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# Succession of phylloplane mycoflora of transgenic bt cotton (JKCH 8836 BG II)

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**Abstract**
Bt cotton, a transgenic crop variety is extensively cultivated in Telangana state especially in Warangal district. This pest resistant variety is reported to be infected by number of diseases. In the present investigations, incidence and succession of phylloplane mycoflora was studied with a premise to assess its role in leaf spot diseases. The results of this study reveal that the incidence of different species varied with the age of the leaf. A total of 39 fungal species representing 24 genera were recorded. Few fungi like *Aspergillus flavus*, *A. niger*, *Cladosporium cladosporioides*, *Rhizopus stolonifer* and *Penicillium chrysogenum*, were recorded in all the stages of the leaf phenology. The percentage of frequency and abundance also varied with the species. The incidence of a large number of fungi from young to senescent leaves indicates that phylloplane fungi play a definite role in leaf spot disease and decomposition of leaf litter.

**Keywords:** Bt cotton, phylloplane, mycoflora, succession

## Introduction
Plant surface i.e., leaves, buds, flowers, branches, stem and roots are colonized by a variety of microorganisms such as fungi, bacteria, actinomycetes. Phylloplane is a natural habitat for a large number of various microorganisms on the surface of the leaf and provide nutrients to microorganisms. They play a significant role in either disease resistance or susceptibility. Some of these microbes are capable of causing leaf spot and post-harvest diseases.

The surface of leaves contain stimulatory or inhibitory substances that regulate the colonization of leaf surface organisms (Pugh and Buckley, 1971) [1]. Plant pathogenic fungi spend a critical period on the phylloplane before they cause infection. Similarly, saprophytes and weak pathogens, which initiate the decomposition of the tissue, are delayed until the host resistance decreases with the onset of senescence that predisposes initial penetration into tissues by the propagules (Potter, 1910) [2]. Non-pathogenic phylloplane organisms may also important in determining the severity of plant diseases caused by pathogenic fungi (Fating and Khare, 1978) [3].

In the present investigations is an attempt to analyze the succession of mycoflora of transgenic Bt cotton during the crop season of 2016-2017 with an objective to assess the role of mycoflora in leaf spot disease incidence, senescence and further decomposition of leaf litter.

## Material and Methods
### Collection of Samples
Mature and healthy leaves were collected randomly from Bt cotton (JKCH 8836 BG II) field in the Warangal district, Telangana State, India and brought to the laboratory in separate polythene bags to avoid contamination.

### Preparation of potato dextrose agar medium (PDA)
Peeled and sliced potatoes of 200g were boiled in 500ml distilled water for 30 minutes till they become soft. The supernatant were decanted and filtered the extract through muslin cloth and squeezed gently. Added dextrose (20g) and agar (20g) transferred into the extract and swirled to dissolve the ingredients. The Medium was made up to 1000 ml, pH was adjusted to 5.6 and then sterilized in autoclave at 15 psi for 15mts.

### Isolation of Fungi
Phylloplane mycoflora of cotton was analyzed by dilution plate method (Dickinson, 1971) [4]. Disc of 3 mm diameter cut from a number of leaves selected randomly, using a sterile cork borer. Fifty discs were placed in a 250ml of conical flask containing 100ml of sterile water thoroughly shaken for half an hour to get a homogeneous suspension. This solution was

further diluted serially (1:1000, 1:10000). The above solution (1ml) was poured aseptically in to sterilized petriplate and then sterilized and cooled PDA (Potato Dextrose Agar) medium was poured by making gentle rotational movements. Such plates after solidification were incubated at $(27\pm2)^0$C for 7 days. Rose bengal was added to the medium to suppress the growth of bacteria. The fungal colonies developed on agar plates were further purified, sub cultured and identified.

**Identification of Fungi**

The isolated fungal species were identified by the examining the colony morphology, structure of mycelium, spores and fruit bodies. The phylloplane mycoflora was identified using the standard taxonomic manuals such as Dematiaceous Hyphomycetes (Ellis, 1971) [5], Illustrated Genera of Imperfect Fungi (Barnett, 1972) [6], and Microbiology of Aerial Plant Surface (Dickinson and Preece, 1976) [7].

The percentage of incidence, frequency and abundance were calculated by using the following formulae.

$$\% \text{ of incidence} = \frac{\text{Number of colonies of a species in all the plates}}{\text{Total number of colonies of all the species in all the plates}} \times 100$$

$$\% \text{ of frequency} = \frac{\text{Number of observations in which a species appeared}}{\text{Total number of observations}} \times 100$$

$$\% \text{ of abundance} = \frac{\text{Total number of colonies of a species in all observations}}{\text{Total number of colonies in all observations}} \times 100$$

**Results**

A critical study of the table-1 reveals that phylloplane of transgenic Bt cotton is colonized by a variety of fungi, right from young leaves to senescent leaves. The incidence of different species varied with the age of the leaf. Many fungal species are sporadic in incidence. However, few fungi like *Aspergillus flavus*, *A. niger*, *Cladosporium cladosporioides Rhizopus stolonifer* and *Penicillium chrysogenum*, were recorded in all the stages of the leaves. The percentage of incidence also varied the fungus as well as age of the leaves. In general, mature leaves were colonized more fungal species followed by senescent and young leaves. This phenomenon can be understood as the young leaves take more time to be colonized by different fungal species. On the other hand mature leaves are metabolically active thus contributing to the exudates required for phylloplane fungi. Senescent leaves do not provide the nutrients required for the fungi. A total of 39 fungal species representing 24 genera were recorded. Among these, *Aspergillus* species (eight) dominated followed by *Fusarium* species. Percentage of frequency and abundance also varied with the species.

**Discussions**

Phylloplane micro flora varies in size and diversity depending on the impact of several biotic and abiotic factors, which affect their growth and survival (Bakker *et al.*, 2002) [8]. The factors include host, genotype, leaf age, external nutrients and interactions between populations of different microorganisms (Blakeman, 1985) [9]. The environmental variables such as temperature, moisture, humidity, and pH, different sampling periods have also been reported to affect the changes in population of specific phyllosphere fungi (Breeze and Dix, 1981) [10]. *Alternaria alternata*, *Aspergillus niger*, *A. fumigatus* and *Cladosporium oxysporum* were most frequently fungi on the phylloplane of *Ocimum sanctum* (Sharma, 2011) [11]. Fungi like *Alternaria alternata*, *A. tenuissima*, *A. flavus*, *A. fumigatus*, *A. terreus*. *Cladosporium cladosporioides*, *C. herbarum*, *Curvularia lunata*, *Fusarium equiseti* and *Penicillium purpurogenum* and white sterile forms were recorded on mature leaves of *Butea monosperma* (Deepika Chauhan *et al*., 2014) [12]. The incidence of phylloplane fungi have been reported in some vegetable crops and *Cladosporium cladosporoides* showed the highest incidence (Mari Bhat and Anusree, 2015) [13].

The variations observed in species richness and compositions of phyllosphere mycoflora on different leaf types during various sampling months can be assumed as the differences in competitive abilities, life cycle characteristics, potentialities to utilize residual organic chemical resources between the species present thereon (Osono, 2006) [14]. Different leaves of one and the same plant showed variation in the phyllosphere microbial population based on the position and age of the leaf (Ruinen, 1961) [15]. Larger amounts of nutrients are leached from ageing leaves than the young ones (Kerling, 1964) [16].

It is evident that there is no relation between percentage incidence, frequency and abundance of phylloplane mycoflora of Bt cotton and its age-group of the leaves. However, there are some species with greater percentage of incidences but found to be less in their frequencies. Similarly, the species with equal frequencies differed in their abundances.

**Table 1:** Succession of phylloplane mycoflora of bt cotton (JKCH 8836 BG II)

| Name of the fungi | % of Incidence | | | | | | | | % of Frequency | % of Abundance |
|---|---|---|---|---|---|---|---|---|---|---|
| | July | August | September | October | November | December | January | February | | |
| | Young leaves | | | Mature leaves | | | Senescent leaves | | | |
| *Alternaria alternata* | | 10 | | 4.16 | 2.77 | 4.83 | | 7.40 | 62.5 | 1.83 |
| *Alternaria macrospora* | 10.55 | | | 2.77 | 1.38 | | | | 37.5 | 1.22 |
| *Alternaria tenuis* | | | 2.08 | 2.77 | 3.22 | | 5.40 | | 50 | 1.37 |
| *Ascochyta gossypii* | | | | 4.16 | 3.22 | 4.83 | | | 37.5 | 1.83 |
| *Aspergillus flavipes* | | | | | 2.77 | 1.61 | | | 25 | 0.45 |
| *Aspergillus flavus* | 26.31 | 12.5 | 16.66 | 5.55 | 7.66 | 9.81 | 13.6 | 18.5 | 100 | 10.45 |
| *Aspergillus fumigatus* | | | | 5.55 | 1.38 | | | | 25 | 1.06 |
| *Aspergillus nidulans* | | 2.5 | 4.16 | 1.38 | 2.77 | | | | 50 | 2.44 |
| *Aspergillus niger* | 21.0 | 12.5 | 10.41 | 7.15 | 5.55 | 8.06 | 8.10 | 14.8 | 100 | 10.98 |
| *Aspergillus parasiticus* | | 5 | | 1.38 | | | | | 25 | 1.67 |
| *Aspergillus terreus* | | | 2.08 | 4.16 | 4.16 | 1.61 | 5.40 | 7.40 | 75 | 2.75 |
| *Aspergillus versicolor* | 5.26 | 5 | 4.16 | 2.77 | 4.16 | 1.61 | | | 75 | 2.74 |
| *Aureobasidium pullulans* | | | 4.16 | | 1.38 | | | | 25 | 0.61 |
| *Cercospora gossypii* | | 7.5 | 2.08 | | 2.77 | 1.61 | | | 50 | 1.22 |
| *Corynespora cassicola* | 5.26 | | 2.08 | | | 3.22 | | | 37.5 | 1.06 |
| *Chaetomium globosum* | | 7.5 | 4.16 | | 1.38 | | | 3.70 | 50 | 1.67 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Choanephora cucurbitarum* | | 7.5 | | 1.38 | 2.77 | | | | 37.5 | 1.22 |
| *Cladosporium cladosporioides* | 10.55 | 15 | 2.08 | 11.11 | 11.11 | 11.29 | 8.10 | 11.2 | 100 | 13.25 |
| *Cladosporium herbarum* | | | 4.16 | | | 4.83 | | | 25 | 1.22 |
| *Colletotrichum gossypii* | | | | | 2.77 | | 2.70 | 3.70 | 37.5 | 1.83 |
| *Curvularia lunata* | | | 4.16 | 2.77 | 2.77 | 4.83 | | 3.70 | 62.5 | 1.83 |
| *Drechslera spicifera* | | | 2.08 | 2.77 | | 1.61 | 2.70 | | 50 | 1.67 |
| *Fusarium equiseti* | | 5 | | 2.77 | 2.77 | 3.22 | | 7.40 | 62.5 | 2.74 |
| *Fusarium moniliforme* | | | | | 1.38 | | | 3.70 | 25 | 0.76 |
| *Fusarium oxysporum* | | | | 4.16 | 5.55 | 4.83 | 5.40 | | 50 | 1.83 |
| *Fusarium solani* | | | 2.08 | 1.38 | 2.77 | 1.61 | | | 50 | 1.67 |
| *Memnoniella echinata* | | | 4.16 | | 2.77 | | | | 25 | 1.06 |
| *Mucor sp.* | 5.26 | | 2.08 | 1.38 | | | | 3.70 | 50 | 1.06 |
| *Myrothecium roridum* | | | | 4.16 | 2.77 | 4.83 | | 3.70 | 50 | 3.35 |
| *Neurospora crassa* | | | | | | 3.22 | 8.10 | | 25 | 1.37 |
| *Nigrospora oryzae* | | | | | | 3.22 | | | 12.5 | 0.61 |
| *Penicillium chrysogenum* | 10.55 | 5 | 10.41 | 4.16 | 4.16 | 3.22 | 5.40 | 3.70 | 100 | 5.80 |
| *Penicillium citrinum* | | | | 1.38 | 1.38 | 1.61 | 2.70 | | 50 | 2.74 |
| *Rhizoctonia solani* | | | | 2.77 | 4.16 | 3.22 | 5.40 | | 50 | 1.37 |
| *Rhizopus stolonifer* | 5.26 | | 4.16 | 2.77 | 2.77 | 3.22 | 8.10 | 3.70 | 87.5 | 1.83 |
| *Trichoderma harzianum* | | | | 1.38 | 1.38 | | | | 25 | 1.06 |
| *Trichoderma viride* | | 5 | 6.36 | 2.77 | | 3.22 | 2.70 | | 62.5 | 3.05 |
| *Trichothecium roseum* | | | 2.08 | 2.77 | | 1.61 | 5.40 | 3.70 | 62.5 | 1.83 |
| *Verticillium dahliae* | | | 2.08 | 5.55 | 1.38 | | 5.40 | | 50 | 1.52 |
| *White sterile mycelium* | | | 2.08 | 2.77 | 2.77 | 3.22 | 5.40 | | 62.5 | 1.98 |

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