Applied Ecology and Environmental Sciences, 2020, Vol. 8, No. 3, 128-134
Available online at http://pubs.sciepub.com/aees/8/3/8
Published by Science and Education Publishing
DOI:10.12691/aees-8-3-8

Isolation and Morphological Identification of Culturable Endophytic Fungal Species from Mangrove Ecosystem

Jeyabalan Sangeetha*, Revathy Unnikrishnan, Habeeb Jasmin, Simmi Maxim Steffi

Department of Environmental Science, Central University of Kerala, Tejaswini Hills, Periye 671316, Kasaragod, Kerala, India
*Corresponding author: drjsangeetha.cuk@gmail.com

Received March 23, 2020; Revised April 28, 2020; Accepted May 08, 2020

Abstract Endophytic fungi inhabit healthy plant tissues, and are now considered as ubiquitous symbionts of plants. Besides having the positive effects on the plants in phytostimulation, production of pigments, enzymes and bioactive compounds and nutrient cycling, they are also responsible for the destruction of host tissues and toxic production within the host. The endophytic fungi can be found in any part of the plant such as scale primordial, meristem, resin ducts, petiole, buds, stem, root, shoot, leaves, barks and even in the pneumatophores. Mangroves act as a host for plenty of endophytic fungal populations. The endophytic fungi obtained from mangroves have certain specialized characteristics and are resistant to several environmental stress conditions. Aim of the present study is to isolate and identify endophytic fungi inhabiting mangrove plant species that are abundantly found along the state of Northern Kerala, India. The collected plant specimens were surface sterilized and ground to paste form to earn the extract, which was then inoculated into the medium to obtain fungal culture. Among seven plant specimens collected, six types of endophytic fungi were morphologically identified as *Aspergillus* sp. (two different species), *Penicillium* sp., *Alternaria* sp., *Fusarium* sp. and *Sarocladium* sp.

Keywords: *endophytic fungi; mangroves, morphology, Aspergillus sp., Penicillium sp., Alternaria sp., Fusarium sp., Sarocladium sp*

Cite This Article: Jeyabalan Sangeetha, Revathy Unnikrishnan, Habeeb Jasmin, and Simmi Maxim Steffi, "Isolation and Morphological Identification of Culturable Endophytic Fungal Species from Mangrove Ecosystem." *Applied Ecology and Environmental Sciences*, vol. 8, no. 3 (2020): 128-134. doi: 10.12691/aees-8-3-8.

1. Introduction

Mangroves are coastal rainforests regarded for their adaptability to harsh environmental conditions such as high salinity, temperature fluctuation, strong winds, extreme tides and even low oxygen levels. They are also known as tidal forests or coastal woodlands. Mangroves are tropical and subtropical forest systems which include flora from different genera sharing the common characteristics to grow in marine ecosystem including estuarine [1]. The word mangrove is a combination of Portuguese word "Mangue" and English word "Grove" [2]. In India, mangroves cover an area of 4,639 sq. km, which is 0.14% of the total geography of the country. However, the mangrove cover in India represents 3% of the global mangrove cover and 8% of Asian mangrove coverage [3,4]. East coast along with Bay of Bengal occupies about 60% of the Indian mangroves, West coast in Arabian Sea possesses around 27% and Andaman and Nicobar Islands show13% of mangroves. In India, the maximum mangrove cover is in Sundarbans, followed by Gujarat and Andaman and Nicobar Islands [5].

The state of Kerala provides favorable environment for the rich growth of mangroves with its coastal line of around 560 km and 41 rivers, emptying into the Lakshadweep Sea. Out of all 14 districts of Kerala, 10 districts have mangrove covers in the coastal environment. Districts like Idukki, Pathanamthitta, Palakkad and Wayanad are places having no coastal areas and hence do not have mangrove forests. Among the districts having mangrove cover, Kannur has the richest floristic diversity of mangroves [6]. The district has the distribution of about 83% of mangrove stocks of Kerala. The mangrove species that are abundantly found in Kerala are *Rhizophora mucronata*, *Rhizophora apiculata*, *Avicennia officinalis*, *Avicennia marina*, *Bruguiera cylindrica*, *Kandelia candal*, *Sonneratia alba*, *Sonneratia caseolaris*, *Excoecaria agallocha* and *Aegicera scorniculatum* [7,8].

Fungi could be either endophytes or ectophytes. Ectophytes are those that reside outside the host body, whereas endophytes are organisms that grow inside the body of the host organism. Endophytic fungi are a group of highly diverse fungi falling in the class ascomycetes. They are found within the plant tissues without causing any notable effects [9-11]. Nearly 3,00,000 species of higher plants exist on earth and each plant contains diversity of endophytes [11]. In most cases, fungi induce some diseases on the host organisms or produce reproductive structures at host's expense. But in case of endophytic fungi, they do not show any external signs of growth on the plants. These endophytic fungi could be isolated and identified by microscopic observation or

molecular analysis according to laboratory protocols. Endophytes prevent the growth and development of pathogenic organisms within the plant tissues. They are classified into two groups as clavicipitaceous and non-clavicipitaceous based on the differences in their selection of the host plant, ecology, evolution and taxonomy. Clavicipitaceous are those endophytes that can infect only grasses whereas non clavicipitaceous are endophytes found in the asymptomatic tissues of non-vascular and vascular plants. Endophytes enhance plant growth, distribute soil nutrients and increase resistance to abiotic stress in plants [12]. *Aspergillus* sp. was found to be the dominant endophytes among the various fungal species isolated from the mangrove ecosystems [13].

The other commonly found endophytes isolated from mangroves are *Fusarium* sp. and *Penicillium* sp. Endophytes may produce abundant potential substances that can be used in agriculture, industry and modern medicine such as novel antibacterial, antifungal and anticancer compounds and immunosuppressants [14,15]. Some of the factors that influence the endophytic fungal communities include geographical area, climatic conditions, host physiology and specificity of the colonized plant tissue [5,16]. Endophytic fungi commonly protect their hosts against pathogens, insects and pests [17]. Seena and Sridhar [18] worked on two sand legumes from south coast of India to study the endophytic fungal diversity. They concluded in their study that there is no significant difference in the endophytic fungi colonization between plant species. Suryanarayanan et al. [19] isolated endophytic fungal species from the leaves of *Rhizophora apiculate* Bl. and *Rhizophora mucronata* Lamk., collected from Pichavaram mangrove at Tamil Nadu. They compared the richness index of isolated endophytic fungal diversity between two plant species collected in two different time periods. They observed that the presence of the endophytic fungal diversity is higher in rainy days than in summer days.

2. Materials and Methods

2.1. Sample Collection

The samples were collected from two mangrove sites at Valiyaparamba of Kasargod district and Kunhimangalam and Ezhimala of Kannur district. Healthy and mature plant species were chosen for sampling. The bark samples were obtained aseptically and were stored in sterile polythene bags [19].

2.2. Isolation of Fungal Endophytes

The plant materials were rinsed with tap water to remove dust and debris and then washed with sterilized water to cut into small pieces aseptically. The cut pieces were surface sterilized with 70% ethanol for three minutes and washed again five times using sterile distilled water. These surface sterilized samples were weighed. About 0.5g of each sample were ground using a sterile mortar and pestle by adding 2ml of sterile distilled water and a paste of the sample was obtained. About 0.1 ml paste of the sample was then plated onto Potato Dextrose Agar (PDA) and was incubated at room temperature for 4 days [12,18].

2.3. Morphological Identification of Fungal Species

The fungal colonies were stained using lactophenol cotton blue dye. The prepared slides were viewed under trinocular light microscope. The fungal species were identified on the basis of their morphological characteristics. The different fungal isolates were described on the basis of their macroscopic (topography, texture and colour) as well as microscopic (structure of hyphae, conidia, conidiophores and phialides) characteristics [20,21].

3. Results and Discussions

3.1. Sample Collection

The study areas selected were Valiyaparamba situated at 12.1510°N latitude and 75.1420°E longitude in the belt of Kasaragod district, Kunhimangalam situated at 12.0718°N latitude and 75.2285°E longitude and Ezhimala situated at 12.0213°N latitude and 75.2071°E longitude in the belt of Kannur district. Table 1-2 shows the seven mangrove plants species that were obtained from the sampling sites and its characteristics respectively. The collected plant species were identified based on literature [2,5] as *Avicennia officinalis*, *Bruguiera cylindrica*, *Excoecaria agallocha*, *Acanthus ilicifolius*, *Kandelia candel*, *Rhizophora apiculata* and *Aegicera scorniculatum* (Figure 1).

Table 1. Plant species collected and used for the study

Species	Family	Habit	Known as	Local name	Mangrove forest
Avicennia officinalis L.	Avicenniaceae	Tree	Indian mangrove	Uppatti	Ezhimala
Bruguiera cylindrica L.	Rhizophoraceae	Tree	White Burma mangrove	Kuttikandal	Ezhimala
Excoecaria agallocha L.	Euphorbiaceae	Tree	Blinding tree	Kannambotti	Kunhimangalam
Acanthus ilicifolius L.	Acanthaceae	Shrub	Holly mangroves	Chullikandal	Kunhimangalam
Kandelia candel L.	Rhizophorazeae	Shrub	Narrow Leaved Kandelia	Cherukandal Nallakandal	Kunhimangalam
Rhizophora apiculata Blume.	Rhizophoraceae	Tree	Tall Stilted Mangrove	Vallikandal	Valiyaparamba
Aegicera scorniculatum Blanco.	Myrsinaceae	Small tree	Black/River mangrove	Pookandal	Valiyaparamba

Avicennia officinalis

Bruguiera cylindrica

Excoecaria agallocha

Acanthus ilicifolius

Kandelia candel

Rhizophora apiculata

Aegicera scorniculatum

Figure 1. Plant species collected from sampling sites

Table 2. Characteristics of the plant species collected for endophytic fungal isolation

Name	Stem/bark	Leaves	Flowers	Fruits /seeds	Roots
Avicennia officinalis	Thin, smooth, brownish or grey in colour	Opposite obovate or broadly oblong, thick and leathery appearance with the edges slightly rolled downwards	Orange yellow coloured, having a bell shaped structure, bearing 2 to 12 flowers together	Seeds are large and flattened, without a seed coat, and germinates in water	Much branched and occasional stilt roots
Bruguiera cylindrica	Grey and smooth bark	Simple, thin, light green in colour having elliptical shape and oppositely arranged structure	Pale greenish colour and small in clusters of 2 to 5	Fruits a berry, enclosed by the persistent, sometimes enlarged calyx tube. Seed solitary produced in large numbers	Mostly underground producing numerous knee roots
Excoecaria agallocha	Bark is in greyish brown colour with warty appearance	Simple with papery and slightly fleshy leaf blades	Minute and yellow	Fruits are small, dark and in capsular form	Shallow and surface running
Acanthus_ilicifolius	Scarcely branched stems which often produce adventitious aerial roots	Leaves having spiny edges	Light violet colour which appear at the branch tips	Fruits are green, long and kidney shaped	Shallow tap roots and occasionally develops a stilt root
Kandelia Candel	Dark brown bark	Simple, mostly elliptical and oppositely arranged showing pale green colour.	White coloured	Only one seed, which is spindle shaped and slightly curved with pointed radicle	Sometimes appear braided at the base of the trunk
Rhizophora apiculata	Dark grey bark	Elliptical, smooth, leathery and dark green	Pale yellow coloured occurring in pairs	Shows viviparity	Conspicuous and arching stilt roots, sometimes having aerial roots
Aegicerascorniculatum	Grows as a shrub or small tree	Long and oval shaped. Possess glands that secretes salt	Small and white, arranged in bunches and smell like rotten bananas	The fruits are green and later ripen to red.	No obvious above ground roots

Table 3. Macroscopic and microscopic characteristics shown by the fungal species isolated from mangrove

Fungal Species	Macroscopic features		Microscopic features			
	Texture	Colour	Hyphae	Conidia	Conidiophores	Phialides
Isolate 1	Powdery, granular	Yellowish green, become green with age and appear creamish yellow on reverse side	Hyphae are septate and hyaline	Globose to subglobose	Heavy walled, long, uncoloured, coarsely roughened	Uniseriate or biseriate
Isolate 2	Granular	Blackish brown	Stipe long, smooth, hyaline to brownish	Spherical and roughens with maturity	Smooth	Biseriate
Isolate 3	Velvety	Grayish green	Septate and hyaline	Globose	Simple or branched	Flask-shaped, consisting of a cylindrical basal part and a distinct neck
Isolate 4	Web like	Ashy white	Septate, brown hyphae	Conidia are long ellipsoid in size, septate, slightly constricted near some septa, with few longitudinal septa, moderately long chains	Simple or solitary, multicellular and elongated conidiophores	Uniseriate or biseriate
Isolate 5	Velvety to cottony	White to cream colored	Hyphae are septate and hyaline	Hyaline, thin walled, 3-5 septate, falcate to almost straight	Branched or unbranched	Cylindrical, solitary
Isolate 6	Fluffy	White creamy with orange to pink in reverse	Submerged or slightly fasciculate aerial hyphae	Ellipsoidal conidia	Long and straight conidiophores	Long slender phialides

3.2. Fungal Endophytes Isolated from Mangrove Tree Barks

Each species of plants harbor a specific kind of endophytic fungi. In the current study, fungal species were isolated from barks of the specimens. Plant parts of seven broad ranges of mangrove plant species were collected and processed for the isolation of endophytic fungi. Six different fungal species were observed under the trinocular microscope. The characteristics shown by each fungal species microscopically and macroscopically were noted and identification was done on the basis of their observed characteristics (https://mycology.adelaide.edu.au) [22]. Macroscopic and microscopic views of the isolated fungal species are given in Figure 2 and Figure 3 respectively. Table 3 explains the macroscopic and microscopic characteristics of the isolated fungal species. A total of six fungal strains were obtained from the samples of which 3 belong to the class Eurotiomycetes and each one out of the remaining three are from the classes, Dothideomycetes, Sordariomycetes, and Ascomycetes. The isolated endophytic fungi were confirmed with their microscopic and macroscopic features as *Penicillium* sp., *Alternaria* sp., *Fusarium* sp., *Sarocladium* sp. and two *Aspergillus* sp. (Table 4). Table 5 shows the identified fungal endophytes and the respective plants from where they have been isolated.

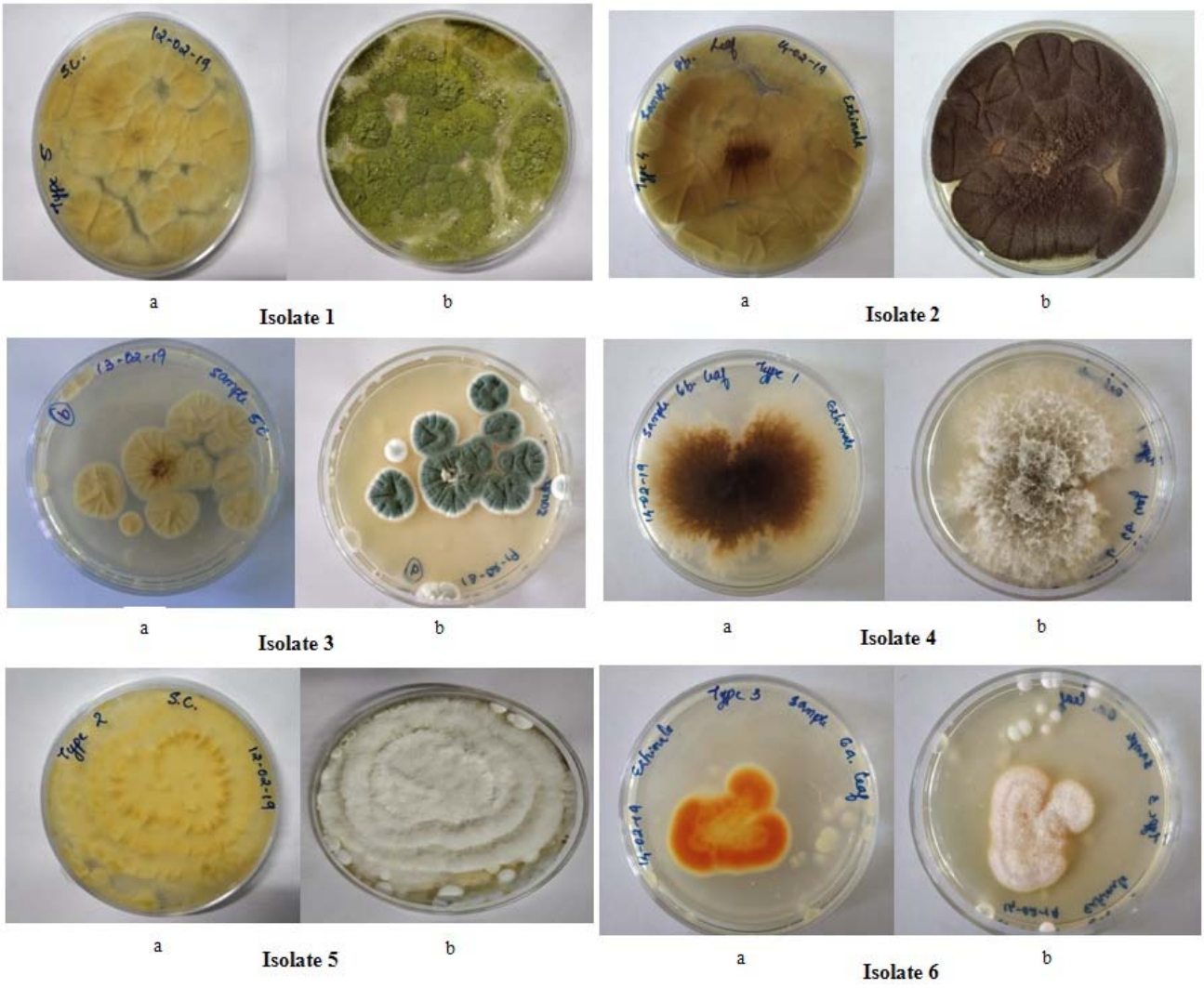

Figure 2. Endophytic fungal species identified from mangrove plant species on PDA (a) Reverse side of the Petri Plate (b) Front view of the Petri Plate

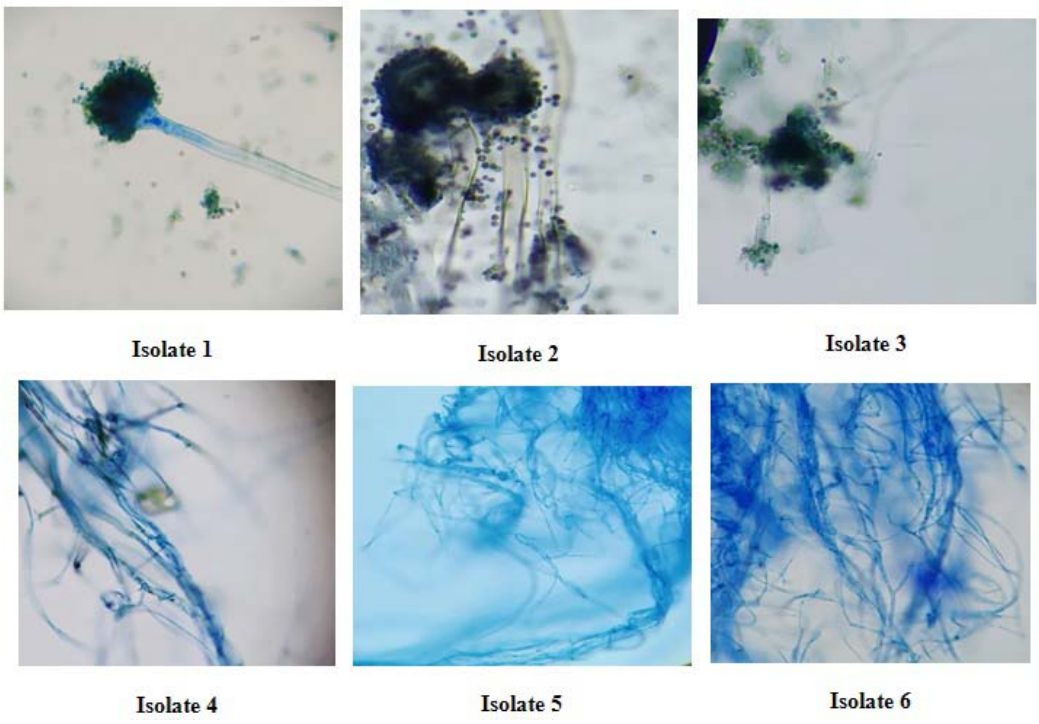

Figure 3. Microscopic view of isolated fungal species from mangroves trees

Table 4. Identified endophytic fungal species based on macroscopic and microscopic features

Fungal Isolates	Fungal Name
Isolate1	*Aspergillus* sp.
Isolate 2	*Aspergillus* sp.
Isolate 3	*Penicillium* sp.
Isolate 4	*Alternaria* sp.
Isolate 5	*Fusarium* sp.
Isolate 6	*Sarocladium* sp.

Table 5. Morphologically identified endophytic fungal species isolated from mangrove plants

Plant Species	Isolated Endophytic Fungi
Avicennia officinalis	*Fusarium* sp., *Penicillium* sp.
Bruguiera cylindrica	*Fusarium* sp., *Penicillium* sp., *Aspergillus* sp.
Aegiceras corniculatum	*Alternaria* sp., *Penicillium* sp., *Fusarium* sp., *Sarocladium* sp.
Rhizophora apiculata	*Alternaria* sp., *Fusarium* sp., *Penicillium* sp., *Aspergillus* sp.
Excoecaria agallocha	*Sarocladium* sp., *Fusarium* sp., *Alternaria* sp.
Kandelia candel	*Penicillium* sp., *Fusarium* sp.
Acanthus ilicifolius	*Fusarium* sp., *Penicillium* sp. *Aspergillus* sp.

From the surface sterilized bark samples collected from seven mangrove species, six endophytic fungi were identified. Among the six endophytic fungi, *Fusarium* sp. was found to be the most dominant fungi due to its presence in all seven plant species obtained. Second most dominant species was *Penicillium* which was isolated from all collected plant species except *Excoecaria agallocha*. *Aspergillus* sp. was obtained from *Bruguiera cylindrica* and *Rhizophora apiculata*. The genus *Alternaria* was obtained from *Aegiceras corniculatum*, *Rhizophora mucronata* and *Excoecaria agallocha*. *Sarocladium* sp. was found only in *Aegiceras corniculatum* and *Excoecaria agallocha*. Another different species of *Aspergillus* from previously mentioned was isolated from only *Acanthus ilicifolius*.

Though the endophytic population is highly diverse, it shows uniqueness depending upon the host plant it occupies. Mangroves being peculiar kind of plants growing in saline and moist environment, houses a wide variety of endophytic fungal population. Endophytes act as mutualists [23] and are present in almost all parts of the plant. The fungal diversity present in each plant part also varies. This study identifies the endophytic fungal population present in the bark samples of the mangrove plants abundantly seen along Northern Kerala.

4. Conclusion

The endophytic fungi create their own habitat and ecological niche inside the plant cells. They reside inside the plant cells without causing any effect on the plants. These fungi can interact positively and negatively with their environment. They have great potential in the drug and pharmaceutical industry as most of the fungal strains possess antimicrobial, antibacterial and anticancerous properties. Studies regarding endophytes have proved that they can decrease insect herbivory, enhance plant growth, increase drought resistance and disease resistance. Endophytic fungi are also recognized as a major source of novel bioactive compounds and secondary metabolites useful in biological control [24]. With regard to all these, endophytes prove that they have beneficial impacts on plants, environment and humans in different ways. They can be used to fight against harmful pathogenic organisms in the agricultural, pharmacological and drug development fields. In conclusion, the present study was conducted during summer season which may be the reason for less endophytic fungal species isolation. It is supported by Suryanarayanan et al. [19]. They stated that diversity richness of endophytic fungi would be higher during rainy months than summer. The present study will be strengthened by carrying out the analysis throughout the year in different seasons with molecular and phylogenitic analysis in near future.

Statement of Competing Interest

The authors have no competing interests.

References

[1] Sarma, V.V., Hyde, K.D. and Vittal, B.P.R. (2001). Frequency of occurrence of mangrove fungi from the east coast of India. Hydrobiologia, 455: 41-53.

[2] Vaiga, M. and Joseph, S. (2016). Identification of mangrove and mangrove associates in Kannur district of Kerala including their economic - ecological linkages. International Journal of Botany Studies, 1(5): 22-31

[3] FAO. (2007), Mangroves of Asia 1980-2005: Country Reports. Forest Resources Assessment Working, Paper No. 136. Rome.

[4] SFR, (2009), Forest Survey of India 2007, India State of Forest Report, Dehradun, pp. 27-31.

[5] Vidyasagaran, K. and Madhusoodanan, V. (2014). Distribution and plant diversity of mangroves in the west coast of Kerala, India. Journal of Biodiversity and Environmental Studies, 4(5): 38-45.

[6] Kattany, V. and Madhusoodanan, V.K. (2014). Distribution and plant diversity of mangroves in the west coast of Kerala, India. Journal of Biodiversity and Environmental Sciences, 4(5): 38-45.

[7] Khaleel, K.M. (2005). Study of the quantitative structure of true mangroves present in the Mangal forests of Tellicherry, Pappinissery and Kunhimangalam of Kannur district. The Indian Forester, 13(1):81-89.

[8] Radakrishnan, C., Gopi, K.C. and Palot, J. M. (2006). Mangrove and their faunal associates in Kerala, with special reference to northern Kerala. Records of Zoological Survey of India, Calcutta, Occasional paper No. 246:1-81.

[9] Baccon, C.W. and White, J.F. (2000). Microbial Endophytes, Marcel Dekker Inc., New York, N.Y

[10] Hyde, K.D. and Soytong, K. (2008). The fungal endophyte dilemma. Fungal Diversity, 33: 163-173.

[11] Aly, A.H., Abdessamad, D., Kjer, J. and Chaidir, C. (2010). Fungal endophytes from higher plants: a prolific source of phytochemicals and other bioactive natural products. Fungal Diversity, 41(1): 1-16.

[12] Jariwala, B. and Desai, B. (2018). Isolation and identification of endophytic fungi from various medicinal plants. BMR Microbiology, 4(1): 1-7.

[13] Gilna, V.V. and Khaleel, K.M. (2011). Diversity of fungi in mangrove ecosystem. Journal of Experimental Sciences, 2(2): 22-31.

[14] Arnold, A.E., Maynard, Z., Gilbert, G.S., Coley, P.D. and Kursar, T.S. (2000). Are tropical fungal endophytes hyperdiverse? Ecology Letters, 3(4): 267-274

[15] Mitchell, A.M., Strobel, G.A., Hess, W.M., and Vargas, P.N. (2008). *Muscodor crispans*, a novel endophyte from *Ananas ananassoides* in the Bolivian Amazon. Fungal Diversity, 31: 37-43.

[16] Amrita, A., Sindhu, P., Swetha, J., Vasanthi, N.S. and Kannan, K.P. (2012). Enumeration of endophytic fungi from medicinal plants and screening of extracellular enzymes. World Journal of Science and Technology, 2(2): 13-19.

[17] Anitha, D., Vijaya, T., Pragathi, D., Reddy, N.V., Mouli, K.C., Venkateswarulu, N. and Bhargav, D.S. (2013). Isolation and characterization of endophytic fungi from endemic medicinal plants of Tirumala Hills. International Journal of Life Sciences, Biotechnology and Pharma Research, 2(3): 367-373.

[18] Seena, S. and Sridhar, K.R. (2004). Endophytic fungal diversity of 2 sand dune wild legumes from the southwest coast of India. Canadian Journal of Microbiology, 50(12): 1015-1021.

[19] Suryanarayanan, T.S., Kumaresan, V. and Johnson, J.A. (1998). Foliar fungal endophytes from two species of the mangrove *Rhizophora*. Canadian Journal of Microbiology, 44: 1003-1006.

[20] Alsohaili, S.A. and Bani-Hasan, B.M. (2018). Morphological and Molecular Identification of Fungi Isolated from Different Environmental Sources in the Northern Eastern Desert of Jordan. Jordan Journal of Biological Sciences, 11(3): 329-337.

[21] Gaddeyya, G., Niharika, P.S., Bharathi, P. and Kumar, P.K.R. (2012). Isolation and identification of soil mycoflora in different crop fields at Salur Mandal. Adv. Appl. Sci. Res., 3:2020-2026.

[22] Rodrigues, P., Soares, C., Kozakiewicz, Z., Paterson, R.R.M., Lima, N. and Venâncio, A. (2007). Identification and characterization of *Aspergillus flavus* and aflatoxins. Communicating Current Research and Educational Topics and Trends in Applied Microbiology, Mendez-Vilaz, A. (ed)., Formatex, Badajoz, 527-534.

[23] Carroll, G. (1988). Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. Ecology, 69: 2-9.

[24] Strobel, G.A. (2003). Endophytes as sources of bioactive products. Microbes and Infection, 5:535-544.