistan.

Materials and methods

Experimental site and sampling

This field experiment was conducted in the kharif season of the year 2013. Two well-known *Bt*-cotton varieties (CIM-602 and CIM-599) Bollgard-I and non-*Bt* varieties (CIM-591 and CIM-573), were evaluated with a randomized complete block design in triplicates at four different locations in cotton belt districts of Punjab (Central Cotton Research Institute-Multan (30°12′N, 71°28′E, alt. 123 m), Mouza Naseer Pur-Shujababad (29°53′N, 71°18′E, alt. 152 m), Kot Lal Shah-Lodhran (29°32′N, 71°38′E, alt. 111 m), and Cotton Research Station-Bahawalpur (29°24′N, 71°41′E, alt. 252 m). Four districts were selected to eliminate the transient effects of physicochemical properties of soil and environmental conditions. Soil fertility status was analyzed and recommended doses of fertilizer were applied i.e. N, P_2O_5 and K_2O 161, 58 and 50 kg/acre respectively. Soil samples were collected from the rhizosphere of the *Bt* and non-*Bt* cotton at peak flowering stage (70 DAS). Bed furrow planting technique was adopted with planting geometry (P × P ′1, R × R ′2.5) and plot size 30 × 10 ft. The representative 2–3 cotton plants were carefully uprooted from the each plot and soil adhering to the roots was separated and composite sample stored in sealable plastic bags. The samples were passed through 2 mm sieve and processed in the laboratory for the determination of microbiological and biochemical attributes and stored at 4 °C for 7 days before analysis. However, soil samples were quickly processed for enzymatic analysis within 3–4 days of collection (Mina & Chaudhary, 2012).

Soil microbiological analysis

Bacteria were isolated from rhizosphere samples by dilution plate technique. Glucose peptone agar medium (GPAM) was used as the growth medium for bacteria (Wollum, 1982). Inoculated plates were incubated at 28 ± 1 °C for 72 h. Colony forming units (CFU) per gram of soil were calculated (Mafham et al., 2002). Microbial respiration of rhizospheric soil was measured as *in vitro* static CO_2 evolution. The CO_2 evolution was measured by acid–base titration and expressed as mg CO_2–C kg^{-1} d^{-1} (Stotzky, 1965).

Phosphatase activity (alkaline) in the rhizosphere was evaluated as proposed by Tabatabai and Bremner (1969). Alkaline phosphatase catalyzes the hydrolysis of *p*-Nitrophenyl phosphate (*p*NPP) to *p*-Nitrophenol. *p*NPP is colorless but *p*-Nitrophenol has a strong absorbance at 405 nm. The rate of increased absorbance at 405 nm is proportional to the enzyme activity. Dehydrogenase activity was determined as described by Min et al. (2001). For this purpose, a sample of 5 g field-moist soil (collected from rhizosphere) was incubated for 12 h at 37 °C in 5 mL triphenyl tetrazolium chloride (TTC) solution (5 g TTC in 0.2 M Tris–HCl buffer, pH 7.4). Two drops of concentrated sulfuric acid were added immediately after the incubation to terminate the reaction. Then samples were blended with 5 mL of toluene and shaken for 30 min at 250 rpm, followed by centrifugation at 4500 rpm for 5 min to extract Triphenyl formazon (TPF). The optical density of the supernatant was measured at 492 nm using a spectrophotometer. Soil dehydrogenase activity was expressed as µg TPF g^{-1} soil.

Cation exchange capacity (CEC) was evaluated as proposed by US, Salinity Lab. Staff (1954). Soil organic matter contents were determined by adopting the method as described by Moodie et al. (1965). Total carbohydrates in soil were determined following the methodology of Safarik and Santruckova (1992). While nitrogen was estimated as described by Jackson (1962), total and extractable phosphorus by Olsen and Sommers (1982), extractable potassium by Carson (1980), and DTPA extraction method for Extractable Iron and Zinc developed by Lindsay and Norvell (1978).

Statistical analysis

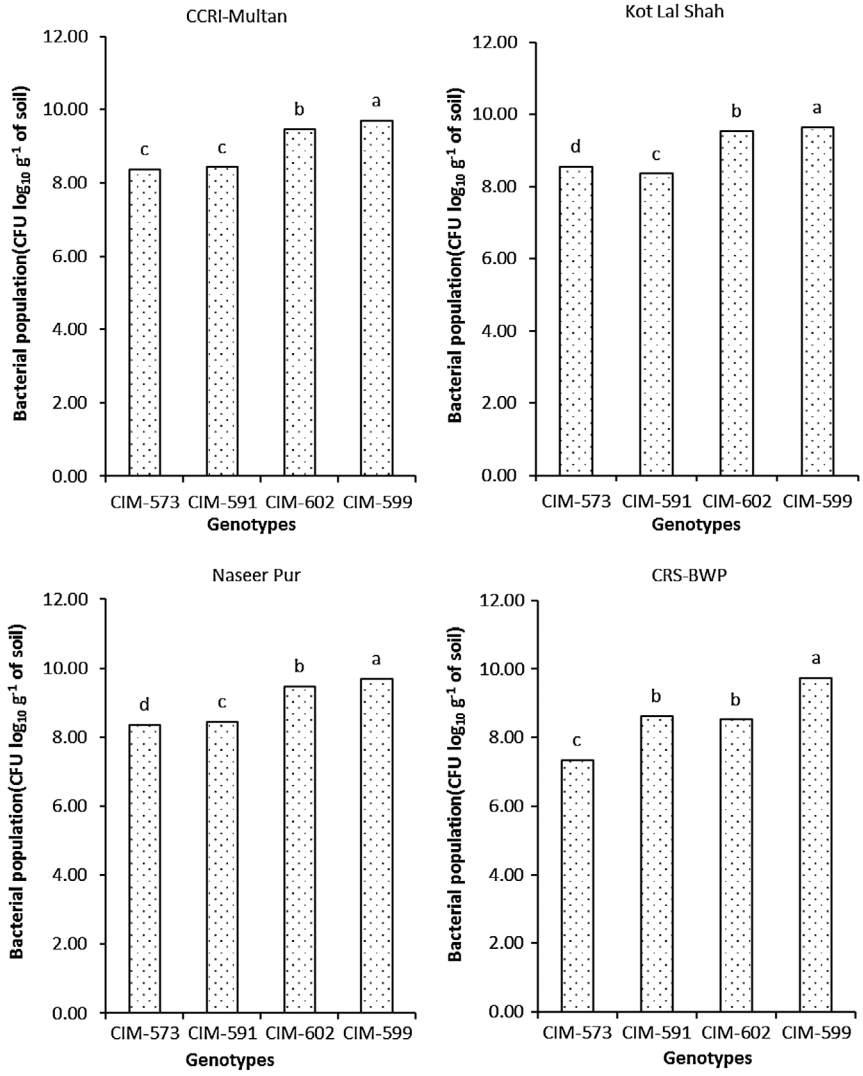
Multivariate procedures (SAS Institute, 2008) were used for data analysis and appropriate interpretation of results. These procedures provide the most up-to-date capabilities

for repeated measures of analysis of variance and analysis of covariance to separate simple and interactive effects of predictor variables on dependent variables using the Tukey's *post hoc* test. Data were transformed to detect the response of independent variables on dependent variables.

Results and discussion

Impact of Bt-cotton on soil culturable microbial population

Bacterial population was higher in *Bt*-cotton rhizosphere compared with non-*Bt* varieties (Figure 1). Maximum bacterial population observed in rhizosphere of CIM-599, a *Bt*-genotype at Naseer Pur as well as CRS-BWP, while minimum population was observed in non-*Bt* variety CIM-573 at CRS-BWP. The increase in microbial population indicates no adverse effects of growing *Bt*-cotton on soil microbial community and their activity. The differences in the bacterial population of *Bt* and non-*Bt* cotton varieties might be attributed to variations in root exudates quantity, composition, and root characteristics. Brusetti et al. (2005), Hu et al. (2009), and Saxena and Stotzky (2001) reported no difference in the bacterial populations of rhizosphere of both *Bt* and non-*Bt* cotton cultivars. But Yan et al. (2007) stated that the root exudates of *Bt*-cotton strongly affect the structure of bacterial populations in the rhizosphere. Petras and Casida (1985) revealed that *Bt* crops increase in the microbial community structure as the bacteria, actinomycetes, fungi, and nematodes use the crystal protein as substrate, when *Bacillus thuringiensis* subsp. *kurstaki* was added to the soil. Similarly, Donegan et al. (1995) reported a transient increase in the culturable bacteria population and fungi as a result of soil incorporation of



Bars sharing the same letters are statistically non-significant for each parameter (Tukey's test, $p < 0.05$)

Bt-varieties: CIM-599, CIM-602 **Non-Bt varieties:** CIM-573, CIM-591

Figure 1. Bacterial population (CFU $\log_{10}$ g^{-1} of soil) at four locations.

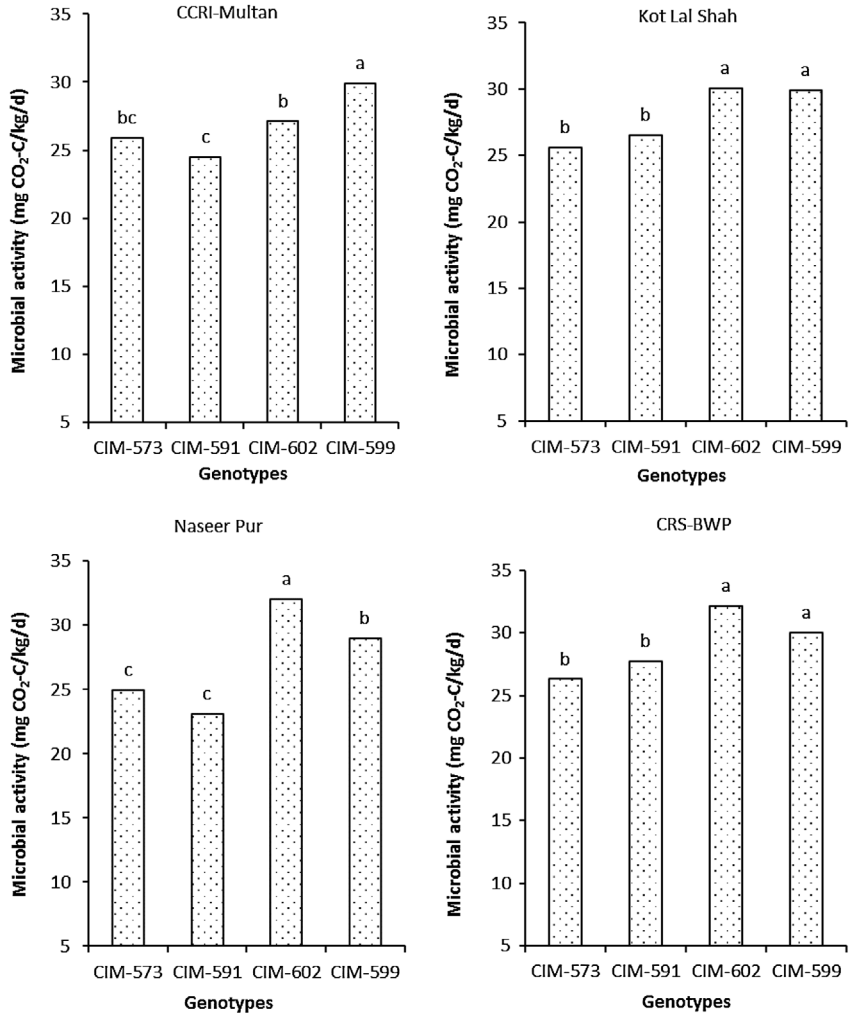


Figure 2. Microbial activity (mg CO_2-C/kg/d) at four locations.

Bt-cotton (*Gossypium hirsutum* L.) leaves expressing *cry1Ac* protein. Using the Biolog system (Biolog, Hayward, CA), the *Bt* cotton had no oppositional effect on the richness and diversity of soil microbial community compared to near-isogenic non-*Bt*-cotton (Shen et al., 2006; Zhang et al., 2013). Similarly, Hu et al. (2013) and Li et al. (2011) revealed no adverse effects on soil microbial population due to transgenic *Bt* crops and reduction in rhizobacterial community structure is possibly due to climatic factors rather than the presence of the *Bt* gene but no variation was observed in the microbial diversity between non-*Bt* and *Bt* maize utilizing the next generation sequence (Barriuso et al., 2012).

Impact of Bt cotton on soil microbial activity

The rhizosphere of *Bt*-varieties had more microbial activity as compared to non-*Bt* varieties (Figure 2). CIM-602 *Bt*-variety showed maximum microbial activity of 32.18 mg CO_2–C/kg/d at CRS-BWP. Minimum microbial activity 24.48 mg CO_2–C/kg/d found in non-*Bt* variety CIM-591 at CCRI-Multan. Improvement in microbial growth and activity might be correlated with the higher soil respiration.

Impact of Bt-cotton on soil dehydrogenase and phosphatase activities

Both *Bt*-varieties (CIM-602, CIM-599) had more dehydrogenase activity in rhizosphere ranging from 20.70 to 22.55 µg TPF g^{-1} h^{-1} than non-*Bt* varieties (Figure 3). Maximum dehydrogenase activity (22.55 µg TPF g^{-1} h^{-1}) was recorded in *Bt*-variety CIM-602 at CCRI-Multan, while minimum (20.70 µg TPF g^{-1} h^{-1}) was exhibited by non-*Bt* variety CIM-591 at Kot Lal Shah.

In present study, microbe-dependent phosphatase activity significantly increased in rhizosphere of *Bt*-varieties

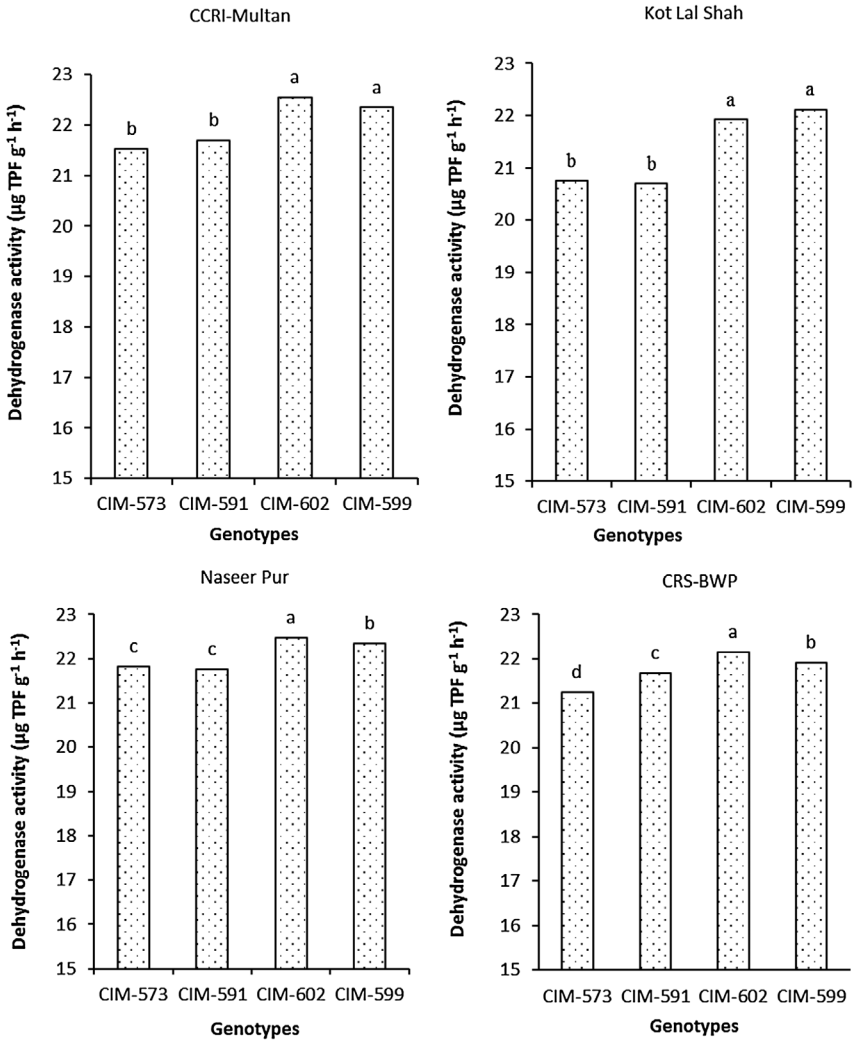


Figure 3. Dehydrogenase activity (µg TPF g^{-1} h^{-1}) at four locations.

as compared to non-*Bt* varieties (Figure 4). The maximum phosphatase activity of 184.08 µg *p*-Nitrophenol g^{-1} h^{-1} was observed in CIM-602 (*Bt*-Cotton) at CCRI-Multan, while lowest activity of 178.51 µg *p*-Nitrophenol g^{-1} h^{-1} was seen in non-*Bt* rhizosphere of CIM-591.

The higher soil enzyme activities might be due to more organic matter contents, microbial activity, and available nutrients compared to non-*Bt* (Dick & Tabatabai, 1992; He et al., 2007; Singh et al., 2013). *Bt*-toxin had no adverse effect on dehydrogenase activity in soil (Singh et al., 2013). More dehydrogenase activity in *Bt*-cotton rhizosphere in contrast to non-*Bt* rhizosphere could be attributed to the presence of higher bacterial biomass. Dehydrogenase activity is also often used as an alternative to substrate-induced respiration and has been found to be correlated with microbial activity (Chaperon & Sauve, 2007; Kraigher et al., 2006). Higher alkaline phosphatase activity might be due to the increase in microbial biomass, because alkaline phosphatase is associated with micro-organisms, while the acid phosphatase is predominantly due to plants (Kebrabadi et al., 2014). Sarkar et al. (2009) demonstrated that the growth of *Bt*-cotton had positive impact on most of the microbial and biochemical indicators, as microbial biomass carbon, microbial biomass nitrogen, microbial biomass phosphorous, and a range of soil enzyme activities, Sun et al. (2007) also reported that *Bt*-cotton plant material had positive effect on acid and alkaline phosphatase activities and alkaline activity was much higher than acid phosphatase activity.

Impact of Bt-cotton on soil nutrient dynamics

Maximum contents of total carbohydrates (406.8 mg kg^{-1}) were observed in CIM-599 (*Bt*) at CRS-BWP. Minimum

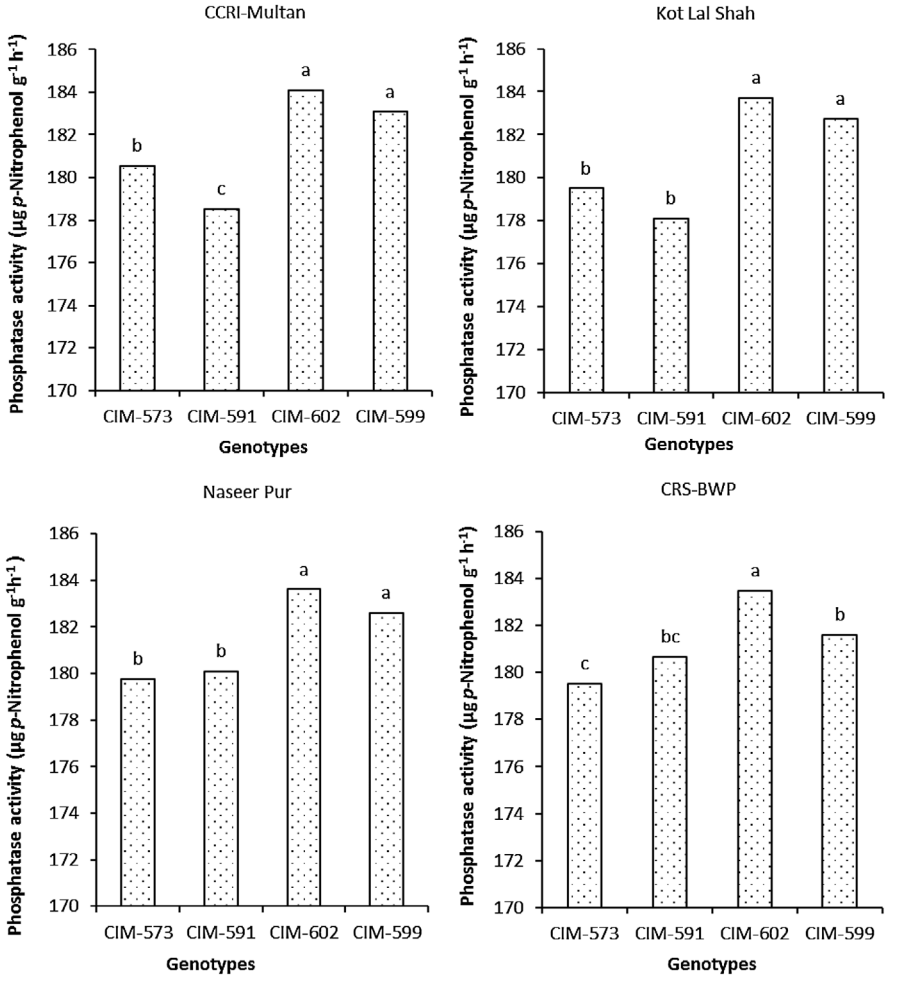


Figure 4. Phosphatase activity (µg *p*-Nitrophenol g^{-1} h^{-1}) at four locations.

contents of 304.8 mg kg^{-1} were analyzed at CCRI-Multan in CIM-573 (non-*Bt*). *Bt*-genotypes had maximum total carbohydrates contents among all genotypes (Table 1).

Bt-varieties (CIM-602, CIM-599) showed significantly higher CEC as compared to non-*Bt* varieties (Table 1). Maximum CEC 4.2 (C mol_c kg^{-1}) was found in *Bt*-variety CIM-602 at CRS-BWP while minimum 2.5 C mol_c kg^{-1} CEC was observed in CIM-591 at Kot Lal Shah as well as Naseer Pur.

At Naseer Pur, the maximum DTPA-Zn contents (1.57 mg kg^{-1}) were perceived in CIM-602 (*Bt*) rhizosphere, while minimum value of DTPA-Zn contents 1.01 mg kg^{-1} were observed in non-*Bt* variety (CIM-573) at CCRI-Multan. Zn contents remained in range of 1.01–1.57 at all location in all genotypes (Table 1).

Rhizosphere of *Bt*-varieties had high DTPA-Fe (mg kg^{-1}) contents with respect to non-*Bt* (Table 1). DTPA-Fe ranged from 1.87 to 2.55 mg kg^{-1} at all locations. Maximum 2.55 mg kg^{-1} was observed at Naseer Pur in rhizosphere of *Bt*-genotype CIM-599, while minimum (1.87 mg kg^{-1}) was found at location of CRS-BWP in non-*Bt* variety CIM-591.

Bt-rhizosphere had higher N contents with respect to non-*Bt* at all locations except CCRI-Multan. Maximum contents of nitrogen (0.138%) were observed in rhizosphere of CIM-602 (*Bt*) at location CRS-BWP, while minimum (0.088%) N contents found at location CCRI-Multan in rhizosphere of CIM-591 (non-*Bt*) (Table 1). *Bt*-genotypes had high N contents as compared to non-*Bt* genotypes.

Non-*Bt* varieties had higher total phosphorous contents in cotton rhizosphere with respect to *Bt*-varieties while all varieties showed statistically similar results at Naseer Pur (Table 1). Highest total phosphorous contents were recorded in CIM-591 (non-*Bt*) followed by CIM-573 (non-*Bt*) at Naseer Pur. Lowest phosphorous contents observed as 422.5 mg kg^{-1} in *Bt* variety CIM-602 at Kot Lal Shah.

Extractable-P contents were higher in *Bt*-varieties at all four locations compared with non-*Bt* varieties (Table 1). P contents ranged from 9.28 to 15.50 mg kg^{-1} and maximum

Table 1. Chemical attributes of rhizosphere of *Bt* and Non-*Bt* Genotypes of cotton on various locations of Punjab, Pakistan.

	Non-*Bt* Genotype		*Bt* Genotype		Non-*Bt* Genotype		*Bt* Genotype		Non-*Bt* Genotype		*Bt* Genotype	
	CIM-573	CIM-591	CIM-602	CIM-599	CIM-573	CIM-591	CIM-602	CIM-599	CIM-573	CIM-591	CIM-602	CIM-599
Location	Total Carbohydrates (mg kg^{-1})				CEC (C mol$_c$ kg^{-1})				DTPA-Zn (mg kg^{-1})			
CCRI-Multan	304.8 c	320.3 b	333.2 a	334.9 a	3.5 b	3.6 b	4.1 a	4.0 a	1.01 b	1.03 b	1.15 a	1.21 a
Kot Lal Shah	372.6 b	374.5 b	376.3 b	388.9 a	3.3 a	2.5 b	3.9 a	3.5 a	1.19 b	1.18 b	1.28 a	1.29 a
Naseer Pur	330.6 c	322.0 d	353.8 b	382.2 a	2.6 b	2.5 b	3.6 a	3.5 a	1.43 b	1.41 b	1.57 a	1.55 a
CRS-BWP	390.1 bc	385.1 c	394.5 b	406.8 a	3.0 c	3.0 c	4.2 a	3.4 b	0.6 c	0.66 b	1.11 a	1.08 a
	DTPA-Fe (mg kg^{-1})				Nitrogen (%)				Total P (mg kg^{-1})			
CCRI-Multan	2.35 b	2.33 b	2.49 a	2.51 a	0.09 b	0.088 b	0.122 a	0.124 a	530.25 a	525.5 a	509.0 b	504.75 b
Kot Lal Shah	2.25 b	2.23 b	2.35 a	2.38 a	0.118 b	0.111 c	0.122 b	0.132 a	455.50 a	456.5 a	422.5 b	427.75 b
Naseer Pur	2.38 b	2.37 b	2.52 a	2.55 a	0.122 *ab*	0.118 b	0.122 *ab*	0.124 a	575.50 a	581.25 a	569.5 a	565.0 a
CRS-BWP	1.89 c	1.87 c	1.98 b	2.07 a	0.116 b	0.113 b	0.138 a	0.132 a	476.75 a	471.75 a	437.0 b	434.75 b
	Extractable P (mg kg^{-1})				Extractable K (mg kg^{-1})				Organic matter (%)			
CCRI-Multan	9.28 c	9.47 b	11.45 a	11.45 a	177.66 b	176.66 b	181.66 a	181.33 a	1.01 b	1.03 b	1.16 a	1.15 a
Kot Lal Shah	12.60 d	13.48 c	13.58 b	14.55 a	213.66 c	209.33 d	222.33 a	219.33 b	1.33 b	1.31 b	1.54 a	1.57 a
Naseer Pur	9.61 b	9.52 b	10.55 a	10.48 a	119.33 c	119.00 c	123.33 b	126.00 a	0.56 b	0.48 c	0.70 a	0.74 a
CRS-BWP	14.52 c	14.54 c	15.40 b	15.50 a	189.00 b	191.00 b	205.33 a	210.00 a	1.12 b	1.07 c	1.17 a	1.07 c

Note. Means sharing the same letters for locations are statistically non-significant for each parameter (Tukey's test, $p < 0.05$).

value 15.50 mg kg^{-1} was observed in *Bt*-variety CIM-599 at location CRS-BWP. The minimum P contents (9.28 mg kg^{-1}) were found in CIM-573 (non-*Bt*) at CCRI-Multan.

At locations of CCRI-Multan and CRS-BWP extractable-K was increased in *Bt*-varieties compared to non-*Bt* varieties (Table 1). *Bt*-genotypes had maximum potassium contents at all locations, while it was minimum at Naseer Pur in CIM-591 (Non-*Bt* genotype).

Maximum organic matter was observed in *Bt*-varieties rhizosphere as compared to non-*Bt* varieties (Table 1). Rhizosphere of CIM-599 presented maximum organic matter (1.57%) at Kot Lal Shah, while minimum (0.48%) was found where non-*Bt* CIM-591 was grown Naseer Pur.

Maximum organic matter contents were observed in *Bt*-rhizosphere with higher value of cation exchange capacity and increased availability of nutrients. Results revealed that more microbial diversity and activity in *Bt*-rhizosphere instead of non-*Bt* rhizosphere. Transgenic *Bt*-crops may affect nutrient cycling, either through the products of introduced genes or modifying rhizosphere chemistry (Hu et al., 2013). Increase in nutrients availability might be due to the non-targeted physiological changes (e.g. content of starch, soluble N, proteins, carbohydrates, and lignin) and high amount of root exudates in transgenic plants (Motavalli et al., 2004). Genetic transformation in plants has been shown to cause positive changes in N content, C:N ratio, lignin, fructose, and carbohydrate contents. *Bt* stubble had a higher N content and lower C:N ratio and the differences in percent C, N, and C:N ratio between *Bt* cotton and isoline and their interactions with other environmental factors also influence the decomposition which have positive impact on nutrient availability (Mahmood et al., 2014; Oostherius et al., 2013). The increase in lignin content in *Bt* rhizosphere might cause the slow release of nutrients by affecting the decomposition which ultimately affects the biogeochemical cycles (Mina et al., 2008).

Phosphorus availability in soil is generally influenced at the main interaction zone between the plant and soil biota near the root surface in the rhizosphere (Saleem et al., 2011). The increase in extractable phosphorous in the rhizosphere of *Bt* might be due to higher microbial population having more phosphatase activity as compared to non-*Bt*. Both plant roots and soil micro-organisms could increase the soil phosphorous availability through root exudates containing organic acids, H^+ ions, sugars, and phosphatases that facilitates the solubilization and desorption of mineral phosphorous (Ryan et al., 2001). Alterations in the composition and quantity of root exudates through the introduction of new genetic traits affect the processes such as mineral phosphorous or fixed phosphorous solubilization, availability of phosphorous through changes in the activity of rhizosphere micro-organisms (Shen et al., 2006). Changes in *Bt*-cotton rhizospheric conditions such as more phosphatase activity (Mina & Chaudhar, 2012) might result in enhanced phosphorous availability. *Bt*-genotypes showed more response to phosphorous contents as the total phosphorous contents observed low in *Bt*-rhizosphere. These results are consistent with phosphorous availability in the rhizosphere of transgenic alfalfa which might be attributed to high release of citrate, oxalate, malate, succinate, and acetate type root exudates (Tesfaye et al., 2001). Exogenous application and organic acid exudation from roots improve phosphorous availability in *Bt*-rhizosphere (Bucio et al., 2000; Koyama et al., 2000). Citrate and oxalate appeared to be the most efficient components of root exudates with respect to mobilization of phosphorous from those soils which are low in readily available

phosphorous (Kaya et al., 2007). In the present study, extractable potassium contents were found to be higher in *Bt*-rhizosphere compared to non-*Bt*. Higher potassium contents in *Bt*-cotton rhizosphere might be due to the more microbial activity and higher soil enzyme activities. These microbes decompose silicate minerals such as K-feldspar and mica. They transform solid K in the soil into available K that can be directly absorbed by plants, and they secrete active substances that promote plant growth (Sheng, 2005). The use of potassium solubilizing bacteria as a biological fertilizer is a hot spot in the study of agriculture and environmental conservation (Deng et al., 2003). In *Bt*-rhizosphere, micronutrient DTPA-Zn and Fe contents were observed higher as compared to non-*Bt*. Significant increase in the available Zn and Fe in the soil under *Bt*-cotton over non-*Bt* cotton was due to higher root biomass-mediated exudation and this might the most important reason for the increase (Beura & Rakshit, 2011).

In conclusions, *Bt*-cotton expressing *cry1Ac* gene producing *Bt*-toxin had no detrimental effects on soil microbiological activities such as culturable bacterial population, microbial activity via respiration, dehydrogenase and phosphatase activities. The nutrient dynamics also showed positive impact of growing *Bt*-cotton. Spatial and temporal variations perceived in *Bt* and non-*Bt* cotton varieties were attributable to differences in genetic makeup of cotton varieties rather than the *Bt*-gene expression. Superstitions around the world, spreading that GMO's have deleterious effects on soil microbial ecology but our study demonstrates that *Bt*-cotton varieties expressing *cry1Ac* gene producing *Bt*-toxin do not stance any detrimental effects on microbial population counts, enzymatic activity, and nutrient dynamics of soil. However, further work is needed for estimating the impact of *Bt*-cotton on soil insect's ecology and soil-dwelling invertebrates before recommending the cultivation of *Bt*-cotton in Pakistan which will reduce the cost to input ratio, ultimately leading the county's economy to the bloom.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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