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

Sanaullah Yasina, Hafiz Naeem Asghara, Fiaz Ahmadb, Zahir Ahmad Zahira and Ejaz Ahmad Waraichc

aInstitute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan; bPhysiology/Chemistry Section, Central Cotton Research Institute, Multan, Pakistan; cDepartment of Agronomy, University of Agriculture, Faisalabad, Pakistan

**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 revealed 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.

**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 biodiversity 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.
(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, P₂O₅ and K₂O 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 CO₂ evolution. The CO₂ evolution was measured by acid–base titration and expressed as mg CO₂–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 (pNPP) to p-Nitrophenol, pNPP 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

![Figure 1. Bacterial population (CFU log_{10} g^{-1} of soil) at four locations.](image-url)
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 CO₂-C/kg/d at CRS-BWP. Minimum microbial activity 24.48 mg CO₂-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 2.** Microbial activity (mg CO₂-C/kg/d) at four locations.
as compared to non-\textit{Bt} varieties (Figure 4). The maximum phosphatase activity of 184.08 \(\mu\text{g} \ p\text{-Nitrophenol g}^{-1} \ h^{-1}\) was observed in CIM-602 (\textit{Bt}-Cotton) at CCRI-Multan, while lowest activity of 178.51 \(\mu\text{g} \ p\text{-Nitrophenol g}^{-1} \ h^{-1}\) was seen in non-\textit{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-\textit{Bt} (Dick & Tabatabai, 1992; He et al., 2007; Singh et al., 2013). \textit{Bt}-toxin had no adverse effect on dehydrogenase activity in soil (Singh et al., 2013). More dehydrogenase activity in \textit{Bt}-cotton rhizosphere in contrast to non-\textit{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 \textit{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 \textit{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 \textit{Bt}-cotton on soil nutrient dynamics**

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

![Figure 3. Dehydrogenase activity (\(\mu\text{g TPF g}^{-1} \ h^{-1}\)) at four locations.](image)

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
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

---

**Figure 4.** Phosphatase activity (μg \(p\)-Nitrophenol g\(^{-1}\) h\(^{-1}\)) at four locations.
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 isolate and their interactions with other environmental factors also influence the decomposition which has positive impact on nutrient availability (Mahmood et al., 2014; Oosterhuis 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

---

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

| Location         | Non-Bt Genotype | Bt Genotype | Non-Bt Genotype | Bt Genotype | Non-Bt Genotype | Bt Genotype |
|------------------|-----------------|-------------|-----------------|-------------|-----------------|-------------|
|                  | Total Carbohydrates (mg kg\(^{-1}\)) | CEC (C molc kg\(^{-1}\)) | DTPA-Zn (mg kg\(^{-1}\)) | Nitrogen (%) | Total P (mg kg\(^{-1}\)) | Organic matter (%) |
| CCRI-Multan      | 304.8 c          | 320.3 b    | 332.2 a         | 344.9 a     | 3.5 b           | 4.1 a       | 4.0 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           | 3.9 a       | 3.5 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 a       | 3.6 a         | 3.6 a         | 1.43 b         | 1.41 b         | 1.57 a         | 1.55 a         |
| CRS-BWP          | 390.1 b          | 385.1 c    | 394.5 b         | 406.8 a     | 3.0 c           | 3.0 a       | 4.2 a         | 4.2 a         | 0.6 c          | 0.66 b         | 1.11 a         | 1.08 a         |
|                  |                 |             |                 |             |                 |             |              |              |                |                |                |                |
|                  |                 |             |                 |             |                 |             |              |              |                |                |                |                |
|                  |                 |             |                 |             |                 |             |              |              |                |                |                |                |
|                  |                 |             |                 |             |                 |             |              |              |                |                |                |                |
|                  |                 |             |                 |             |                 |             |              |              |                |                |                |                |
|                  |                 |             |                 |             |                 |             |              |              |                |                |                |                |
|                  |                 |             |                 |             |                 |             |              |              |                |                |                |                |
|                  |                 |             |                 |             |                 |             |              |              |                |                |                |                |
|                  |                 |             |                 |             |                 |             |              |              |                |                |                |                |

**Note.** Means sharing the same letters for locations are statistically non-significant for each parameter (Tukey’s test, \(p < 0.05\)).
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 be 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 stand 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 country’s economy to the bloom.

Acknowledgements
This research work was conducted in ISES, UAF, field trials were conducted at four locations Central Cotton Research Institute, Multan, Cotton Research Station, Bhawalpur, Mouza Kot Lal Shah, and Mouza Naseer Pur with financial assistance of Higher Education Commission (HEC) Islamabad and Centre of Environmental Protection Agency (CERA) USA. The assistance from HEC and CERA, USA is highly acknowledged.

Disclosure statement
No potential conflict of interest was reported by the authors.

References
Barriuso, J., Valverde, J. R., & Mellado, R. P. (2012). Effect of Cry1Ab protein on rhizobacterial communities of Bt-maize over a four year cultivation period. Plos ONE, 7, 35–48.
Benedict, J. H., & Altman, D. W. (2001). Commercialization of transgenic cotton expressing insecticidal crystal proteins. In J. N. Jenkins & S. Saha (Eds.), Genetic improvement of cotton: Emerging technologies (p. 137). Enfield, NH: Science.
Beura, K., & Rakshit, A. (2011). Effect of Bt cotton on nutrient dynamics under varied soil type. Italian Journal of Agronomy, 6, 25–28.
Brusetti, L., Francia, P., Bertolini, C., Pagliuca, A., Borin, S., Sorlini, C., … Daffonchio, D. (2005). Bacterial communities associated with the rhizosphere of transgenic Bt 176 maize (Zea mays) and its non transgenic counterpart. Plant Soil, 266, 11–21.
Bucio, L. J., Martinez de la Vega, O., Guevara-Garcia, A., & Herrera-Estrella, L. (2000). Enhanced phosphorus uptake in transgenic tobacco plants that overproduce citrate. Nature Biotechnology, 18, 450–453.
Carson, P. L. (1980). Recommended potassium test. In W. C. Dahneke (Ed.), Recommended chemical soil test procedures for the North Central Region (pp. 12–13). Fargo, ND: North Central Region Publication 221 (revised). N.D. Agri. Exp. Stn.
Chaperon, S., & Sauvé, S. (2007). Toxicity interaction of metals (Ag, Cu, Hg, Zn) to urease and dehydrogenase activities in soils. Soil Biology and Biochemistry, 39, 2329–2338.
Deng, S. B., Bai, R. B., Hu, X. M., & Luo, Q. (2003). Characteristics of a bioflocculant produced by Bacillus mucilaginosus and its use in starch wastewater treatment. Applied Microbiology and Biotechnology, 60, 588–593.
Dick, R. P., & Tabatabai, M. A. (1992). Potential uses of soil enzymes. In F. B. Meeting, Jr. (Ed.), Soil microbial ecology: Applications in agricultural and environmental management (pp. 95–127). New York, NY: Marcel Dekker.
Donegan, K. K., Palm, C. J., Fieland, V. J., Porteous, L. A., Ganio, L. M., Schaller, D. L., … Seidler, R. J. (1995). Changes in levels, species and DNA fingerprints of soil microorganisms associated with cotton expressing the Bacillus thuringiensis var. kurstaki endotoxin. Applied Soil Ecology, 2, 111–124.
Gasson, M. J. (2000). Gene transfer from genetically modified food. Current Opinion in Biotechnology, 11, 505–508.
Hartmann, M., Niklaus, P. A., Zimmermann, S., Schmutz, S. J., Kremer, & Abarenkov, K. (2014). Resistance and resilience of the forest soil microbiome to logging-associated compaction. The ISME Journal, 8, 226–244.
He, J. Z., Shen, J.-P., Zhang, L.-M., Zhu, Y.-G., Zheng, Y.-M., Xu, M.-G., & Di, H. (2007). Quantitative analyses of the abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea of a Chinese upland red soil under long-term fertilization practices. Environmental Microbiology, 9, 2364–2374.
Hu, H., Xie, M., Yu, Y., & Zhang, Q. (2013). Transgenic Bt cotton tissues have no apparent impact on soil microorganisms. Plant, Soil and Environment, 8, 366–371.
Hu, H. Y., Liu, X. X., Zhao, Z. W., Sun, J. G., Zhang, Q. W., Liu, X. Z., & 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.
Jackson, M. L. (1962). Chemical composition of soil. In F. E. Bean (Ed.), Chemistry of soil (pp. 71–144). New York, NY: Van Nostrand Reinhold.
James, C. (2002). Preview: Global status of commercialized transgenic crops: 2002. ISAAA Briefs No. 27, Ithaca, NY. Retrieved from http://www.ISAAA.org/S

Karuri, H., Amata, R., Amugune, N., & Waturu, C. (2013). Effect of Bt cotton expressing Cry1Ac and Cry2Ab2 protein on soil nematode community assemblages in Mwea, Kenya. Journal of Animal and Plant Sciences, 19, 2864–2879.

Kaya, C., Tuna, A. L., Ashraf, M., & Altunlu, H. (2007). Improved salt tolerance of melon (Cucumis melo L.) by the addition of proline and potassium nitrate. Environmental and Experimental Botany, 60, 397–403.

Kebrabadi, B. Z., Matinizadeh, M., Ghodskhah, M., Davaryi, & Salehi, A. (2014). Changes in acid and alkaline phosphatase enzyme activity in rhizosphere ash Fraxinus rotundifolia and its correlation with soil and plant phosphorus. Journal of Biodiversity and Environmental Sciences, 4, 233–238.

Kowalchuk, G. A., Bruinsma, M., & van Veen, J. A. (2003). Assessing responses of soil microorganisms to GM plants. Trends in Ecology & Evolution, 18, 403–410.

Koyama, H., Kawamura, A., Kittara, T., Hara, T., Takita, E., & Shibata, D. (2000). Overexpression of mitochondrial citrate synthase in arabidopsis thaliana improved growth on a phosphorus-limited soil. Plant and Cell Physiology, 41, 1030–1037.

Kraighe, B., Stres, B., Hacin, J., Ausic, L., Mahne, I., Van Elsas, J. D., & Mandic-Mulec, I. (2006). Microbial activity and community structure in two drained fen soils in the Ljubljana Marsh. Soil Biology and Biochemistry, 38, 2762–2771.

Li, X. G., Liu, B., Cui, J. J., Liu, D. D., Ding, S., Gilna, B., … Han, Z. M. (2011). No evidence of persistent effects of continuously planted transgenic insect-resistant cotton on soil microorganisms. Plant and Soil, 339, 247–257.

Lindsay, W. L., & Norvell, W. A. (1978). Development of a DTPA soil test for zinc, iron, manganese and copper. Soil Science Society of America Journal, 42, 421–428.

Lynch, J. M., Benedetti, A., Insam, H., Nuti, M. P., Smalla, K., Torsvik, V., & Nannipieri, P. (2004). Microbial diversity in soil: ecological theories, the contribution of molecular techniques and the impact of transgenic plants and transgenic microorganisms. Biology and Fertility of Soils, 40, 363–385.

Mafham, P. J., Boddy, L., & Randerson, P. F. (2002). Analysis of microbial community functional diversity using sole-carbon-source utilization profiles—a critique. FEMS Microbiology Ecology, 42, 1–14.

Mahmood, T., Arshad, M., Jahangir, G. Z., Nasir, I. A., & Iqbal, M. (2014). Disease free and rapid mass production of sugarcane cultivars. Advanced Life Science, 1, 171–180.

Min, H., Ye, Y. F., Chen, Z. Y., Wu, W. X., & Yufeng, D. (2001). Effects of butachlor on microbial populations and enzyme activities in paddy soil. Journal of Environmental Science and Health, Part B, 36, 581–595.

Mina, U., & Chaudhary, A. (2012). Impact of transgenic cotton varieties on activity of enzymes in their rhizosphere. Indian Journal Biochemistry and Biophysics, 49, 195–201.

Mina, U., Khan, A. S., Chaudhary, A., Choudhary, R., & Aggarwal, P. K. (2008). An approach for impact assessment of transgenic plants on soil ecosystem. Applied Ecology and Environmental Research, 6, 1–19.

Moodie, C. D., Smith, H. W., & McCrery, R. A. (1965). Laboratory manual for soil fertility. Washington, DC: State College of Washington Pullman.

Motavalli, P. P., Kremer, R. J., Fang, M., & Means, N. E. (2004). Impact of genetically modified crops and their management on soil microbially mediated plant nutrient transformations. Journal of Environment Quality, 33, 816–824.

Muchaonyerwa, P., Waladde, S., Nyamugafata, P., Mpepekre, S., & Ristori, G. G. (2004). Persistence and impact on microorganisms of Bacillus thuringiensis proteins in some Zimbabwean soils. Plant and Soil, 266, 41–46.

Olsen, S. R., & Sommers, L. E. (1982). Phosphorus. In A. L. Page (Ed.), Methods of soil analysis, Agron. No. 9, Part 2: Chemical and microbiological properties (2nd ed., pp. 403–430). Madison, WI: American Society of Agronomy.

Oosterhuis, D. M., Loka, D. A., & Raper, T. B. (2013). Potassium and stress alleviation: Physiological functions and management of cotton. Journal of Plant Nutrition and Soil Science, 176, 331–343.

Palm, C. J., Seidler, R. J., & Schaller, D. L. (1996). Persistence in soil of transgenic plant produced Bacillus thuringiensis var. kurstaki δ-endotoxin. Canadian Journal of Microbiology, 42, 1258–1262.

Petas, S. F., & Casida, L. E. (1985). Survival of Bacillus thuringiensis spores in the soil. Applied and Environmental Microbiology, 50, 28–35.

Ryan, P. R., Delhaize, E., & Jones, D. L. (2001). Function and mechanism of organic anion exudation from plant roots. Annual Review of Plant Physiology and Plant Molecular Biology, 52, 527–560.

Sabir, H. M., Tahir, S. H., & Khan, M. B. (2011). Bt cotton and its impact on cropping pattern in Punjab. Pakistan Journal of Social Sciences, 31, 127–134.

Safařík, I., & Šantrůčková, H. (1992). Direct determination of total soil carbohydrate content. Plant and Soil, 143, 109–114.

Saleem, M. F., Cheema, M. A., Bilal, M. F., Anjum, S. A., Shahid, M. Q., & Khurshid, I. (2011). Fiber quality of cotton (Gossypium hirsutum) cultivars under different phosphorus levels. The Journal of Animal & Plant Sciences, 21, 26–30.

Sarkar, B., Patra, A. K., Purakayastha, T. J., & Megharaj, M. (2009). Assessment of biological and biochemical indicators in soil under transgenic Bt and non-Bt cotton crop in a sub-tropical environment. Environmental Monitoring and Assessment, 156, 595–604.

SAS Institute. (2008). SAS online doc 9.13. Cary, NC: SAS Institute.

Saxena, D., & Stotzky, G. (2001). Bacillus thuringiensis (Bt) toxin released from root exudates and biomass of Bt corn has no apparent effect on earthworms, nematodes, protozoa, bacteria, and fungi in soil. Soil Biology and Biochemistry, 33, 1225–1230.

Saxena, D., Flores, S., & Stotzky, G. (2001). Bt toxin is released in root exudates from 12 transgenic corn hybrids representing three transformation events. Soil Biology and Biochemistry, 34, 133–137.

Shen, R. F., Cai, H., & Gong, W. H. (2006). Transgenic Bt cotton has no apparent effect on enzymatic activities or functional diversity of microbial communities in rhizosphere soil. Plant Soil, 285, 149–159.

Sheng, X. (2005). Growth promotion and increased potassium uptake of cotton and rape by a potassium releasing strain of Bacillus edaphicus. Soil Biology and Biochemistry, 37, 1918–1922.

Singh, R. J., Ahlawat, I. P. S., & Singh, S. (2013). Effects of transgenic Bt cotton on soil fertility and biology under field conditions in subtropical inceptisol. Environmental Monitoring and Assessment, 185, 485–495.

Stotzky, G. (2004). Persistence and biological activity in soil of the insecticidal proteins from Bacillus thuringiensis, especially...
from transgenic plants. *Plant Soil*, 266, 77–89.

Stotzky, G. (1965). Microbial respiration. In C. A. Black (Ed.), *Methods of soil analysis* (pp. 2: 1551–1572). Madison, WI: American Society of Agronomy.

Sun, C., Qi, H., Sun, J., Zhang, L., & Miao, L. (2007). Photosynthetic characteristics of Bt or CpTI-Bt transgenic cotton at seedling stage. *Acta Agronomica Sinica*, 33, 469–475. (In Chinese with English abstract).

Tabatabai, M. A., & Bremner, J. M. (1969). Use of p-nitro-phenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry*, 1, 301–307.

Tapp, L., Calamai, L., & Stotzky, G. (1995). Insecticidal activity of the toxins from *Bacillus thuringiensis* subspecies *kurstaki* and *tenebrionis* adsorbed and bound on pure and soil clays. *Applied and Environmental Microbiology*, 61, 1786–1790.

Tesfaye, M., Temple, S. J., Allan, D. L., Vance, C. P., & Samac, D. A. (2001). Overexpression of malate dehydrogenase in transgenic alfalfa enhances organic acid synthesis and confers tolerance to aluminum. *Plant Physiology*, 127, 1836–1844.

US, Salinity Lab. Staff. (1954). Diagnosis and improvement of saline and alkali soils. USDA Agric Handbook 60. (pp. 160).