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Screening of leaf surface mycoflora over *Barleria prionitis* Linn: A seasonal study

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ABSTRACT

The percentage frequency and contribution of leaf surface mycoflora of Barleria prionitis was observed fortnightly with the help of gravity petriplate method. The result shows that Chaetomium aureum, Curvularia clavata, Alternaria citri, Alternaria alternata, Cladosporium oxysporum, Cladosporium cladosporioids, Mycelia sterila were most frequent fungi. Maximum percentage contribution were observed in winter season (39.62), minimum percentage contribution were (25.71) in summer season and (35.12) moderate in rainy season. Month wise percentage contributions of the leaf surface mycoflora were also recorded. Maximum percentage contribution (32) were observed in the month of November while minimum percentage (14.60) in month of May. Role of Environmental factor on the percentage frequency and contribution of leaf surface mycoflora were also discussed.

Key words: Leaf surface Mycoflora, Percentage contribution, Frequency, Anamorphic fungi.

INTRODUCTION

Fungi are known to be ubiquitous in nature, growing where organic material is available .The presence of fungal species and consequently their concentration in the atmosphere are the result of the action of many biological and environmental factors. Fungal spores constitute a significant fraction of airborne bioparticles. Last (1955) introduced term phyllosphere,[1] to denote the leaf surface of plants. The report of the intensive investigations on leaf surface mycoflora has been reported by Last and Deighton (1965), [2]. The present investigation carried out on *Barleria prionitis Linn.* a medicinal plant belongs to family Acanthaceae. *Barleria prionitis Linn*, widely distributed throughout India, Sri Lanka, Africa and tropical Asia [3]. The crude extract of this plant is commonly used in folk medicine to treat whooping cough. The plant extract has also shown its potential applications as diaphoretic and expectorant [4,5]. The plant has also shown anti-respiratory syncytial virus, anti-arthritic, anti-inflammatory and anti-fertility activities. Its leaves are known to contain 6-Hydroxyflavone, one of the chemical compounds that are a noncompetitive inhibitor of the protein [6]. Aspect related to leaf surface mycoflora associated with several plant have been studied by Gregory (1971), Diemh(1973), Abdel-Hafez (1981), Blackman (1981), Ayachi and Tiwari (1993), Tiwari and sahu (1991,1997), Uddin (2005) [7-14].The aim of the study was to determine seasonal variation in concentration of selected fungal species types and the relationship to meteorological factors.

MATERIALS AND METHODS

Sample Collection

Barleria prionitis Linn was cultivated in Botanical Garden of Govt. Science College Raipur. During present studies of leaf surface mycoflora of above plant were observed fortnightly with the help of gravity petriplate method. For the survey of leaf surface mycoflora, the fresh leaves were collected randomly in sterilized polythene bags then brought into the laboratory.

Media Preparation

Composition of Potato Dextrose Agar Medium
Potato (peeled) - 250gm
Dextrose - 15 gm
Agar - 18 gm
Distilled water - 1000 ml

Isolation and identification of fungi

To isolation of the leaf surface mycoflora, in the laboratory the collected leaves were placed in conical flask containing 75ml of sterilized distilled water. The flask was hand shaken for 30 minutes to make a homogenous suspension of fungal species.1ml of this suspension poured into the petriplate containing Potato Dextrose Agar (PDA) medium. 5 petriplate were used at a time in each experiment. Then the plates were incubated at 26 ± 1^0C in incubation chamber for 6-7 days. After the incubation, the fungal colonies were counted.

Lacto Phenol Cotton Blue Mounting

A portion of the mycelium of the representative colonies was picked up with the help of a pair of needles and semi permanent slides were prepared using lactophenol cotton blue (20g – phenol (crystal); 20g lactic acid; 40g glycerin; 20 ml water; cotton blue a few drops). The slide was gently heated in a sprit lamp so as to release the air bubbles, if any present inside the cover glass. The excess stain was removed using tissue paper and the cover glass was sealed and then identified and photographs were taken using Nikon microscope with Nikon Camera (fig 1).

For the environment study on leaf surface mycoflora, meteorological data (temperature, relative humidity and rainfall) obtained from the meteorological department of Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.).

RESULTS AND DISCUSSION

During this work 568 fungal colonies, representing 38 species and 17 genera were isolated. Among 38 fungal species, 3from zygomycotina, 2 from Ascomycotina and 33 from Anamorphis fungi were observed (Table.1). During investigation, 30 fungal species were observed in winter, 25 in rainy season and 20 in summer season. The percentage frequency of the leaf surface mycoflora was also observed. *Chaetomium aureum*, *Aspergillus niger*, *Aspergillus* flavus, *Penicillium rugulosum*, *Curvularia lunata*, *Curvularia clavata*, *Alternaria citri*, *Alternaria alternata*, *Cladosporium oxysporum* , *Cladosporium cladosporioides*, Mycelia sterile while were most frequent fungi.On the contrary moderate ferquent fungi were *Choaenephora cucurbitarum*, *Mucor sp. Rhizopus rhizopodiformis*, *Aspergillus ustus*, *Asp. awamori*, *Asp.nidulans*, *Curvularia eragrostidis*, *Cuv.oryzae*, *Nigrospora sphaerica*, *Nigrospora oryzae*, *Fusarium oxyspourm*, *Colletotrichum dematium*, Mycelia sterila black. While least frequent fungi were *Thielavia boothi*, *Asperillus stellatus*, *Aspergillus nidulans var.acristatus*, *Alternaria chlamydospora*, *Penicillium notatum*,*Curvularia senegalensis*, *Drechslera australiensis*, *Drechsler halodes*, *Drechslera hawaiiensis*, *Fusarium moniliforme*, *Phoma sp* (Table.2).

In winter season (39.61) maximum percentage contribution of the fungal species were observed, while minimum percentage contribution (25.17) were in sumer season and (35.21)were in rainy season (Fig.2). In November month maximum percentage contribution (13.25) were observed while minimum percentage contribution (3.65) were observed in May month (Fig.3). In rainy season, the percentage contribution 2.50, 1.00, 96.50 in Zygomycotina, Ascomycotina and Anamorphic fungi were observed (Fig.4). In winter season, percentage contribution of Zygomycotina were 2.66, Ascomycotina were 0.88 and Anamorphic fungi were 96.44(Fig.4).

Similary, the percentage contribution in summer season, Zygomycotina were .66, Ascomycotina were 1.39 and Anamorphic fungi were 97.90 (Fig.4). Month wise percentage contribution of each class in different month was

also observed. The percentage contribution in July was 7.24 from Zygomycotina 2.89 from Ascomycotina and 89.80 from Anamorphic Fungi (Table.3). In August, 100.00 of Anamorphic Fungi. In September, 100.00 of Anamorphic fungi. In October, 100.00 of Anamorohic Fungi were recorded (Table.3). In August, September and October month,Zygomycotina and Ascomycotina were totally absent. In November, 2.70 from Zygomycotina and 93.20 from Anamorphic Fungi in December 100 from Anamorphic Fungi, Zygomycotina and Ascomycotina were totally absent, in January, 3.40 from Zygomycotina, 3.40 from Ascomycotina and 93.10 from Anamorphic Fungi . In Feb 5.12 from Anamorphic Fungi, In March 100 from Anamorphic Fungi, Zygomycotina and Ascomycotina were totally absent , In April,100 from Anamorphic Fungi, Zygomycotina and Ascomycotina were totally absent , In May 4.76 From Zygomycotina 4.76 Ascomycotina and 90.40 from Anamorphic Fungi in June, 2.77 from Ascomycotina, 97.20 from Anamorphic fungi were recorded observed(Table-3). Month wise percentage contribution of each class of leaf surface mycoflora was also observed. In Zygomycotina the maximum percentage contribution (41.60) were observed in July while the minimum percentage contribution (8.33) were observed in May month. In Ascomycotina, the maximum percentage contributions (33.33) were observed during the July and January months, while the minimum percentage contributions were recorded in May and June. In Anamrphic fungi, the maximum percentage contributions 12.9 were observed in during November month while minimum percentage contributions 3.45 were recorded during June month. The percentage contributions were 9.45 in March then decrease gradually up to May (Table-2).Environmental factor was the important physical factor, which effect the fungal population and contribution present on the leaf surface, maximum percentage contribution (39.61) were recorded in winter season,(max tem 28.46^0c min 13.30^0c RH- 87.2 and Avg. Rainfall 190mm) due to favorable temperature, relative humidity and rainfall, similar result were also observed by Gegory (1971)[7],Tiwari and Godheja (1985),Farah et.al (2011)[15,16]. Minimum fungal contribution(25.17) have recorded in summer season (max temp 38.4^0 c min28^0c RH- 55.6 and Avg. rainfall 10-15 mm) due to unfavorable condition, also observed by Tiwari et.al(1997)[13] and Strzelczak et.al[17].

Table.1 Table showing the different name of fungi present in leaf surface mycoflora of *Barelria prionitis L.*

S .NO.	NAME OF FUNGI
	ZYGOMYCOTINA
1	*Rhizopus rhizopodiformis*
2	*Choaenephora cucurbitarum*
3	*Mucor sp.*
	ASCOMYCOTINA
4	*Thielavia boothi*
5	*Chaetomium aureum*
	ANAMORPHIC FUNGI
6	*Alternaria radicina*
7	*Alt.citri*
8	*Alt.alternata*
9	*Alt.chlamydospora*
10	*Aspergillus niger*
11	*Asp.fumigatus*
12	*Asp. ustus*
13	*Asp .stellatus*
14	*Asp. flavus*
15	*Asp. nidulans var acristatus*
16	*Asp. awamori*
17	*Asp.nidulans*
18	*Cladosporium oxysoprum*
19	*Cladosporium cladosporioids*
20	*Curvularia lunata*
21	*Curvularia clavata*
22	*Cur. eragrostidis*
23	*Cur.senegalensis*
24	*Cur.oryzae*
25	*Colletotrichum dematium*
26	*Drechslera australiensis*
27	*Dre.halodes*
28	*Dre.hawaiiensis*
29	*Fusarium moniliforme*
30	*Fus. oxyspourm*
31	*Nigrospora sphaerica*
32	*Nigrospora oryzae*

33	*Penicillium notatum*
34	*Penicillium rugulosum*
35	*Pithomyces graminicola*
36	*Phoma sp.*
37	*Mycelia sterilia black*
38	*Mycelia sterilia white*

Table.2 Showing Percentage Frequency of Leaf Surface mycoflora of Barleria prionitis.

1	*Rhizopus rhizopodifomis*	66.66
2	*Choaenephora cucurbitarum*	66.66
3	*Mucor sp.*	66.66
4	*Thielavia boothi*	33.33
5	*Chaetomium aureum*	100.00
6	*Alternaria radicina*	100.00
7	*Alternaria citri*	100.00
8	*Alternaria alternata*	100.00
9	*Alternaria chlamydospora*	100.00
10	*Aspergillus niger*	66.66
11	*Aspergillus fumigates*	66.66
12	*Asp. ustus*	33.33
13	*Asp. stellatus*	100.00
14	*Asp. flavus*	33.33
15	*Asp nidulans var acristatus*	33.33
16	*Asp. awamori*	66.66
17	*Asp. nidulans*	66.66
18	*Penicillium notatum*	33.33
19	*Pencillium rugulosum*	100.00
20	*Curvularia lunata*	100.00
21	*Curvularia clavata*	100.00
22	*Curvularia eragrostidis*	66.66
23	*Curvularia senegalensis*	33.33
24	*Curvularia oryzae*	66.66
25	*Drechslera australiensis*	33.33
26	*Drechslera halodes*	33.33
27	*Drechslera hawaiiens*	33.33
28	*Cladosporium oxysporum*	100.00
29	*Cladosporium Cladosporioides*	100.00
30	*Nigrospora sphaerica*	66.66
31	*Nigrospora oryzae*	66.66
32	*Fusarium moniliforme*	33.33
33	*Fusarium oxyspourm*	66.66
34	*Colletotrichum dematium*	66.66
35	*Pithomyces graminicola*	66.66
36	*Phoma sp.*	33.33
37	*Mycelia sterilia black*	66.66
38	*Mycelia sterilia white*	100.00

Table.3 The month wise percentage contribution of each class of Leaf surface mycoflora of *Barleria prionitis linn*.

S.NO.	Name of Class	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	March	April	May	June
1	Zygomycotina	7.24	-	-	-	2.7	-	3.4	5.12	-	-	4.76	-
2	Ascomycotina	2.89	-	-	-	-	-	3.4	-	-	-	4.76	2.77
3	Anamorphic	89.8	100	100	97.2	100	93.1	93.1	94.8	100	100	90.4	97.2

Table. 4 Total percentage contribution of each class of Leaf surface mycoflora of *Barleria prionitis* L.

S.No	Name of Class	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	March	April	May	June
1	Zygomycotina	41.66				16.6		16.66	16.66			8.33	
2	Ascomycotina	33.30						33.30				16.60	16.6
3	Anamorphic	11.30	8.72	6.18	8.90	12.90	10	9.80	6.72	9.45	6.18	3.45	6.36

Fig:1 Photographs of some fungi

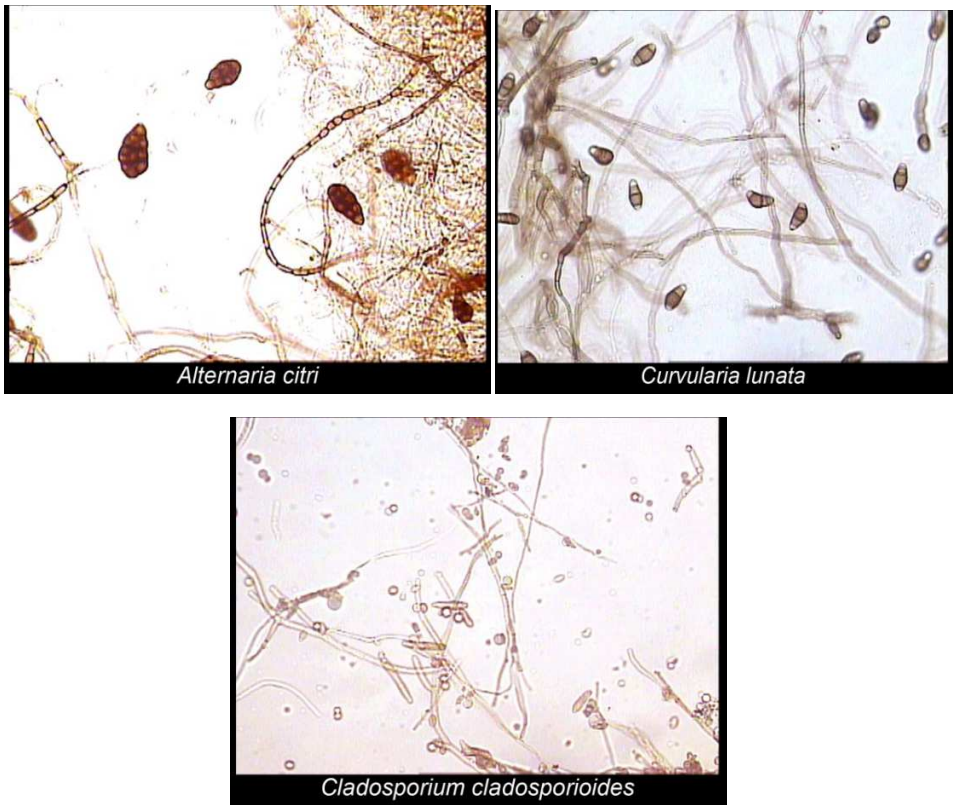


Fig:2 Different Seasonwise Percentage Contribution of Leaf surface mycoflora of *Barleria prionitis* .

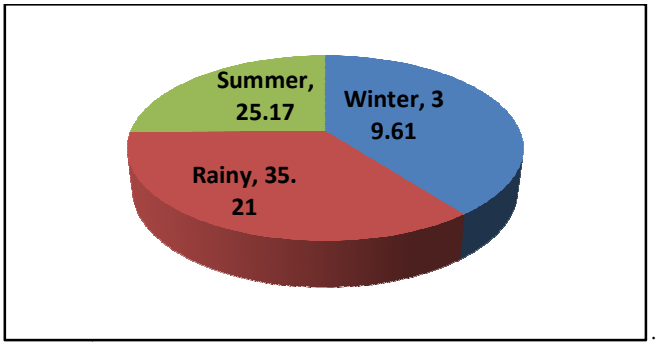


Fig:-3 Months wise percentage contribution of leaf surface mycoflora of *Barleria prionitis*

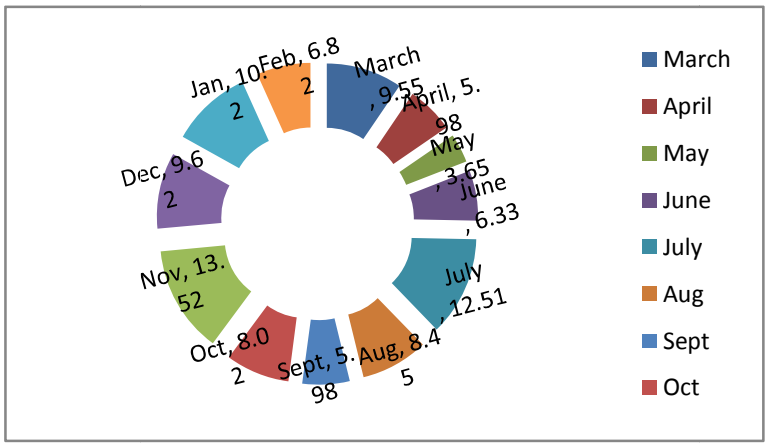
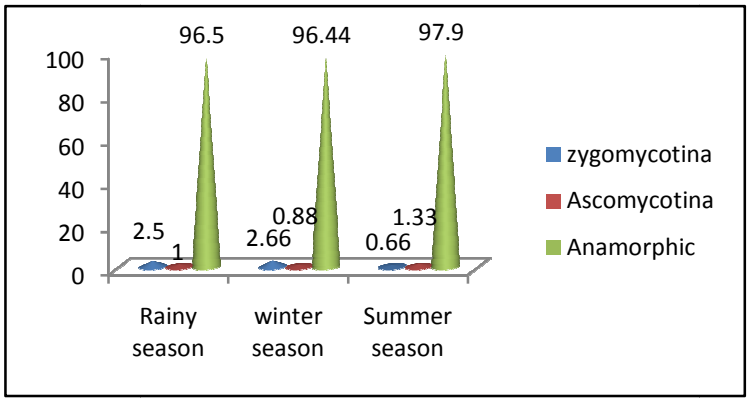


Fig-4 Different Season and Class wise Percentage Contribution of Leaf Surface Mycoflora of *Barleria prionitis*



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