Research Article

# MORPHOLOGICAL STUDY OF ENDOPHYTIC FUNGI INHABITING LEAVES OF *MELIA AZEDARACH* L.

**Kaushal Kanwer Shekhawat, D. V. Rao and Amla Batra**
Biotechnology lab, lab no. 5, Department of Botany, University of Rajasthan, Jaipur, India.
***Corresponding author's E-mail:** kaushal.botany@gmail.com

**Received on:** 10-11-2010; **Finalized on:** 22-12-2010.

**ABSTRACT**

Endophytic fungi were isolate from leaves of *Melia azedarach* L. (Meliaceae), which is an exotic tree with a widespread distribution and often cultivated. In Brazil *Melia azedarach* L. widely used medicinal plant in Indian sub-continent, was Investigated for endophytic mycoflora. Single mycelium method was used to isolate endophytic fungi from surface sterilized of medicinal plant. Seven hundred twenty segments of leaves of *Melia azedarach* L., collected from "Botanical garden of University of Rajasthan during 2009-2010 were processed for the presence of endophytic fungi. According to morphological charatersities, 3 fungal species of Aspergillus viz., *Aspergillus flavus*, *Aspergillus niger*, Aspergillus sp. and one species of Nigrospora sp. were isolate and identified. Interestingly all the fungi were isolate only from leaves and overall colonization frequency from surface sterilized leaves was found to be 10.8% in response to plant samples.

**Keywords:** Endophytic fungi, morphological study, *Melia azedarach* L.

## INTRODUCTION

Endophytic fungi, at the beginning were applied for any organism found within the plant. The term endophytic was revered to the diverse group of fungi which live asymptomatic within photosynthetic tissue of plant for the all or some part their life cycle[1,2]. Recent studies have shown that fungal endophytes are ubiquitous in plant species[3,4]. Endophytic fungi infect and inhabit primarily the aerial tissue of the host plant without causing detectable symptoms. The relationships between endophytes are thought to be symbiotic, such as those endophytes obtained nutrients and protection from the host but contribute to effective host defense mechanism against pathogens herbivores or abiotic stress[5,6]. Globally, there are at least one million species of endophytes in all plant parts[7]. One of the important roles of endophytic fungi is to initiate the biological degradation of dead or dying host plant which is necessary for nutrient recycling[8]. Endophytic fungi are reported from plants grow in various environmental including tropic[9], temperate[7], 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. Usually several to hundred of endophytes species can be isolated from a single plant[13].

*Melia azedarach L*. also known as chinaberry or pride of India. It is native to India and has long been recognized for its medicinal and insecticidal properties[14]. 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[15]. It has long ethno botanical history and extensive uses in traditional medicine. It is a deciduous tree, drought – resistant high and salt tolerance moderate.

The present study was carried out to determine endophytic mycoflora in *Melia azeadarch* L. by simple morphological characters.

### Isolation of endophytic fungi from *Melia azedarach* L.

Endophytic fungi were isolated from the *Melia azeadrach* L. leaves of the plants were sampled for the present investigation of endophytic fungal communities.

### Sampling

Healthy (showing no visual disease) and mature plant were carefully chosen for sampling. Fresh mature leaves of *Melia azedarach* L. (meliaceae) were collected from a healthy plant grown in "Botanical garden" of University of Rajasthan. The plant material was brought to the laboratory in sterile bags and processed within a few hours after sampling. Fresh plant materials were used for isolation work to reduce the chance of contamination.

### Isolation of Entophytic Fungi

The plants were rinsed gently in running water to remove dust and debris. After proper washing, leaves were selected for further processing under aseptic condition. Highly sterile condition was maintained for the isolation of endophytes. All the work was performed in the laminar air flow (Shivam Institute, New Delhi. Modal No. HLF-3). Sterile glassware (Conical Flask) and mechanical things such as scissor, forceps, scalpel, and blades were used in all experiment. Leaves were cut into 3-4 mm in diameter and 0.5-1.0 cm in length with and without midrib.

The isolation of endophytic fungi was done according to the method described by Petrini (1986) [1].

The surface sterilization was done by sodium hypochlorite (Naocl) and 75% ethanol. The time of treatment and concentration of sodium hypochloride was changed according to the type of tissue (E.g. Higher concentration was used for root samples). The concentration of NaOCl was used 1-13% and time of sterilization was 3-10 minutes. Each set of plant material was treated with 75% ethanol for 30 seconds. Later the segments were rinsed three times with sterile distilled water. The plant pieces were blotted on sterile blotting paper. The efficiency of surface sterilization procedure was ascertained for every segment of tissue following the imprint method[16]. In each Petri plats 5-6 segments were placed on potato dextrose agar (PDA) supplemented with penicillium- G (a) 100 units/ml and streptomycin (a) 100 micro/ ml concentration. The dishes were sealed with Para film and incubated at 27°C for 4-6 weeks in culture chamber. When liquid medium was used, single segment was inoculated in each test tube. Most of the fungal growth was initiated within two weeks of inoculation. The incubation period for each fungus recorded was almost similar for the same species. The day of first visual growth was observed from plating date was considered as an incubation period for growth. Isolation from the master plates was done by the transfer of hyphal tips to fresh Potato Dextrose Agar (PDA) plates without addition of antibiotics to obtained pure culture for identification endophytes isolates were identified on the basis of culture characteristics morphology of fruiting body and spores.

### Calculation of colonizing frequency

Colonization frequency (CF) was calculated as described by Suryanarayanan, et al., (2003). Briefly, proper time of incubation was given for colonizing frequency counting. Colonization frequency (%) of an endophyte species was equal to the number of segments colonized by a single endophyte divided by the total number of segments observed x 100.

$$\text{Colonization frequency} = \frac{\text{Number of segment colonized by fungi}}{\text{Total number of segment observed}} \times 100$$

## RESULTS

Seven hundred twenty (720 leaf samples) segments of *Melia azedarach* were processed for the isolation of endophytic fungi three species from Aspergillus's and one species of Nigrospora were isolate (Table 1). All the isolates fungi from healthy tissue of *Melia azedarach* were belong to the class hypomycetes. Preliminary results shows only two genera of fungi produced only sterile mycelium. The most common genera found were Aspergillus's in initial stages of investigation. Both fungal genera are cosmopolitan in nature and usually epiphytic, but may also occur endophytically[24].

**Table 1:** Name and colonizing frequency of Endophytic fungi isolate from *Melia azedarach.*

| S. No. | Fungi Class - Hypomycetes | Isolate from | % Frequency of colonization | Number of isolates |
|---|---|---|---|---|
| 1. | Aspergillus's flavus | leaves | 6.6% | 2 |
| 2. | Aspergillus's niger | leaves | 13.3% | 3 |
| 3. | Aspergillus's spp. | leaves | 16.6% | 1 |
| 4. | Nigrospora spp. | leaves | 3.3% | 1 |

**DESCRIPTION OF ENDOPHYTIC FUNGI:** Morphological description of isolate endophytic fungi from *Melia azedarach (L.).*

| Plant organ | Taxon | Fungi | Morphological description |
|---|---|---|---|
| Leaves | hyphomycetes | Aspergillus's flavus | Colonies: light greenish-yellow<br>Reverse side colonies: primary stage -yellowish, mature stage- brownish. Conidiophores arose from submerged hyphae.<br>Conidiophores walls: pitted, rough and uncolored. Conidial head: hemispherical<br>Conidia: pyrifrom and globose.<br>Conidia size:3-4 |
| Leaves | hyphomycetes | Aspergillus's niger | Colonies: carbon black submerged mycelia.<br>Conidiophores walls: smooth with thick and uncolored. Conidial head: fuscous black, globose.<br>Conidial chain was present over the entire surface of vesicles.<br>Conidia: rough and globose.<br>Conidia size:3-4 |
| Leaves | hyphomycetes | Aspergillus's sp. | Colonies: hairy-powdery. Reverse side colonies: brown red.<br>Conidiophores: slightly granular and colorless.<br>Conidial head: blue green radiate, uniseriate.<br>Conidia: smooth globose or subglobose. |
| Leaves | hyphomycetes | Nigrospora sp. | Colonies: white initially and then becomes grey with black areas and turns to black eventually from both frin and reverse. Conidiophores: septate hyaline hypha, hyaline and slightly pigmented.<br>Conidia: visualized. Conidia: black, solitary, unicellular. Conidia size: 14-20 micros in diameter. |

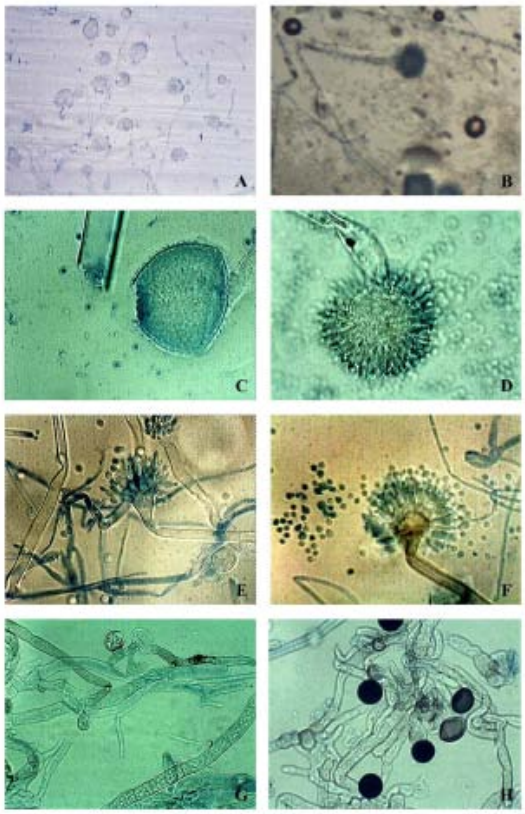

Fig. A: Older conidiophores with the tip slightly swollen to from a young vesicle in Aspergillus's flaves spp.
Fig. B: A portion of mycelium bearing conidiophores, an archicarps.
Fig. C: Older vesicle of Aspergillus's niger sp.
Fig. D: A mature conidiophores Bearing sterigmata in Aspergillus's niger sp.
Fig. E-F: Mature conidiophores bearing sterigmata and chain of conidia in Aspergillus's sp.
Fig. G-H: Eucarpic mycelium bearing sporangiophores and sporangia in Nigrospora sp.

## DISCUSSION

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[17].

Fungi have been widely investigated as a source of bioactive compounds. An excellent example of this is the anticancer drug, taxal, which had been previously suppared to occur only in the plants[12].

Endophytic fungi from medicinal plants can therefore be used for the development of drugs. Endophytic flora, both numbers and types, differ in their host and depends on host geographical position[18,19].

*Melia azedarach* is a well known medicinal plant and different part of tree, such as the bark and the leaves were used in folk medicine[20]. Where the tree leaf juice is anthlmintic, antilithic and diuretic. The root is highly effective against ringworm and parasitic and skin diseases[21]. The medicinal properties of the plant could be attributed to their endophytic fungi. Therefore, the present work was initiated to find out endophytic flora associated with in this widely used medicinal plant.

A study of endophytic biodiversity of the temporal variation in Plumeria rubra leaves was conducted by the T.S. Suryanarayanan and S. Thennarasan (2004)[22]. They reported temporal pattern of endophytic infection in leaves of Plumeria rubra and isolate harbor fungal species in this plant throughout the year. The result shows fungal diversity was in September 2.6 and in October 2.66.

In the preliminary study shows 2 different genera with Aspergillus's (6.6%, 13.3%, 16.6%) and Nigrospora (3.3%) colonization frequency were isolate from *Melia azedarach*. This is slightly less than the above cited report by (T.S. Suryanarayanan & S.Thennarasan, 2004)[22]. During the present study, mainly Aspergillus's sp. and Nigrospora sp. were isolate as endophytic fungi in *Melia azedarach*. All the isolate species of this plant belong to hyphomycetes class. Hypomycetes fungi were largely prevalent and showed the resistance too many pathogens. The genera Aspergillus's found most conman in this plant. There were the dominant genera or endophytic fungi found in this study. Similar to the findings reported previously in *Calotropis Procera* and *Withania Somnifera*[23].

Attempts have also been made to isolate pharmaceutical substances from plants and their endophytic fungi, as endophytes are considered to be a rich source of novel compounds[12]. Studies were also carried out on endophytic fungi to screen them for antibiotic, antiviral and anticancer, antioxidants, insecticidal and immunomodulatory compounds[13]. The endophytic fungi isolate from *Melia azedarach* will also be investigated for potential bioactive compounds, in future studies.

**Acknowledgement:** Authors are thankful to provide financial assistance to by the UGC for sanction the major research project 34-169/2008 SR to Prof. Amla Batra, Department of Botany, University of Rajasthan, Jaipur. Special thanks to Roop Narayan Verma for helping me in making photoplate and Savita Sagwan & Kshipra Dhabhai for critical reading of the manuscript.

## REFERENCES

1. Petrini, O. (1986).Taxonomy of endophytic fungi of aerial plant tissues. In: Microbiology of the phylospere. Fokkenna N.J. Van Den Heuvel J. (eds) Cambridge University Press, Cambridge.pp. 175-187.
2. Arnold, A.E. (2007). Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers, Fungal Biology Reviews 21:51-66.
3. Faeth SH, Hammon KE (1997) Fungal endophytes in oak trees: long-term patterns of abundance and association with leafminers.Ecology 78:810-819.
4. Huang WY, Cai YZ, Xing J, and Corke H, Sun M 2007 A potential antioxidant resource: endophytic fungi isolate from traditional Chinese medicinal plants.Econ Bot (in press)
5. Redman RS, Sheehan KB, Stout RG, Rodriguez RJ,Henson JM (2002) Thermotolerance generated by plant/fungal symbiosis, Science 298:1581

6. Arnold A.E., and Herre, E. A. (2003).Canopy cover and leaf age affect colonization by tropical fungal endophytes:Ecological pattern and process in *Theobroma cacao* (Malvaceac).*Mycologia*.95 (3):388-398.
7. Ganley RJ, Brunsfeld SJ, Newcombe G (2004) A community of unknown, endophytic fungi in western white pine.Proc Nat Acad Sci USA 101:10107-10112.
8. Strobel, G. A. (2000). Microbial gifts from rain forests. *Canadian Journal of plant pathology*. 24:14-20.
9. Mohali, S., Burgess, T, I., and Wingfield , M. N. (2005). Diversity and host association of the tropical tree endophytic *Lasiodiplodia theobrome* revealed using simple sequence repeat markers. *Forest pathology*.35 (6):385-396.
10. Suryanarayanan, T.S., Wittlinger, S.K.J., and Faeth, S.H. (2005). Endophytic fungi associated with cacti in Arizona. *Mycological Research*. 109 (5):635-639.
11. Sraj-Krzic, N., Pongrac, P., Klemenc, M., Kladnik, A., Regvar, M., and Gagerscik, A. (2006).Mycorrizal colonization in plants from intermittent aquatic habitats. Aquatic Botany. 85 (4): 340-346.
12. Strobel, G., and Daisy, B. (2003). Bioprospecting for microbial endophytes and their natural products. *Microbiology and Molecular Biology Reviews*.67 (4):491-502.
13. Tan, R. X., and Zou, W. X. (2001).Endophytes: a rich source of functional metabolites. Natural Product Reports.18: 448-459.
14. Bohnenstengel F. I., Wray V., Witte L., Srivastava R.P., and Proksch P.1999.Insecticidal meliacarpin (C Saco limonoids) from *Melia azedarach*. Phytochemistry 50,977D982.
15. Kim M., Kim S.K., Park B.N., Lee K.H., Min G.H., Seoh J.Y., Park C. G., Hwang E. S., Cha C.Y., and Kook Y.H.1999.Antiviral effects of 28-deacetylsendanin on herpes simplex virus-1 replication Phytochemistry 43:103-112.
16. Schulz, B., Wanke, U., Draeger, S., AND Aust, H. – J. (1993). Endophytic from herbaceous plants and shrubs: effectiveness of surface sterilization method. *Mycological Research*.97:1447-1450.
17. Clay, K.and Schardl, C. (2002). Evolution origins and ecological consequences of endophytes symbiosis with grasses. American Naturalist.160: 99-127.
18. Gange, A, C., S. Dey, A.F. Currie and B.V.C. Sutton. 2007. Site- and species-species differences in endophytic occurrence in two herbaceous plants. *Journal of Ecology*, 95 (4):614-622.
19. Arnold AE, Mejia LC, Kyllo D, Rojas EI, Maynard Z, Robbins N, Herre EA (2003) Fungal endophytes limit pathogen damage in a tropical tree. Proc Natl Acad Sci USA 100: 15649-15654.
20. Hadidi and L.Boulos, In: The street trees of Egypt, The American University in Cairo Press, 1988.
21. J.A. Duke and E.S. Ayenser, In: medicinal plant of china, Ref.Pub.Inc.1985.
22. Suryanarayanan, T.S and Thennarasan S, (2004). Temporal variation in endophytic assemblages of *Plumeria rubra* leaves. Fungal Diversity.15:197-204.
23. Rezwana khan, Saleem Shahzad, M.Iqbal Choudhary, Shekeel A Khan and Aqeel Ahmad (2007).Biodiversity of the endophytic fungi isolate from *Calotropis Procera* (AIT) R BR. Pak.J.Bot., 39(6): 2233-2239.
24. Schulthess. F.M. & Faeth, S.H.1998 Distribution, abundance and associations of the endophytic fungal community of Arizona fescue (Festuca arizonica). *Mycologia* 90, 569-578.



**About Corresponding Author: Ms. Kaushal Kanwer Shekhawat**

Ms. Kaushal is Post graduated and graduated from University of Rajasthan, Jaipur, India. She is currently pursuing her Ph.D under the guidance of Dr. D.V.Rao and Prof. Amla Batra, University of Rajasthan, Jaipur, India.