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Screening of Phylloplane mycoflora of some members of Amaranthaceae by leaf imprint method

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Abstract

Phylloplane mycoflora of some members from Amaranthaceae are investigated by leaf imprint method. Studies on the selected plants [*Achyranthes aspera* L., *Amaranthus tricolor* L., *Alternanthera sessilis* (L.) R. Br., *Celosia argentea* L.] revealed that the total number of fungal isolates were increased with the plant age and were found more on adaxial surface of leaves than on abaxial surface. Aspergillus niger has been reported as one of the most dominant inhabitants of leaf surface mycoflora followed by *Cladosporium cladosporioides, Curvularia species, Alternaria alternata,* and *Penicillium species.*

Keywords: Phylloplane, Mycoflora, Leaf imprint, Amaranthaceae

Introduction

Phylloplane, being very rich in nutrients, plays a needy role for the colonization of various fungal species. Phylloplane fungal diversity are also influence by host leaf epidermal attributes including its size, stomatal index, density of hairs and trichomes and architect of leaf surface; as well as host plant surroundings such as temperature, humidity, light intensity, presence of air pollutants [1,2]. Epiphytic microorganisms including pathogens are responsible for a number of phenomenons on the leaf surface viz. climate change, seasonal fluctuations, their change in nature of leachates, changes in biochemical characters of host tissues, such diverse variations affect the whole leaf surface ecology. Microbes present on the leaf surface represent an intricate relationship with one another. The entire system including microbes, non living

entities and leaf surface are in dynamic equilibrium, and if disturb, it may alter the associated parameters [3-5].

Fungal colonization of leaf surface expresses complex inter-relationships. Living host leaf is an ideal landing stage for spores, conidia and other propagules, deposited by various means. Once reaching the leaf surface, unless washed off by the rain, they derive the benefits of nutrients by diffusion from the leaf. Environment is not completely favourable for early colonizers, due to fluctuations in temperature, high rates of transpiration, exposure to ultraviolet radiations, etc. Aerial leaf surfaces with spores and hyphae of fungi forms interkingdom runway, subject to the impact of range of abiotic factors and morpho-physiological changes in the plants [6,7].

Leaves, being vital substratum, morphology and nature become an important factor influencing phylloplane mycoflora. Majority of the phylloplane microbiota constitutes yeasts and filamentous fungi. Filamentous fungi from the phylloplane are diverse in nature like parasites, saprophytes, endophytes or epiphytes [8,9]. Epiphytic fungi, without causing damage, exist on the leaf surfaces, however photosynthetic potential may get reduced [6,10]. Existence of positive or negative complex interactions between epiphytes and pathogenic microorganisms, may results in beneficial or harmful relationship. In contrast to phytopathogens, few phylloplane fungi protect host leaves against infections [11-13].

In the present investigation, phylloplane mycoflora of some members from Amaranthaceae *[Achyranthes aspera L., Amaranthus tricolor L., Alternanthera sessilis (L.) R. Br., Celosia argentea L]* were quantified by leaf imprint method.

Material and Methods

Depending on the target mycoflora, different techniques and various nutrition media were used and standardized by researchers [6-10, 12, 13]. Isolation of phylloplane carried out by Leaf imprinting, Leaf washings and dilution plate methods, Agar plate method (Leaf discs), Moist chamber methods; [14] out of which leaf imprint method on PDA media is used in present studies.

For Phylloplane microflora investigations, healthy leaf samples were collected from several different locations of Sakoli taluka, in different stages of growth (Vegetative, Flowering, senescence). Healthy, asymptomatic leaf samples were collected and immediately put into sterile ziplock bags, and brought to the laboratory for further processing. Sampling of aerial leaf surfaces from the selected plants was done and all the samples were brought to the laboratory. Leaf samples were processed for the leaf imprint method, within 1 hour of sample collection.

Adaxial and abaxial surfaces of leaves were gently pressed for a short time, separately against solid sterilized PDA media in sterilized petriplates at the center of each plate. The plating of different leaves of host plants was done in triplicates. After imprinting, plates were allowed to incubate at 27±1°C for a week and then examined for the appearance of fungal colonies. Numbers of fungal isolates (number of colonies of individual fungi) were recorded. A comparison for the fungal populations on both the surfaces was done. Fungal isolates were broadly identified on the basis of macro-morphological and colony characteristics, followed by their sub-culturing and pure cultures on Czapek-Dox medium. After getting pure cultures, slides were made for individual species for their identification, with the help of micro-morphological characteristics, and then were verified further with ready reference from literature, flora and keys.

Results and Discussion

Leaf imprinting results in the higher densities and diversities adaxially, although differences between abaxial and adaxial imprints were not always significant. Total number of mycoflora found on adaxial surface of leaves were higher than on abaxial surface at

all the stages of plant growth, as observed from vegetative to flowering stages. On both adaxial and abaxial surfaces, highest number of fungal isolates found in *Amaranthus,* followed by *Achyranthes, Celosia,* and *Alternanthera* [Table 1].

Plants from different sites were selected for phylloplane mycoflora by leaf imprint method, and it revealed that the total number of fungal isolates were increased with the plant age and were found more on adaxial surface of leaves than on abaxial surfaces, independent of nearby geographical locations of Sakoli taluka.

Leaf imprint from selected plants results into the isolations of some common and dominant phylloplane fungi; *Alternaria alternata, Aspergillus niger, Aureobasidium pollulans, Cladosporium cladosporioides, Curvularia lunata and Penicillium species* was encountered throughout the investigations. However, *Aspergillus niger* has been reported as one of the most dominant inhabitants of aerial leaf surface mycoflora followed by *Cladosporium cladosporioides, Curvularia sp, Alternaria alternata, Aureobasidium pullulans,*and *Penicillium species.* Similar findings have been recorded in earlier investigations [6-9].

Table 1. Phylloplane Mycoflora of selected plants by Leaf Imprint method

SN	Name of Fungal species isolated	Number of Fungal Isolates found by leaf imprint (Mean)							
		Achyranthes		Alternanthera		Amaranthus		Celosia	
		Ad	Ab	Ad	Ab	Ad	Ab	Ad	Ab
1	*Alternaria alternata*	5	3	4	3	8	2	4	-
2	*Alternaria tenuissima*	4	-	-	-	4	1	-	-
3	*Aspergillus flavus*	3	-	2	-	5	2	3	2
4	*Aspergillus fumigates*	2	2	-	-	-	-	-	-
5	*Aspergillus niger*	9	3	4	3	13	4	4	2
6	*Aspergillus terreus*	1	-	-	-	2	1	2	-
7	*Aurobasidium pollulans*	2	-	2	1	2	-	1	-
8	*Cladosporium cladosporioides*	6	2	4	3	8	2	4	1
9	*Curvularia clavata*	2	-	-	-	2	-	-	-
10	*Curvularia lunata*	4	3	2	1	2	1	3	2
11	*Curvularia brachyspora*	-	1	-	1	2	-	1	-
12	*Dreschlera species*	2	1	1	2	1	-	2	2
13	*Fusarium semitectum*	3	1	-	-	4	-	1	-
14	*Fusarium moniliforme*	-	-	2	-	2	-	-	-
15	*Nigrospora oryzae*	-	-	-	-	2	1	-	-
16	*Penicillium notatum*	2	-	2	1	-	-	2	1
17	*Penicillium sp.*	3	-	1	-	2	1	2	2
18	*Rhizopus stolonifer*	-	2	-	2	-	1	-	-
19	*Trichoderma viridae*	2	-	3	-	-	2	2	1
20	*Sterile mycelia*	+	+	+	+	+	+	+	+
Total		**50**	**18**	**27**	**17**	**59**	**19**	**31**	**13**

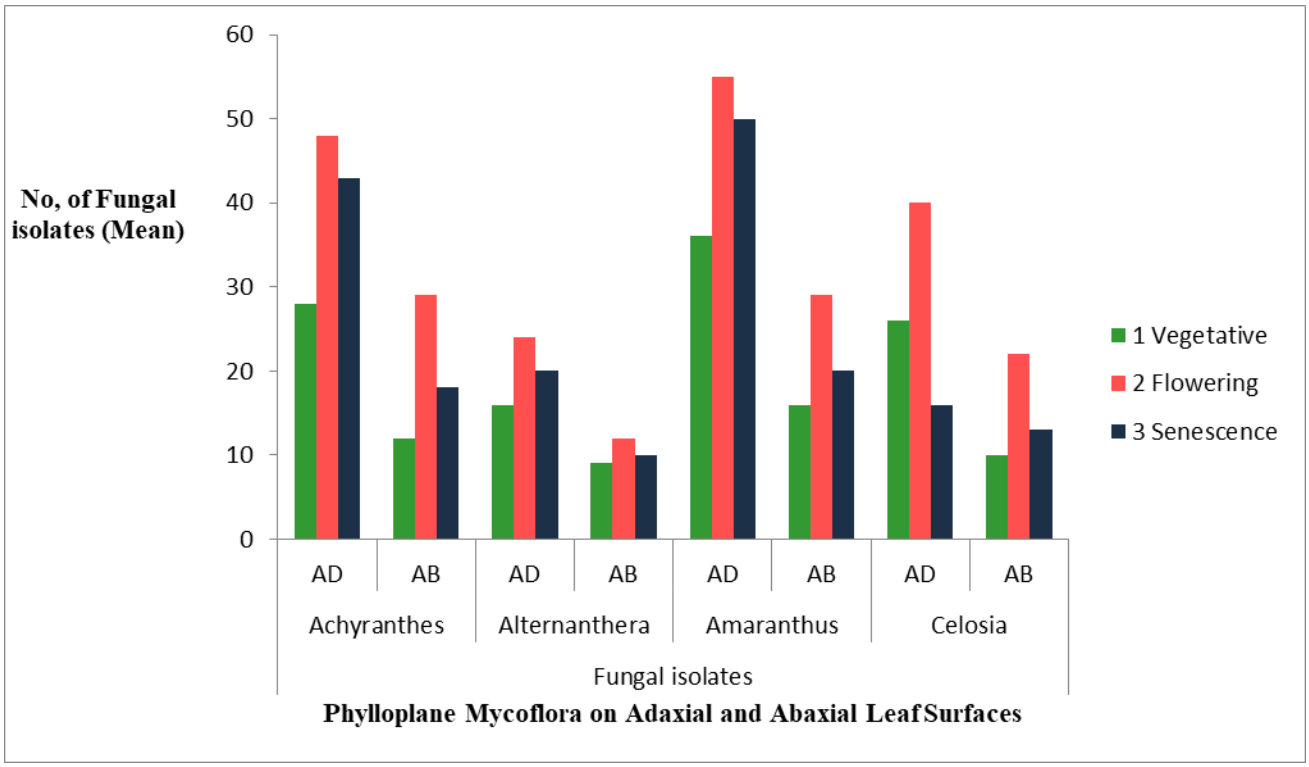

Figure 1. Phylloplane Mycoflora of selected plants by Leaf Imprint method

Numbers of fungal isolates in all the selected plants displayed common pattern of changes in fungal populations, as minimum at pre-flowering / vegetative phase, elevated continuously upto the flowering phase, and thereafter steady fungal populations with sharp decline were recorded at senescent stage [Graph 1]. Populations of filamentous phylloplane fungi is directly proportional to the age of leaf. Low population of microbial colonizers during early stage of plant growth may be due to the low nutrient content on aerial leaf surfaces. On attaining maturity, the leaching of nutrients by leaves elevate and favorable environmental conditions promote the increase of population of colonizers [15,16].

Fusarium was frequently found in all the host plants. The 'sterile forms' were partly due to some obligate basidiomycetous parasites viz. rusts and smuts, whereas, the pattern of prevalence of fungi was found to be similar with the phyllosphere in cases of *Cladosporium, Aspergillus, Penicillium, Alternaria, Curvularia, Drechslera* and '*sterile forms*'. Anamorphic fungi was reported as the most dominant group on phylloplane of various host plants, by many research groups [17-22]. Significant observations are also confirmed in the present study.

Overall, *Amaranthus tricolor* showed the highest degree of colonization and species richness, followed by *Achyranthes aspera, Celosia argentea*, and least species reported in *Alternanthera sessilis*.

Conclusion

The observations from plants of Amaranthaceae revealed that, adaxial surfaces of leaves represented higher diversities than abaxial surfaces by leaf imprint method, and similar results were noted earlier, on other plants as well. The aerial surfaces of leaves form a stage facing the air, inviting the air mycoflora to settle on adaxial surface instead of abaxial surface; interpretated as one of the prime reason behind high population of fungi on adaxial surfaces. More fungal propagules

encountered on adaxial surface of leaves leads to the greater hyphal development on the mature leaves on the adaxial surfaces.

Conflicts of interest: The author stated that no conflicts of interest.

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