Research Journal of Biological Science

MEDWELL PUBLICATIONS

Diversity of Endophytic Fungi from Leaves of *Cenetlla asiactica* L. and *Melia azedarach* L.

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Key words: *Centella asiatica, Melia azedarach*, leaves, Czapek dextrose agar, endophytic fungi, diversity

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Page No: 22-25
Volume: 15, Issue 2, 2020
ISSN: 1815-8846
Research Journal of Biological Sciences

Abstract: Endophytic fungi are symbiotic association and the harbor of plants. It is living in inner tissues and without causing any apparent symptoms. In the present investigation, the endophytic fungi were isolated from leaves of *Cenetlla asiatica* and *Melia azedarach*. These 2 plants are highly medicinal properties. Totally eight different genera were isolated from both the leaves. Generally, *Absidia repens, Alternaria* sp., *Aspergillus flavus, A. terreus, Chaetomium globosum, Curvularia* sp., *Fusarium solani* and *Humicola* sp. The maximum percentage of endophytic fungi and diversity indices were recorded in *Melia azedarach* leaves fragment when compared to *Centella asiatica* leaves.

INTRODUCTION

Endophytes are ubiquitous in nature and are found in every plant. Endophytic fungi are microorganisms found inside plant species[1] and may play a key role in plant development by controlling endophytic microbes and herbivorous insects or producing growth promoting substances[2]. Endophytic fungi also have functions related to saprophytic microorganisms and many species are involved in the process of maturation and leaf decomposition[3, 4]. Endophytic fungi infect and inhabit primarily the aerial tissue of the host plant without causing detectable symptoms. The relationship between endophytes are thought to be symbiotic such as those endophytes to get nutrients and protection from the host but contribute to effective host defense mechanism against pathogens herbivores or abiotic stress[5, 6].

One of the important role of endophytic fungi is to initiate the biological degradation of dead or dying host plant which is necessary for nutrient recycling[7]. Endophytic fungi are reported from plants grow in various environmental including tropic[8] temperate[9] xerophytic[10] and aquatic environment[11]. Medicinal plants are reported to harbour endophytes[12] which in turn provide protection to their host from infection agents and also, provide adaptability to survive in adverse environmental condition. It is therefore, important to determine the endophytic fungi diversity of medicinal plants. Recently, the knowledge about endophyte biodiversity is becoming more apparent.

Centella Asiatica (CA) is a very important medicinal herb used in the orient[13] which is also, becoming popular in the West[14]. It is a tasteless, odourless plant that thrives in and around water. It has small fan-shaped green leaves with white or light purple-to-pink or white flowers and it bears small oval fruit. The whole plant is used for medicinal purposes[15]. It is widely used as a blood purifier as well as for treating high blood pressure for memory enhancement and promoting longevity. In Ayurveda, *Centella asiatica* is one of the main herbs for revitalizing the nerves and brain cells.

Melia azedarach is a species of deciduous tree in the Mahogany family, Meliaceae that is an evergreen tree, cultivated in various parts of the Indian subcontinents. Neem has a long history of use in the traditional medical systems of India (Ayurvedic, Unani-Tibb). Leaves have

been used as a natural insecticide to keep with stored food but must not be eaten as they are highly poisonous. A diluted infusion of leaves and trees has been used in the past to induce uterus relaxation. Extracts from neem leaf, seed and bark possess a wide spectrum of antibacterial action[16, 17]. Although, the fruits are poisonous part of the tree, they have been used traditionally for the treatment of a variety of diseases, especially, dermatitis and rubella[18].

MATERIALS AND METHODS

Sample collection: Collection, identification and authentication of leaf materials of *Melia azedarach* L. and *Centella asiatica* L. were collected from Thanjavur District (the latitude in such as 10.6590°N and longitude in 79.2451°E) Tamil Nadu, India. The plants were identified by taxonomist for St. Joesph's College, Triuchirappalli (Fig. 1).

Isolation of endophytes: The endophytic fungi were isolated from leaves of *Melia azedarach* L. and *Centella asiatica* L. as described by Strobel and Daisy (2003). In first instance, the leaves were cleaned by thorough washing in running tap water, followed by Deionized (DI) water. Clean leaves were cut into small pieces were sterilized in series of solution; 70% ethanol, 1% sodium hypochlorite (v/v) again 70% ethanol for 1 min each. Finally, they were again rinsed with sterile distilled water two times. After surface sterilization, the tissues were dried on blotting sheets and cut into 1 cm square pieces. These sterile small pieces were placed on Czapek Dextrose Agar (CDA) plates containing streptomycin (250 µg mL^{-1}) to inhibit the bacterial growth. The plates were incubated at 25±2°C after wrapping with parafilm and observed daily. The fungal mycelia which started growing from the tissues were sub-cultured on new CDA plates. These endophytes were stored in paraffin at 4°C.

Colony frequency: Colonization Frequency (CF %) was calculated as described by Suryanarayanan *et al*.[19] and Photita *et al*.[20]. The colonies appearing on petriplates were sub-cultured into the tube containing Czapek dextrose agar medium for identification. Morphological identification was done according to the standard taxonomic key included colony diameter, texture, color and the dimensions and morphology of hyphae and conidia:

$$\text{Percentage of colony} = \frac{\text{No. of colonies in individual segment}}{\text{Total number of colonies in the sample}} \times 100$$

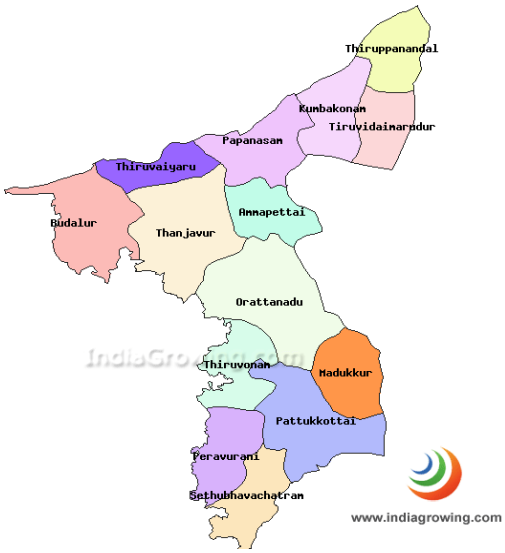

Fig. 1: Map showing the study site

$$\text{Percentage of CF} = \frac{\text{No. of segment colonized by fungi}}{\text{Total number of segment observed}} \times 100$$

Diversity index: The species diversity of the endophytic fungi was measured using the diversity indices with species richness and relative species abundance. The species evenness which assesses the contribution of the individuals to the community also, calculated.

The diversity indices used for the isolates were the Shannon-Wiener index (H) (formula: [(ni/n) ln(ni/n)]) Simpson's diversity index (1-D) (formula: 1-[D = (ni/n) 2]) and Simpson's dominance index (D) where ni is the number of distinct species (i) and (n) is the abundance of each species in the community. The equitability was calculated by the following formula; $E = H/H_{max}$ where H is the Shannon-Wiener index and $H_{max,}$ is the ln of ni. The diversity analyses were performed the MS Excel program[21].

RESULTS AND DISCUSSION

In the present study, 11 isolates were obtained from 7 tissue fragments from 2 medicinal plant species. The 10 different types of genera were isolated, the maximum number of colonies of *Absidia repens* and *Aspergillus terreus* were observed in *M. azedarach* and *A. terreus* in *C. asiatica* leaves fragment. The number of colony, percentage of colony and percentage of colony frequency were recorded Table 1.

Endophytic fungal diversity is measured in terms of Shannon, Margalef's index, Menhinick's species richness,

Table 1: Isolation of endophytic fungi from the leaf samples

	M. azedarach			*C. asiactica*		
Name of the fungi	No. of colony	CP (%)	Colony frequency	No. of colony	CP (%)	Colony frequency
Absidia repens	5	20	71.42	1	6.25	20
Alternaria sp.	1	4	14.28	1	6.25	20
Aspergillus flavus	4	16	57.14	2	12.5	40
A.terreus	5	20	71.42	4	25	80
Chaetomium globosum	1	4	14.28	1	6.25	20
Curvularia geniculata	1	4	14.28	-	0	0
C.lunata	-	0	0	1	6.25	20
Curvularia sp.	2	8	28.57	2	12.5	40
Fusarium solani	3	12	42.85	3	18.75	60
Humicola sp.	2	8	28.57	1	6.25	20
Melanospora sp.	1	4	14.28	-	0	0
Total	25	100	357.14	16	100	320

Table 2: Diversity indices of isolated endophytic fungi

Diversity indices	*M. azedarach*	*C. asiactica*
No. of species	25	16
No. of individuals	10	9
Genera	10	8
Margalef's index (d)	6.463	4.039
Menhinick's Species Richness (SR)	5.735	3.671
Pielou's Evenness (J)	0.086	0.081
McIntosh's Evenness (McE)	0.141	0.188
Shannon's Diversity (H)	0.277	0.224
Margalef's Diversity (Dmg)	12.427	14.427
Fisher's apha	6.17	8.5

Margalef's Diversity, McIntosh's Evenness, Simpson and Pielou's evenness and Fisher's apha indices. The diversity indices were calculated in the endophytic fungi of both the plants. The highest species richness present in *M. azedarach* when compared to *C. asiatica.* The Margalef's Diversity is 14.427 in *C. asiatica* leaves and 12.427 in *M. azedarach* (Table 2).

Endophytic microorganism, especially, fungi established a symbiotic relationship with plants while living inside the plant and synthesize metabolites that often helps their host to survive and adapt in adverse environment[22]. In addition, they are the group of organism with very good potential for application in plant improvement and disease control[23]. A study of endophytic biodiversity of the temporal variation in *Plumeria rubra* leaves was conducted by the Suryanarayanan and Thennarasan[24].

In the similar study 2 different genera of *Aspergillus* (6.6) and *Nigrospora* (3.3%) the colonization frequency were isolate from *Melia azedarach*. This is slightly less than the above cited report by Suryanarayanan and Thennarasan[24]. During the present study, *Absidia* sp., *Alternaria* sp., *Aspergillus* sp., *Chaetomium* sp., *Curvularia* sp., *Fusarium* sp., *Humicola* sp. and *Melanospora* sp. were isolated from *Melia azedarach* and *Centella asiatica*. All the species belong to Hyphomycetes class. These class fungi were largely prevalent and showed the resistance too many pathogens. The geneus *Aspergillus* found most common in plant.

Similar to the findings reported previously in *Centella asiatica* leaves, the higher colonization of endophytic fungi in mature leaf as compared to young leaf over all the 3 seasons suggests that mature leaf provides more favourable environment for the establishment of endophytic fungi[25]. Nascimento *et al*.[26] reported *Phaeoramularia calotropidis* (63.5%) as dominant endophyte in *Calotropis procera* leaves.

The diversity of the endophytic fungal populations isolated from leaves of *C.asiatica* and *Melia azedarach* such as was evaluated by various indices such as Fisher alpha diversity index (α) Shannon index H, Simpson's Diversity index (1-D) and Margalef richness index (Dmg) (Table 2). Similarly, Mehinick's index is similar, conceptually, to Margalef's richness index for analyzing species richness.

CONCLUSION

The assume diversity values from each of the 2 indices showed similar patterns in these results because of the endophytes, Shannon's diversity index (H')[27] and Simpson's Diversity index (D)[28] were not used. The species richness (Dmn) was highest in the leaves (4.24) followed by the flowers (2.82) and roots (1.41)[29].

ACKNOWLEDGEMENT

The researchers thank the principal of A.V.V.M Sri Pushpam College (Autonomous), Poondi, Thanjavur and the Director, Indian Biotrack Research Institute, Thanjavur for extending the lab facilities to carry out the research.

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