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Phylloplane of *Habenaria foliosa*- the threatened terrestrial orchid

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Abstract: *The present investigation deals with the Phylloplane of Habenaria foliosa which is threatened terrestrial orchid. The leaf samples were collected from five different districts of Maharashtra viz. Gadchiroli, Chandrapur, Bhandara, Amravati & Sindhudurg. The phylloplane fungi were isolated using leaf dilution method. Seven different media were used for the isolation of fungi viz. PDA, CzA, RBA, MEA, SA, CMA and WkM. A total 33 species representing 20 genera were found to be associated with H. foliosa. Aspergillus niger was the most frequent species isolated from leaves. The maximum diversity of fungi was obtained from the Gadchiroli district whereas it was least from the Sindhudurg district. Pestolotiopsis glandicola, Nigrospora oryzae, Torula herbarum, Phoma terricola were only reported from Gadchiroli and Chandrapur district over the all 7 media.*

Key Words: *Phylloplane, Habenaria, PDA, Leaves, Aspergillus, RBA, orchid.*

1. INTRODUCTION:

Habenaria foliosa is a threatened terrestrial orchid belonging to Orchidaceae, one of the largest family of Angiosperms. *Habenaria foliosa* is a annual herb growing in the tropical and subtropical regions with the tuberous root system and having flowering period of August-September. Generally this plant is found in the shades of the forest. Two varieties of these taxa are located in Maharashtra out of which the present study is on *Habenaria foliosa* var. *foetida*. Day by day the population of the orchids is decreasing because of poor seed germination, herbivores and anthropogenic activities and these taxa are facing the threats [1].

The external surface of the leaf, an environment for microorganisms has been termed the phyllosphere or phylloplane. Phylloplane is found to be a good platform for the growth of saprophytic fungi since there is a limited availability of nutrients, strong sun irradiation and variation in water availability [2].

The phylloplane fungi play a vital role in the life processes of plant. It plays a key role in cutline degradation, more enzyme production, defense mechanism and also may lead to disease in the plant. So considering the importance of phylloplane fungi, the aim of this research is to determine the main constituents of the mycoflora on the leaves of *Habenaria foliosa*.

2. MATERIALS AND METHODS:

2.1 Plant collection

For the analysis of Phylloplane of *Habenaria foliosa*, the leaves were collected from five different districts of Maharashtra state of India. Leaf samples were taken from Chandpur (N21^{0}28.795’ E079^{0}08.865’) of Bhandara district, Wadegao (N20^{0}13'3.2592' E80^{0}8'41.442') of Gadchiroli district, Amboli (N15^{0}55.853' E074^{0}00.838') of Sindhudurg district, Melghat (N21^{0}24.853' E77^{0}08’56.990') of Amravati district and Ghodazari (N20^{0}35.853' E079^{0}38.208') of Chandrapur district.

2.2 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.

2.3 Isolation of Phylloplane fungi

For to access the Phylloplane diversity, seven different fungal growth media viz. Potato Dextrose Media (PDA), Czapex Dox Agar Media (CzA), Malt extract Peptone Dextrose Media (MEA), Rose Bengal Agar Media (RBA), Sabouraud’s Agar (SA), Corn Meal Agar (CMA) and Waksman’s Medium (WkM) were used. The leaf dilution method was employed for the isolation of fungal flora as suggested by the [3].

2.4 Identification of fungi

The isolated fungi were identified using the standard literature like A Manual of Aspergilli [4], Illustrated Genera of Imperfect Fungi [5], Handbook of Soil Fungi [6], Manual of Soil Fungi [7], Hyphomycetes [8], Dematiaceous Hyphomycetes [9].

2.5 Data analysis

The isolated fungal colony count was noted in the form of tabulated data and were further analysed for frequency, Shannon Diversity Index and Simpson Diversity Index by using following formulas.

$$\text{Frequency} = \frac{\text{Number of individual colony}}{\text{Total number of all fugal colonies}} X\ 100$$

$$\text{Shannon index H}' = -\sum_{i=1}^{R} \text{Pi lnPi}$$

$$\text{Simpson's Index(D)} = \frac{\Sigma\ \text{n(n}-1)}{\text{N(N}-1)}$$

3. RESULTS AND DISCUSSION:

The present study reveals that a total 33 fungal species comprises of 20 genera were found to be reported from the leaves of Habenaria foliosa over seven different media which were belongs to five different localities. Out of 33 species, two species were found to be unidentified and they were reported as unknown-1 and unknown-2. As far as the diversity and dominance is concerned, the maximum diversity of species was found to be of *Aspergillus, Penicillium and Fusarium* while they also show the dominance among all others. The similar results were also reported by other researcher [10, 11, 12].

A total 222 fungal colonies comprises of 16 species were isolated over seven different media from the Ghodazari (Chandrapur district) sample. The maximum colony count was recorded over PDA i.e. 22% while that of minimum was at SA (11.71%). *Pestolosiopsis glandicola* and *Fusarium semitactum* was found to be the dominant in the Ghodazari region (Table 1). Amboli (Sindhudurg district) leaf sample was represented by only 8 fungal species which comprise of only 110 fungal colonies. *Trichoderma viride* was dominant one (Table 2).

A total 172 fungal colonies were isolated from Melghat (Amravati district) comprising only 12 species whereas 214 colonies with 11 species were reported from Chandpur (Bhandara district). It was found that the maximum diversity of fungi were associated with the leaf samples of H. foliosa collected from Gadchiroli district. A total 506 fungal colonies were associated with the leaves comprising 24 fungal species. From the same sample maximum colony count was isolated over the PDA i.e. 19% (Table 3, 4, 5).

Aspergillus niger was the dominant one over Potato Dextrose Agar followed by *Penicillium oxalicum*. Over this medium the maximum diversity was observed from the samples of Gadchiroli. The same dominance nature of *Aspergillus* and *Penicillium* was recorded over remaining media. The fungal genera like *Pestolotiopsis, Phoma, Torula, Curvularia* were only reported from the Gadchiroli and Chandrapur district. The members of *Aspergillus* nigri group were not isolated from Rose Bengal Agar medium. Altogether 24 species were reported from Gadchiroli district while 11 from Bhandara, 12 from Amravati, 8 from Sindhudurg and 16 from the Chandrapur district. Simpson (1-D) index was found to be almost same i.e. 0.8 from the entire site except Gadchiroli where it was noted to be 0.9. Shannon (H') diversity index was maximum at Gadchiroli while it was least at Sindhudurg (Table 6).

4. CONCLUSIONS

The maximum fungal diversity associated with leaves of *Habenaria foliosa* was reported from the Gadchiroli and Chandrapur districts in terms of fungal count and species diversity. Over the all seven media under study, maximum fungal isolation was of from Gadchiroli whereas it was least from Sindhudurg. A total 33 fungal species comprising of 20 genera were isolated over seven different media from five different localities. *Pestolotiopsis glandicola, Nigrospora oryzae, Torula herbarum, Phoma terricola* are only reported from Gadchiroli and Chandrapur district over the all the 7 media. *Acremonium* spp and *Curvularia lunata* are isolated from Gadchiroli. Only the members of *Aspergillus* nigri group were not isolated over RBA media. Simpson (1-D) index was found to be almost 0.8 of all sites whereas Shannon (H') index was to be found in range of 1.9 to 2.8. The present study showed that the phylloplane of *Habenaria foliosa* supports the growth of diversified fungi.

Table 1: Phylloplane of *Habenaria foliosa* from Ghodazari (Chandrapur district)

Sr. No.	Fugal taxa	PDA	CzA	RBA	SA	CMA	MEA	WkM	Total colony	Frequ-ency
1	*Aspergillus awamori*	7	6	0	6	7	7	8	**41**	18.47

3	*Aspergillus flavus*	0	0	0	0	0	2	0	**2**	0.90
4	*Aspergillus fumigatus*	0	0	0	0	0	2	0	**2**	0.90
5	*Aspergillus ochraceus*	0	0	9	0	0	0	0	**9**	4.05
6	*Aspergillus niger*	6	0	0	2	0	4	0	**12**	5.41
7	*Fusarium oxysporum*	7	0	9	0	0	0	0	**16**	7.21
8	*Fusarium semitactum*	0	10	0	2	7	2	4	**25**	11.26
9	*Mucor heimalis*	0	0	0	1	0	0	0	**1**	0.45
10	*Nigrospora oryzae*	1	0	0	0	0	4	0	**5**	2.25
11	*Penicillium digitatum*	13	0	0	0	6	0	0	**19**	8.56
12	*Penicillium spp*	0	0	12	0	0	0	0	**12**	5.41
13	*Pestolosiopis glandicola*	4	5	0	10	0	9	1	**29**	13.06
14	*Trichoderma viride*	1	5	0	0	0	2	0	**8**	3.60
15	*White sterile mycelium*	7	0	3	2	0	0	8	**20**	9.01
16	*Black sterile mycelium*	3	0	4	3	4	3	2	**19**	8.56
	Total colony	**49**	**28**	**37**	**26**	**24**	**35**	**23**	**222**	100.00
	Frequency	22.07	12.61	16.67	11.71	10.81	15.77	10.36	100	

Table 2: Phylloplane of *Habenaria foliosa* from Amboli (Sindhudurg district)

Sr. No.	Fugal taxa	PDA	CzA	RBA	SA	CMA	MEA	WkM	Total colony	Freq.
1	*Aspergillus niger*	0	1	0	3	1	2	2	**9**	8.18
2	*Gliocladium penicillatum*	1	2	0	1	3	2	2	**11**	10.00
3	*Penicillium chrysogenum*	1	1	1	2	2	3	3	**13**	11.82
4	*Penicillium oxalicum*	1	0	0	0	0	2	2	**5**	4.55
5	*Trichoderma viride*	2	1	2	4	4	6	2	**21**	19.09
6	Unknown-2	0	0	0	2	2	5	2	**11**	10.00
7	Black sterile mycelia	1	0	0	2	2	4	2	**11**	10.00
8	White sterile mycelia	3	2	10	3	2	6	3	**29**	26.36
	Total colony	**9**	**7**	**13**	**17**	**16**	**30**	**18**	**110**	100
	Frequency	8.18	6.36	11.82	15.45	14.55	27.27	16.36	100	

Table 3: Phylloplane of *Habenaria foliosa* from Melghat (Amravati district)

Sr.No.	Fugal taxa	PDA	CzA	RBA	SA	CMA	MEA	WkM	Total colony	Frequency
1	*Aspergillus awamori*	1	12	0	2	0	0	5	**20**	11.63
2	*Aspergillus niger*	2	3	0	4	5	2	7	**23**	13.37
3	*Aspergillus ochraceus*	0	0	1	0	0	0	0	**1**	0.58
4	*Aspergillus sulphureus*	0	0	2	0	0	0	0	**2**	1.16
5	*Blakeslea trispora*	1	0	0	0	0	0	0	**1**	0.58
6	*Fusarium oxysporum*	0	0	5	5	0	0	6	**16**	9.30

7	*Michrodochium dimerum*	0	0	2	2	5	0	0	**9**	5.23
8	*Penicillium oxalicum*	0	2	0	3	0	2	5	**12**	6.98
9	*Trichoderma viride*	6	4	6	3	0	7	0	**26**	15.12
10	Unknown- 1	0	0	28	3	0	0	2	**33**	19.19
11	White sterile mycelia	4	10	0	2	2	0	2	**20**	11.63
12	Black sterile mycelia	4	0	0	0	0	5	0	**9**	5.23
	Total colony	**18**	**31**	**44**	**24**	**12**	**16**	**27**	**172**	100
	Frequency	10.47	18.02	25.58	13.95	6.98	9.30	15.70	100	

Table 4: Phylloplane of *Habenaria foliosa* from Chandpur (Bhandara district)

Sr.No.	Fugal taxa	PDA	CzA	RBA	SA	CMA	MEA	WkM	Total colony	Frequency
1	*Aspergillus awamori*	5	7	0	4	2	0	0	**18**	8.41
2	*Aspergillus japonicus*	9	12	0	11	0	4	0	**36**	16.82
3	*Aspergillus niger*	11	13	0	13	8	7	6	**58**	27.10
4	*Aspergillus sulphureus*	0	0	6	2	0	0	0	**8**	3.73
5	*Fusarium monoliformae*	0	0	3	0	0	0	0	**3**	1.40
6	*Penicillium citrinum*	0	0	0	0	0	0	2	**2**	0.93
7	*Penicillium oxalicum*	7	4	0	2	3	0	0	**16**	7.47
8	*Phycomyces nitens*	4	0	2	0	0	0	0	**6**	2.80
9	*Trichoderma spp-2*	2	0	0	0	0	4	3	**9**	4.20
10	White sterile mycelia	2	3	10	0	0	3	3	**21**	9.81
11	Black sterile mycelia	7	0	8	3	13	6	0	**37**	17.2
	Total colony	**47**	**39**	**29**	**35**	**26**	**24**	**14**	**214**	100
	Frequency	21.96	18.22	13.55	16.35	12.14	11.21	6.54	100	

Table 5: Phylloplane of *Habenaria foliosa* from Gadchiroli (Gadchiroli district)

Sr.No.	Fugal taxa	PDA	CzA	RBA	SA	CMA	MEA	WkM	Total colony	Frequency
1	*Acremonium spp*	19	14	16	11	9	16	11	**96**	18.97
2	*Alternaria alternata*	0	0	3	0	0	0	0	**3**	0.59
3	*Aspergillus ochraceus*	2	3	2	1	1	3	2	**14**	2.77
4	*Aspergillus japonicus*	11	8	0	8	7	5	7	**46**	9.09
5	*Aspergillus niger*	12	8	0	10	2	3	9	**44**	8.70
6	*Aspergillus sulphureus*	1	2	8	2	0	1	2	**16**	3.16
7	*Aspergillus wentii*	0	0	2	0	0	0	0	**2**	0.40
8	*Blakeslea trispora*	3	3	1	1	0	2	3	**13**	2.57
9	*Cladosporium macrocarpum*	1	1	0	2	3	2	1	**10**	1.98
10	*Curvularia lunata*	5	2	0	0	1	3	3	**14**	2.77
11	*Fusarium monoliformae*	2	2	5	5	4	2	2	**22**	4.35
12	*Fusarium oxysporum*	1	2	5	2	3	3	3	**19**	3.75

13	*Gliocladium penicillatum*	5	3	3	3	3	3	3	**23**	4.55
14	*Mucor heimalis*	2	3	2	0	2	1	3	**13**	2.57
15	*Mucor mucedo*	2	1	1	0	0	0	1	**5**	0.99
16	*Penicillium chrysogenum*	2	3	0	0	0	1	4	**10**	1.98
17	*Penicillium oxalicum*	15	7	7	8	9	6	11	**63**	12.45
18	*Pestolotiopsis glandicola*	3	1	1	5	2	2	4	**18**	3.56
19	*Phoma terricola*	1	2	3	3	2	2	2	**15**	2.96
20	*Phycomyces nitens*	2	0	1	0	1	0	1	**5**	0.99
21	*Torula herbarum*	3	4	2	2	1	6	5	**23**	4.55
22	*Trichoderma spp-2*	0	0	2	0	0	1	3	**6**	1.19
23	White sterile mycelium	3	5	2	1	2	2	2	**17**	3.36
24	Black sterile mycelium	1	2	1	1	0	1	3	**9**	1.78
	Total colony	**96**	**76**	**67**	**65**	**52**	**65**	**85**	**506**	100
	Frequency	18.97	15.01	13.24	12.84	10.27	12.84	16.79	100	

Table 6: Diversity index

	Chandrapur	Sindhudurg	Amravati	Bhandara	Gadchiroli
Simpson(1-D)	0.8986	0.8413	0.8762	0.8418	0.9166
Shannon(H')	2.453	1.956	2.199	2.047	2.801

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