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Original Article

Mycoendophytes Isolated from *Mimusops elengi*. L - A First Report

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Endophytes are organisms present in the internal tissues living in symbiotic association with plants. These organisms may be used as alternative source of many secondary metabolites production. The present study was conducted to explore the endophytic fungi that colonize the inner tissues of medicinal plant *Mimusops elengi* L. Out of 100 plant segments of both leaves and twigs of *Mimusops elengi*. L were surface sterilized and inoculated in potato dextrose agar plates and incubated for 4 weeks. A total of 17 endophytic fungi were isolated. Endophytic infection rate of *Mimusops elengi* L. was 88% in both leaves and twigs segments. Among them 10 were sterile morpho species differentiated by their morphological characteristics. The remaining 7 species were classified into 6 genera, with 2 species from *Phoma* genera and belonging to Coelomycetes, and 5 species from 5 genera belonging to Hyphomycetes. *Nigrospora sphaerica* showed the highest frequency of colonization of 42% and 34% in leaves and twigs, respectively, followed by sterile form IS6, *Alternaria* Sp., and sterile form IS4. Shannon diversity index was higher in twigs (H'= 0.35) and lower in leaves (H'= 0.18). Shannon evenness and relative index for Shannon were predominated in twigs (E'= 0.153 and RIH'= 0.093, respectively) followed by leaves (E'= 0.079 and RIH'= 0.048, respectively). Both twigs and leaves showed similar Gleason index (HG') and relative index of Gleason (RIG') equal to 2.378 and 0.20, respectively. The enumeration of biodiversity of endophytic fungi were dominated by *Nigrospora sphaerica*, Sterile form IS6, Sterile form IS4, *Alternaria* Sp., and *Phoma* Sp.

Keywords: Endophytic fungi, *Mimusops elengi*. L, *Nigrospora sphaerica*, *Alternaria* Sp

The microorganisms colonizing the internal tissues of higher plants are called endophytes. The presence of endophytes may not cause any harmful symptoms to plants, but they rather live in symbiotic association with the host plant (1-2). Under *in vitro* fermentation conditions, endophytic fungi may produce some bioactive secondary metabolites with pharmaceutical applications (3).

Mimusops elengi. L (Magizham in Tamil, and Bakul in Hindi) is a medicinal plant of high pharmac eutical importance. Almost all parts of this plant, namely leaf, root, fruit, seed, bark and flower are used to cure various kinds of disorders as mentioned in 'Ayurveda' medicine. The bark of the plant is used in the Ayurvedic formulations and is applied as a tooth powder (4). Similarly, the leaves are well known for their analgesic and antipyretic properties (5). The endophytic fungi *Taxomyces andreanae* is able to produce Taxol (6). Similarly, the endophytic fungus *Entrophospora infrequens* which belongs to the Phycomycetes family, was isolated from the inner bark of *Nothapodytes foetida* and can

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synthesize an anticancerous alkaloid called Camptothecin (7).

The present study was carried out to isolate endophytic fungi from twigs and leaves of *Mimusops elengi* L. and to study briefly the diversity of endophytic mycobiota. This is the first report of *Mimusops elengi* L. from Western Ghats of Sathyamangalam region.

Materials and methods

Plant material collection

Healthy and fresh plant parts (leaves and twigs) of *Mimusops elengi* L. were collected from herbal garden of Bannari Amman institute of technology campus, Sathyamangalam region, India during the month of August 2016. The plant materials were collected in sterile paper bags and processed immediately to reduce the risk of contamination (8).

Isolation of endophytic fungi

The procedure to isolate endophytic fungi has been followed from previous studies (9) with little modification. Surface sterilization was performed to eradicate epiphytic microorganisms following the procedure of Araujo et al. (10). First, the plant materials were washed with running tap water and allowed to air dry followed by successive immersions in 70% ethanol for 2 min, 4% sodium hypochlorite for 2-3 min (2 min for leaves, 3 min for twigs), 75% ethanol for 30 s, and were then washed again in sterilized distilled water. Then, explants were allowed to dry in autoclaved paper towels. Afterward, the plant samples were cut into 0.5 cm^2 (leaves) and 0.5 cm (twigs) pieces and transferred into petri plates containing potato dextrose agar (PDA) (200 g/l potato extract, 20 g/l dextrose, and 20 g/l Agar Agar type I) supplemented with 250 mg/l streptomycin as an antibiotic source, as described by Siqueira et al. (11) with slight modification. The plates were incubated at 25± 2 °C for 4 weeks and monitored every day for the growth of endophytic fungi. The endophytic fungi were sub cultured in a fresh PDA plates for further experimentation.

Identification of endophytic fungi

Endophytic fungi grown on petri plates were identified based on the morphological characteristics of their colony culture and spores (12-13). The fungal spores were stained with lacto phenol cotton blue stain on a sterile glass slide and observed under bright field microscope (14-15). Microscopic examination showed vegetative and reproductive structures which were useful for fungi identification according to the literature (16-18). Unknown sterile isolates were sorted into sterile morpho species based on the colony surface texture, hyphal pigmentation, exudates, and growth rates (19).

Preservation of endophytic fungi

The purified endophytic fungal strains were stored as slants at 4 °C in endophytic fungal culture collection (EFCCC) for further studies (20).

Statistical analyzes

The endophytic fungal diversity was proved with the help of various statistical tools.

Colonization frequency (%CF) was determined according to the formula below (21):

$$CF\ (\%) = \frac{\text{Number of segments colonized by an endophytic species}}{\text{Total number of segments}} \times 100$$

The relative percentage occurrence (RPO) of each group of fungi was determined according to the formula below (22):

$$RPO = \frac{\text{Density of colonization of one group}}{\text{Total Density of colonization}} \times 100$$

The endophytic infection rate was estimated as follows:

$$EIR\ (\%) = \frac{\text{Total number of infected segments}}{\text{Total number of segments screened}} \times 100$$

Biodiversity indices

The fungal diversity of endophytic myco-populations was estimated using different diversity indices. The strengths of these indices allowed the prediction of the complete structure of different myco-populations. Shannon diversity index (H′) and

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Shannon evenness index (E′) were used for the evaluation of fungal species richness (23), with H′ = –Σ (pi ln pi) where pi was the proportion of individuals that species i contributed to the total.

The evenness was expressed by J′ = H′/H′max = H ln S where H′max was the maximum value of diversity for the number of species (24).

The relative index for Shannon was RIH′= H S /H Smax = H S / ln Ni.

The Gleason index was HG′ = N_p -1/ln N_i.

The relative index for Gleason was RIH′ = H G / H Gmax =Np -1/ Ni -1.

Where Ni was the total number of individuals, N_p was the number of species identified among these isolates, H G_{max} and H S_{max} were the greatest possible values of HG and HS in a sample of N_i individuals.

Results

Morphological characteristics of isolated endophytic fungi

A total of 17 endophytic fungi were isolated from 100 segments of both leaves and twigs parts of *Mimusops elengi* L. Figure 1 shows the Photomicrographs of some isolated endophytic fungi. Among the isolated fungi, 10 were sterile morpho species differentiated by their morphological characteristics. The remaining 7 species were classified into 6 genera, with 2 species in *Phoma* genera and belonging to Coelomycetes, and 5 species in 5 genera belonging to Hyphomycetes (Table 1).

Colonization frequency and relative percentage occurence

The highest frequency of colonization was observed with *Nigrospora sphaerica* which was present in 42% and 34% of examined the colonized segments of leaves and twigs, respectively, followed by sterile form IS6, *Alternaria* Sp., and sterile form IS4 (Fig. 2 and 3). The relative percentage occurrence of leaves of endophytic fungi was dominated by sterile forms (50%), followed by Coelomycetes and Hyphomycetes (25% each), while twigs of endophytic fungi were dominated by sterile forms (60%) followed by Hyphomycetes (40%).

Biodiversity indices of endophytic fungi

Shannon diversity index (H') was higher in twigs (H'= 0.35) and lower in leaves (H'= 0.18). Shannon evenness (E') was predominated in twigs (E'= 0.153) followed by leaves (E'= 0.079). Relative index for Shannon (RIH') was predominated in twigs (RIH'= 0.093) and lower in leaves (RIH'= 0.048). Gleason index (HG') in twigs and leaves were 2.378. Relative index of Gleason (RIG') in twigs and leaves were 0.20. Endophytic infection rate of *Mimusops elengi* L. was 88% in both leaves and twig segment.

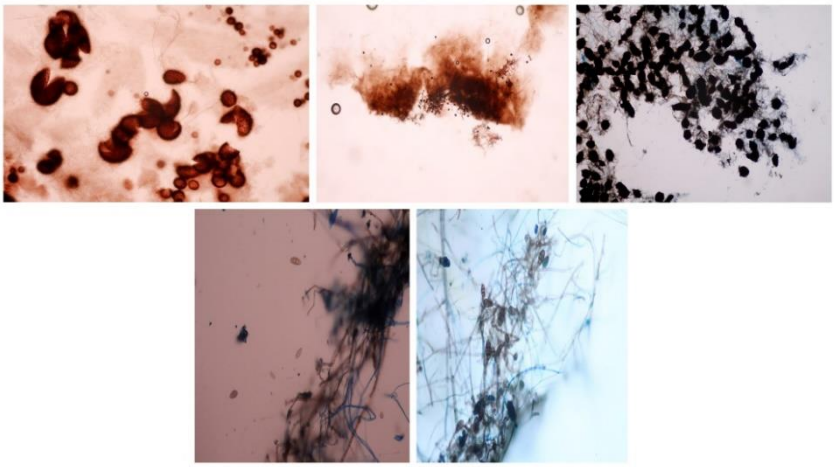

Figure 1. Micrographs of endophytic fungi from *Mimusops elengi* L. (a) *Phoma* Sp.; (b) *Nigrospora sphaerica*; (c) *Phoma nebulosa*; (d) *Dreschlera biseptata;* (e) *Alternaria* Sp.

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Table 1. Endophytic fungi isolated from *Mimusops elengi*. L

Endophytic fungi	Number of segments infected	
Hyphomycetes	**Twigs**	**Leaves**
Nigrospora sphaerica	21	17
Dreschlera biseptata	1	-
Alternaria Sp.	4	-
Myxocyclus polycistis	1	-
Penicillium Sp.	-	4
Coelomycetes		
Phoma Sp.	-	1
Phoma nebulosa	-	2
Sterile forms/Non- Sporulating		
IS1 (black cottony)	1	-
IS2 (blackish grey)	3	-
IS3 (pale brown)	2	-
IS4 (blackish spongy)	4	-
IS5 (brown cottony)	1	-
IS6 (white cottony)	6	-
IS7 (blackish white)	-	3
IS8	-	6
IS9 (greenish white)	-	1
IS10 (grey white)	-	10

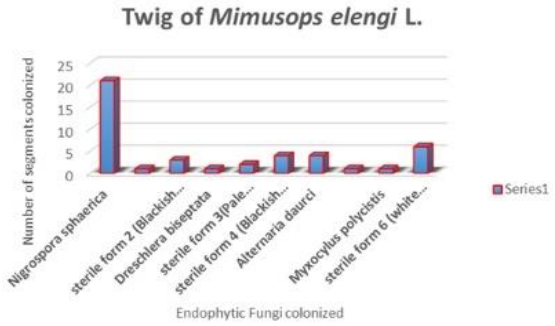

Figure 2. Colonization frequency of endophytic fungi recorded form twig of *Mimusops elengi* L.

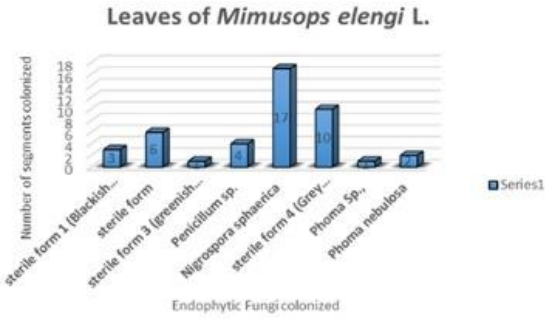

Figure 3. Colonization frequency of endophytic fungi recorded form leaves of *Mimusops elengi* L.

Discussion

The present study investigated the endophytic fungal diversity of *Mimusops elengi* L. The endophytic fungal diversity was proved through various statistical analyzes. The plant parts of leaves and twigs were associated with endophytic fungi of mycelia sterilia IS6 and IS4 forms acting as symbionts. *Nigrospora sphaerica* was predominantly interlinked in both leaves and twigs, whereas *Alternaria Sp.*, and *Dreschlera biseptata* were present in twig parts. *Penicillium* Sp., was only present in leaves. The present study highlighted the

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endophytic fungal diversity of *Mimusops elengi* L. in Sathyamangalam region during the August month. Due to temperature variation, nutritional defects occurence, some endophytic fungi may have not sporulated. Further investigations may help to induce sporulation of the sterile mycelial fungi.

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Conflict of interest

The authors declared no conflict of interest.

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