

Diversity of Phylloplane Fungi Associated with *Habenaria foliosa* A. Rich. (Orchidaceae) from Bhandara and Chandrapur districts of Maharashtra

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Abstract: The phylloplane of *Habenaria foliosa*- the endangered terrestrial orchid was studied from the two different localities of two different districts for to access the diversity of fungi over four different fungal media, present on the leaves. For the present investigation, the plants were collected from the Chandpur (Bhandara district) and Kurkheda (Gadchiroli district) in the months of July-August, 2015. In the present study, a total 22 fungal species with 13 genera were reported over the leaf surface. *Penicillium* and *Aspergillus* are found to be dominant followed by the Black sterile mycelia.

Keywords: Anamorphic fungi, *Aspergillus*, Forest, leaf surface, *Penicillium*.

I. INTRODUCTION

Phylloplane is the term first time used for to denote the leaf surface environment. Phylloplane is a natural habitat on the leaf surface which supports heterogeneous population comprising both pathogenic and non-pathogenic microorganisms [1]. Plant leaf surface is always witnessed the external atmospheric changes occurred in the environment. The leaf surface constitutes a habitat for numerous micro-organisms like fungi and bacteria. The fungi growing on the leaf surface are referred as Phylloplane fungi [8]. Phylloplane fungi are classified into two groups viz. residents and casuals [11]. Sah et al 2013 [12] stated that resident type of fungi can multiply on the surface of healthy leaves without affecting the host but casual species land on the surface of the leaf but cannot multiply themselves. Orchidaceae, one of the largest family of Angiosperms, represented by the beautiful colours and natural beauty of flowers. Their demand in the floriculture market is only because of their beautiful flowers and potted plants [7]. They are distributed in diverse habitat with modified vegetative and floral structures [6].

The populations of orchids are decreasing day by day because of their poor seed germination, low propagation rate and extensive collection from nature. Because of this, so many orchid taxa are now facing the threat of endangered category [9]. *Habenaria foliosa* is a tuberous annual herb growing in the tropical and subtropical regions with flowering period of August-September [15]. The phylloplane microorganisms are found to play a role in nitrogen fixation, lignin and wax degradation, promote to senescence, production of growth regulators, and contribute in plant defence [5]. Therefore the present investigation is undertaken to know the diversity of Phylloplane fungi.

II. MATERIALS & METHODS

A. Plant collection

To analyse the diversity of fungi on *Habenaria foliosa* leaves, the materials were collected from two different districts of Maharashtra state of India. The first site Chandpur, is located in Bhandara district at N21°28.795' E79°08.865' and the second site Kurkheda, is located at N20°13.259' E80°41.442' in Gadchiroli district.

B. Leaf sampling and processing

The leaves of *Habenaria foliosa* were collected in the sterile ziplock bags from sites mentioned above. Care was taken to avoid contact of external environment during travelling. The leaf samples were brought to the laboratory as soon as possible so as to avoid the shrinkage of leaves after being detached from the mother plant.

C. Isolation of Phylloplane fungi

For to access the Phylloplane diversity, four different fungal growth media viz. Potato Dextrose Media (PDA), Czapek Dox Agar Media (CzA), Malt extract Peptone Dextrose Media (MEA), Rose Bengal Agar Media (RBA) were used. The leaf dilution method was employed for the isolation of fungal flora as suggested by the Aneja 2004 [1].

D. Identification of fungi

The isolated fungi were identified using the standard literature like A Manual of Aspergilli[16], Illustrated Genera of Imperfect Fungi[2] and Handbook of Soil Fungi[10].

E. Data analysis

The data generated from observations was used to calculate Frequency, Shannon Diversity Index and Simpson Diversity Index.

III. RESULTS AND DISCUSSION

The output of the present investigation is that a total of 111 fungal colonies were isolated from the leaf surface of *Habenaria foliosa* which was collected from two different districts of Maharashtra. The total mycoflora comprises of the 22 fungal taxa representing 13 genera. The isolated fungal taxa were representing three groups viz. Zygomycota, Anamorphic fungi and the Sterile Group.

Zygomycota contribute 4.5% to the total mycoflora. In this group only three species were isolated out of which *Phycomyces nitens* were found to be dominant over other two. Anamorphic fungi isolated at the frequency of 72.1%. The diversity of this group is noted by 17 fungal species and 8 genera. The *Penicillium* was found to be predominant with 28.8% followed by the *Aspergillus* which was isolated at the frequency of 19.8%. Six different species of *Aspergillus* were isolated. *Aspergillus niger* shows its dominance with 6.3% (Table: I).

Four different fungal growth media were used for the present investigation. Bhandara district contribute near about 29% while those of Gadchiroli district contribute 71%. From the Gadchiroli district 19 fungal colony counts was observed for PDA, 23 for CzA, 23 for MEA and 14 for RBA. The sterile mycelia group was present at the rate of 23% (Table: I).

Diversity index

The diversity index was calculated using PAST 3.12. The maximum Simpson Diversity index was reported from Gadchiroli district over CzA i.e. 0.8922 and the minimum in Bhandara district over CzA i.e. 0.625. The Shannon Diversity index also showed similarity with Simpson index with respect to the media, district, min and max (Table: II).

The study reveals that the overall Phylloplane microbial diversity was found to be associated with the *Habenaria foliosa* was represented by the 22 fungal species including 13 fungal genera. The diverse isolates obtained over the different fungal media proved that the Leaf Dilution method is good one for the present type of study which was also supported by Wahab 1975 [17], Borgohain et al 2014 [4].

Every fungus needs some basic elements and carbon source for the growth and development which it obtains from the respective environment. The growth response of the every fungus is varying to the respective media. Fungi are nutritionally so diverse, no single medium could be used for isolation of all the fungi. Some fungi require specific elements for the growth and sporulation so the diversity of fungi must be recorded over diverse fungal media as like we recorded in present study like PDA, CzA, MEA, RBA. The use of these media for the microfungus study was also done by Banakar et al 2012 [3].

Habenaria foliosa harbour greater Phylloplane diversity. It is represented by the 111 fungal colonies over 4 different growth media from the 2 different districts of Maharashtra state of India. In our investigation we encountered only three members of Zygomycota, Actinomucor and Rhizopus were found to be present only in the Gadchiroli district whereas *Phycomyces nitens* was isolated from the both the districts and it is dominated over other two genera at the frequency of 2.7% (Table-I).

Out of total isolated fungi, maximum diversity was noted in the group of Anamorphic fungi. Altogether 17 species are encountered out of 22 from this group. *Penicillium* showed the maximum dominance at the frequency of 28.8% followed by the *Aspergillus* (19.8%) over all other fungal species. The maximum dominance of these fungi in the Phylloplane is probably because of their high spore production and easy dispersion in the forest. *Habenaria foliosa* is small plant, grown in the rainy season at the strata of forest floor.

Plant height and the leaf sheath are the probable factors that increase the chances of spore settlement over leaf surface. The forest floor is highly nutritious for the microbial growth. The highest dominance of *Aspergillus* and *Penicillium* from the forest area was also reported by the Saravanakumar & Kaviyaran 2010 [13], Banakar et al 2012 [3], similar dominance of *Aspergillus* and *Penicillium* was also recorded in our investigation.

Aspergillus showed its dominance by six different species. Out of that only two, *Aspergillus niger* and *Aspergillus sulphureus*, were isolated from Bhandara district whereas all six species were dominantly present in the Gadchiroli

district. *Penicillium* was isolated with three different species out of which *P. oxalicum* was found to be the most dominant in both the sites. Other fungal genera like *Alternaria*, *Curvularia*, *Fusarium*, *Pestalotiopsis*, *Torula*, *Trichoderma* were isolated at moderate frequency. Sterile mycelia both white and black were found to be dominant after *Aspergillus* and *Penicillium* (Table-I).

The statistical analysis of isolated fungal flora showed that the values of Simpson's diversity index and Shannon diversity index for both the districts were significantly different from each other. Diversity index clearly justifies that the diversity of Phylloplane fungi is more in Gadchiroli district as compared to the Bhandara district. The forest of Gadchiroli harbours the tremendous diversity of Plants and Animals [14]. It is bestowed with the good soil profile, heavy rain fall and thick dense forest. All these factors lead to increase the humus over the forest floor which ultimately supports the maximal microbial population. This is the most probable reason to get the more fungal diversity from the Gadchiroli forest.

IV. CONCLUSIONS

From the present investigation it was concluded that the Phylloplane of *Habenaria foliosa* harbours the tremendous diversity of fungi. A total of 22 fungal species belonging to 13 genera were isolated from the Phylloplane of respective plant. The leaf dilution method was also found to be the good one for the isolation of Phylloplane fungi. The view that different fungi need different requirements was also proved from the use of different media for isolation. Among the isolates, *Aspergillus* and *Penicillium* were recorded as the most dominant fungi followed by the black sterile mycelia. Other fungi like *Alternaria*, *Curvularia*, *Fusarium*, *Pestalotiopsis*, *Trichoderma* and *Torula* are at moderate frequency. The overall data and its statistical analysis revealed that the Phylloplane mycoflora was more diverse in plants from the forest of Gadchiroli as compared to that of Bhandara.

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REFERENCES

- [1] Aneja KR. 2004. Experiments on Microbiology, Plant Pathology and Biotechnology. 4th ed., New Delhi: New Age International Pvt. Ltd..
- [2] Barnett HL, Hunter BB. 1972 - Illustrate Genera of Imperfect Fungi. Burgess publishing company.
- [3] Banakar S, Thippeswamy B, Thirumalesh B, Naveenkumar K, 2012 - Diversity of soil fungi in dry deciduous forest of Bhadra Wildlife Sanctuary, Western Ghats of southern India. *Journal of Forestry Research* 23, 631-640.
- [4] Borgohain A, Das R and Chutia M. 2014 - Fungal Diversity in phylloplane of castor plant (*Ricinus communis* L.): the primary food plant of Eri Silkworm. *Scholarly Journal of Agricultural Science* 4, 82-86.
- [5] Fenn M, Paul H, Dunn P, Durall D. 1989 - Effects of Ozone and Sulfur Dioxide on Phyllosphere Fungi from Three Tree Species. *Applied and Environmental Microbiology* 55, 412-418.
- [6] Jalal JS, Jayanthi J. 2012 - Endemic orchids of peninsular India: a review. *Journal of Threatened Taxa* 4(15), 3415-3425.
- [7] Joshi V, Janarthanam M. 2004 - The diversity of life-form type, habitat preference and phenology of the endemics in the Goa region of the Western Ghats, India. *Journal of Biogeography* 31, 227-237.
- [8] Langvad F. 1980 - A simple and rapid method for qualitative and quantitative study of the fungal flora of leaves. *Canadian Journal of Microbiology* 26, 666-670.
- [9] Machaka-Houri N, Al-Zein MS, Westbury DB, Talhouk SN. 2012 - Reproductive success of the rare endemic *Orchis galilaea* (Orchidaceae) in Lebanon. *Turkish Journal of Botany* 36, 677-682.
- [10] Nagamani A, Kunwar IK, Manocharachary C. 2006 - Handbook of Soil Fungi. I K International Pvt. Ltd.
- [11] Norse D. 1972 - Fungal populations of tobacco leaves and their effect on the growth of *Alternaria longipes*. *Transactions of the British Mycological Society* 59, 261-271.
- [12] Saha A, Ray A, Das P. 2013 - Fungal Colonization of Phylloplane of *Psidium guineense* Sw. Growing in Suryamaninagar, Tripura, Northeast India. *International Journal of Basic and Applied Chemical Sciences* 3 (1), 62-67.
- [13] Saravanakumar K, Kaviyaran V. 2010 - Seasonal distribution of soil fungi and chemical properties of montane wet temperate forest types of Tamil Nadu. *African Journal of Plant Science* 4, 190-196.
- [14] Setiya A, Narkhede S, Dongarwar N. 2016 - Exploration and Documentation of Some Scarcity Food Plants Used by the Aborigines from Gadchiroli District (M.S.) India. *International Advanced Research Journal in Science, Engineering and Technology* 3, 69-73.
- [15] Singh NP, Karthikeyan S, Sharma BD. 1996 - Flora of Maharashtra state - Monocotyledons. BSI Calcutta.
- [16] Thom C, Raper KB. 1945 - A manual of *Aspergilli*. The Williams and Wilkins Company, Baltimore.
- [17] Wahab A. 1975 - Phyllosphere Microflora of Some Egyptian Plants. *Folia Microbiol.* 20, 236-245.

Table I: Phylloplane mycoflora of *Habenaria foliosa* from two different districts of Maharashtra.

Sr no	Fungal organism	Bhandara				Total	Gadchiroli				Total	Total colonies (S-I + S-II)	Frequency	
		PD A	CzA	MEA	RB A	(S-I)	PDA	CzA	ME A	RB A	(S-II)		specie s	gener a
	Zygomycota	1	-	-	1	2(1.8)*	-	1	2	-	3(2.7)*	5	4.5	
1.	Actinomucorel egans	-	-	-	-	-	-	-	1	-	1(0.9)	1	0.9	0.9
2.	Phycomycesnit ens	1	-	-	1	2(1.8)	-	-	1	-	1(0.9)	3	2.7	2.7
3.	Rhizopus stolonifer	-	-	-	-	-	-	1	-	-	1(0.9)	1	0.9	0.9
	Anamorphic fungi	4	1	4	4	13(11.7)	17	19	18	13	67(60.4)	80	72.1	
4.	Alternaria alternata	-	-	-	-	-	-	-	-	1	1(0.9)	1	0.9	3.6
5.	Alternaria brassicicola	-	-	-	-	-	-	1	2	-	3(2.7)	3	2.7	
6.	Aspergillus aculeatus	-	-	-	-	-	-	2	-	-	2(1.8)	2	1.8	19.8
7.	Aspergillus caespitosus	-	-	-	-	-	-	1	-	-	1(0.9)	1	0.9	
8.	Aspergillus japonicus	-	-	-	-	-	1	-	-	-	1(0.9)	1	0.9	
9.	Aspergillus niger	1	-	2	-	2(1.8)	2	-	2	-	4(3.6)	7	6.3	
10.	Aspergillus ochraceous	-	-	-	-	-	-	-	-	5	5(4.5)	5	4.5	
11.	Aspergillus sulphureus	-	-	-	3	3(2.7)	-	1	-	2	3(2.7)	6	5.4	
12.	Curvularia lunata	-	-	-	-	-	1	-	2	-	3(2.7)	3	2.7	2.7
13.	Fusarium moniliformae	-	-	-	1	1(0.9)	-	1	-	2	3(2.7)	4	3.6	4.5
14.	Fusarium oxysporum	-	-	-	-	-	-	1	-	-	1(0.9)	1	0.9	
15.	Penicillium oxalicum	2	1	-	-	3(2.7)	13	3	8	2	26(23.4)	29	26.1	28.8
16.	Penicillium roqueforti	-	-	-	-	-	-	2	-	-	2(1.8)	2	1.8	
17.	Penicillium spp.	-	-	-	-	-	-	-	1	-	1(0.9)	1	0.9	
18.	Pestoliopsisglan dicola	-	-	-	-	-	-	5	3	-	8(7.2)	8	7.2	7.2
19.	Torula herbarum	-	-	-	-	-	-	2	-	-	2(1.8)	2	1.8	1.8
20.	Trichoderma viride	1	-	2	-	3(2.7)	-	-	-	1	1(0.9)	4	3.6	3.6
	Other Types	4	3	4	6	17(15.3)	2	3	3	1	9(8.1)	26	23.4	
21.	White sterile mycelia	1	1	2	3	7(6.3)	1	1	1	-	3(2.7)	10	9	9
22.	Black sterile mycelia	3	2	2	3	10(9)	1	2	2	1	6(5.4)	16	14.4	14.4
	TOTAL	9 (8.1)	4 (3.6)	8 (7.2)	11 (9.9)	32 (28.8)	19 (17.1)	23 (20.7)	23 (20.7)	14 (12.6)	79 (71.1)	111	99.9	99.9

* Values in the parenthesis indicate Frequency. S-I= Site I & S-II= Site II.

Table – II : Diversity Index									
	Simpson (1-D) Diversity Index				Shannon (H') Diversity Index				
	PDA	CzA	MEA	RBA	PDA	CzA	MEA	RBA	
Bhandara	0.7901	0.625	0.75	0.7603	1.677	1.04	1.386	1.499	
Gadchiroli	0.5097	0.8922	0.8242	0.7959	1.117	2.401	2.028	1.767	