



Studied on the impact of Bt-cotton cultivation of rhizospheric and non-rhizospheric bacterial and fungal population in contrast to non Bt-cotton in natural system

Uma Singh¹, IfraZoomi¹, Ovaaid Akhtar², Dheeraj Pandey¹, Kanhaiya Lal Chaudhary¹, Harbans Kaur Kehri¹

¹Sadasivan Mycopathology Laboratory, Department of Botany, University of Allahabad, Prayagraj, Uttar Pradesh, India

²Kamla Nehru Institute of Physical and Social Sciences, Sultanpur, Uttar Pradesh, India

Abstract

Cotton is one of the most important crop worldwide and in India. Genetically modified cotton crop was officially introduced in the year 2002. Genetically modified crop varieties have various advantages over traditional varieties. This cotton carried genes from the bacterium *Bacillus thuringiensis* (Bt), therefore it is called as Bt cotton. *Bacillus thuringiensis* is a gram-positive, spore-forming bacteria, generates a number of insecticidal crystal proteins (ICPs) that are poisonous to larvae of lepidopterans, dipterans, and coleopterans. In recent years, transgenic Bt cotton varieties have been widely grown, which effectively controls cotton bollworm and reduce the application of insecticides. Continuous cultivation of Bt-cotton is known to have negative impact on soil microflora. It has been reported that Bt toxin leach out in the rhizosphere and inhibits the growth of soil microbes. Therefore, the present study was undertaken to study the impact of Bt cotton and non-Bt cotton cultivation on soil microflora at different stages of cotton plants in Diksai, Jalgoan and Maharashtra. The obtained results revealed that Bt-cotton crop adversely affected the below ground biodiversity of microbial population.

Keywords: fungi, cotton, insecticidal crystal proteins, *Bacillus thuringiensis*, microflora

Introduction

Soil is a complex and dynamic environment where microorganisms regulate most of the biological processes. These soil microorganisms show many beneficial effects, like fixation of atmospheric nitrogen, deposition of organic matter, breakdown of metabolic byproducts, and increase in the availability of phosphates, sulphates, nitrates, and essential metals (Bridge and Spooner, 2001) ^[5]. Microbial communities present in the rhizospheric zone can remarkably affect the development of phytopathogens (Nehl *et al.*, 1997; Glick, 1995) ^[22, 12], the acquisition of nutrient, heavy metal resistance (Bradly *et al.*, 1981; Shetty *et al.*, 1994) ^[31], and the overall ecological fitness of plants.

Plants that have been genetically engineered have potential to affect soil microbial populations, and hence key ecosystem activities such as carbon cycling, nutrient solubilization, and the prevalence of soil-borne plant disease (Beura and Rakshit, 2013; Sarkar *et al.*, 2009) ^[3, 25]. However, it is unclear whether these effects are caused directly by the newly imported gene or indirectly by the GM plants' altered rhizosphere chemistry (McGregor and Turner, 2000) ^[19]. Cotton (*Gossypium hirsutum* L.) be a member of the family Malvaceae, subfamily Malvoideae, and tribe Gossypieae. Based on evolutionary lineage, *Gossypium* emerged before 11-14 million years ago (Wendel and Cronn, 2003) ^[39]. Cotton is one of the most significant fiber crops grown worldwide, commercially in over 70 nations within temperate and tropical climates, covering a total area of 33 million hectares (Smith, 1999). It is also known as "white gold" and the premier commercial cash crop in India and it plays a crucial role in the Indian economy.

Cotton is highly vulnerable to insects; particularly to the larvae of lepidopteran pests, which adversely affect cotton production. More than 50% of the total insecticides are used in the country for the protection of cotton from the pests. More than 1200 crores estimated cotton loss due to pest attack in the cotton field and damage all cotton crops. Use of chemical for the suppression of cotton insect pests is proving ineffective for the reason that of the high level of resistance of these pests. Therefore, there are been many reasons behind the adoption of Bt-cotton that could help in the protection of crop potentially against the most destructive bollworms and thus decrease the risk of crop failures. *Helicoverpa armigera*, commonly referred to as American Bollworm, is used to cause significant crop damage, resulting in low yield. Therefore, Mahyco (Maharashtra Hybrid Seed Company) introduced Bt cotton technology into India in partnership with Monsanto. Bt-cotton is the first genetically modified crop to be grown in India. The production of cotton in India increased rapidly with the introduction of Bt cotton in the year 2002. Globally, cotton is projected to cover 80.80 million acres (32.7 million hectares) and produce 121.6 million bales of 217.72 kilograms each in 2021-22. The production of cotton

is predicted to increase in most major producing countries except China in 2021-22. Among the top, five cotton producer countries are India (28 million bales), China (26.80 million bales), the United States (18.3 million bales), Brazil (13.2 million bales), and Pakistan (5.7 million bales).

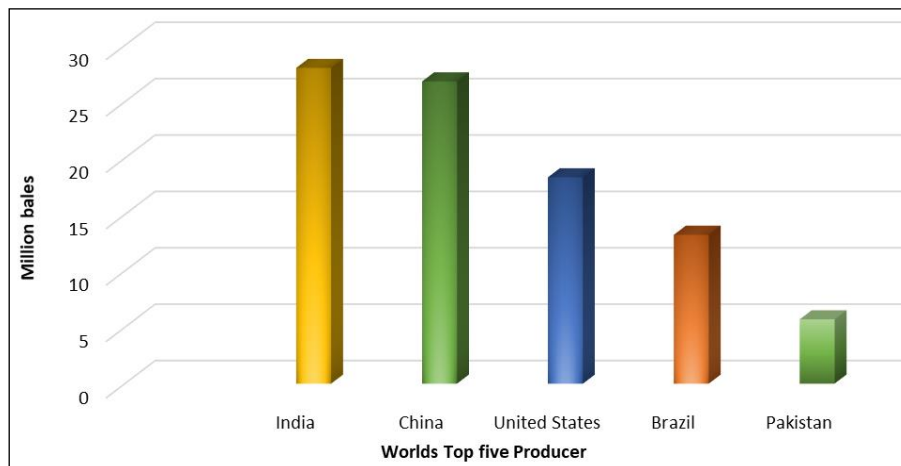


Fig 1

According to the statistics released on 17th September 2021, the cotton area under cultivation for 2021-22 was 119.66 lakh ha, down from 126.97 lakh ha in 2020-21. State-wise, Maharashtra leads the way with 39.41 lakh ha, followed by Gujarat (22.51 lakh ha), Telangana (18.78 lakh ha), Rajasthan (7.08 lakh ha), and Haryana (6.88 lakh ha). The lowest cotton-producing state is Tamil Nadu with just five lakh bales of cotton. (Agricultural Market Intelligence Centre, PJTSAU, January 2022). Initially, it was approved in India on March 26th, 2002, for commercial cultivation in six states of India belonging to central and southern zones of the country. Cotton containing the Bt gene contains the Cry1Ac gene that was extracted from the soil bacterium *Bacillus thuringiensis* var. kurstaki, thus resulting in the development of the Cry1Ac protein, which gives bollworm resistance (Barwale *et al.*, 2004) [2]. Bt-toxin from Bt cotton plants enters the soil via two mechanisms: biomass assimilation and root exudates (Saxena and Stotzky, 2001; Mina *et al.*, 2008) [27, 21]. Bt toxin is adsorbed or bonded on clay particles, humic components, or organic mineral complexes in the soil, where it is subsequently protected against destruction by soil microbes (Tapp *et al.*, 1995) [35]. Although Bt toxin is naturally found in many soils but continuous growing of Bt crops in the same location raises its levels to a point where it can affect the composition and activity of soil microbial communities (Donegan and Seidler, 1999; Stotzky, 2004; Wei *et al.*, 2006, Griffiths *et al.*, 2007; Rui *et al.*, 2005) [7, 28, 38, 13, 24] and soil biochemical properties (Rui *et al.*, 2005; Fang *et al.*, 2007; Sun *et al.*, 2007; Sarkar *et al.*, 2009) [24, 10, 33, 25]. Several experiments were carried out to determine the impact of Bt cotton on flora and fauna in various agro-ecosystems (Zhang *et al.*, 2000; Li *et al.*, 2002; Liu *et al.*, 2002; Men *et al.*, 2003; Bai *et al.*, 2003) [41, 16, 20]. Some studies have found that Bt cotton has no detrimental impacts on soil flora and fauna, and may even be helpful (Saxena and Stotzky, 2001; Sarkar *et al.*, 2009, Kapur *et al.*, 2010) [27, 25, 15], while others have found negative consequences (Saxena and Stotzky, 2001; Sarkar *et al.*, 2009) [27, 25, 15]. (Cui and Xia, 2000; Tan *et al.*, 2002) [6, 34]. Cry proteins released in root exudates or decomposing plant tissue from Bt corn and other Bt crops can enter soil via root exudation throughout the plant's life cycle (e.g., Palm *et al.*, 1996; Saxena *et al.*, 1999; Saxena and Stotzky, 2000; Saxena *et al.*, 2002b Zwahlen *et al.*, 2003a; Saxena and Stotzky, 2002, 2004) [23, 29, 26, 30, 42 28, 30]. As a result, non-target creatures, such as beneficial insects, soil bacteria, and fungus, may be at danger (Tsatsakis *et al.*, 2017) [37].

Material and Methods

Selection of Survey Site

For comparative studies of Bt and non-Bt cotton microbiological biodiversity from rhizospheric and non-rhizospheric soil of both cottons were taken. I selected Maharashtra (India), which is separated into six regions: Amravati, Nashik, Pune, Konkan, Nagpur, and Aurangabad. For our research, I choose only the different villages of Jalgaon which come under north Maharashtra (Nashik division), which contains five districts: Nashik, Dhule, Nandurbar, Jalgaon, and Ahmednagar. The goal of this study was to investigate the role of below-ground microbial population diversity in Bt and non-Bt cotton from both the rhizospheric and non-rhizospheric zones. Microbial population was investigated on the basis of their morpho taxonomy.

Collection of Soil Samples

An extensive survey has been undertaken to access the biodiversity of soil microflora (bacterial and fungal populations). The soil sample collected from rhizosphere and non-rhizosphere soils of Bt cotton and non-Bt-cotton plants from Diksai, Jalgaon, Maharashtra, India. Collected soil sample were properly placed in sterile zip-lock bags, transported to the laboratory, and kept at 4° C until further analysis. Samples were collected 3 times in a year. The first sampling was done in the month of Aug-Sep (seedling stage), second sampling was

done in the month of Oct-Nov (vegetative stage) and third sampling was done in the month of Dec-Jan (vegetative stage).

Physiochemical Properties of Soil

Physicochemical properties of the rhizospheric and non-rhizospheric soil of Non-Bt-cotton and Bt-cotton which is collected from Diknai of Jalgaon, Maharashtra has been presented in Table 1.

Table 1: Physicochemical properties of soil samples both rhizospheric and non-rhizospheric of Diknai from Bt-cotton and non-Bt-cotton

| Sample | pH | EC MS/cm | Organic carbon % | P ₂ O ₅ (kg/h) | K ₂ O (kg/h) | B (ppm) | Fe (ppm) | Mn (ppm) | Zn (ppm) | Cu (ppm) | S (ppm) |
|--------|-----|----------|------------------|--------------------------------------|-------------------------|---------|----------|----------|----------|----------|---------|
| NBt | | | | | | | | | | | |
| R | 7.8 | 0.30 | 0.45 | 15 | 269 | 0.5 | 10.42 | 11.70 | 1.1 | 1.98 | 29 |
| NR | 7.4 | 0.37 | 0.25 | 10 | 269 | 0.5 | 10.52 | 11.98 | 1.08 | 1.44 | 24 |
| Bt | | | | | | | | | | | |
| R | 7.6 | 0.46 | 0.65 | 11 | 269 | 0.7 | 10.16 | 13.02 | 0.68 | 1.24 | 26 |
| NR | 7.7 | 0.50 | 0.50 | 10 | 269 | 0.6 | 10.46 | 13.04 | 1.16 | 1.34 | 20 |

NBt: Non-Bt-Cotton, Bt: Bt-Cotton, R: Rhizospheric, NR: Non-rhizospheric

Determination of Total Fungal Population

Unknown amount of air dried soil sample was added into 100 ml double distilled water and shaken it properly. Serially diluted it up to 10⁻⁴ using by dilution plate method (Timonin, 1940) [36]. 0.5 ml solution poured in Petri plate containing potato dextrose agar. Inoculated Petri plates were incubated in BOD for 2-4 days at a temperature of 25°C. After 2-4 days of serial dilution fungal colony was observed and numbers of colonies were note down. Types of colony, texture of colony, colour of colony and every aspects of morphological identification was done. Cotton blue staining was used and after staining fungus were examined under the light microscope at appropriate magnification (4X, 10X, 40X, 100 X) identification of fungus by using compound microscope. Fungus were isolated and confirmatory identification was done by using “manual of soil fungi” (Gilman, 1957) [11].

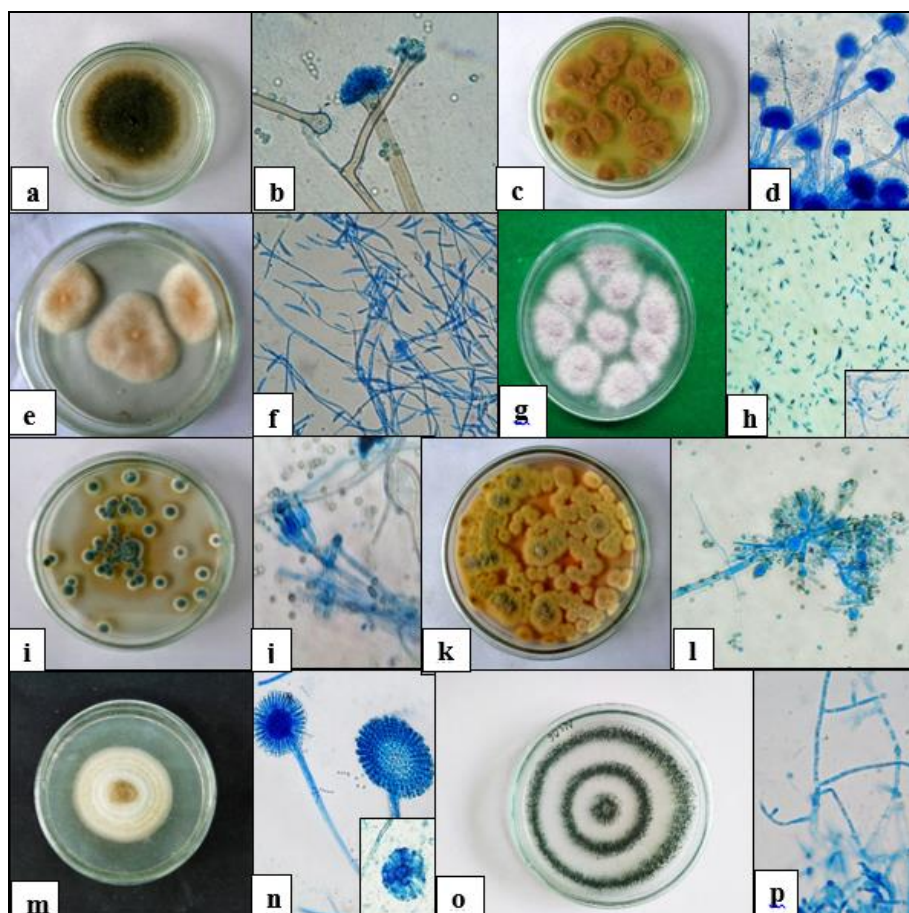


Plate 1: Isolated fungus from Cotton Plants isolated through enriched culture method.

a-b:*Aspergillus nidulans*, c-d:*Aspergillus terreus*, e-f:*Fusarium dimerum*, g-h:*Fusarium oxysporum*, i-j:*Penicillium chrysogenum*, k-l:*Penicillium marneffei*, m-n:*Syncephalastrum* sp., o-p:*Trichoderma* sp.

Determination of Total Bacterial Population

Add quantity of air dried soil sample was added into 100 ml double distilled water and shaken it properly. Serial diluted it up to 10^{-5} using by dilution plate method (Timonin, 1940) [36]. 0.1 ml solution poured in Petri plate containing respective media according to bacterial isolation like: Nutrient agar (Total Bacteria), Yeast extract mannitol agar (*Rhizobium* sp.), Kings B (*Pseudomonas* sp.) and Ashby's Mannitol Agar (*Azotobacter* sp.). Inoculated Petri plate were incubated in BOD at temperature $35^{\circ}\text{C} \pm 2$ for 24 hours. After 24 hours of serial dilution bacterial colony observe and number of colony by using bacterial colony counter (MAK Comp.), texture of colony, colour of colony and every aspects of morphological identification was noted.

Results and Discussion

Total bacterial population per gram soil in different stages at different survey site have been presented in Table 2. Maximum population was recorded in flowering stage and lowest in seedling stage in Bt-cotton and Non Bt-cotton. The results which depicted in table 2 is clearly showed bacterial population is more in Non-Bt-cotton as compare to Bt-cotton in both rhizospheric soil and non-rhizospheric soil.

Table 2: Per gram population of total bacterial from rhizospheric and non-rhizospheric soil from Non-Bt-cotton and Bt -cotton fields at different stages in Jalgaon (Diksal), Maharashtra, India

| Bacteria | Population of Isolated Bacteria($1 \times 10^5 \text{ g}^{-1}$) | | | | | | | | | | | |
|------------------------|---|-------|------|------|------------|-------|------|------|-----------|-------|------|------|
| | Seedling | | | | Vegetative | | | | Flowering | | | |
| | NBt-R | NBt-N | Bt-R | Bt-N | NBt-R | NBt-N | Bt-R | Bt-N | NBt-R | NBt-N | Bt-R | Bt-N |
| Total Bacteria | 4.2 | 1.8 | 2.3 | 1.4 | 7.4 | 2.6 | 4.9 | 1.9 | 11.2 | 4.0 | 7.9 | 2.8 |
| <i>Rhizobium</i> sp. | 3.8 | 1.6 | 2.2 | 0.9 | 6.7 | 2.0 | 2.3 | 1.5 | 9.5 | 3.4 | 7.8 | 2.6 |
| <i>Azotobacter</i> sp. | 1.8 | 0.8 | 1.4 | 0.8 | 2.6 | 1.0 | 1.7 | 1.0 | 4.7 | 1.6 | 3.3 | 1.1 |
| <i>Pseudomonas</i> sp. | 1.9 | 0.9 | 1.3 | 0.5 | 3.1 | 1.1 | 2.3 | 1.0 | 5.9 | 2.0 | 4.6 | 1.5 |

NBt: Non-Bt-Cotton, Bt: Bt-Cotton, R: Rhizospheric soil, NR: Non Rhizospheric soil All data shows average of 3 sampling (n=3)

Table 3,4,5,6 show the results of mycoflora diversity in Bt and non-Bt cotton both rhizospheric non-rhizospheric soil sample. The results show that non-Bt cotton has a greater diversity of mycoflora than Bt cotton both in rhizospheric and non-rhizospheric soil. *Fusarium* species were the most common fungi in both Bt and non-Bt cotton of rhizospheres soil. Other frequent fungus detected in the rhizosphere of both types of plants included *Aspergillus*, *Alternaria*, *Curvularia*, *Rhizopus*, and *Penicillium*, however their abundance was highest in non-Bt cotton rhizosphere soil when compared to Bt cotton. Several studies have found that genetically engineered plants alter microbial rhizosphere activity. The results clearly suggest that non-Bt cotton plant growth is greater than Bt cotton plant growth, and a non-Bt cotton rhizosphere soil sample revealed an increased number of fungus populations. The results of the microbial population analysis show that Bt plants influenced the fungus in the cultural soil. According to the findings, Bt cotton has a negative impact on soil microflora and is not safe for rhizosphere organisms. *Aspergillus*, *Penicillium* and *Fusarium* showing highest frequency which is presented in Table 7.

Table 3: List of Fungi Isolated from the rhizospheric soil of Non-Bt-cotton fields at different stages in Diksal, Jalgaon, Maharashtra, India

| Isolated Fungus | Population of filamentous fungi ($1 \times 10^4 \text{ g}^{-1}$ oven dry soil) | | | | | | | | | | | |
|--------------------------------|---|-----|-----|---------|------------|-----|-----|---------|-----------|------|------|---------|
| | Seedling | | | | Vegetative | | | | Flowering | | | |
| | I | II | III | Average | I | II | III | Average | I | II | III | Average |
| <i>Alternaria alternata</i> | 2.8 | 3.1 | 1.8 | 2.5 | 2.8 | 1.1 | 2 | 1.9 | 6.6 | - | 1.2 | 2.6 |
| <i>Aspergillus flavus</i> | - | - | 2.8 | 0.9 | 1.4 | 2.3 | 1 | 1.5 | - | 8.5 | 1.2 | 3.2 |
| <i>Aspergillus fumigatus</i> | 6.0 | - | 6.5 | 5.8 | - | - | 3.1 | 2.2 | - | 5.7 | - | 1.9 |
| <i>A. nidulans</i> | 4.8 | 5.1 | 2.8 | 4.2 | - | 3.4 | - | 1.1 | 3.3 | - | 1.3 | 1.5 |
| <i>A. niger</i> | 7.2 | 4.1 | 4.6 | 5.3 | 11.4 | 5.7 | 6.2 | 7.7 | 6.8 | 12.3 | 5.5 | 8.2 |
| <i>Aspergillus terreus</i> | 6.4 | 0.3 | 0.3 | 2.3 | 5.7 | 2.3 | 6.2 | 4.7 | 8.3 | 12.3 | 7.8 | 9.4 |
| <i>Aspergillus</i> sp. | 2.4 | 2 | - | 1.4 | 4.2 | - | - | 1.4 | 5 | - | 2.4 | 2.4 |
| <i>Aspergillus</i> sp.1 | - | - | - | - | - | - | 3.7 | 1.2 | 3.3 | - | - | 1.1 |
| <i>Curvularia lunata</i> | - | 1 | 1.8 | 0.9 | 4.2 | - | - | 1.4 | 4.4 | - | 2.4 | 2.2 |
| <i>Fusarium oxysporum</i> | 2.4 | 1 | - | 1.1 | 4.7 | 1.9 | - | 2.2 | - | 4.2 | 3.6 | 2.6 |
| <i>Fusarium dimerum</i> | 1.2 | - | 1.8 | 1.0 | 1.9 | 3.4 | 1 | 2.1 | 14.4 | 10.4 | 3.6 | 9.4 |
| <i>F. udum</i> | - | - | - | - | - | 3.4 | 1.0 | 1.4 | 3.6 | - | 4.2 | 2.6 |
| <i>Microsphaeropsis</i> sp | - | - | - | - | - | - | 3.4 | 1.1 | 3.2 | 2.4 | - | 1.8 |
| <i>Penicillium chrysogenum</i> | 2.4 | - | - | 0.8 | 4.2 | - | 2.4 | 2.2 | 1.6 | 5.7 | - | 2.4 |
| <i>Penicillium marneffeii</i> | 4.8 | 1.0 | 5.0 | 3.6 | 5.7 | 3.4 | 2 | 3.7 | 8 | 7.6 | 11.2 | 8.9 |
| <i>Penicillium</i> sp. | 2.4 | 5.1 | - | 2.5 | 7.1 | 3.4 | 2 | 4.1 | 13.3 | - | - | 4.4 |
| <i>Penicillium</i> sp. 1 | - | - | - | - | - | - | - | - | 6.6 | 1.4 | 8.4 | 5.4 |

| | | | | | | | | | | | | |
|----------------------------|-----|-----|-----|------|-----|-----|-----|------|-----|-----|-----|-------|
| <i>Penicillium</i> sp. 2 | - | - | - | - | 2.3 | 3.4 | - | 1.9 | - | - | - | - |
| <i>Penicillium</i> sp.3 | - | - | - | - | - | - | 2.4 | 0.8 | 1.6 | - | - | 0.5 |
| <i>Rhizoctonia</i> sp. | 2.4 | - | - | 0.8 | 1.9 | - | 1.0 | 0.9 | 3.3 | - | - | 1.1 |
| <i>Rhizopus stolonifer</i> | - | 1 | 1.8 | 0.9 | 2.8 | 1.1 | - | 1.3 | 3.3 | - | - | 1.1 |
| <i>Syncephalastrum</i> sp. | 1.2 | - | 1.8 | 1.0 | 1.4 | 2.3 | - | 1.2 | - | 1.4 | 3.6 | 1.6 |
| <i>Trichoderma</i> sp. | - | - | - | - | 2.8 | - | 0.6 | 1.1 | - | 1.4 | 2.4 | 1.2 |
| Sterile mycelium | 7.2 | 1.0 | - | 2.7 | 2.8 | 3.4 | 4.1 | 3.4 | 8.8 | 1.9 | 4.8 | 5.1 |
| Total | | | | 37.7 | | | | 50.5 | | | | 80.66 |

Table 4: List of Fungi Isolated from the non-rhizospheric soil of Non -Bt-cotton fields at different stages in Diksai, Jalgaon, Maharashtra, India

| Isolated Fungus | Population of filamentous fungi ($1 \times 10^4 \text{ g}^{-1}$ oven dry soil) | | | | | | | | | | | |
|--------------------------------|---|-----|-----|---------|------------|-----|-----|---------|-----------|-----|-----|---------|
| | Seedling | | | | Vegetative | | | | Flowering | | | |
| | I | II | III | Average | I | II | III | Average | I | II | III | Average |
| <i>Alternaria alternata</i> | - | 0.8 | 0.6 | 0.4 | - | 0.3 | - | 0.1 | - | - | 0.9 | 0.3 |
| <i>Aspergillus flavus</i> | 0.9 | - | - | 0.3 | 3.2 | 5.0 | 0.9 | 3.03 | - | - | 1.2 | 0.4 |
| <i>A. fumigatus</i> | - | - | - | - | - | 0.3 | - | 0.1 | - | - | 2.4 | 0.8 |
| <i>A. nidulans</i> | 0.3 | - | - | 0.1 | - | 2.8 | 1.6 | 1.4 | - | 3.2 | - | 1.0 |
| <i>A. niger</i> | 7.2 | 6.2 | 3.6 | 5.6 | 3.3 | 4.0 | 6.2 | 4.5 | 4.6 | 3.9 | 8.8 | 5.7 |
| <i>Aspergillus terreus</i> | 2.2 | 2 | 0.8 | 1.6 | 2.7 | - | 1.8 | 1.5 | 0.9 | - | - | 0.3 |
| <i>Aspergillus</i> sp.1 | - | - | - | - | - | 2.3 | 2.0 | 1.4 | 9 | - | - | 3.0 |
| <i>Curvularia lunata</i> | - | 0.9 | - | 0.3 | - | - | 1.5 | 0.5 | 0.9 | - | - | 0.3 |
| <i>Fusarium dimerum</i> | - | - | - | - | - | - | 0.3 | 0.1 | - | - | 2.4 | 0.8 |
| <i>Penicillium chrysogenum</i> | 2.4 | - | 1.2 | 1.2 | - | - | - | 0 | - | - | 0.9 | 0.3 |
| <i>Penicillium marneffeii</i> | 4 | 4.9 | - | 2.9 | 0 | 0.9 | 3.8 | 1.56 | 2 | - | - | 0.6 |
| <i>Penicillium</i> sp. | 1.2 | - | - | 0.4 | 0.8 | 1.2 | 0 | 0.66 | 3.4 | - | - | 1.1 |
| <i>Penicillium</i> sp. 2 | - | - | 0.9 | 0.3 | - | - | - | - | 2.3 | 3.7 | - | 2.0 |
| <i>Rhizopus stolonifer</i> | 0.9 | - | - | 0.3 | 0 | 0.3 | 0 | 0.1 | 1.2 | - | - | 0.6 |
| Sterile mycelium | 1.2 | - | - | 0.4 | 4.1 | 2.7 | 0.9 | 2.5 | 4 | 2.3 | 3.4 | 3.2 |
| Total | | | | 13.80 | | | | 17.45 | | | | 20.40 |

Table 5: List of Fungi Isolated from the rhizospheric soil of Bt-cotton fields at different stages in Diksai, Jalgaon, Maharashtra, India

| Isolated Fungus | Population of filamentous fungi ($1 \times 10^4 \text{ g}^{-1}$ oven dry soil) | | | | | | | | | | | |
|--------------------------------|---|-----|-----|---------|------------|-----|-----|---------|-----------|------|-----|---------|
| | Seedling | | | | Vegetative | | | | Flowering | | | |
| | I | II | III | Average | I | II | III | Average | I | II | III | Average |
| <i>Alternaria alternata</i> | 1.1 | - | - | 0.3 | - | 3.4 | - | 1.1 | - | - | 1.0 | 0.3 |
| <i>Aspergillus flavus</i> | - | 0.6 | 1.2 | 0.6 | - | 0.9 | 2.1 | 1.0 | - | 2.0 | - | 0.6 |
| <i>A. fumigatus</i> | 3.0 | - | 2.9 | 1.9 | - | - | 1 | 0.3 | - | - | - | - |
| <i>A. nidulans</i> | 3.3 | - | - | 1.1 | 6.3 | - | - | 2.1 | 5.1 | - | - | 1.7 |
| <i>A. niger</i> | 8.1 | 2.2 | 5.8 | 5.3 | 2 | 3 | 5.4 | 3.4 | 4.8 | 1.2 | 2.2 | 2.7 |
| <i>A. terreus</i> | - | 1.1 | 2.5 | 1.2 | 8.7 | 8.2 | 9.3 | 8.7 | 14.5 | 11.3 | 9.4 | 11.7 |
| <i>Aspergillus</i> sp.2 | - | - | - | - | - | - | 2.3 | 0.7 | 1.4 | 2.5 | 3.7 | 2.5 |
| <i>Curvularia lunata</i> | - | - | - | - | - | 1 | - | 0.3 | 3.1 | - | 2.8 | 1.9 |
| <i>Fusarium oxysporum</i> | 1.9 | - | 2.2 | 1.3 | 1 | - | - | 0.3 | 2.1 | 1.5 | - | 1.2 |
| <i>F. dimerum</i> | 1.3 | 0.2 | 1.0 | 0.8 | 2.8 | 1.9 | 2.2 | 2.3 | - | - | 2.6 | 0.8 |
| <i>Penicillium chrysogenum</i> | 2.4 | - | - | 0.8 | - | 1.5 | 3.8 | 1.76 | - | - | 2.2 | 0.7 |
| <i>Penicillium marneffeii</i> | 1.7 | 3.0 | - | 1.5 | - | 2.4 | 3.1 | 1.8 | 6.8 | - | 8.3 | 5.0 |
| <i>Penicillium</i> sp. | 1.8 | - | - | 0.6 | - | 0.9 | 1.1 | 0.6 | 8.4 | 3.6 | - | 4.0 |
| <i>Penicillium</i> sp. 2 | 2.0 | - | - | 0.6 | 0.9 | - | - | 0.3 | - | - | - | - |
| <i>Rhizoctonia</i> sp. | - | 2.3 | 1.0 | 1.1 | - | 2.1 | - | 0.7 | - | - | - | - |
| <i>Rhizopus stolonifer</i> | - | 0.9 | - | 0.3 | - | - | 2.8 | 0.9 | 4.2 | - | - | 1.4 |
| Sterile mycelium | 0.6 | 0.9 | 4.0 | 1.8 | 2.2 | 3.4 | 2.4 | 2.6 | 6.2 | - | 3.4 | 3.2 |
| Total | | | | 19.2 | | | | 29.36 | | | | 37.7 |

Table 6: List of Fungi Isolated from the non-rhizospheric soil of Bt-cotton fields at different stages in Diksai , Jalgaon, Maharashtra, India

| Isolated Fungus | Population of filamentous fungi (1x10 ⁴ g ⁻¹ oven dry soil) | | | | | | | | | | | |
|--------------------------------|---|-----|-----|---------|------------|-----|-----|---------|-----------|-----|-----|---------|
| | Seedling | | | | Vegetative | | | | Flowering | | | |
| | I | II | III | Average | I | II | III | Average | I | II | III | Average |
| <i>A. flavus</i> | 0.9 | 0.8 | 1.5 | 1.0 | - | 0.9 | - | 0.3 | 1 | - | - | 0.3 |
| <i>A. nidulans</i> | 3.2 | - | - | 1.0 | 0.8 | - | - | 0.2 | - | 8.9 | 3.8 | 4.2 |
| <i>A. niger</i> | 4.6 | 3.9 | 4.8 | 4.4 | 4.2 | 3.2 | 2.8 | 3.4 | 8.9 | 3.5 | 8.2 | 6.8 |
| <i>Aspergillus terreus</i> | 3.4 | 2.3 | 1.9 | 2.5 | 6.2 | 8.3 | 7.7 | 7.4 | - | - | 4.1 | 1.3 |
| <i>Aspergillus</i> sp.1 | - | - | - | - | - | 0.3 | 1 | 0.4 | - | - | - | - |
| <i>Aspergillus</i> sp.2 | - | 1.8 | - | 0.6 | - | - | - | - | - | - | - | - |
| <i>Penicillium chrysogenum</i> | - | 1.5 | - | 0.5 | - | 0.9 | - | 0.3 | - | 3.9 | 1.8 | 1.9 |
| <i>Penicillium marneffeii</i> | - | 1.1 | - | 0.3 | 1.3 | - | - | 0.4 | 2.8 | - | - | 0.9 |
| <i>Penicillium</i> sp. | 3.2 | - | 2 | 1.7 | - | 1.5 | 1.0 | 0.8 | 1.8 | - | 2.3 | 1.3 |
| <i>Penicillium</i> sp.2 | - | - | - | - | 4.8 | 3.2 | - | 2.6 | - | - | 1.2 | 0.4 |
| <i>Rhizopus stolonifer</i> | - | - | - | - | - | - | 0.9 | 0.3 | 2.3 | - | - | 0.7 |
| Sterile mycelium | 2.1 | 1.9 | 2.3 | 2.1 | 0.4 | 1.2 | - | 0.5 | - | 2.4 | 3.4 | 1.9 |
| Total | | | | 14.4 | | | | 16.8 | | | | 19.7 |

Table 7: Frequency of isolated fungus at Diksai, Jalgaon, Maharashtra

| Isolated Fungus | Non- Bt-R | | | % | Non-Bt-N | | | % | Bt-R | | | % | Bt-N | | | % |
|--------------------------------|-----------|-----|-----|-------|----------|-----|-----|-------|------|-----|-----|-------|------|-----|-----|-------|
| | S | V | F | | S | V | F | | S | V | F | | S | V | F | |
| <i>Alternaria alternata</i> | +++ | +++ | ++ | 88.88 | ++ | + | + | 44.44 | + | + | + | 33.33 | - | - | - | - |
| <i>Aspergillus flavus</i> | + | +++ | ++ | 66.66 | + | +++ | + | 55.55 | ++ | ++ | + | 55.55 | +++ | + | + | 55.55 |
| <i>A. fumigatus</i> | ++ | + | + | 44.44 | - | + | + | 22.22 | ++ | + | - | 33.33 | - | - | - | - |
| <i>A. nidulans</i> | +++ | + | ++ | 66.66 | + | ++ | + | 44.44 | + | + | + | 33.33 | + | + | ++ | 44.44 |
| <i>A. niger</i> | +++ | +++ | +++ | 100 | +++ | +++ | +++ | 100 | +++ | +++ | +++ | 100 | +++ | +++ | +++ | 100 |
| <i>A. terreus</i> | +++ | +++ | +++ | 100 | +++ | ++ | + | 66.66 | ++ | +++ | +++ | 88.88 | +++ | +++ | + | 77.77 |
| <i>Aspergillus</i> sp. | ++ | + | ++ | 55.55 | - | - | - | - | - | - | - | - | - | - | - | - |
| <i>Aspergillus</i> sp.1 | - | + | + | 22.22 | - | ++ | + | 22.22 | - | - | - | - | - | ++ | - | 22.22 |
| <i>Curvularia lunata</i> | ++ | + | ++ | 55.55 | + | + | + | 33.33 | - | + | ++ | 33.33 | - | - | - | - |
| <i>Fusarium oxysporum</i> | ++ | ++ | ++ | 66.66 | - | - | - | - | ++ | + | ++ | 55.55 | - | - | - | - |
| <i>Fusarium dimerum</i> | ++ | +++ | +++ | 88.88 | - | - | - | - | +++ | +++ | + | 77.77 | - | - | - | - |
| <i>Fusarium udum</i> | - | ++ | ++ | 44.44 | - | - | - | - | - | - | - | - | - | - | - | - |
| <i>Microsphaeropsis</i> sp. | - | + | ++ | 33.33 | - | - | - | - | - | - | - | - | - | - | - | - |
| <i>Penicillium chrysogenum</i> | + | ++ | ++ | 55.55 | ++ | - | + | 33.33 | + | ++ | + | 44.44 | + | + | ++ | 44.44 |
| <i>P. marneffeii</i> | +++ | +++ | +++ | 100 | ++ | ++ | + | 55.55 | ++ | ++ | ++ | 66.66 | + | + | + | 33.33 |
| <i>Penicillium</i> sp. | ++ | +++ | + | 66.66 | + | ++ | + | 44.44 | + | ++ | ++ | 55.55 | ++ | ++ | ++ | 66.66 |
| <i>Penicillium</i> sp. 1 | - | - | +++ | 33.33 | - | - | - | - | - | - | - | - | - | ++ | ++ | 44.44 |
| <i>Penicillium</i> sp. 2 | - | ++ | - | 22.22 | + | - | ++ | 33.33 | + | + | - | 22.22 | - | ++ | + | 33.33 |
| <i>Penicillium</i> sp.3 | - | + | + | 22.22 | - | - | - | - | - | - | - | - | - | - | - | - |
| <i>Rhizoctonia</i> sp. | + | ++ | + | 44.44 | - | - | - | - | - | - | - | - | - | - | - | - |
| <i>Rhizopus stolonifer</i> | ++ | ++ | + | 55.55 | + | + | + | 33.33 | + | + | + | 33.33 | - | + | + | 22.22 |
| <i>Syncephalastrum</i> sp. | ++ | ++ | ++ | 66.66 | - | - | - | - | - | - | - | - | - | - | - | - |
| <i>Trichoderma</i> sp. | - | ++ | ++ | 33.33 | - | - | - | - | - | - | - | - | - | - | - | - |
| Sterile mycelium | ++ | +++ | +++ | 88.88 | + | +++ | +++ | 77.77 | +++ | +++ | ++ | 88.88 | +++ | ++ | ++ | 77.77 |

S: Seedling, V: Vegetative, F: Flowering R: Rhizospheric Soil, N: Non-rhizospheric Soil

Conclusion

A significant difference in Rhizosphere and non-Rhizosphere soil fungus and bacterial count between Bt and non-Bt cotton clearly demonstrated the transgenic influence of Bt-cotton on soil flora. Microbiological abundance in non-Bt soil revealed the non-toxication effect while the toxic effect of the Cry1AC gene reduced

the microbial population. Therefore, Bt may not be safe for the non-targeted microbes including rhizosphere and non-rhizosphere mycoflora.

Acknowledgements

The authors are thankful to University Grant Commission (UGC), New Delhi and Head of Botany Department of University of Allahabad, Prayagraj, India for providing financial assistance and laboratory facilities. Uma Singh also thankful to Prof. Harbans Kaur Kehri, Department of Botany, University of Allahabad, Prayagraj, India, Dr. IfraZoomi, Dr. Ovaaid Akhtar, Dr. Dheeraj Pandey and Kanhaiya Lal Chaudhary, Department of Botany University of Allahabad for their valuable suggestion during manuscript preparation.

References

- Bai L, Zhang L, Chen X, Feng H. Composition and diversity of the weed community in transgenic Bt cotton (four Bollgard strains) fields. *Acta Phytoecol Sin*,2003;27:610-616.
- Barwale RB, Gadwal VR, Zehr U, Zehr B. Prospects for Bt cotton technology in India, 2004.
- Beura K, Rakshit A. Bt cotton influencing enzymatic activities under varied soils. *Open Journal of Ecology*, 2013.
- Bradley R, Burt AJ, Read DJ. Mycorrhizal infection and resistance to heavy metal toxicity in *Calluna vulgaris*. *Nature*,1981;292(5821):335-337.
- Bridge P, Spooner B. Soil fungi: diversity and detection. *Plant and soil*,2001;232(1):147-154.
- Cui J, Xia J. Effects of transgenic Bt cotton R93-6 on the insect community. *Acta EntomologicaSinica*,2000;4:343-351.
- Donegan KK, Seidler RJ. Effects of transgenic plants on soil and plant microorganisms (pp. 415-424). US Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Western Ecology Division, 1999, 415-424.
- Dunfield KE, Germida JJ. Diversity of bacterial communities in the rhizosphere and root interior of field-grown genetically modified *Brassica napus*. *FEMS Microbiology Ecology*,2001;38(1):1-9.
- Dunfield KE, Germida JJ. Impact of genetically modified crops on soil-and plant-associated microbial communities. *Journal of environmental quality*,2004;33(3):806-815.
- Fang M, Motavalli PP, Kremer RJ, Nelson KA. Assessing changes in soil microbial communities and carbon mineralization in Bt and non-Bt corn residue-amended soils. *Applied Soil Ecology*,2007;37:150-160.
- Gilman JC. A manual of soil fungi. Oxford and Ibh Publishing Co., New Delhi, 1957.
- Glick BR. The enhancement of plant growth by free-living bacteria. *Canadian journal of microbiology*,1995;41(2):109-117.
- Griffiths BS, Birch ANE, Caul S, Thompson J, Heckmann LH, Krogh PH *et al*. The role of laboratory, glasshouse and field scale experiments in understanding the interactions between genetically modified crops and soil ecosystems: A review of the ECOGEN project. *Pedobiology*,2007;51(3):251-260.
- Icoz I, Stotzky G. Fate and effects of insect-resistant Bt crops in soil ecosystems. *Soil Biol. Biochem*,2008;40:559-586.
- Kapur M, Bhatia R, Pandey G, Pandey J, Paul D, Jain RK. A case study for assessment of microbial community dynamics in genetically modified Bt cotton crop fields. *Current microbiology*,2010;61(2):118-124.
- Li J, Liu Q, Jin X, Liu J, Liu Y. The niches of major pests and natural enemies between Bt cotton and common cotton. *Entomology Journal of East China*,2002;11:51-54.
- Liu N, Zhu P, Peng C, Kang L, Gao H, Clarke NJ *et al*. Effect on soil chemistry of genetically modified (GM) vs. non-GM maize. *GM crops*,2010;1(3):157-161.
- Liu W, Wan F, Guo J. Structure and seasonal dynamics of arthropods in a transgenic Bt cotton field. *Acta EcologicaSinica*,2002;22:729-735.
- McGregor AN, Turner MA. Soil effects of transgenic agriculture: biological processes and ecological consequences. *NZ Soil News*,2000;48(6):166-169.
- Men X, Ge F, Yin X, Cai D. Diversities of arthropod community in transgenic Bt cotton and nontransgenic cotton agroecosystems. *Chinese Journal of Ecology*,2003;22:26-29.
- Mina U, Khan SA, Choudhary A, Choudhary R, Aggarwal PK. An approach for impact assessment of transgenic plants on soil ecosystem. *Appl Ecol Environ Res*,2008;6(3):1-19.
- Nehl DB, Allen SJ, Brown JF. Deleterious rhizosphere bacteria: an integrating perspective. *Applied Soil Ecology*,1997;5(1):1-20.
- Palm CJ, Schaller DL, Donegan KK, Seidler RJ. Persistence in soil of transgenic plant-produced *Bacillus thuringiensis* var. *kurstaki* δ -endotoxin. *Can. J. Microbiol*,1996;42:1258-1262.
- Rui YK, Yi GX, Zhao J, Wang BM, Li ZH, Zhai ZX *et al*. Changes of Bt toxin in the rhizosphere of transgenic Bt cotton and its influence on soil functional bacteria. *World Journal of Microbiology and Biotechnology*,2005;21(6):1279-1284.
- Sarkar B, Patra A. T. P.-E. monitoring, undefined. Assessment of biological and biochemical indicators in soil under transgenic Bt and non-Bt cotton crop in a sub-tropical environment. *Springer*,2009;156(1-4):595-604. <https://doi.org/10.1007/s10661-008-0508-y>

26. Saxena D, Stotzky G. Insecticidal toxin from *Bacillus thuringiensis* is released from roots of transgenic Bt corn *in vitro* and *in situ*. *FEMS Microbiology Ecology*,2000;33(1):35-39. <https://doi.org/10.1111/J.1574-6941.2000.TB00724.X>
27. Saxena D, Stotzky G. *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*,2001;33(9):1225-1230.
28. Saxena D, Stewart CN, Altosaar I, Shu Q, Stotzky G. Larvicidal Cry proteins from *Bacillus thuringiensis* are released in root exudates of transgenic *B. thuringiensis* corn, potato, and rice but not of *B. thuringiensis* canola, cotton, and tobacco. *Plant Physiol. Biochem*,2004;42:383-387.
29. Saxena D, Flores S, Stotzky G. Transgenic plants: Insecticidal toxin in root exudates from Bt corn. *Nature*, 1999, 402:480.
30. Saxena D, Flores S, Stotzky G. Bt toxin is released in root exudates from 12 transgenic corn hybrids representing three transformation events. *Soil Biol. Biochem*,2002b;34:133-137.
31. Shetty KG, Hetrick BAD, Figge DAH, Schwab AP. Effects of mycorrhizae and other soil microbes on revegetation of heavy metal contaminated mine spoil. *Environmental pollution*,1994;86(2):181-188.
32. Stotzky G. Persistence and biological activity in soil of the insecticidal proteins from *Bacillus thuringiensis*, especially from transgenic plants. *Plant and Soil*,2004;266:77-89.
33. Sun CX, Chen LJ, Wu ZJ, Zhou LK, Shimizu H. Soil persistence of *Bacillus thuringiensis* (Bt) toxin from transgenic Bt cotton tissues and its effect on soil enzyme activities. *Biology and Fertility of Soils*,2007;43(5):617-620.
34. Tan S, Chen X, Li D. Progress in the studies on *Helicoverpa* spp Resistance to transgenic Bt cotton and its management strategy. *Acta Entomologica Sinica*,2002;45:138-144.
35. Tapp H, Stotzky G. Insecticidal activity of the toxins from *Bacillus thuringiensis* subspecies *kurstaki* and *tenebrionis* adsorbed and bound on pure and soil clays. *Applied and Environmental Microbiology*,1995;61(5):1786-1790.
36. Timonin MI. The interaction of higher plants and microorganisms I. microbial population of rhizosphere of seedlings of certain cultivated plants. *Can. J. Res*,1940;18:307.
37. Tsatsakis AM, Nawaz MA, Kouretas D, Balias G, Savolainen K, Tutelyan VA *et al*. Environmental impacts of genetically modified plants: a review. *Environmental research*,2017;156:818-833.
38. Wei XD, Zou HL, Chu LM, Liao B, Ye CM, Lan CY. Field released transgenic papaya affects microbial communities and enzyme activities in soil. *Plant and Soil*,2006;285(1-2):347-358.
39. Wendel JF, Cronn RC. Polyploidy and the evolutionary history of cotton. *Advances in agronomy*,2003;78(13986):78004-8.
40. Yaqoob A, Shahid AA, Samiullah TR, Rao AQ, Khan MAU, Tahir S *et al*. Risk assessment of Bt crops on the non-target plant-associated insects and soil organisms. *Journal of the Science of Food and Agriculture*,2016;96(8):2613-2619.
41. Zhang Q, Li S, Chen Z, Fu H, Yang Y. Study of the population dynamics changes of the beneficial and injurious living beings on the field of Bt cotton. *Journal of Hebei Normal University. Natural Science Edition*,2000;24:4521-523.
42. Zwahlen C, Hilbeck A, Gugerli P, Nentwig W. Degradation of the Cry1Ab protein within transgene, 2003a.