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Isolation and Characterization of Endophytic Fungi from *Boswellia Ovalifoliolata.* An endemic medicinal plants of Tirumala hills.

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ABSTRACT

The aim of the work is to isolate endophytic fungi from *Boswellia ovalifoliolata* an endemic medicinal plant from Eastern Ghats of Tirumala hills, India. Six endophytic fungi were isolated from leaf and stem samples. Based on morphological identification they were identified as *Xylaria* sp, *Fusarium equiseti* , *Cladosporium* sp, *Lasiodiplodia theobromae* , *Aspergilus niger* , *Cheatomium* sp. The over all colonization frequency of both stem and leaf was 17.5%.Among the endophytic fungi *Fusarium equiseti* was found to be the core group of fungus with colonization frequency of 30%.

Keywords: Endophytic fungi, endemic plant, Colonization frequency.

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INTRODUCTION

Fungal endophytes are symbiotic organisms, and colonize the inner tissues of healthy plants to establish a harmonious relationship with their host without causing any visible disease symptoms [1-2]. Fungal endophytes have been isolated from various plant species, ranging from herbaceous crop plants to higher giant woody forest trees, suggesting their universal presence in nearly all higher plants [3-5]. They may contribute substantially to global fungal diversity but the proportion of this ecological group to total species richness in kingdom fungi remains unknown [6-7]. Previous studies have indicated that the association of fungal endophytes can significantly enhance plant growth, including biomass as well as production yield [8-9]. The beneficial effects of associated fungi (both endophytic fungi and ecto-mycorrhizae) on host plants mitigate biotic and abiotic stresses by increasing nutrient availability, enhancing tolerance to contaminants, and competing with and essentially inhibiting pathogenic organisms [10-11].

MATERIAL AND METHODS

Collection of plant material

Boswellia ovalifoliolata an endemic medicinal plant from Eastern Ghats of Tirumala hills, India. Plant parts such as leaves and stems were used for isolation of endophytic fungi.

Isolation of endophytic fungi

Endophytic fungi isolation was followed by Hallman [12]. Leaves were washed thoroughly under running tap water and then with liquid detergent teepol for 1-2 min to remove the adhered dust particles. Then the leaves were rinsed with Milli-Q water for two minutes and then transferred to laminar airflow (LAF). Under LAF, surface sterilization of leaves was carried out using 5% H_2O_2 for 45 sec and then the leaves were rinsed using 80% alcohol for 1 min. Finally, the leaves were rinsed thoroughly with Milli-Q water for 5 min. Moisture on the leaves was removed by placing them on sterile blotting paper and then cut into small segments of 5X5 mm. Leaf segments were placed on potato dextrose agar (PDA) plates and the plates were incubated at 28 ± 2°C for 8-10 days.

Colonization Frequency

The Colonization Frequency (CF) was calculated using the method Suryanarayanan [13]

$$\text{Colonisation frequency} = \frac{\text{No of species isolated}}{\text{No of segments screened}} \times 100$$

Morphological identification

All cultures were incubated at 28^0 c for 7 days. Colonies were identified based on color of conidia, mycelia, reverse colors, texture, color , zonation and sporulation. All the isolates were subjected to microscopic analysis for characterization and identification.

RESULTS

Isolation of endophytic fungi

A total of 160 leaf and stem samples of *Boswellia ovalifoliolata* were inoculated on PDA medium(Table:1). Among them 28 isolates of six different fungal species were isolated . Out of 28 isolates, six were found to be *Xylaria* sp, six were found to be *Fusarium equiseti* , four were found to be *Cladosporium* sp. Two were found to be *Lasiodiplodia theobromae*. Eight were found to *Aspergilus* sp and two were found to be *Cheatomium* sp. *Fusarium* sp showed highest colonisation frequency (C.F) of 30% followed by *Aspergilus* sp and *Xylaria* sp (C.F=20%), *Cladosporium* (C.F=16%) , *Lasiodiplodia theobromae* (C.F=13%) and the lowest colonisation frequency of 6% was observed by *Cheatomium* sp.The total isolation frequency was found to be 17.5%.

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Table 1: colonization frequency of endophytic fungi isolated from *Boswellia ovalifoliolata*

s.no	species	Site of isolation	N.O of samples	Fungi isolates	Colonization frequency
1.	*Xylaria* sp	leaf	30	6	20
2.	*Fusarium equiseti*	leaf	20	6	30
3.	*Cladosporium* sp	stem	25	4	16
4.	*Lasiodiplodia theobromae*	leaf	15	2	13
5.	*Aspergilus* sp	stem	40	8	20
6.	*Cheatomium* sp	leaf	30	2	6
	Total	-	160	28	17.5

Morphological identification

***Xylaria* sp**

Fruiting bodies were black in colour grown up to 2-3 cm in length (Fig.1.a) often growing in groups of three clustered into finger-like subcylindric at first, becoming flattened upper branches appear powdered white, finally tipped black when mature, stalk black and hairy. The mycelia was initially white and turned in to red in colour with irregular margins. Hyphae are thin-walled and branched (Fig.2.a).

Fusarium equiseti

Colonies are usually fast growing white , cottony, flat (Fig.1.b). Conidiophores are short, single, lateral monophialides in the aerial mycelium, later arranged in densely branched clusters. Chlamydospores are terminal, hyaline , smooth and short hyphal branches (fig.2.b).

***Cladosporium* sp**

The colony was velvety to suede like in texture , slightly heaped and greyish green in colour (fig.1.c), edges of the colony are feathery. The colonies are diffuse and mycelia form mats and grown upward and seem velvety. Hyphae are erect and septated , conidia are small, oval shaped, single celled and smooth walled (fig .2.c).

Lasiodiplodia theobromae

Colonies with abundant aerial mycelium reaching to the lid of Petri plate, aerial mycelium is black in colour (fig.1.d). Isolates had irregularly shaped colonies with dense, fluffy and a moderate growth rate. The fungus produced stromata and pycnidia. From fruiting bodies liquid exudates. solitary, globose, thick-walled, non-papillate with a central ostiole. *Paraphyses* hyaline, cylindrical, thin-walled, aseptate and branched (fig.2.d).

***Aspergillus* niger**

The colour of the colony was green in colour(fig.1.e) consisting of a dense felt of erect conidiophores. Conidiophores terminate in a vesicle covered with layer of subtending cells which bear small whorls of phialides (fig.2.e). The vesicle, phialides, metulae and conidia form the conidial head. Conidia are one-celled, smooth walled, hyaline pigmented are produced in long dry chains which may be divergent.

***Cheatomium* sp**

Chaetomium sp colonies are cottony and white in colour, the colony reverse is brown (fig.1.f) Hyphae are septated Perithecia are large, dark brown to black, fragile and globose to flask shaped and have filamentous, hair-like, brown to black appendages (setae) on their surface. Ascocarps covered with pale, thin-

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walled, flexuous hairs (fig.2.f) .Terminal hairs sparse, olivaceous brown and fading towards the tips, punctuate and erect .

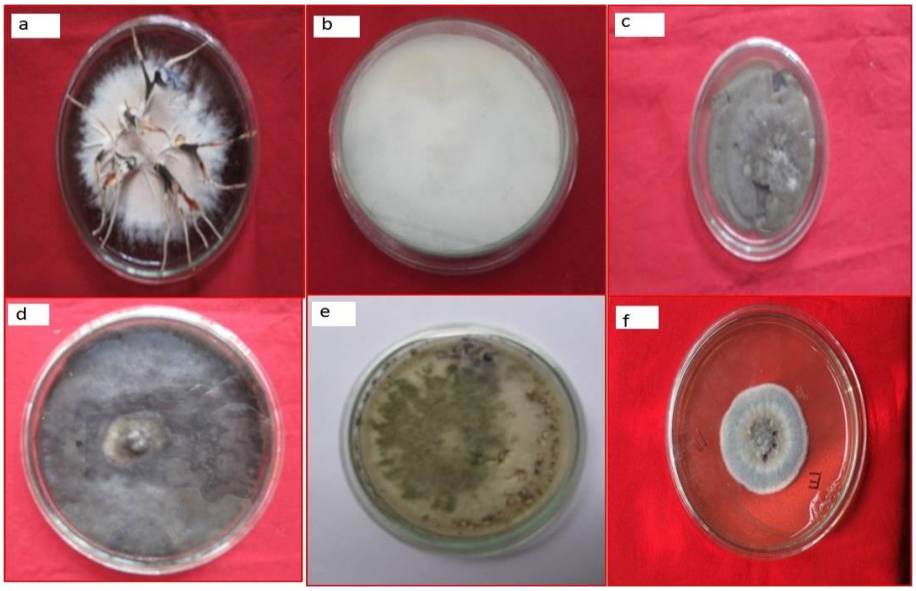

Fig 1: Colony morphology of endophytic fungi isolated from *Boswellia ovalifoliolata*
a. *Xylaria* sp b. *Fusarium equiseti* c. *Cladosporium* sp d. *Lasiodiplodia theobromae* e. *Aspergilus niger*
f. *Cheatomium* sp.

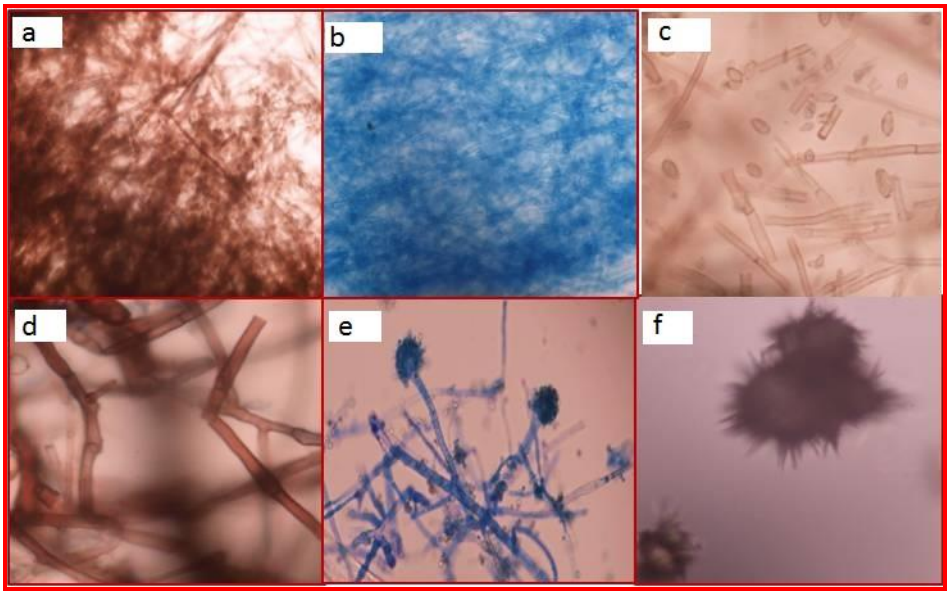

Fig 2: Microscopic identification of endophytic fungi
a. *Xylaria* sp b. *Fusarium equiseti* c. *Cladosporium* sp d. *Lasiodiplodia theobromae*
e. *Aspergilus niger* f. *Cheatomium* sp.

DISCUSSION

Endophytes are residing asymptomatically in internal tissues of all higher plants are of growing interest as promising sources of biologically active agents [14]. The protection mechanism of the endophytes is exerted directly by releasing metabolites to attack any antagonists, or indirectly by inducing host defence mechanisms [15]. Endophytes can also promote plant growth through different mechanisms like production of phytohormones ,synthesis of siderophores [16], nitrogen fixation, solubilisation of minerals [17], ethylene suppression [18] or via assisting phytoremediation [19]. However only a few plants have been studied for their

endophytic diversity and their potential to produce bioactive compounds because they occupy unique biological niches as they grow in so many environments [20] . In the present study altogether of six endophytic fungi were isolated from leaves and stems of *Boswellia ovalifoliolata* an endemic medicinal plant of Tirumala hills. Some hyphomycetous forms viz., *Cladosporium* .sp, *Lasiodiplodia theobromae* , *Aspergilus* .sp, [21-22]. were isolated as endophytes in the present study. A significant variation was observed in the colonization frequency. In this investigation low rate of colonization of endophytic fungi may be attributed due to the secretion of the certain antifungal and antibacterial components [23].Previous studies reported that Eighteen different endophytic fungi were isolated from different tissues of bark, stem and leaf segments of five medicinal plants found within in Kudremukh range of Western Ghats of India, the dominant species isolated were *Curvulana clavata, C.lunata, C.pallescens* and *F.oxysporum*. The highest species richness as well as colonization frequency was found in the leaf segments of the host plant species [24].

CONCLUSION

In this study a total of 28 fungal endophytes were isolated from 160 leaf and stem explants (95 leaf explants and 65 stem explants) of *Boswellia ovalifoliolata* an endemic medicinal plant from Eastern Ghats of Tirumala hills, India. The isolated 28 fungal endophytes belongs to six different genera which includes .The leaf sample shows colonization frequency of 16.85 where as stem samples shows colonization frequency of 18.46%. *Xylaria* sp, *Fusarium equiseti* , *Cladosporium* sp, *Lasiodiplodia theobromae* , *Aspergilus niger* , *Cheatomium* sp. Colonization frequency (%) of fungal endophytes isolated from leaf and stem samples of *Boswellia ovalifoliolata* was ranged from 6 to 20%. Total colonization frequency of 17.5% was determined from *Boswellia ovalifoliolata*.

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