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STUDY OF ENDOPHYTIC FUNGAL COMMUNITY OF *MORINGA OLEIFERA* FROM OMALUR REGION – SALEM

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ABSTRACT: Endophytic fungi are ubiquitous in nature residing inside all the plant species contributing to their host plants by producing a plenty of substances that provide protection and survival value to the plant. Many researchers have proven that endophytes are a new and potential source of novel natural products for exploitation in modern medicine. This present study is undertaken to isolate and identify the potential endophytic fungi from *Moringa olifera*, a medicinal plants which is traditionally known and reported to posses various biological properties. A total of 72 fragments each 18 from the stem, leaves, flower and calyx were collected, surface sterilized and was inoculated on to Sabouraud dextrose agar (SDA) plates. Based on the macroscopic & microscopic features, the fungal isolates were identified. The most predominant endophytic fungal species isolated belonged to the genus *Aspergillus spp.* (53.3%) followed by *Bipolaris spp.* (6.6%). The Colonization Frequency (CF %) and Endophytic Infection Rate (EIR %) was found to be 83.31% and 20.83% respectively. The stem segments showed a maximum repository for endophytic fungi than the leaf and flower segments. The results of the study suggest that Endophytic fungi are a novel source of bioactive compounds. The rich biodiversity of these medicinal plants have to be preserved for the future years for the benefit of the mankind.

INTRODUCTION: Medicinal plants have been used in developing countries for thousands of years. World Health organization (WHO) estimates that 70-80% of the population living in India and other developing nations depend on traditional healthcare systems for primary healthcare. Medicinal plants and herbal medicines form an important part of the treatment in the indigenous medicine systems such as Ayurveda, Unani and Siddha [1].

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Endophytes are microbes that colonize the internal tissues of plants living without causing any harm to their host. However recent studies have revealed the ubiquity of these fungi with an estimate of at least one million species of endophytic fungi [2]. These fungi have proven themselves to be invaluable sources of natural product for industrial as well as biomedical development for decades.

These Endophytic fungi improve the resistance of the host plants to adversity by secretion of various bioactive metabolites of unique nature which includes alkaloids, benzopyranones, chinones, flavonoids, phenolic acids, quinones, steroids, terpenoids, tetralones and xanthones which find a wide range of application in agrochemicals,

industries, antibiotics and as immunosuppressants, antiparasitics, antioxidants and anticancer agents [3].

Moringa oleifera is a medicinal plant, belonging to monogeneric family Moringaceae. This plant (root, bark, gum, leaf, pods, flowers, seeds and seeds oil) have been used for the treatment of various ailments in the indigenous medicine. It has been known to posses' anti-helminthic activity, antimicrobial activity, detoxifier, immune booster and anti-parasitic activity [4]. Hence in this present study an attempt has been made to study the endophytic mycoflora of *Moringa oleifera* from Omalur region located in the plains of Salem district.

MATERIALS AND METHODS:

Collection of samples: Healthy and mature trees were carefully chosen for sampling. Fresh mature leaves, stem, flowers and calyx of *Moringa oleifera* Lam (Moringaceae) without symptoms of ripening and disease were collected from Omalur region of Salem district. The plant materials were packed in sterile zip-lock bags transported in to the laboratory. The samples were then processed immediately to reduce the chance of contamination.

The plants were rinsed in running tap water to remove the soil particles and unwanted debris. After washing the leaves, flowers, calyx, and stem were selected for further processing under aseptic conditions. Highly sterile conditions were maintained for the isolation of endophytes and the entire process was carried out inside the laminar air flow. Sterile glassware (conical flask) and mechanical things such as scissor, forceps, scalpel, and blades were used in sterile conditions for this Study. The stem and leaves were cut into segments (0.5-1cm) by the use of sterile lancet blades.

Surface sterilization of Samples: The samples were surface sterilized by following the method described by [5]. The segments of stem and leaves were immersed in 70% ethanol for 5s. The branch portions were further sterilized sequentially in 4% sodium hypochlorite solution (Merck Laboratories) for 90s, and then rinsed in sterile distilled water for 10s. The excess moisture was blotted on a sterile filter paper.

Inoculation of samples on SDA: The surface sterilized segments were placed in petridishes containing Sabouraud dextrose agar (SDA) medium supplemented with chloramphenicol (5mg/ml). Six segments were placed for one plate. The petridishes were incubated at 25ºC to 27º C for 72 hrs in dark condition and they were monitored everyday to check the growth of endophytic colonies from the segments.

Isolation and Identification of Endophytic fungi: 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 plate was done by the transfer of hyphal tips to fresh Potato Dextrose Agar (PDA) plates. The fresh PDA plates were incubated at 25ºC to 27ºC for 72 hrs & periodically checked for purity. The pure cultures were maintained on PDA slants. The endophytic fungi were identified according to their macroscopic (front and reverse side of fungal colonies) and microscopic characteristics such as the morphology of fruiting structures and spore morphology under a bright-field microscope (10x and 40x).

Statistical Analysis: The colonization frequency (CF) and Endophytic Infection Rate (EIR) were calculated as described by [6]. Samples were incubated and growth was examined daily during 6 weeks and colonization frequency was calculated by the following formula.

Colonization frequency (CF %):

$$\mathbf{CF} = \frac{\text{No. of individual fungi recorded}}{\text{Total no. of segments screened}} \times 100$$

Endophytic Infection Rate (EIR %):

$$\mathbf{EIR} = \frac{\text{Total no. of endophytic fungi recorded}}{\text{Total no. of segments screened}} \times 100$$

RESULTS & DISCUSSION:

Many medicinal plants and herbal medicines catapulted into patent war zone as highly valuable commodities through modern technologies. To date, only a few plants have been extensively

investigated for their endophytic biodiversity [7]. The study of the endophytic fungi occupies an important part in fungal biology as these endophytes play a very important and tremendous role in the production of secondary metabolites with pharmaceutical significance [8].

This study also showed such a trend of diversity of endophytic fungi which was apparent with the leaves, stem, flowers and calyx parts of the medicinal plant *Moringa oleifera*. Among the array of domestic medicinal plants which are used in day today life, *Moringa oleifera* Lam is one of the best known and highly distributed medicinal plants throughout the Asian continent and a list of medicinal properties conferred by this medicinal plant was described by [9].

Hence, an attempt was made to study the diversity of endophytic fungi of *M. oleifera* of Omalur region, Salem. Out of 72 segments plated, each 18 from leaf, stem, flowers and calyx, 15 different endophytic fungal isolates were obtained. The stem segments showed a maximum repository for endophytic fungi than the other segments. As shown in **Table 1** the Colonization Frequency (CF) and the Endophytic Infection Rate (EIR %) was found to be 80.31% and 20.83%. This is comparatively lesser than the one reported by the study of [10] indicating that the colonization of these endophytic fungi also varies with the geographical distribution.

TABLE 1: COLONISATION FREQUENCY AND ENDOPHYTIC INFECTION RATE OF ENDOPHYTIC FUNGI COMMONLY ISOLATED FROM PARTS OF *MORINGA OLEIFERA*

S. No	Plant Part	Endophytes Isolated	Number of endophytes Isolated	Colonization Frequency (%)	Endophytic Infection Rate (%)
1	Leaves	*Aspergillus spp,* *Aspergillus terreus* *Mycelia sterilia* (1)	3	16.66%	
2	Stem	*Bipolaris spp,* *Aspergillus flavus* *Aspergillus versicolor* *Mycelia sterilia* (2)	5	27.77%	20.83%
3	Flowers	*Aspergillus niger* *Aspergillus ochraceus* *Aspergillus spp.* (2)	4	22.22%	
4	Calyx	*Aspergillus spp* *Mycelia sterilia* (2)	3	16.66%	
Total			**15**	**83.31%**	**20.83%**

The endophytic fungal genera isolated were predominantly *Aspergillus spp.*, namely *Aspergillus ochraceus* **(Fig. 1A),** *Aspergillus niger* **(Fig.1B),** Aspergillus *flavus* **(Fig.1C),** *Aspergillus versicolor*, *Aspergillus terreus*, and *Bipolaris spp* **(Fig.1D).** Many unidentified sterile mycelial forms were also found which were grouped under the class *Mycelia sterilia* **Table 2**. These results obtained in these studies were similar to the studies of [10], which also reported *Aspergillus spp.*, However there were variation with regard to the diversity of these endophytic fungi reported in these studies. The plant used in the present study were collected from the plains than the one reported by [10], which was collected from the hilly regions. The studies of [10], also reported *Alternaria spp.*, *Bipolaris spp.*, *Exosphiala spp.*, *Nigrospora spp.*, and *Penicillium spp* in *Moringa oleifera.* However in this study there were no other endophytic fungi other than the *Aspergillus spp* except Bipolaris *spp*

TABLE 2: ISOLATED ENDOPHYTES IN RELATION TO FUNGAL GROUP

S. No	Isolated Endophytes	Fungal Class	Description
1.	*Bipolaris spp.*	Dematiaceous Hyphomycetes (Dueteromycetes)	Colonies: grey to blackish brown, suede-like to floccose, Conidiophores: branched, short, geniculate, or Zig-zag rachis conidia: ellipsoidal rounded at both ends, smooth
2.	*Aspergillus niger*	Hyaline hyphomycetes (Dueteromycetes)	Colonies: white or yellow basal felt covered by a dense layer of dark-brown to black conidial heads. Conidiophores are smooth-walled, hyaline or turning dark towards the vesicle. Conidial heads are biseriate with the phialides borne on brown, often septate metulae.
3.	*Aspergillus flavus*	Hyaline hyphomycetes	Colonies: granular, flat, often with radial grooves, yellow.

		(Dueteromycetes)	Conidiophore: Stipeses are hyaline and coarsely roughened. Conidia are globose to subglobose (3-6 µm in diameter), pale green and conspicuously echinulate.
4.	*Aspergillus terreus*	Hyaline hyphomycetes (Dueteromycetes)	Colonies: suede, cinnamon-buff to sand brown in color with a yellow to deep dirty brown reverse. Conidial heads are compact and biseriate. Conidiophores are hyaline and smooth-walled. Conidia are globose to ellipsoidal, hyaline to slightly yellow and smooth-walled.
5	*Aspergillus versicolor*	Hyaline hyphomycetes (Dueteromycetes)	Colonies: center initially floccose and outer areas velvety, at first white, passing through shades of yellow with reverse brown. Conidia: globose conspicuously spinulose, green.
6	*Aspergillus ochraceus*	Hyaline hyphomycetes (Dueteromycetes)	Colonies: granular powdery tan to buff colour.Conidia: elliptical, pitted, yellowish.
7	*Mycelia sterilia*	Sterile forms	Many fungidonot produce any recognizable sexual/ asexual conidia state in culture.

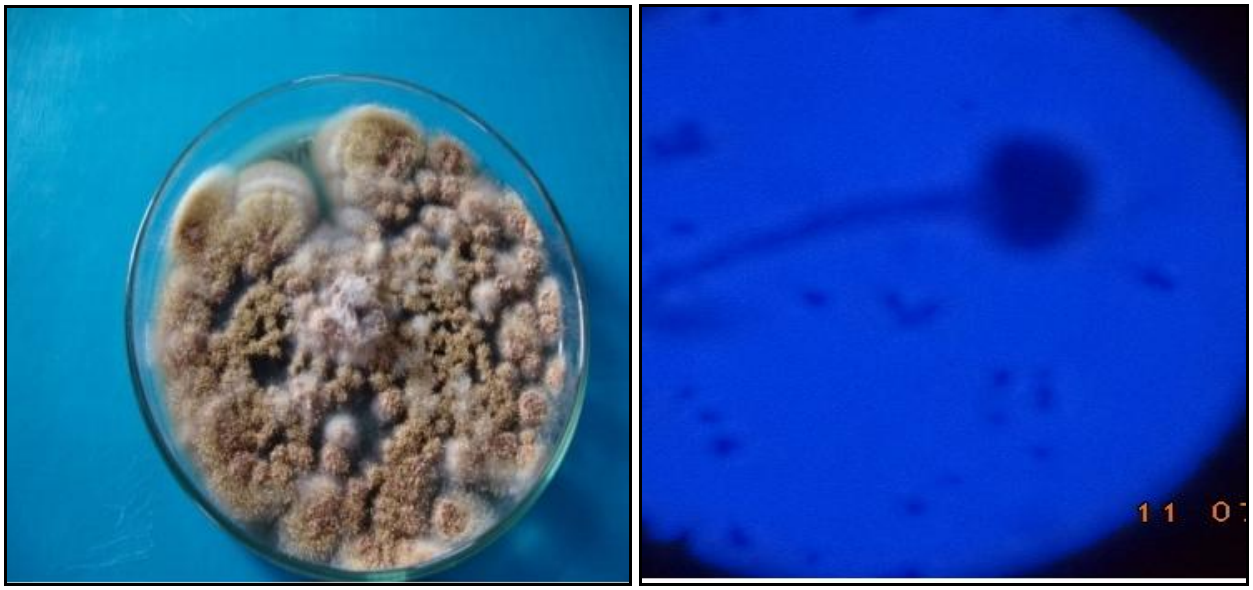
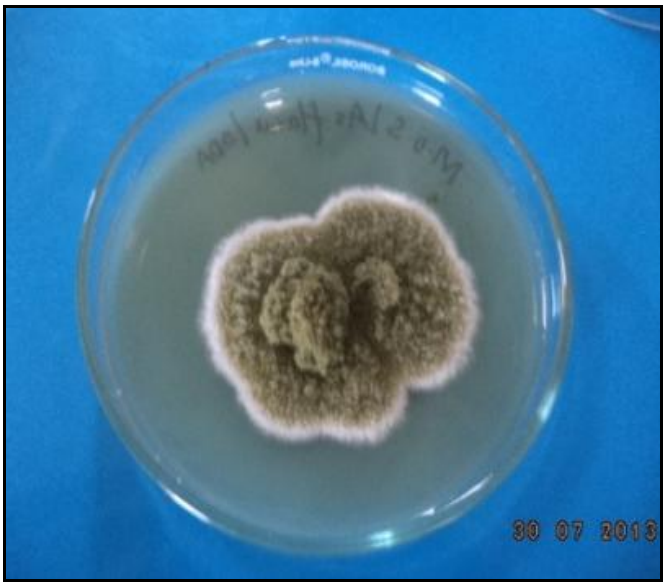


FIG.1A: YELLOW-BROWN COLONIES OF *A. OCHRACEUS ON SDA*

FIG.1B: BROWN-BLACK CONIDIOSPORES BEARING CONIDIOPHORES OF *A. NIGER*



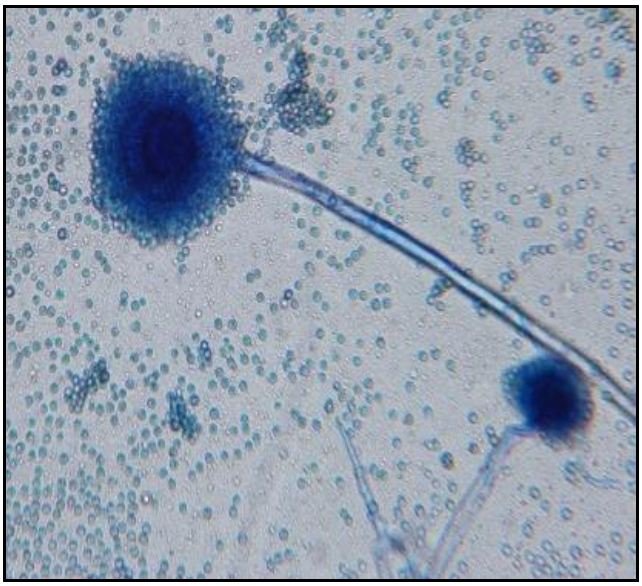


FIG.1C: OLIVE-GREEN COLONY OF *A. FLAVUS* SHOWING BISERIATE CONIDIOSPORES

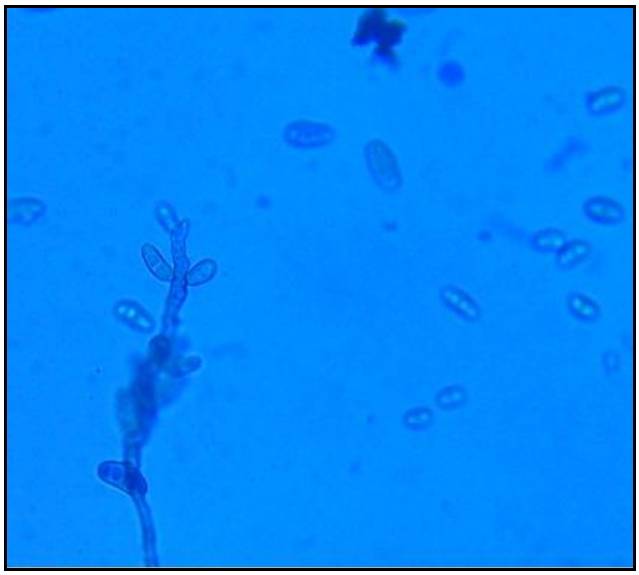


FIG.1D: SYMPOIDIAL ARRANGEMENT OF CONIDIA ON ZIG-ZAG RACHIS OF *BIPOLARIS SPP.*

CONCLUSIONS: The result of the study suggests that the medicinal plants are a rich source of endophytic fungi. However, future studies are required to screen the compounds produced by these endophytic fungi and for testing their activity both *in vitro* as well as *in vivo* to rule out the emerging drug resistant pathogens through the modern technologies in pharmaceutics. The diversity of these medicinal plants has to be preserved for the rich flora, as well as for studying their endophytic biodiversity which will help in the production of novel compounds for the benefit of the mankind.

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