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Original Research Paper

Seasonal Occurrence of Endomycophytes from Inner Bark of *Barringtonia acutangula* (L.) Gaertn

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ABSTRACT

Endophytic fungi from inner bark of *Barringtonia acutangula* (L.) Gaertn were studied in three different seasons during 2008-2009. A total of 23 endophytes were recorded during rainy season, followed by 30 endophytes in winter season and 14 fungi in summer season. *Aspergillus niger* and *Aspergillus flavus* were found to be dominant endophytes followed by *Verticillium* and *Cladosporium* species.

INTRODUCTION

Endophytic microorganisms are those that inhabit interior of plants especially in branches, bark and stems showing no apparent harm to host (Azevedo 1998). Endophytes include fungi that have one or more of a variety of interaction with their host plant. Some fungi are widespread and found on many different plant species, while others are highly specific to single host in single environment.

Endophytes show a diverse array of interaction between plant and fungus, and a large number of fungi may be isolated from and one host. It seems possible that endophytes will have one or more range of functions, most of which are unknown at present (Redlin & Carris 1996, Ahlholm et al. 2002). Endophytic organisms stimulate greater resistance to stress condition, alteration in physiological properties, production of phytohormones and other compounds of biotechnological interests (Daniella et al. 2004). Thus, endophytes advocate a good tool for the protection of host by various pathways. Therefore, an attempt was made to screen out endophytic fungi in the bark of *Barrigtonia acutangula* (L.) Gaertn, a medicinal plant.

Barringtonia acutangula, commonly known as Sumudraphalli, is widely distributed in tropical countries. The roots of this tree possess antipyretic, stimulant and emetic properties. The fruits are acrid, bitter, cooling, anti-helminthic and antipyretic used in vitiated conditions of 'pitta'. Again, it is used in skin diseases, leprosy, bronchitis, intermittent fever, lumbago, etc. Saponins and triterpenoid are polar constituents of plant, many of which exhibit a broad spectrum of biological activities. The acidic saponin mixture is isolated along with secondary metabolites from this plant (Sahu & Achari 2001).

MATERIALS AND METHODS

The bark pieces of the *Barringtonia acutangula* were collected from Town Hall garden, Kolhapur periodically in three different seasons. The bark pieces were cut at 1-2 metres above the ground level

and to the depth of 1-1.5 cm in the trunk. The collected bark samples were brought to the laboratory and surface sterilized by 70 percent ethanol for 1 minute followed by 1 to 2 minutes in 3.5 percent sodium hypochlorite solution in a beaker, and later rinsed three times in distilled water (Petrini et al. 1986). The outer skin was removed slowly with sterilized knife and inner portion containing cortex was cut into small pieces of 0.2 × 0.8 mm (Mahesh et al. 2005). Approximately 100 segments were cut and plated on Nutrient Agar and PDA media mixed with Septran (100 mg/L) and incubated in a chamber for 21 days at 12 hours light/dark cycles at 28 ± °C. The Petri plates were allowed to grow endophytic fungi and monitored regularly. Isolation was done for pure culture of the fungi from each Petri plate after 18th to 20th day by subculturing on to an appropriate medium. The seasonal endophytic mycoflora were identified based on morphological characters using standard identification manual.

The number of endophytes were calculated in all Petri plates. Percentage of colonizing frequencies were calculated according to the method prescribed by Fisher & Petrini (1987). The dominant fungi in all three seasons were estimated by the method of Kumaresan & Suryanarayan (2002).

RESULTS AND DISCUSSION

Seasonal occurrence of endophytes from inner bark of *Barringtonia acutangula* is depicted in Tables 1, 2 and 3. A few studies have been carried out on endophytic mycoflora of tropical trees (Frohlich & Hyde 1999). A total of 67 fungal species have been recorded in the inner bark of this tree during 2008-2009. The taxa like *Aspergillus* sp., *Bispora* sp., *Geotrichum* sp., *Fusarium* sp., *Rhizopus* sp., *Mucor* sp. and *Verticillium* sp. were recorded during June to October 2008 as endophytes. *Aspergillus niger, A. flavus* and *Rhizopus stolonifer* were dominant fungi during rainy season.

A total of 30 species of hypomyceteous fungi were recorded in the inner bark of *Barringtonia acutangula* (Table 2) during winter season from November 2008 to January 2009. *Verticillium* sp., and *Aspergillus niger* were found to be dominant fungi followed by *Trichothecium roseum, Cladosporium herbarum, Nigrospora* sp., *Fusarium oxysporum* and *Geotrichum* sp. Meanwhile a few endophytes have been recorded during summer period from February 2009 to May 2009 (Table 3). *Diplodia* sp. and *Cunninghamella* sp. were noticed during summer. *Aspergillus niger* was dominant fungus followed by *Cladosporium herbarum* and sterile mycelia.

Table 1: Endophytic fungi isolated from inner bark of *Barringtonia acutangula* during rainy season.

Sr.No.	Endophytic Fungi	Number of Endophytes	Colonization Frequency	Dominant Fungi
1	*Aspergillus niger*	4	4	17.39
2	*Aspergillus flavus*	3	3	13.04
3	*Verticillium tenuissimum*	2	2	8.69
4	*Mucor racemosus*	1	1	4.34
5	*Rhizopus stolonifer*	4	4	17.39
6	*Fusarium solani*	2	2	8.69
7	*Fusarium oxysporum*	2	2	8.69
8	*Geotrichum* sp.	1	1	4.34
9	*Verticillium albo-arum*	1	1	4.34
10	*Bispora punctata*	1	1	4.34
11	*Sterile mycelia*	2	2	8.69
	Total isolation	23	23	

Total Segments-100; Total endophytes-23

Table 2: Endophytic fungi isolated from inner bark of ***Barringtonia acutangula*** during winter season.

Sr.No.	Entophytic Fungi	Number of Endophytes	Colonization Frequency	Dominant Fungi
1	*Aspergillus niger*	3	3	10.0
2	*Aspergillus flavus*	2	2	6.66
3	*Verticillium* sp.	3	3	10.0
4	*Rhizopus stolonifer*	2	2	6.66
5	*Fusarium oxysporum*	2	2	6.66
6	*Geotrichum* sp.	2	2	6.66
7	*Verticillium albo-arum*	1	1	3.33
8	*Bispora punctata*	1	1	3.33
9	*Monilia barringtoniae*	2	2	6.66
10	*Monilia* sp.	1	1	3.33
11	*Trichothecium roseum*	2	2	6.66
12	*Nigrospora* sp.	2	2	6.66
13	*Cladosporium herbarum*	2	2	6.66
14	*Memnoniella* sp.	1	1	3.33
15	*Trichoderma viridae*	1	1	3.33
16	*Cephalosporium inerium*	1	1	3.33
17	*Sterile mycelia*	2	2	6.66
	Total isolation	30	30	

Total Segments-100; Total endophytes-23

Table 3: Endophytic fungi isolated from inner bark of ***Barringtonia acutangula*** during summer season.

Sr.No.	Entophytic Fungi	Number of Endophytes	Colonization Frequency	Dominant Fungi
1	*Aspergillus niger*	3	3	21.42
2	*Aspergillus flavus*	1	1	7.14
3	*Rhizopus stolonifer*	2	2	14.28
4	*Fusarium moniliformis*	1	1	7.14
5	*Cladosporium herbarum*	2	2	14.28
6	*Diplodia* sp.	1	1	7.14
7	*Cunninghamella* sp.	1	1	7.14
8	*Trichothecium roseum*	1	1	7.14
9	*Sterile mycelia*	2	2	14.28
	Total isolation	14	14	

Total Segments-100; Total endophytes-14

The endophytes such as *Acremonium* sp., *Phialocephala* sp., etc. are commonly isolated from tropical and subtropical plants (Koga et al. 1997), whereas *Fusarium* sp. and *Drechslera* sp. are pathogenic to crops. Sometimes they may get modified by mutation and grow into useful non pathogenic endophytes. The toxic products synthesized by endophytes in woody plants were able to modify growth and death rates in larvae of the spruce bud worm *C. fumiferanna* feeding on balsam fir (Calhoun et al. 1992). The endophytes were identified as *Phyllosticta* sp. and *Hormonema dematioides*, and the toxic compounds were mainly heptelidic acid and regulosine, even tremorgenic toxin in tropical woody plants infected with an endophytic fungus from the genus *Phomopsis* (Billis et al. 1992) was recorded. Antibiotic phomol was isolated from fermentation by *Phomopis* sp., an endophytic fungus from *Erythrina crista-galli* (Webber 1981). Thus, endophytes provide protection against pathogens

and are potential biocontrol agents, which could be utilized to protect tissue culture plants before they are transplanted to the field.

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