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SEASONAL RECURRENCE OF ENDOPHYTIC FUNGI OF *Orthosiphon spiralis*

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ABSTRACT
Orthosiphon spiralis, a perennial shrub growing well in tropics was selected for the study of endophytic fungi recurring in different seasons during a year. Foliar endophytes were isolated by culturing the leaf segments aseptically on Potato dextrose agar plates amended with chloramphenicol. The growing colonies were subultured by hyphal tipping and the percentage colonization frequency (CF%) of endophyte species was calculated. The number of endophytes isolated were higher during the wet periods rather than the dry periods. The diversity of endophyte assemblage in this tropical plant was highest comprising various hyphomycete and coelomycete members. Few members like Colletotrichum sp., Phomopsis sp., Cladosporium sp., Trichoderma sp., Curvularia sp., has proven their recurrence with Geotrichum sp., being the dominant genus that can be recurred and harbored during anytime within the year. Due to its prevalent recurrence in this medicinal plant having therapeutic attributes, it can be harveseted at anytime for their potent metabolites, that makes an insight into the area of metabolomics.

KEYWORDS: *Orthosiphon siphalis,* Geotrichum sp., endophyte.

INTRODUCTION

Endophytic fungi, which cause asymptomatic infections in plants, are important organisms as many of them produce novel secondary metabolites of industrial potential and some of them enhance the fitness of their host plants (Schulz *et al*., 2002; Worapong *et al*., 2002). Their occurrence and distribution in the plants growing in tropics are well studied yet not much on the seasonal infection patterns. *Orthosiphon spiralis,* a perennial shrub is one among growing well in tropics, attributed for its therapeutic value.

Orthosiphon spiralis (Lour.) Murr. (Limiaceae), commonly called 'Kidney Tea Plant', is a medicinal plant widely used in many parts of the world. It is an erect, perennial shrub found in North eastern India and Western Ghats and Nicobar Islands. Leaves are coarsely toothed. Flowers are white or purplish in cluster. The plant is grown as an ornamental for its clusters. The plant is mainly valued for its medicinal values.

Leaves are used for the treatment of various kidney and urinary bladder diseases including nephrocirrhosis and phosphaturia. The activity of the leaves is attributed to the presence of a bitter glycoside, orthosiponin (Wealth of India, 1966). *Orthosiphon stamineus* and *O. aristatus* are synonyms of *O. spiralis*. *O. stamineus* exhibits hypoglycemic activity (Mariam et al., 1996) and the plant is also used to treat hepato-renal syndrome and renal ischaemia. For commercial conexploitation, the flowers and the floral buds of O. spiralis are usually removed to enhance the quantity of the active constituent in the leaves. Altogether, the plant is significant of its medicinal value whose biological attributes confers upon their diversified endophytic fungal isolates, core of the present study.

MATERIALS AND METHODS

Host and Site Collection:

Plant samples of *Orthosiphon spiralis* were procured during the year from the Kolli Hills, a part of the Eastern Ghats which is located in central Tamil Nadu in Namakkal district of India (June - Aug, Sep - Nov, Dec - Feb, Mar - May).

Collection of Plant Samples

Leaf samples of *Orthosiphon spiralis* were collected from the plant, placed in sealed plastic bags and kept at 4°C until for the isolation of endophytic fungus.

Treatment of Plant material

Plant leaf samples were thoroughly washed in a tap water for 10 minutes. Leaf segments of 5 –8 mm length were cut using a sterile scalpel. This was followed by surface sterilization of the cut segments by dipping in 75% ethanol for 1 minute, followed by a solution of HgCl2 (0.2%) for 30 seconds. Surface sterilized samples were washed with three changes of sterile distilled water and blotted with sterile tissue paper. The segments were then transferred to Potato Dextrose Agar (PDA) plates amended with chloramphenicol (12mg/100ml) to inhibit bacterial growth. Plates were labeled accordingly and incubated at 24°C with a 12-hour cycle of dark and light (Lacap et al., 2003).

Culturing and Subculturing

The growing colonies were transferred to a fresh PDA plates by hyphal tipping and subcultured. Percentage colonization frequency (CF%) of endophyte species was calculated as number of segments colonized by a single endophyte divided by total number of segments observed * 100 and tabulated.

Induction of sporulation

The cultures which failed to sporulate within 2-3 months of incubation were designated as "mycelia sterilia". Different methods were adapted to induce sporulation. In the first method, isolates were subcultured along with sterilized host parts and incubated at 24°C with a cycle of 12 hours of UV light and dark. In the second method, isolates were cultured on nutrient deficient potato carrot agar.

Identification of Fungal Isolates

Pure cultures were examined periodically for sporulation and identified. Fungal identification methods were based on the morphology of the fungal culture, the mechanism of spore production and characteristics of the spore by following the standard mycological manuals. Wet mounts were also prepared using Lactophenol cotton blue and glycerol for identification.

RESULTS AND DISCUSSION

Orthosiphon spiralis harboured endophytes throughout the year. About 140 fungal isolates were obtained from 100 leaf segments (Fig.1&2). There were 6 recognisable sterile mycelia comprising 50 isolates. These fungal isolates were then identified and colonization frequencies were calculated (Table 1).

TABLE 1: Colonisation frequency% of endophytes from leaves of *Orthosiphon spiralis* Sampled during various seasons.

Endophyte	June – Aug	Sep – Nov	Dec – Feb	Mar – May
Alternaria alternata	1	2.5	-	-
Fusarium sp.,	6	5.5	2	-
Penicillium sp.,	1.5	-	-	3.5
Phomopsis sp.,	16	40.5	25.5	9
Acremonium strictum	13.5	25.5	35	1
Aspergillus niger	-	7.5	2	-
Aspergillus ustus	1.5	2	-	2
Cladosporium oxysporum	-	0.5	-	-
Cladosporium cladosporioides.,	11.5	25	12.5	-
Trichoderma sp.,	-	3	2.5	4.5
Geotrichum sp.,1	3.5	45	20	11.5
Geotrichum sp.,2	2	52.5	35	3.5
Geotrichum sp.,3	6.5	45.2	17	2
Colletotrichum sp.,	11.5	28.5	36	-
Curvularia lunata	6.2	12	5.5	-
Sterile mycelia 1	20	25.5	4.5	-
Sterile mycelia 2	14.5	29	6	-
Sterile mycelia 3	3	1.5	-	-
Sterile mycelia 4	4.5	2	-	-
Sterile mycelia 5	8	5.2	-	3
Sterile mycelia 6	2.5	6.2	-	-

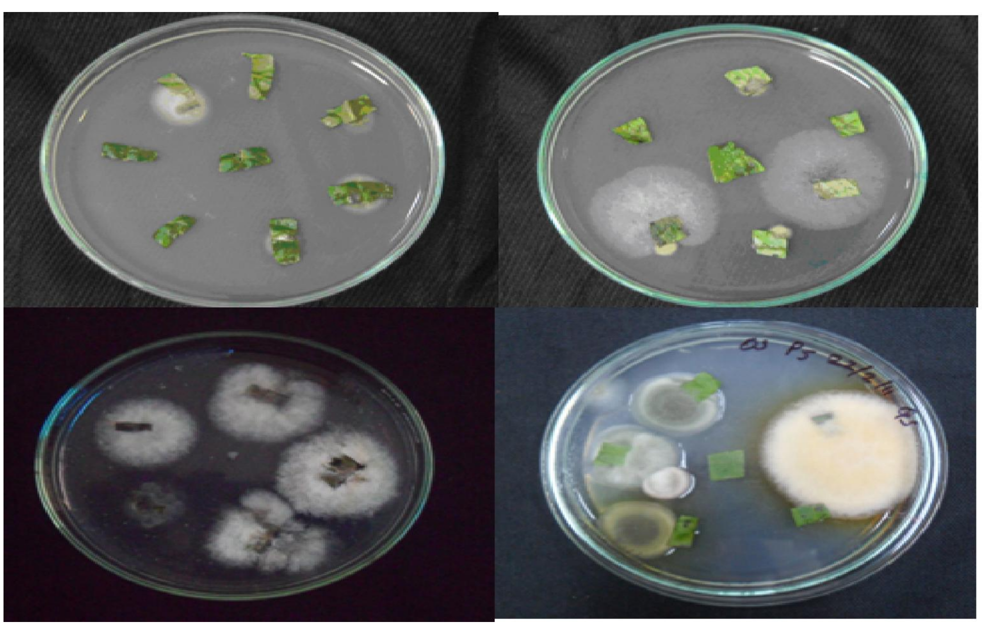

FIGURE 1. Foliar Endophytes from leaf segments of *Orthosiphon spiralis*

FIGURE 2. Endophytic fungal isolates of *Orthosiphon spiralis*

Endophyte assemblages are composed by rare or singleton species which are isolated only once or very few times, and by dominant or plural species which are frequently isolated from a given host species (Neubert et al., 2006). Considering the endophyte assemblage of *Orthosiphon spiralis*, Geotrichum sp., was occurring prevalently in all the seasons being the dominant genus. Further, in a geoclimatic context, endophytic assemblages appear to be richer in tropical than in temperate or cold zones of the world (Fisher et al., 1995; Arnold and Lutzoni, 2007). Colletotrichum sp. and Phomopsis were second dominant genera possessing high colonization frequencies. Henceforth, the diversity of endophyte assemblage in this tropical plant was highest comprising various hyphomycete and coelomycete members

The number of endophytes isolated were higher during the wet periods rather than the dry periods (Fig. 3). Although the susceptibility of the leaves to endophytes increases with age, the leaves continue to recruit only some species of fungi as

endophytes. Further, precipitation could have been an associated factor correlating to infection frequencies of foliar endophytes(Suryanarayanan and Thennarasan, 2004). In many instances, leaves sampled during wet seasons have harbored more endophytes rather than the dry seasons. The same trend has been apparent in *Orthosiphon spiralis* also (Riyaz et al., 2010).

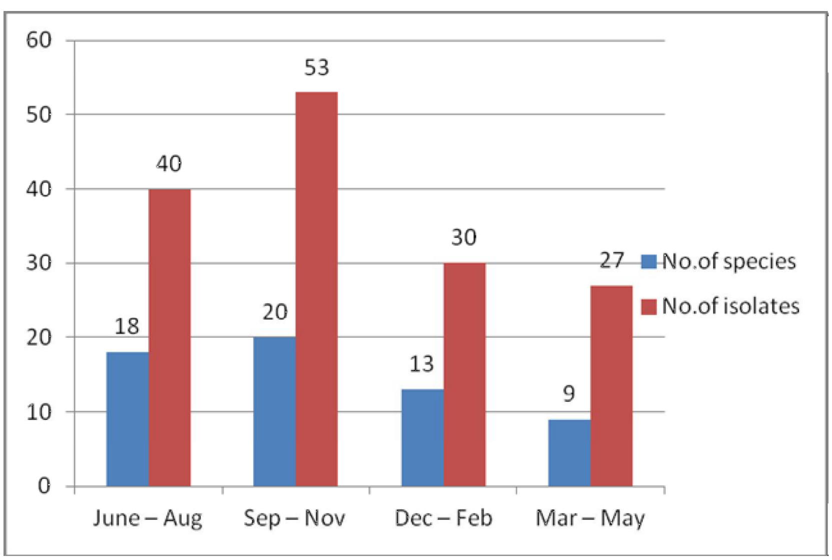

FIGURE 3-Number of species and isolates recovered from *O.spiralis* during various periods

However, few members like *Colletotrichum* sp., *Phomopsi*s sp., C*ladosporium* sp., *Trichoderma* sp., *Curvularia* sp., has proven their recurrence with Geotrichum sp., being the dominant genus that can be recurred and harbored during anytime within the year.

The plant has been attributed towards its therapeutic values especially in treatment of various kidney ailments. Endophytes are also known to produce various secondary metabolites of medicinal value that further studies on this Geotrichum sp., being an isolate of this perennial shrub confer high potential value. Due to its prevalent recurrence in this plant, it can be harveseted at anytime for their potent metabolites, that makes an insight into the area of metabolomics.

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