Int J Pharm Bio Sci 2013 Apr; 4(2): (B) 1259 - 1266

Research Article **Pathology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

DIVERSITY OF THE ENDOPHYTIC FUNGI ISOLATED FROM *SPILANTHES ACMELLA* LINN. - A PROMISING MEDICINAL PLANT

H. C. LAKSHMAN*[1], AND JAYSHREE M. KURANDAWAD[2]

Microbiology Laboratory, P .G. Department of studies in Botany, Karnatak University, Dharwad- 580003 karnatak, India.

ABSTRACT

A total of 300 segments from 18 plants of *Spilanthes acmella* Linn., were collected from Karnatak University Botanical Garden Dharwad. And they were screened for the presence of endophytic fungi. Altogether 8 fungal species; viz *Aspergillus flavipes* Bainer & Sarto*ry*, *Aspergillus niger* Tiegh., *Aureobasidium pullulans* (De Bary.) Arnaud. & Les, *Bipolaris nodulosa* (Bert and Curt. ex. Sacc.) Shoemaker., *Cladosporium epiphyllum* Person., *Colletrichum Cord.*, *Hymenula affinis* (Fautrey and Lambotte)., *Rhizopus nodusus* Namyslowski. they were isolated and identified based on the morphology of the spores. The main isolation of endophytes, scanned out their diversity in the host. The results revealed the growth of endophytes on media PDA media MEA medium, colonization frequency of endophytic fungi significantly highest in leaves and greater activity in host leaves have been documented.

KEYWORDS:Endophytes, Colonization Frequency, PDA, MEA.

H. C. LAKSHMAN
Microbiology Laboratory,P .G. Department of studies in Botany, Karnatak University, Dharwad- 580003, Karnatak, India.

Corresponding author

INTRODUCTION

Endophytic fungi, defined as those species that occur within the living tissues of plants, without causing symptoms and have been isolated from every organ of almost every plant species sampled[1]. Measurement of microbial diversity is one of the greatest challenges in modern microbiology, given the astonishing number of microbial species believed to exist. In fungi 1,500,000 species are estimated to exist, based on a 6: 1 ratio of fungal: plant species[2]. All plants in natural ecosystems appear to be symbiotic with fungal endophytes. These are highly diverse group of fungi can have profound impacts on plant communities through increasing fitness by conferring abiotic and biotic stress, tolerance, increasing biomass and decreasing water consumption, or decreasing fitness by altering resource allocation[3]. Fungal endophytes colonize within plant organs and recently endophytes are viewed as an outstanding source of secondary metabolites, bioactive compounds, antimicrobial natural products and nutrients uptake process[4]. One of the most compelling features of fungal endophytes is their exceptional diversity. Several fungi showed differences in the same plant, but these differences were not consistent between sites [5,6,7]. The aim of this paper is to provide a recent data on diversity of fungal endophytes, colonization frequency of plant and their dominancy on the host of *Spilanthes acmella*.*Spilanthes acmella* Linn. is annual herb, more or less pubescent, the plant grows up to 30-60 cm. The flower heads are chewed to relieve the toothache and other mouth related troubles. Leaves are used externally in treatment of skin diseases. Root decoction is used as purgative. Leaf decoction is used as diuretic and lithotriptic. Whole plant is used in treatment of dysentery. Tincture made from flower heads treat inflammation of Jawbones and caries. It increases the flow of saliva and is useful in fever especially during summers. Leaf decoction is used as diuretic and lithotripic. Whole plant is used in dysentery[8]. The alkaloids spilanthol is effective extremely at low concentrations against blood parasites to most invertebrates. No reports of endophytic studies were recorded on this plant as per our literature survey. Therefore this paper brings a preliminary data on diversity of fungal endophytes and their colonization frequency of endophytes on *Spilanthes acmella* of different sites, where plants are growing in University Botanical garden.

MATERIALS AND METHODS

Spilanthes acmella Linn. is a promising medicinal plant selected for the study of the endophytic fungal diversity where, plants growing in University Botanical Garden. Geographically, the selected area is situated south western part of India. Karnatak University Botanical Garden one of the beautiful Botanical garden measures 40 acres lying in between 14°15¹ to 15°5¹ North longitude and 74°49¹ and 76° 21¹ East lattitude. The climate is marked diurnal temperature difference. The temperature varied 20.2°C in June and high as 34.42°C in March. The annual rain fall is 600-850 mm. Soil is sandy loam covered with a hard, compact crust having dark brown colour.

Collection of plant samples

Eighteen samples of *Spilanthes acmella* Linn., were collected from medium sized healthy plants in the Karnatak University Botanical Garden, Dharwad. The plant parts include; stem, leaves, petiole and roots they were placed in closed sterile polythene bag with labeling.

Isolation of endophytic fungus

All the collected samples were rinsed gently in running tap water to remove dusts and debris. The stem, leaves, petiole, and roots cut into segments (0.5--1cm). The sample were surface sterilized Dobranic (1995)[9] and the surface sterilized plant segments were placed in petridishes containing PDA and MEA medium[20]. The petridishes were sealed using parafilm and

incubated at 26 ± 1^0c at 12 hours light/dark cycle (Jayashree and Lakshman, 2011). The petridishes were monitored every day to check the growth of endophytic fungal colonies from the plant segments. The colonization frequency was calculated from the formula as;

$$CF\% = \frac{\text{Number of segments colonized by an endophyte}}{\text{Total number of segments analyzed}} \times 100$$

The hyphal tips which grew out from the segments were isolated and sub cultured on PDA and MEA medium. The pure cultures were maintained on PDA slants. The endophytic fungi were identified according to their macroscopic and microscopic characteristics such as the morphology of fruiting structures and spore morphology with the help of identification manuals [10,11,12,13].

RESULTS

Altogether 300 segments namely 78 segments are leaves, 74 segments are petiole, 76 segments are stem, 76 segments are roots of *Spilanthes acmella* Linn., samples were collected and they were processed for the isolation of endophytic fungus. A total of 8 fungal species were obtained shown in (Table 1). All the isolated and identified fungal culture was stored in Microbiology Laborotary, Karnatak University Dharwad (MLKUD).

Charcterization of fungi

1: *Aspergillus flavipes Bainier and Sartory*

Colonies on MEA or PDA media white at first, becoming yellowish, in some strains forming more or less abundant, closely woven, yellow masses containing many helicoids to horseshoe-shaped, thick-walled cells. Heads mostly columnar or calyptri form masses, commonly persistently white, but with some strains in pale avellaneous shades to deep avellaneous. Conidiophores300-500× 4-5µ, or up to 2-3mm. in length and 8-10 µ in diameter, smooth; vesicles subglobose or elliptical up to 20×30 µ; phialides in two series; primary 4-7 µ or 8×2µ or 3µ, secondary 5-8 ×1.5-2µ.Conidia 2-3 µ. Smooth, subglobose, colorless or nearly so.

2: *Aspergillus niger Tiegh*

Colonies growing moderately on PDA or MEA, 3.5-4.5cm in 10 days, with abundant submerged mycelium, conidial heads carbon black, exudates lacks, conidial heads large and black, at first globose and then radiate or splitting in well defined columns in age, up to700-800µmin diam; conidiophores arising directly from the substratum, smooth, non septet, thick walled, 1-2mm ×15-20µm; vesicles globose, walls thick, commonly 45-75µm in diam, occasionally longer, bearing two series of fully packed phialides, brownish;metulae mostly 20-30×5-6µm, often reaching 60-80×8-10µm,rarely septate; phialides7-10×3-3.5µm;conidia globose, spinulose with colouring substance, black, 4-5µm; globose to subglobose shown in (Plate I-1

3: *Aureobasidium pullulans (de Bary) Arnaud, Les*

Colonies growing fast, reaching 4cm in 7 days at 24C on MEA, mid to dark brown; Vegitative hyphae hyaline, up to 12µm wide; pigmented hyphae distinctly constricted at the septa; Conidiophores mostly 6-8µm wide, dark brown, with small lateral protuberances which become short, open ended necks of phialides; conidia hyaline ellipsoidal, often with in distinct basal apiculation, variable in size and shape, straight, mostly (7.5-)9-11X(3.5-)4-5.5(-7)µm , but may be bigger in old colonies; secondory conidia and endoconidia similor, but smaller.

4: *Bipolaris nodulosa (Bert. And Curt.ex Sacc.) Shoemaker*.

B.nodulosa (Bert. And Curt.ex Sacc.) Shoemaker. Conidiophores simple, branched, hyaline at tip dark brown at lower parts usually broader toward the apex, swollen and geniculate at the conspicuous circular conidial scars occurring in close succession in the upper

poprtion; conidia straight, ellipsoid or ovate or typically obclavate,. Thin-walled pale to moderately dark brown or olivaceous brown, with a circular basal hilum included within the coutous of theroundedbasal wall and often surrounded by a hyaline area, 3-7 septate, 28-70× 10-18µ, mostly 48.4 ×56.6 ×13-15.7µ(21.64× 12-17 µ mean 48.6× 14µ) in the type 1 produced singly and acrogenously at the tips of the conidiophores. And successive growing points, with a length/ breadth ratio of about 5.5-3.7. Shown in (Plate I-2)

Species 5: Cladosporium epiphyllum Person

Colonies greenish-black, large, thick; conidiophores at first erect, then falling, pale green; conidia very numerous, soon falling from the chain, at first one-celled, then two-to more-celled, olive-green, 10-22µ long×4-6µ thick. Shown in (Plate I-3)

Genus 6: Colletitrichum Corda

Fruting bodies acervuli, developing from the stromatic mass of hyphae, in culture no stroma present; acervulus saucer shaped, surrounded by stiff, blak, unbranched setae; conidiophores closely packed, simple, short, hyaline; phialides in palisade layer; conidia aseptate, fusoid, somewhat curved or sickle shaped or cylindrical, smooth, 1-celled, hyaline, aggregated in cream, orange or red or brownish slimy masses, appearing as glistening mass. Shown in (Plate I-4)

Species 7: Hymenula affinis (Fautrey and Lambotte) Wollenber /fusarium affinis

Conidia straight, somewhat dorsiventral near apex, apedicillate, typically one- septate, 10.2×2.8µ(9-11.4×2.6-3µ) usually in a continuous smooth or slightly roughened, slimy-layer, from hyalin to pale salmon-colored on a globose agar. Conidiophores from simple to sparingly branched, septate. Mycelium hyalin. No chlamydospores Shown in (Plate I-5).

Species 8: Rhizopus nodosus Namyslowski

The mycelium is cottony, white when young, then tinted ochre-yellow. In midst of the mycelium and on the stolons, branches ending in sporangia occur. These branches 1-2mm. in height×12-28µ in diameter have thick, smooth walls, colorless at first, then becoming pale ochre or brown. They are simple or branched, the branches ending in sporangia. The branches may swollen at any point. When these swellings are terminal they give rise to a group of three to five sporangiophores, each terminating in a sporangium. Sporangiophores 1-2mm. high, the sporangia are globose 100-200µ in diameter Shown in (Plate I-6)

Table 1

Colonization frequency (%) of endophytes in Spilanthes acmella Linn., on different media

S.N	Endophytic fungi	(%) of Colonization frequency						Total isolates
		MEA			PDA			
		L	P	S	L	P	S	
1	*Aspergillus flavipes*	6.66	-	20	13.3	20	-	59.96
2	*Aspergillus niger*	-	-	6.66	-	-	26.6	33.26
3	*Aureobasidium pullulans*	-	-	20	-	-	-	20
4	*Bipolaris nodulosa*	13.3	33.3	33.3	33.3	20	33.3	166.5
5	*Cladosporium epiphyllum*	6.66	13.3	13.3	20	6.66	13.3	73.22
6	*Colletitrichum* Corda	-	-	-	-	-	6.66	6.66
7	*Hymenula affinis*	20	6.66	6.66	26.6	6.66	20	86.58
8	*Rhizopus nodosus*	6.66	-	20	6.66	20	33.3	86.62
	TOTAL							532.8

* ***L-Leaf , P-petiole , S-stem***

Figure 1
Colonization frequency (%) of endophytes in Spilanthes acmella Linn. on different media.

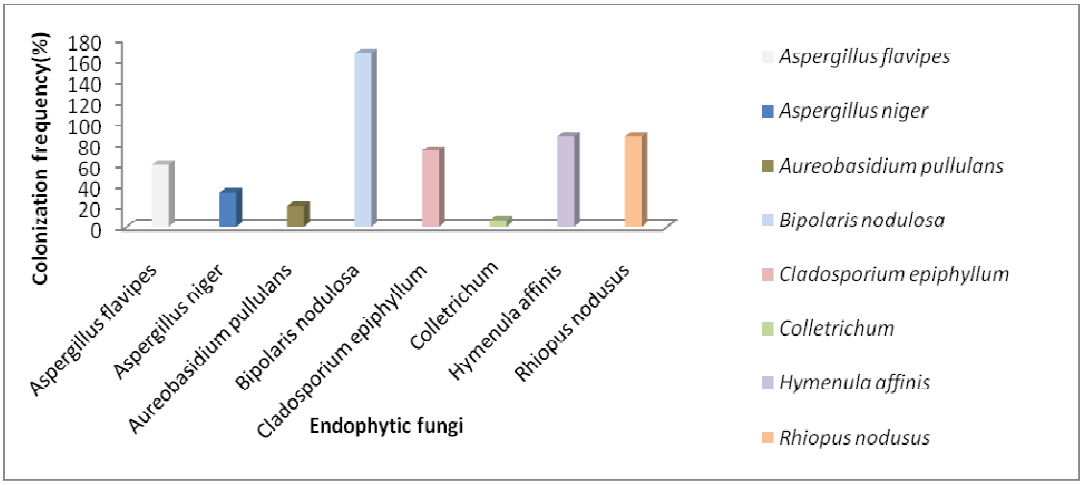


Plate I

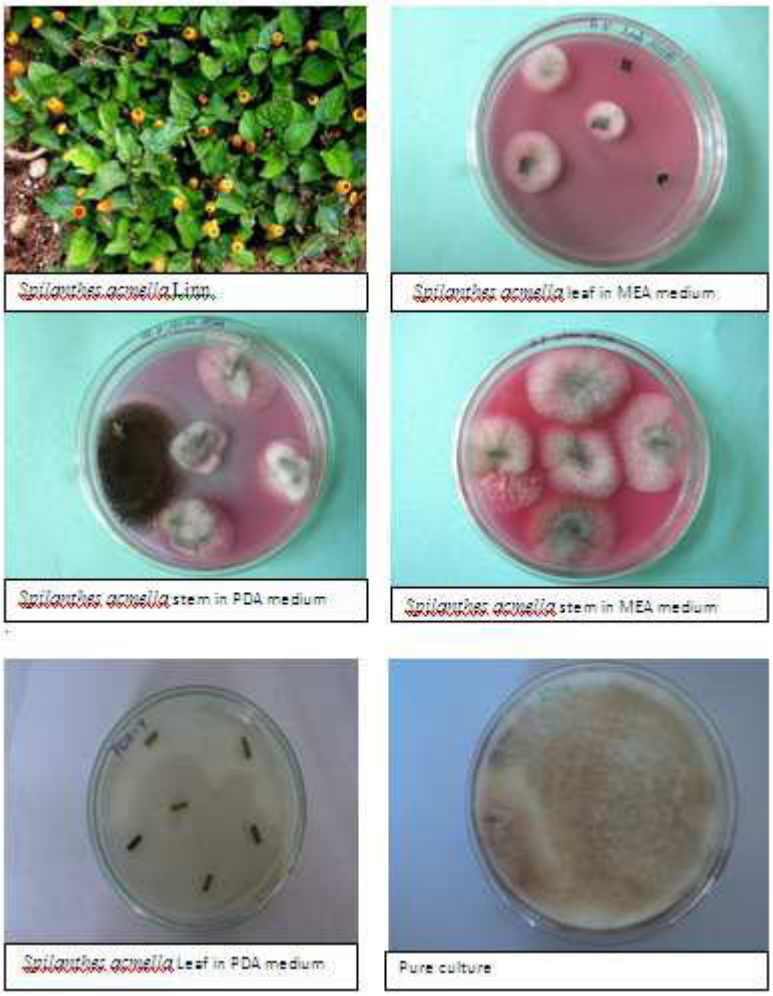
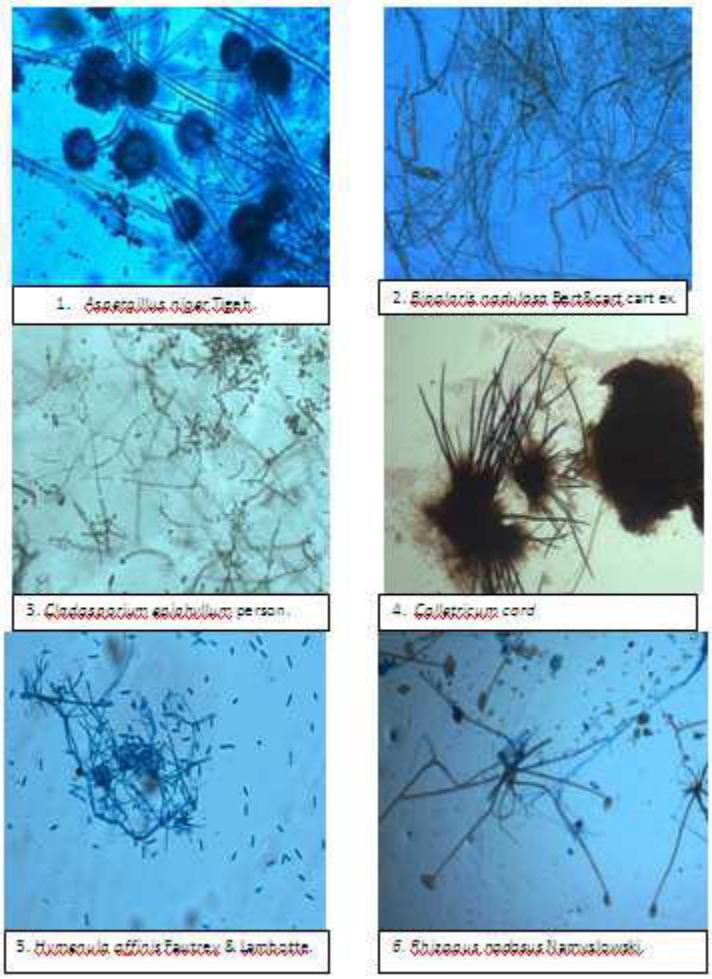


Plate II



DISCUSSION

In the Present study, it was observed that the leaves of the host plant exhibited the highest endophytic diversity than the petiole, stem, root samples, Thus, these endophytes isolated from leaf samples exhibited greater diversity and high colonization frequency compared to the endophytes of the other plant parts examined. Similar findings were reported by (Weber *and* Gamboa)[14,15]. There are reports on single leaves of a tropical forest tree, *manilkara bidentata*, was showed fine scale variation of endophyte isolation rates and identity. Our university Botanical garden is also located in the tropical forest of sahydri hill. And also stated that the overall colonization rate in the leaves was found to be significantly higher than those in root, stem and petiole. These findings are consistent to earlier workers (Okane, Kumar and Hyde,)[5] as, leaf samples finding more number of endophytic diversity in the plants. One of the possible reasons for the differences in the colonization rates between plants is the structure and substrate which influence the colonization and distribution of endophytic fungi. This may be due to that, plants are genetic mosaics because each organ may have a unique combination of genes in its micro biome[16]. However, some endophytes are restricted to single cell and tissues in the leaf endophytes in different tissues may not interact with each other[17]. A recent meta-analysis found that leaf endophytes are indeed more species-

rich in the tropics than in temperate regions[18]. Present studies clearly exhibited the number of endophytic fungi was higher in leaves followed by petiole, stem and roots. However, the overall colonization frequencies differed with different organs. Similar results were reported by Thalavaipandian on diversity of fungal endophytes in medicinal plants of courtallam hills, Western Ghats, India (2011) studied[19].The most prevalent endophytes were recorded among *Bipolaris nodulosa* was dominant followed by *Hymenula affinis* and *Rhizopus nodusus* The total colonization frequency was higher from *Bipolaris nodulosa* (166.5) and very low among *Aureobasidium pullulans* followed by *Aspergillus niger* from (20 and 33.26%). The other recovered endophytes are *Aspergillus flavipes*, *Bipolaris nodulosa, Cladosporium epiphyllum*, *Colletrichum Cord*. All the endophytes were microphotographed and recorded with Photographs (Table 1).

CONCLUSION

we understanding the number of endophytic diversity associated with a *Spilanthes acmella* Linn., plant in different sites of Botanical garden. Significantly endophytes more on leaves than petiole and stem. A significant variation was detected in the colonization frequency of endophytic species on *Spilanthes acmella* medicinal plant. However, examined percent colonization frequency in roots was megar one or two endophytic fungi was observed. Similarly, on different culture media results showed that endophytes growth was significantaly more in MEA, when compare to PDA media. Present investigation suggested that there is need of more research is warranted to understand endophytic fungal activity especially, in extraction of secondary metabolites in each endophytic fungi associated with *Spilanthes acmella*.

ACKNOWLEDGEMENT

First author is thankful to the UGC, New Delhi, for sanctioning major research project on the 'Investigation of AM fungal role in four rare millets of North Karnataka' and for financial support. Second author is indebted to Karnatak University for Financial support under SC/ ST cell for Ph.D students.

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