

Research Paper

**DIVERSITY OF FUNGAL ENDOPHYTES ASSOCIATED WITH AQUATIC
MACROPHYTE: *Limnophyton obtusifolium* (L.) Miq.**

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ABSTRACT

Association of fungal endophytes with the plant kingdom was exploited from the decades for their better bioactive principles to the sustainable development. Aquatic macrophyte *Limnophyton* (L.) Miq. belong to *Alismataceae* family was not yet been screened for their endophytic fungal biota and this work was the first report that put forward the hidden diversified endophytic fungal community. A total of 204 culturable endophytic fungi out of 400 segments belongs to 14 genera were isolated from surface sterilized leaf (108/200) and stem (96/200) of *Limnophyton obtusifolium* (L.) Miq. Among 204 fungal endophytes, *Cladosporium* sp. was the predominant endophytic fungi present, with the colonization frequency of 15.98% followed by *Alternaria* sp. at 13.26%, *Penicillium* sp. at 11.56%, *Bipolaris* sp. at 4.42% and *Colletotrichum* sp. 4.42%. Diversity indices revealed that both the plant tissues don't have much significant differences with the endophytic fungal diversity. Highest Species Richness (S) was observed in leaves (12.0) when compared to the stem (10.0). Further studies required to screen the potential bioactive fungal endophytes residing in aquatic macrophyte *Limnophyton obtusifolium* (L.) Miq.

Keywords: Aquatic macrophyte, *Limnophyton obtusifolium*, *Cladosporium* sp., Diversity indices.

1. INTRODUCTION

Endophytic mycopopulation are the diverse living microorganisms, intensively present within the healthy tissue of plant parts and their presence throughout the lifecycle without affecting the plants [1]. Presenting the host some endophytic fungi are said to

produce and found to be shown the similar of the secondary metabolites that expressed in the host plant simultaneously as the other biologically active ingredients as well as connecting to the new compounds [2]. The term “Endophyte” was first coined by De Bary in the year 1866, it is a word derived from the Greek, *endon*= within and, *phyte*= plant. Endophytes are the living organism found within the tissues of living plants, In the present days, modern mycologists are stated that endophytes that live in the living internal plant tissues without causing apparent harm to their respective host [3]. Fungi, Bacteria, Actinomycetes, and Mycoplasma are the different class of organisms that are unformatted as Endophytes of plants [4]. Endophytes fungi are the rich source of having novel natural compounds with potent biological activities, which actually incorporated with a high number of biodiversity and may also produce several compounds of medicinal significance [5].

Mycopopulation associated with Endophytes are denotative and living with high diversity within plants, every plant species have the versatility of fungal endophytes, diversity variation mainly depends on the type of plant species and geographical area. Till date scientific report suggests that plant growing in rich biodiversity, lodge diverse group of endophytes with bioactive potent activity [3]. As endophytes include *Trichoderma sp.*, *Rhizopus.*, *Penicillium sp.*, *Cladosporium.*, *Aspergillus flavus.*, *Drechslera turcica.*, *Fusarium verticillioides* etc [1][6][7]. Majority of Indian plants has been attributed with potential for medicinal value, which has been aided in plant-based product as therapeutics, endophytes have been played the antibiotic effects against the human pathogens and plant pathogens and human tumor cell lines [8] having antimicrobial, antioxidant, antimalarial, antitumor etc [11] [12] [13]. The plant specimen for the study had been identified as *Limnophyton obtusifolium* (LO) that belong to the family Alimataceae, an aquatic herb that grows at the edge of the ponds, muddy places. They are observed as the common systems of terrestrial and freshwater. Generally blossoms between the months of the December to March that tends to the flowering and fruiting. LO plants are reduced to ashes to obtain a vegetable salt that is taken with food or as an important ingredient for medicines. They are generally widespread in Africa and Asia with no report of any known threats. Here we mainly isolating the endophytes present in the Aquatic plant: *Limnophyton obtusifolium*.

Extracting the endophytes from green plants has become the most provoking strategy in the research field that has the indefinite potential applications in the agricultural and modern medicine aspects.



Figure 1: Picture showing Plant Inflorescence and Plant Flower of *Limnophyton obtusifolium* (L.)

2. MATERIALS AND METHODS:

2.1 Study Species and Sampling

Healthy Stems and Leaves of *Limnophyton obtusifolium* were collected from Madikere, Southeastern Karnataka. The plant was identified by the botanist and taxonomist from Departmental studies of Botany, University of Mysore, Manasagangotri (UoM).

2.2 Surface sterilization of plant Tissue bits

Collected Samples were washed under running tap water to remove dirt particles and air dried. Individual plant parts were cut into 0.5cm² pieces in a sterile condition. Tissue bits were subjected to surface sterilization according to with minor modifications, tissue bits were first treated with 70% ethanol for 1 min, followed by 4 min wash with 4% Sodium hypochlorite (NaOCl) solution and 3-4 subsequent washes with Sterile distilled water and air dried. Endophytic fungi were isolated from surface sterilized tissue bits which were equidistantly plated on water agar plates amended with Chloramphenicol (200mg/L) and incubated for 5-6 days at 25 ± 2° C under the 12h cycle of light and dark.

2.3 Preparation of Pure Culture of Isolated Endophytes

After incubation, PDA plates were prepared and kept for 24-48 hrs under the aseptic condition, for checking individual fungal hyphal tips was picked using the sterile forceps and placed isolation agar PDA plate under sterile condition and incubated at 25 ± 2° C for 5-6 days.

2.4 Identification of Isolated Endophytic fungi from Pure Culture

After the Incubation periods of pure culture is fungal colonies were observed under different magnification of a microscope by mounting the fungal colony on the slide with help of sterile forceps and needle, slide is prepared by the stain Lactophenol cotton blue stain and observed under magnifications of the microscope for morphological identification using the Standard Manual.

2.5 Calculation of Diversity indices of (LO) isolated endophytes

Data Analysis

Isolation rate (IR), the measure of the fungal richness of a sample, was calculated as the number of isolates obtained from the tissue bits placed on the PDA plates, divided by the total number of Tissue bits, and expressed as fractions but not as percentages.

The colonization frequency (CF), expressed as a percentage, was calculated according to Kumaresan and Suryanaryanan[10] as follows:

$$\%CF = \frac{\text{Number of Tissue bits colonized by a Fungus} \times 100}{\text{Total No. of Tissue bits placed}}$$

The percentage of dominant endophytes (D) was calculated based on the %CF divided by the total number of endophytes x 100 [9]. Simpson and Shannon diversity indices were calculated for endophytic fungi from different places, using online Shannon diversity calculator.

FUNGI	<i>Limnophyton obtusifolinum</i>	
	Leaves	Stems
Shannon-Wiener Diversity Index	2.0818765	2.0878112
Species Richness (S)	11.0	10.0
Total Abundance	108	96
1-D:	0.84550756	0.8530816
Evenness	0.8682099	0.9067249

Table 1: Shannon-Wiener Diversity Index

3. RESULTS

3.1 Isolation of Endophytic Fungi.

A total of 204 Endophytic Fungal isolates of which, 108 isolates from leaves, 96 isolates from stem were obtained from 400 segments (Tissue bits) of the aquatic plant: *Limnophyton obtusifolium*. All the obtained mycopopulation are observed under a microscope and identified based on the morphology and conidial characteristics and stated into 13 taxa of Genera. *Alternaria sp.* *Bipolaris sp.* *Ceratocystis sp.* *Cladosporium sp.* *Collectrotrichum sp.* *Fusarium sp.* *Morpho sp. 1*, *Morpho sp. 2*, *Myrothecium sp.* *Penicillum sp.* *Phoma sp.* were isolated from the Leaves and *Alternaria sp.* *Chaetomium sp.* *Ceratocystis sp.* *Cladosporium sp.* *Collectrotrichum sp.* *Morpho sp. 1*, *Morpho sp. 2*, *Penicillum sp.* *Pestalotiopsis sp.* *Phoma sp.* isolated from the stem tissue bits.

Sl. No.	Endophytes Isolated from <i>Limnophyton obtusifolium</i>	Sl. No.	Endophytes Isolated from <i>Limnophyton obtusifolium</i>
1.	<i>Cladosporium sp.</i>	7.	<i>Myrothecium sp.</i>
2.	<i>Collectrotrichum sp.</i>	8.	<i>Bipolaris sp.</i>
3.	<i>Pestiloopsis sp.</i>	9.	<i>Phomopsis sp.</i>
4.	<i>Morpho sp. 1</i>	10.	<i>Fusarium sp.</i>
5.	<i>Morpho sp. 2</i>	11.	<i>Gliocladium sp.</i>
6.	<i>Phomo sp.</i>	12.	<i>Chaetomium sp.</i>
7.	<i>Penicillum sp.</i>		

Table 2: Endophytes isolated from *Limnophyton obtusifolium*

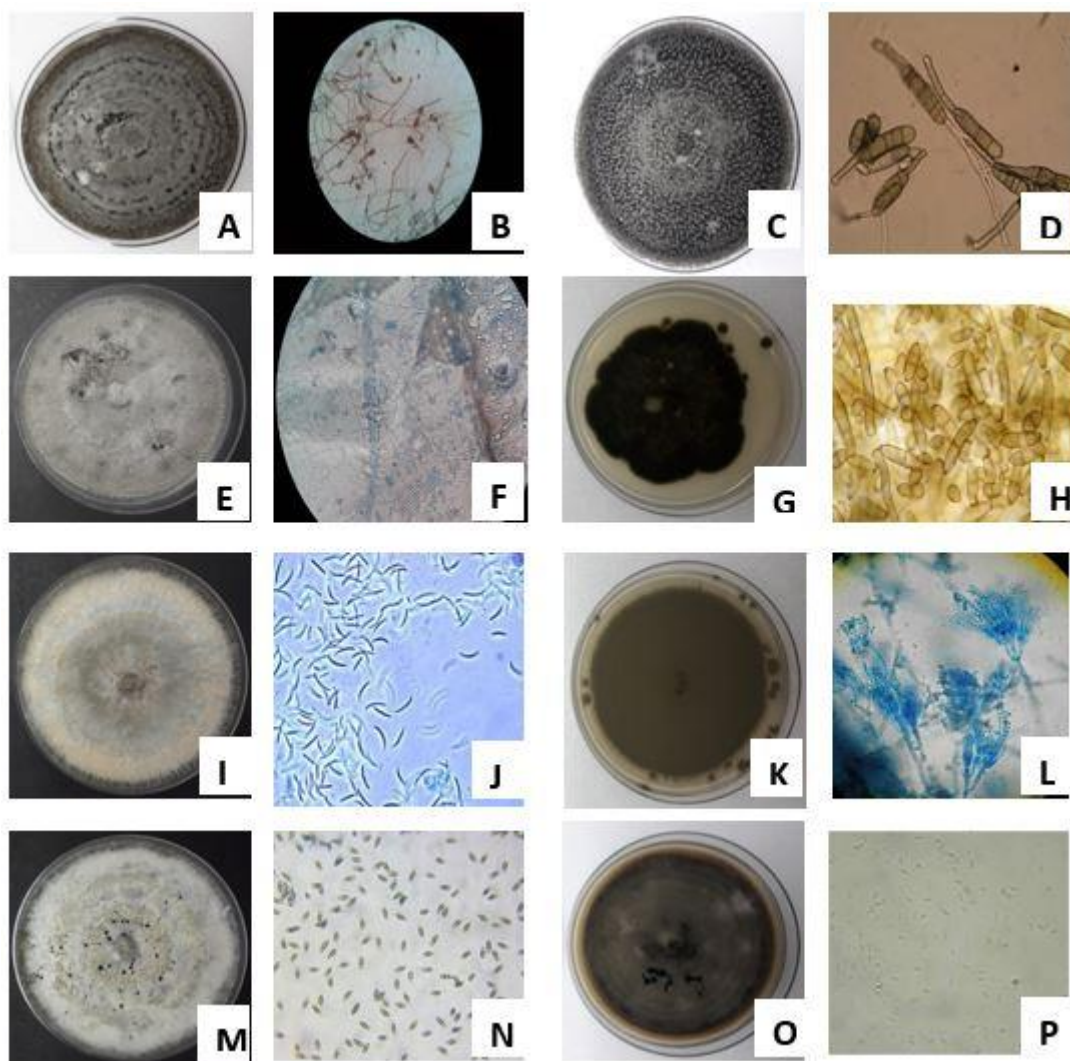


Figure 2: Colony morphology and Microscopic images of isolated fungal endophytes. (A) Colony morphology *Alternaria sp.* (B) Microscopic images *Alternaria sp.* (C) Colony morphology *Biploaris sp.* (D) Microscopic images *Biploris sp.* (E) Colony morphology *Myrothecium sp.* (F) Microscopic images *Myrothecium sp.* (G) Colony morphology *Cladosporium sp.* (H) Microscopic images *Cladosporium sp.* (I) Colony morphology *Collectrotricum sp.* (J) Microscopic images *Collectrotricum sp.* (K) Colony morphology *Penicillium sp.* (L) Microscopic images *Penicillium sp.* (M) Colony morphology *Pestalotiopsis sp.* (N) Microscopic images *Pestalotiopsis sp.* (O) Colony morphology *Phoma sp.* (P) Microscopic images *Phoma sp.*

	<i>Limnophyton obtusifolium</i>		TOTAL
	Leaves	Stems	
No. of Segments	200	200	400
No. of segments yielding endophytic fungi	154	140	294

No. of isolates	108	96	204
Isolation rate (%)	54.0%	48.0%	51.0%
Colonisation rate (%)	77.0%	70.0%	73.5%

Table 3: Calculation of Isolation Rate (%) and Colonisation Rate (%).

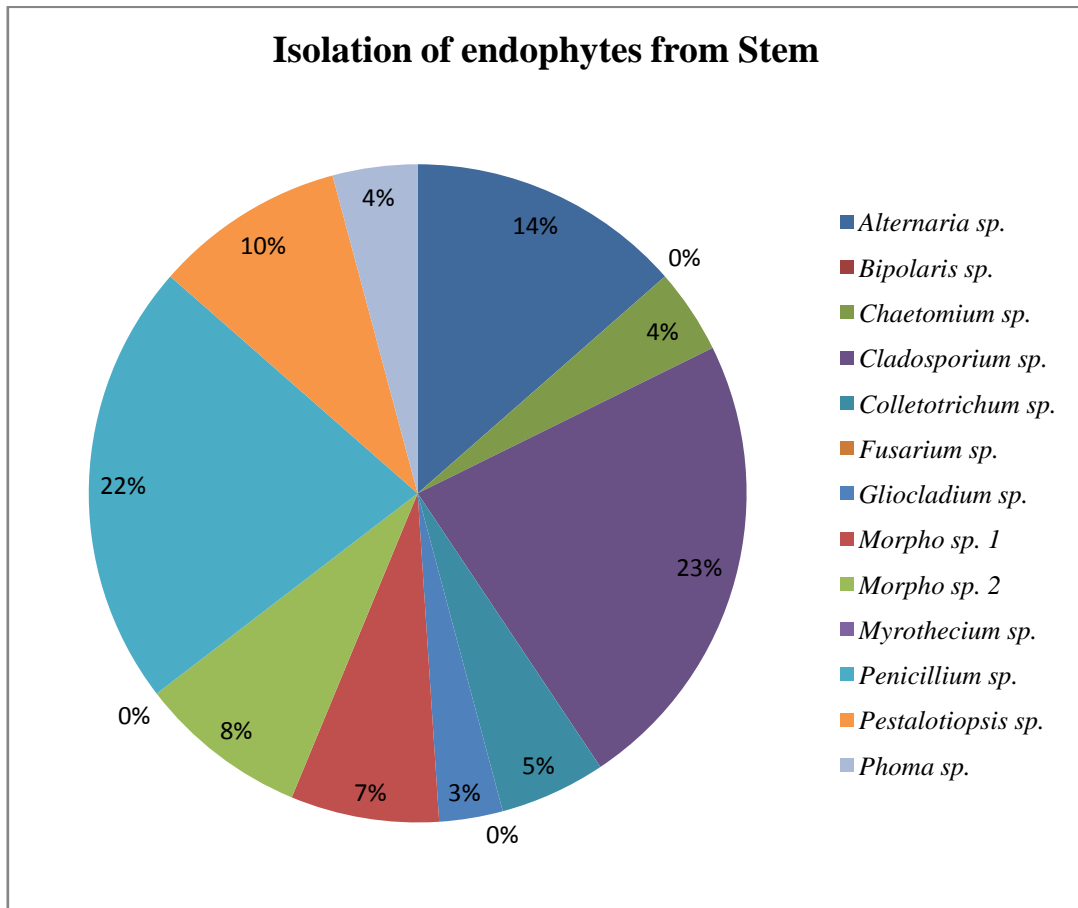


Figure 2: Graph showing isolated Fungi from *Limnophyton obtusifolium* (L)

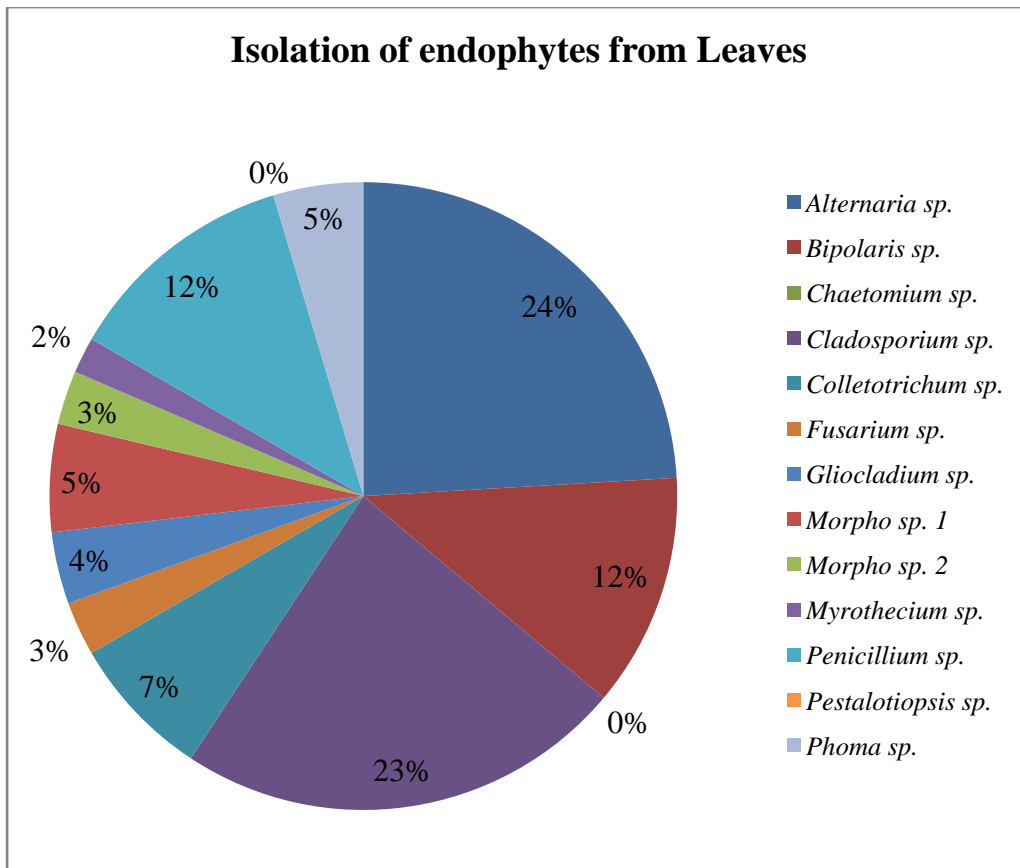


Figure 3: Graph showing isolated Fungi from *Limnophyton obtusifolinum* (L) Leaves.

4. DISCUSSION

In this paper, we have discussed mainly the number of an outcome of endophytes, with respect to particular geographical area. As per the study conveys that diversity versatility mainly depends upon the richness of species as well as geographical area, the distribution of endophytes as result obtain clearly states that the rich biodiversity on the particular geographical area. Comparison between leaves and stems show the high dominance in the leaves that have the high in no. of segments yielding endophytic fungi were they are also carry forward with respect to isolation rate and colonization rate of plant *Limnophyton obtusifolinum*.

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