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First report on Biological Evaluation and Preliminary Screening of Fungal Endophytes from *Spilanthes paniculata*, a Medicinal Herb in Arunachal Pradesh, India

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A B S T R A C T

Endophytes are important source of natural bioactive products because these fungal endophytes are able to take unique biological and ecological niches and grow in non-ordinary environments. Many natural bioactive compounds produced by medicinal plants are reportedly produced by their fungal endophytes. *Spilanthes paniculata* (locally called Marsang in Arunachal Pradesh) is known to possess medicinal properties. In the present study, the endophytic mycoflora of *S. paniculata* was studied. In order to isolate fungal endophytes, 150 segments (50 leaf, 50 stem, and 50 root samples) of 15 different plants of *S. paniculata* were screened. Finally, 110 isolates consisting of twenty different fungal genera, out of which, seven belonged to the class Ascomycetes, twelve (Deuteromycetes) and one (Ulvophycetes). Among the parts studied, the highest species richness as well as frequency of colonization was reported in Leaf. Overall, colonization frequency was determined 73.33% of surface sterilized tissue. *Colletotrichum* was found to be the most dominant endophytic species followed by *Fusarium*, *Manokwaria* and *Oncopodium*. It is worth mentioning that the genera *Pestalotiopsis*, *Drecheslra, Cylindrocladium*, *Aspergillus*, *Nodulisporium* were, first reported from Arunachal Pradesh. The present study was therefore, initiated to study the endophytic fungal population in *S. paniculata,* a commonly used medicinal plant in Arunachal Pradesh, northeast India.

Introduction

Endophytes are microbes, belonging to ascomycetes and anamorphic fungi (Arnold, 2007). Fungal endophytesliving, internal tissues of plants without causing any mistreat to their host (Bacon and White, 2000). Mostly, they represent an important quantifiable component of fungal biodiversity (Krings *et al.*, 2007). Endophytic fungi benefits the infected plants, for example, by increasing resistance to herbivore grazing through the production of various alkaloids (Owen and Hundley, 2004), improving growth and competitive ability by increasing the mineral uptake potential, plant phenotypic traits, temperature and drought tolerance, leaf chemistry, and tolerance of heavy metals in soils (Redman, 2002). In addition, they have the ability to protect host plants from diseases effects, for example, by enhancing the plant defense (Arnold, 2003). Further, these microbes produce a plethora of bioactive metabolites of unique structure, benzopyranones, flavonoids, phenolic acids, steroids, tetralones, xanthones (Tan and Zou, 2001) of potentially immense value in agriculture, industry and medicine (Selim, 2012). Medicinal plants have been known haven for fungal endophytes because they are able to produce bioactive compounds similar to those of their host (Zhang, 2006; Khan, 2010). The universality of these symbiotic fungal endophytes is clear, but host range, variety and geographical distribution have been unknown (Arnold and Engelbrecht, 2007). Thus, only a few numbers of medicinal plants have been studied for their fungal endophyte biodiversity and the potential to produce secondary bioactive metabolites (Khan, 2007).

Arunachal Pradesh also known as the “Paradise of Botanists” is known to have more than 500 species of medicinal plants (Tag *et al.*, 2005). *S. paniculata* belongs to Asteraceae family with a long history of medicinal application is widely used in conventional and modern Medicine, which are known to harbour endophytic fungi that are believed to be associated with the production of pharmaceutical products (Zhang, 2006). The pharmaceutical properties of this plant can be attributed to its endophytes and there appears to be different endophytes with bioeffects. This research was to isolate and identify the endophytic fungi of *S. paniculata* in Arunachal Pradesh.

Materials and Methods

Study area and sample collection

The state of Arunachal Pradesh (29°30' N; 97°30' E) is recognized as the 25th biodiversity hotspot in the world (Chowdhery, 1999) and among the 200 globally important ecoregions (Olson and Dinerstein, 1998). *S. paniculata* was collected from Botanical Garden, Rajiv Gandhi University, Papum Pare District of Arunachal Pradesh (Fig. 1 and 2). The plant material was brought to the laboratory in sterile bags and processed within hours after sampling.

Surface sterilization and incubation

Isolation of fungal endophytes was done according to the method described by Petrini *et al.,* (1986). These medicinal plant samples were washed gently in running tap water to remove soil and debris. Leaf samples were cut into 0.5 cm² pieces while, stems and roots samples were cut into 0.5-1.0 cm. The pieces were then surface-sterilized by dipping them serially in 70% ethanol for 5 second and in 4% NaOCl for 90 second and were finally rinsed in sterile distilled water for 10 second (Suryanarayanan *et al.*, 1998). Of these, only 150 segments were selected randomly and plated on Potato Dextrose Agar (PDA) medium (supplemented with 150 mg/L chloramphenicol) contained in Petridishes and were observed for the growth of endophytes. Each 9 cm diameter Petridish had 20 mL medium and was plated with 10 leaf segments. Petridishes were incubated under near ultraviolet lamps (12 h dark: 12 h light cycle) for 25 days at 28°C, as this treatment was given for induce sporulation in fungi (Suryanarayanan *et al.*, 2015). Colonization Frequency (CF) was calculated as described by Suryanarayanan *et al.,* (2003).

$$\text{CF of endophyte} = \frac{\text{Number of segments colonized by fungi}}{\text{Total number of segment observed}} \times 100$$

For identification, fungi were grown on specified culture media under specified culture condition and identified on the basis of their morphological and cultural characteristics (Domsch *et al.*, 1980; Ellis, 1971; Kenneth *et* *al.,* 1965; Sutton, 1980).

Results and Discussion

The present study is the first report about the endophytic flora of *S. paniculata*, a medicinal plant found in this region. A total of 150 segments including 50 leaf, 50 stem and 50 root segments were screened for the presence of endophytic fungi (Table 2; Fig. 4 and 5). Finally, 110 isolates consisting of twenty different fungal genera, out of which, seven belonged to the class Ascomycetes, twelve (Deuteromycetes) and one (Ulvophycetes). The microscopic pictures of these fungi are depicted in Figure 3. It includes eleven fungi from leaves, nine from stems and seven from roots (Table 1). The observation shows that highest species richness and frequency of colonization, was recorded in leaf followed by stem and root.

In the present study, *Colletotrichum* sp. was found to be the most dominant endophytic species which is not organ-specific. It has been isolated from leaf, stem and root tissues in three replicates. Further, *Colletotrichum* spp., *Cladosporium* spp., *Chaetomiun* spp. were common in all the plant parts; however, *Xylaria* was isolated from stem and roots, and *Aspergillus* could be isolated from leaves and roots, respectively. Among the identified isolates, the most abundant genera were *Colletotrichum*, *Fusarium*, *Manokwaria* and *Oncopodium.* The genera *Pestalotiopsis*, *Drecheslra*, *Cylindrocladium*, *Aspergillus*, *Nodulisporium* were also, first time reported for the first time in this study. Overall, colonization frequency was determined 73.33% of surface sterilized tissues.

Endophytic organisms have received considerable attention as they are found to protect their host against pest, pathogens and even domestic herbivorous (Weber, 1981). Only a few plants have been investigated for their endophytic flora and their potential to produce bioactive compounds. Some studies have been conducted about the endophytic biodiversity, taxonomy, reproduction, host ecology and their effects on host (Redman *et al.,* 2002, Domsch *et al.,* 1980; Dayle *et al.,* 2001; Clay and Schardl 2002). Currently, endophytes are considered as unexplored source of bioactive natural compounds. They have been found to play a crucial role in the production of beneficial chemical compounds. Taxol, an antifungal and anticancer compound, is one such example. It was found to be a product of endophytic fungi that colonizes on *Taxus brevifolia* and other plants (Gangadevi and Muthumary, 2007; Strobel *et al.,* 1996).

The biodiversity of endophytic fungi of medicinal plants are completely overlooked aspect of plant biology of Arunachal Pradesh. The present work was initiated to find out endophytic fungal population in widely used medicinal plant, *S. paniculata.* Various fungal endophytes population was detected to colonize this plant. Twenty fungal genera were isolated. The highest species richness, as well as frequency of colonization was found in leaves. *Cladosporium* sp., however, was found in both leaf and stem, while *Xylaria*, was isolated from roots and stems. In this study, most dominant endophyte was found to be *Colletotrichum.*Overall colonization frequency was determined as 73.33 % in surface sterilized tissues.

In most of the cases, Ascomycetes, Deuteromycetes and Basidiomycetes are reported as endophytic fungi (Petrini, 1986; Clay and Schardl, 2002). A large number of genera and species of fungi, belonging to first two classes, are able to live endophytically in plants. In the present study, the isolated fungi belonged to the class Ascomycetes and Deuteromycetes.

Table.1 Endophytic fungi isolated from different parts of *Spilanthes paniculata*

S. No.	Site of Isolation	Number of Sample	Number of Fungi Isolated
1.	Leaf	50	11
2.	Stem	50	9
3.	Root	50	7
	Total number of isolates	150	27

Table.2 Name and colonizing frequency of Endophytic fungi isolated from *S. paniculata*

S. No.	Name of Endophytic Fungi (sp.)	Isolated From			Frequency (%) of Endophytic Fungi	No. of Isolates
1	*Colletotrichum*	Leaf	Stem	Root	33.33%	17
2	*Oncopodium*	Leaf	-	-	16%	8
3	*Nodulisporium*	Leaf	-	-	10%	5
4	*Cylindrocladium*	Leaf	-	-	4%	2
5	*Fusarium*	Leaf	-	-	20%	10
6	*Cladosporium*	Leaf	Stem	-	14%	7
7	*Aspergillus*	Leaf	-	Root	14%	7
8	*Taeniolellea*	Leaf	-	-	2%	1
9	*Nigrospora*	Leaf	-	-	14%	7
10	*Phaeotrichoconis*	Leaf	-	-	4%	2
11	*Chaetomium*	Leaf	Stem	-	12%	6
12	*Corynespora*	-	Stem	-	6%	3
13	*Lasiodiplodia*	-	Stem	-	2%	1
14	*Dreschslera*	-	Stem	-	2%	1
15	*Alterneria*	-	Stem	-	10%	5
16	*Xylaria*	-	Stem	Root	12%	6
17	*Pestalotiopsis*	-	Stem	-	12%	6
18	*Phomopsis*	-	-	Root	8%	4
19	*Penicillium*	-	-	Root	6%	3
20	*Manokwaria*	-	-	Root	18%	9

Fig.1 Sample collection site of *Spilanthes paniculata* in Arunachal Pradesh, India (Red circle indicates collection site)

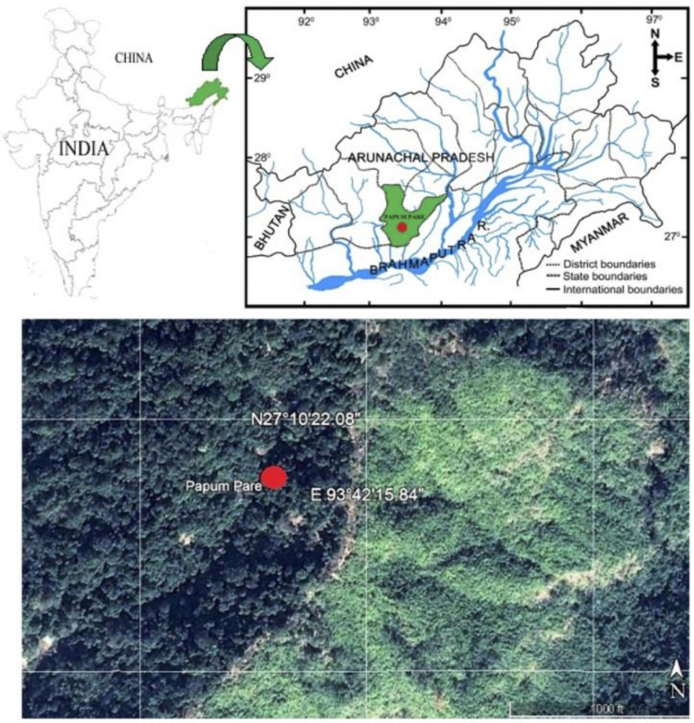


Fig.2 Image of *S. paniculata* (Wall. ex DC.) medicinal plant

Fig.3 Endophytic fungi isolated from the different parts of *S. paniculata*

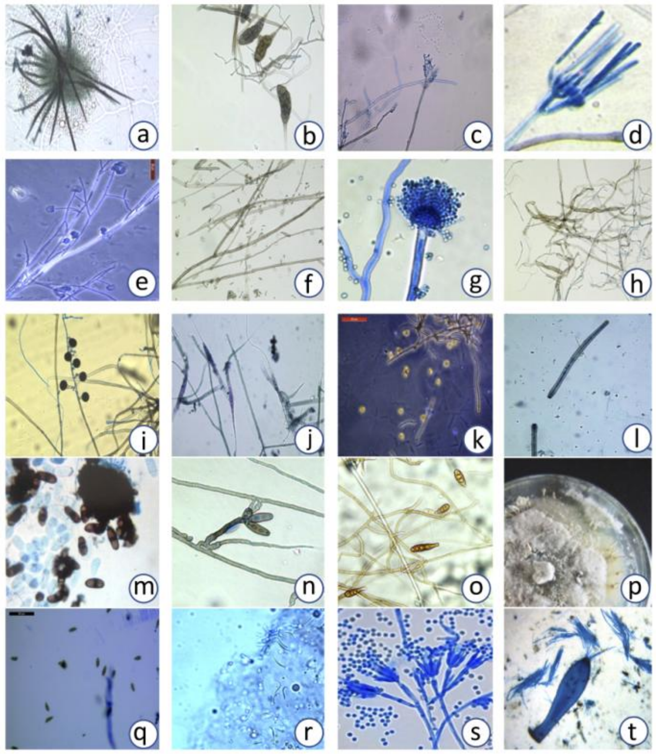


Fig.4 Endophytic fungi (%) isolated from different parts of ***S. paniculata***

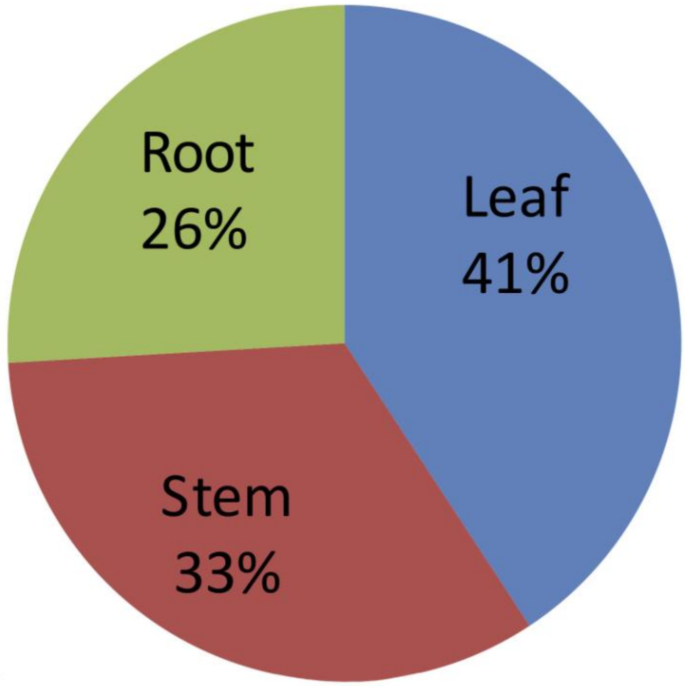
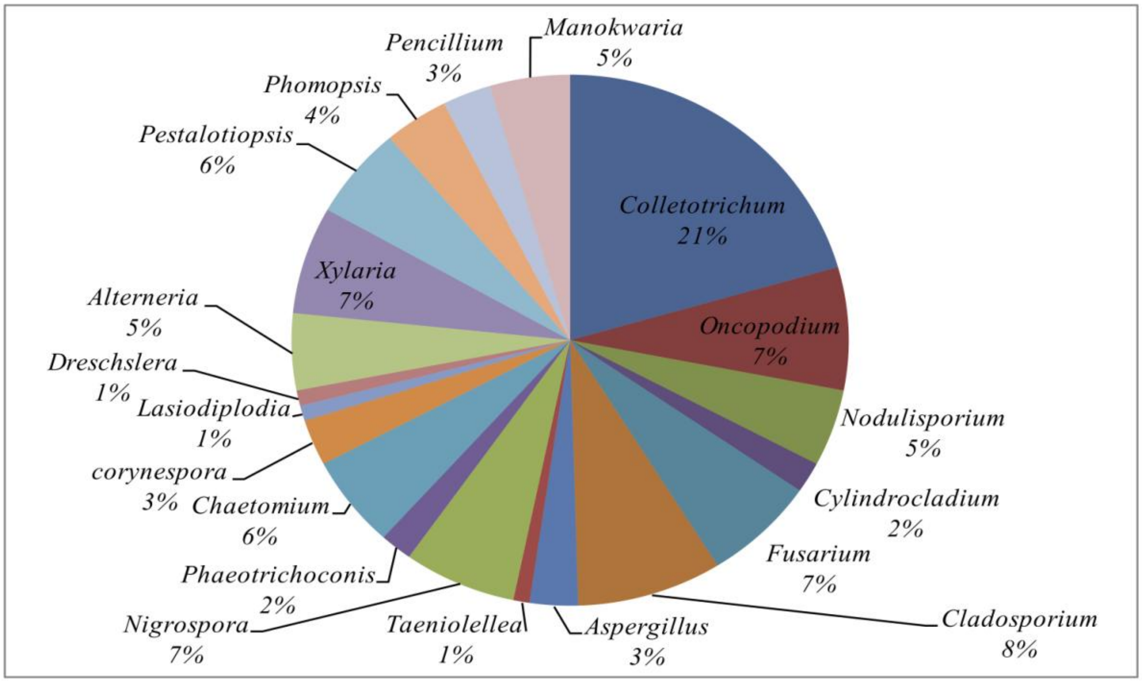


Fig.5 Colonizing frequency (%) of endophytic fungi isolated from *S. paniculate*



In the present study, among the endophytic fungal population, *Colletotrichum* sp. was found to be the most dominant endophytic species. Previously, this fungus was reported as a common endophyte to other plant species like *Triticum aestivum*, *Zea mays* (Zhang *et al.*, 2006; Larran *et al.*, 2002; Fisher *et al.*, 1992). *Colletotrichum* sp. in *Spaniculata* was not organ-specific as the fungus was isolated from stems, leaves and roots. But frequency of colonization was higher in the leaves as compared to stem. There is sufficient evidence that endophytic fungi play an important role in host-plant physiology. They receive nutrition, protection and propagation opportunities from their hosts (Kenneth *et al.*, 1965; Thrower and Lewis, 1973) while host plants are also benefited from this symbiosis. Endophytes provide protection to their hosts from insects, pests, and herbivore, and help their hosts to adapt in different stress conditions (Kenneth *et al.*, 1965; Knop *et al.*, 2007; Malinowski and Belesky, 2006; Clay *et al.*, 2005). However, endophytes also act as opportunistic microorganisms under some conditions (Saikkonen *et al.*, 1998; Faeth *et al.*, 2004).

A good amount of endophytic fungi were collected from this region. *Colletotrichum* was found to be the most dominant endophytic species followed by *Fusarium*, *Manokwaria* and *Oncopodium.* It is worth mentioning that the genera *Pestalotiopsis*, *Drecheslra*, *Cylindrocladium*, *Aspergillus and Nodulisporium* were, first reported from Arunachal Pradesh. The present study was therefore, a humble initiative to explore the potential fungal endophytic population in medicinal plant, that would pave a way for future research opportunities in this sector.

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