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Research Article

FUNGAL COLONIZATION OF PHYLLOPLANE OF *PSIDIUM GUINEENSE* SW. GROWING IN SURYAMANINAGAR, TRIPURA, NORTHEAST INDIA

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ABSTRACT
The phylloplane of *Psidium guineense* was examined for the diversity of fungi in three months. The total of 17 fungal species were identified and one sterile mycelium. Among them, the majority of microrganisms grew in the month of May and the least were found in the month of March and January. *Cladosporium cladosporioides, Gliocladium viride, Mucor racemosus, Penicillum chrysogenum* and sterile mycelium were found to be dominant among all the species. The highest number of species was obtained from the month of May. Shannon diversity index was maximum in the month of May. Simpson index of dominance was high in the month of May and evenness was high in the month of March. Thus the result suggests that insignificant monthly variations in fungal species in the leaves of *P. guineense*.

Key Words: *Phylloplane, Psidium Guineense, Fungi and Diversity*

INTRODUCTION
Phylloplane fungi are the mycota growing on the surfaces of leaves (Langvad, 1980). There are two groups of phylloplane fungi; residents and casuals (Norse, 1972). Residents can multiply on the surface of healthy leaves without noticeably affecting the host whereas casuals land on the leaf surface but cannot grow (Leben, 1965). Phylloplane fungi have been poorly studied as compared to endophytes, saprobes and pathogenic fungi. The interest shown in the last few years in the study of phyllosphere microbes is due principally to their interactions with plants, herbivores and pathogens living on leaves which may be involved in the plant immunity system, reabsorption of organic and mineral matters from leachates, redistribution of nutrients prior to leaf fall and participation in the primary degradation of plant tissues (Carroll *et al*., 1977; Cabral, 1985; Lindow and Brandl, 2003 and Osono, 2006). Most phylloplane fungal studies have been concerned with pathogens or non-parasitic fungi of crops or economically-important trees (Dickinson, 1967; Lamb and Brown, 1970; Pugh and Mulder, 1971; Bainbridge and Dickinson, 1972; Norse 1972; Godfrey, 1974; Mishra and Dickinson, 1981; Cabral, 1985; Vardavakis, 1988 and Carris, 1992).
Psidium guineense Swartz belongs to Myrtaceae, is a under shrubs or shrubs rarely small trees. Bark not exfoliating in thin flakes and fruit small with thin pulp. Natural regeneration is very common but the plant do not seems to be spreading. It is native to South America. In Tripura, the plant is localized in the Sadar sub-division, Agartala (Deb, 1981).
However, there are no studies related to the fungal diversity in the phylloplane of plant species in Tripura, northeast India. In paucity of this kind of studies, it is our interest to analyse microbial diversity particularly fungi from the phylloplane of *P. guineense* in Tripura University Campus, Suryamaninagar, Tripura, north east India.

MATERIALS AND METHODS
Site Description
To analyse the diversity of phylloplane fungi, leaf samples were collected from *P. guineense* growing in Tripura university campus, Suryamaninagar, Tripura (west). It is situated between $22^0 57'$ and $24^0 32'$ N and $91^0 10'$ and $92^0 20'$ E.

Leaf Sampling and Processing

The leaf samples with fungi in their phylloplane were collected from *P. guineense* from Tripura university campus. The samples were taken in such a way that the leaves were not directly exposed to the outer environment and micro organisms present in the leaf surface remains intact. The leaf was brought to the lab and was then washed with 100ml of sterile distilled water in a conical flask thoroughly with the help of a glass rod in a beaker (sample- A). Then 1ml of sample A was taken and mixed with another 99 ml of sterile distilled water in another conical flask (sample-B). The sample B is then used for further inoculation of fungi in the prepared media by pouring 0.1 ml in each petriplates. After washing the leaves, it was measured for the determination of leaf area in the graph paper.

Isolation of Fungi

The culture media was used for the analysis was Czapek Dox Agar. After preparing the media it was then sterilized in an autoclave and then 1g of antibiotic (streptomycin) was added to it for preventing it from the contamination of bacteria. After the media is prepared, it was then poured into the sterile plates. Then it was allowed to cool at room temperature. Inoculation was done by pouring 0.1 ml of the inoculum to the petriplates. The inoculum was then spread well in the media plates with the help of a spreader. Then it was incubated at 25^0C for 4-6 days till the colonies grow well. The colonies were then identified with cotton blue and lacto glycerol with the help of a microscope. After the incubation period, the colony forming units were counted and expressed as CFU/30cm^2 of leaf area. Isolates which did not sporulate were placed under near UV light (black light for 12h dark: 12h light) in an attempt to stimulate sporulation. Isolates which did not produce spores were treated as sterile mycelium (Lacap *et al*., 2003). Cultures that failed to sporulate were recorded as mycelia sterilia. All isolates were numbered and are maintained in Culture Collection Centre of Department of Botany, Tripura University, Tripura, India.

Identification of Fungi

The fungal species were identified on the basis of cultural characteristics and morphology of fruiting bodies and spores by using standard texts and keys. The species was then identified by using the identification manual (Ellis, 1993; Domsch *et al*., 1980 and Watanabe, 2002).

Data Analysis

Means with standard error was calculated average number of colonies in the petri dishes. Isolation frequency (IF) of the fungi species was calculated using the following formula:

Isolation frequency (IF) = Ni/Nt ×100

Where Ni is the number of petri dishes from which a given species was isolated and Nt is the total number of petri dishes used for isolation. Diversity analysis was conducted using Past software (Hammer *et al*., 2001).

RESULTS AND DISCUSSION

The colony forming unit (CFU) of the fungal population in three different months was obtained (Table 1). The CFU value in the month of May is the highest and March is the lowest. Fungal population was maximum in the month of May and minimum in the month of March.

Table 1: Fungal population in the phylloplane of *Psidium guineense*

Months	**January**	**March**	**May**
CFU/30cm^2 of leaf area	19.4x10^5	11.8x10^5	48.8x10^5

In the month of January, seven species were isolated from the total of 18 species isolated from all the studied months. *Penicillum chrysogenum* was found to be highest followed by sterile mycelium. *Mucor racemosus* was found to be lowest colonies. In the month of March, the average number of colonies of different fungal species was found to be decreased in comparison with that of the first month (January). Both in the month of January and March, only 7 species of fungi was observed. The colonies were dominated by the species of *Penicillum chrysogenum, Gliocladium viride, Pythium ultimum,*

Mucorracemosus and mostly were sterile. In the third and last month (May), it was observed that the number of fungal colonies was much increased in comparison to the first two months and 11 species were isolated out of 18 species observed in all the studied months. The culture media was dominated by the species of *Cladosporium cladosporioides, Cladosporium macrocarpum, Gliocladium viride* and mostly were sterile and some were mixed colonies *Gliocladium viride* and sterile mycelium were found in all three months whereas *Cladosporium cladosporioides* and *Penicillum chrysogenum* were found in two months (Table 2).

Table 2: Average number of fungal colonies isolated from different months

Phylloplane fungi	January	March	May
Acremonium butyric	0	0	0.2±0.2
Acremonium murorum	0	0.4±0.4	0
Alternaria alternate	0	0.2±0.2	0
Arthrobotrytis arthrobotryoides	0	0	0.4±0.4
Bactrodesmium spilomeum	0	0	1.2±1.2
Cladosporium cladosporioides	1.6±1.6	0	11.2±5.77
Cladosporium macrocarpum	0	0	12±11.75
Gliocladium viride	1.4±0.87	1.6±1.6	4.8±4.8
Mortierilla polycephala	0.8±0.8	0	0
Mucor racemosus	0.4±0.4	0.8±0.8	0
Paecilomyces carneus	0	0	0.8±0.8
Penicillium brevicompactum	0	0	1.4±1.4
Penicillium jensenii	0	0	1.2±1.2
Penicillium thomii	0	0	0.6±0.6
Penicillum chrysogenum	7.2±5.43	4±4	0
Pythium ultimum	0	0.8±0.8	0
Sterile mycelium	5.8±2.99	3.4±3.15	15±13.75
Trichoderma viride	1.4±0.87	0	0

The present study of phylloplane fungal diversity of *Psidium guineense* isolated a total of 18 species; *Cladosporium cladosporioides, Gliocladium viride, Mucor racemosus, Penicillum chrysogenum,* and sterile mycelium were found to be dominant among all the species. In general, these species were extensively reported as common primary saprobes and ubiquitous hypomycetes from attached leaf surface of wide varieties of plants throughout the world (Breeze and Dix, 1981) which can withstand an adverse condition such as desiccation, UV radiation and microbial lysis by producing thick walled pigmented multicellular spores (Hudson, 1968 and Sadaka and Ponge, 2003). Among the species recorded some are able to utilize cellulosic components and also found to play an important role in the degradation of plant tissues. From the present study, it was found that phyllosphere fungi showed preferences in different months.

Sterile mycelium exhibit the maximum isolation frequency whereas *Cladosporium cladosporioides, Mortierilla polycephala* and *Mucor racemosus* shows the least isolation frequency in the month of January. Out of seven species in the month of March, six species exhibit the 20 % isolation frequency and 40 % by sterile mycelium. In the month of May, the isolation frequency of 8 species possesses 20 %, one 40 %, one 60 % and sterile mycelium 100 % (Table 3).

Table 3: Isolation frequency (%) of phylloplane fungi in different months

Phylloplane fungi	January	March	May
Acremonium butyri	-	-	20
Acremonium murorum	-	20	-
Alternaria alternata	-	20	-
Arthrobotrytis arthrobotryoides	-	-	20
Bactrodesmium spilomeum	-	-	20
Cladosporium cladosporioides	20	-	60
Cladosporium macrocarpum	-	-	40
Gliocladium viride	40	20	20
Mortierilla polycephala	20	-	-
Mucor racemosus	20	20	-
Paecilomyces carneus	-	-	20
Penicillium brevicompactum	-	-	20
Penicillium jensenii	-	-	20
Penicillium thomii	-	-	20
Penicillum chrysogenum	40	20	-
Pythium ultimum	-	20	-
Sterile mycelium	80	40	100
Trichoderma viride	40	-	-

Maximum isolation frequency was observed in the month of May and exhibited by sterile mycelium (Fig. 1). The month of May was found to be highest in terms of species richness and individuals. Shannon diversity index was maximum in the month of May. Simpson index of dominance was high in the month of May and evenness was high in the month of March (Table 4).

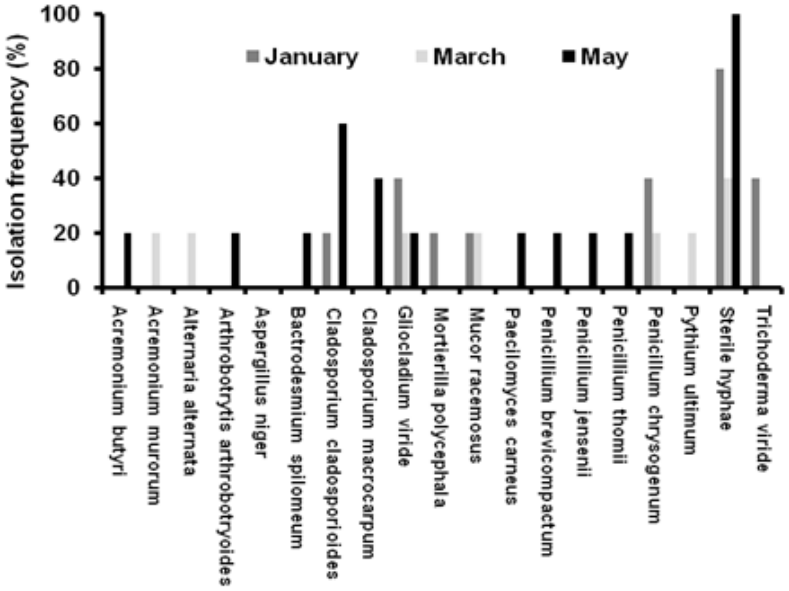

Figure 1: Isolation frequency (%) of phylloplane fungi in different months

Table 4: Diversity indices of fungi from the phylloplane of *Psidium guineense*

Diversity indices	January	March	May
Taxa_S	07	07	11
Individuals	15	08	45
Dominance_D	0.4123	0.4937	0.2585
Shannon_H	1.654	1.735	1.8
Simpson_1-D	0.5877	0.5063	0.7415
Evenness_e^H/S	0.7469	0.8096	0.55

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). Besides this prevailing environmental variables such as temperature moisture humidity pH, during different sampling periods have also been reported to affect the changes in population of specific phyllosphere fungi (Breeze and Dix, 1981). These fungi are normally encountered as epiphytes, but some can also occur as endophytes (Petrini, 1991). As in our study, *Alternaria alternata*, *Penicillium* sp and *Cladosporium* sp were found which are also found to be endophyte in certain study (Sun *et al*., 2011).

According to Kayini and Pandey (2010), in the phylloplane of *Alnus nepalensis*, *Alternaria alternata* was absent in the month of January which is similar to the present investigation. The absence of *Aspergillus niger* in all the three months also shows similarity with the present study. *Trichoderma viride* were found to be present in January but absent in March and May which was observed in this work similarly. Sterile mycelium was also found to be present in March and May in this investigation.

In conclusion, the monthly variation in fungal species isolated was insignificant in the leaves of *P. guineense*. The month of May exhibits high diversity with less dominance. Most of the fungal cultures did not sporulate. However, morphological identification of sterile mycelium was inadequate and their identification based exclusively on molecular characters could through more light on the diversity of phylloplane.

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