ECOLOGICAL AND ENDOPHYTE DIVERSITY STUDIES ON SELECTED MANGROVE PLANT SPECIES AT CHORAO ISLAND, GOA

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DECLARATION

I, Apurva Satyavan Sawant hereby declare that this thesis represents work which has been carried out by me and that it has not been submitted, either in part or full, to any other University or Institution for the award of any research degree.

Place: Taleigao Plateau. Apurva Satyavan Sawant

Date :

CERTIFICATE

I hereby certify that the above Declaration of the candidate, Apurva Satyavan Sawant is true and the work was carried out under my supervision.

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Chapter 1: Introduction

1.1: Mangrove ecosystem

India is one of the 17 mega-diverse countries in the world, having ten biodiversity regions. Significant ecological habitats include forests, wetlands, deserts, grasslands, and coastal marine ecosystems. Approximately 25% of the world's coastline is dominated by mangroves, distributed in 118 countries and territories encompassing an area of 181,000 km^2 (Spalding *et al*., 1997). Of this, 90% of mangroves are distributed in South-East Asia, America, and Africa. Over 59 mangroves have been recorded worldwide, of which 45 species are found in India. Of these, 12 species are recorded in the saline waters of Goa. These species, along with their expanded roots from all sides, adapt to different tidal fluctuations and constant changes in temperature. They mainly consist of high organic content deposition, which helps protect the coast from high wave action (Xing *et al*., 2011). Goa is the smallest state in India, covering 3702 km^2 area drained by seven major rivers. The Mandovi and Zuari with the Cumbarjua Canal form the largest estuarine complex. Along the entire coast, there exists an intricate network of creeks and backwaters. A luxuriant growth of mangroves (some of which are degraded) and associated swamps can be observed along most of the water bodies within the estuarine reaches. The total area covered by the estuaries in Goa, including the significant Mandovi Zuari estuarine complex, is approximately 12,000 ha, of which the mangrove forest occupies 2000 ha. The constant influx of fresh and seawater makes the ecosystem unique, adapting mangroves to grow in various environmental niches.

The mangrove plant species grow under varying climatic conditions throughout the tropics (Dodd *et al*., 1995). It is one of the most productive ecosystems, consisting of macro- and micro-habitat. It is an integral part of the food web, providing a micro-community for species reproduction. The ecosystem supports diversity and protects coastal flora (Kathiresan and Bingham, 2001). The prop-roots and pneumatophores of mangrove trees extend into the inter-tidal and sub-tidal, where they become a rare feature with hard substrata in soft sediment (Ellison and Farnsworth, 1992), which provide shelter for most of the living organisms. The salinity may vary from place to place, impacting the degree to which species can survive and multiply (Ellison and Farnsworth, 2001).

1.2: Mangrove adaptation:

Mangrove vegetation depends on species tolerance against various tidal variations, pH gradient, and salinity levels (Din *et al*., 2002). In some species, high salinity can be a limiting factor for species survival (Kathiresan and Rajendran, 2005). Hence, a homologous patch of species is noticed in a few mangroves. Since the mangrove vegetation regenerates in a saline environment, many workers have attempted to compare salinity as one of the parameters to correlate it with the standing biomass (Chen and Twilley, 1999, Ukpong, 1991).

Along with salinity, pH is also one of the factors responsible for the growth of plant species, basically due to the changes in primary and secondary essential elements (Slattery *et al*., 1999). High tide brings in salt water, and when the tide recedes, solar evaporation of the seawater in the soil leads to a further increase in salinity. The return of waves can flush out these soils, bringing them back to salinity levels comparable to that of seawater (https://en.wikipedia.org/wiki/Mangrove). Thus, for a species to survive in such broad changes, it must tolerate constant moisture, temperature, and salinity changes. Salt-tolerant forest ecosystems are a dynamic ecotone between terrestrial and marine habitats and have significant ecological, economic, and social significance (Gopal and Chauhan, 2006).

1.3: Mangrove colonizers

Mangroves, along with their associated flora, adapt to periodic fluctuations in various biotic and abiotic factors, harbouring groups of secondary metabolites as a mechanism for adaptation. These adaptations can result from several plant colonizers that directly serve in the growth of the plant species (Holguin *et al*., 2006). Besides plants, the ecosystem comprises groups of fungal species crucial in transporting mineral nutrients, decomposition, and recycling organic matter. It is estimated that the ecosystem hosts millions of such important fungal species, some of which are still not identified. There are several studies on microorganisms that act as plant colonizers, one of them being the endophytic fungi (Thatoi *et al*., 2013). Fossil records suggest that endophytes colonized plants over 400 million years ago (Krings *et al*., 2007) and played an essential role in shaping plant evolution on earth (Rodriguez *et al*., 2009). Aerial parts in mangroves are exposed to salt spray, and these fungi attain resistance to salt stress. However, factors such as substrate diversity, salinity, and periodic inundation contribute to the variety of fungi in mangroves, and daily changes in water level due to tides provide further niche differentiation (Kohlmeyer and Kohlmeyer, 1979).

1.4: Diversity of Endophytic fungi

Fungi are vital components of tropical ecosystems; their diversity depends on the host habitat and particular ecosystem. They can be categorized into epiphytic fungi, endophytic fungi, and litter fungi. Each category plays a significant role in the ecosystem (Jeewon *et al*., 2003; Kodsueb *et al*., 2008; Selvi *et al*., 2014). Endophytes are microorganisms associated with a wide variety of plant species and can invade interior plant tissues without causing any adverse effect on the parent body (Xiang and Liang-Dong, 2012).

The term "endophytes" was coined by German botanist Heinrich Friedrich Link in 1809 to define the occurrence of bacteria and fungi inside plant tissue (Pablo *et al*., 2015). Endophytes hence can be defined as microorganisms that act as micro-endosymbionts within the plant biota, colonizing healthy parts without causing any adverse effect on the host. The colonization may be intra- or inter-cellular, depending on the organism colonizing the plants (Kaul *et al*., 2016). The term endophyte was derived from the Greek word endon: within and phyte: plant (Khiralla *et al*., 2017). De Bary in 1966 first reported their occurrence within the plant biota as non-symptomatic and non-pathogenic.

It is estimated that every plant species is being colonized by at least two endophytes (Wang *et al*., 2014). Over one hundred thousand fungal species have been reported, and their numbers keep increasing (Clay and Holah, 1999). Endophytes are unique symbiotic microorganisms inhabiting different parts of the plant. The research on endophytes paved attention in recent years due to its application in various fields of biology. The study might help in understanding the evolutionary history and ecological aspects of plant communities (Saikkonen, 2007).

1.5: Diversity and colonization of endophytic fungi within-host

Endophytes colonize the internal parts of the plant without causing any adverse effects on the host plant (Koide *et al*., 2005). They are reported to be present in almost all parts of the plant. The number of endophytes recovered from leaf tissue has been shown to increase with the age of leaves in several hosts, including Douglas-fir (Stone, 1987), coastal redwood (Espinosa-Garcia and Langenheim, 1990), *Trachycarpus fortunei* (Taylor *et al*., 1999) and *Azadirachta indica* (Rajagopal and Suryanarayanan, 2000). The increased density of colonization of older leaves is due to repeated reinfection of the blade over time, probably from airborne inoculum (Rodrigues *et al*., 1993). Due to changing environmental conditions in the estuarine ecosystem, plants develop adaptive mechanisms to acclimatize. Hence, endophytes must continuously modify their secondary metabolites to penetrate the host plant through its chemotactic process.

To overcome such responses, endophytes secrete certain detoxifying enzymes like cellulase, lactase, and xylanase, which match the plant's enzymes to attain colonization. After penetration in the parent host, it is assumed that endophytes undergo three different life stages: neutralism /quiescent stage (at this stage, endophytes experience a latent stage which may be even for a lifetime), mutualistic stage (at this stage, the host and the endophyte share mutual benefits from each other without any harm), and the antagonistic stage (Min *et al*., 2016).

Several studies on the endophytic community have confirmed their presence in almost all the biomes, such as tundra, deserts, marine substrata, and tropical rainforests (Liu *et al*., 2010; Jeewon *et al*., 2003; 2017; Doilom *et al*., 2017), liverworts, hornworts, mosses, lycophytes, equisetopsids, ferns, and seed plants (Bacon and White, 2000). Their colonization depends upon several factors, such as geography, environment, age of the host plant, and type of tissue (Gange *et al*., 2007; Selosse and Schardl, 2007).

1.6: Production of secondary metabolites by endophyte and its role in the host plant

Mangrove endophytic fungi are increasingly recognized for the production of bioactive compounds. Endophytes as endo-symbionts showed a remarkable role in the production of secondary metabolites in the plant, having a potent role in the host species. Bioactive compounds isolated have been found to possess anti-cancer, anti-diabetic, and many other properties that are useful in biomedical research and drug development (Lu *et al*., 2010; Changi, 2015).

They are chemical synthesizers within plants as they are supposed to be used by host plant species for chemical defense against various stresses (Wang *et al*., 2008). It is estimated that almost all the plant species on earth are being colonized by endophytic bacteria/fungi. Ecological niches like mangroves deserve exploration as they are bound to have novel microbial species and biotypes, particularly endophytes, which are relatively less explored (Elsa and Bhima, 2012). The metabolites detected from endophytes showed a clear difference from those of the host, having diverse and unique structural characteristics (Khare *et al*., 2018).

Because of the global drug resistance problem, the need to discover novel drug sources, particularly antibiotics, cannot be over-emphasized (Costelloe *et al*., 2010). Fungal endophytes have yielded alkaloids, terpenoids, quinines, isocoumarin derivatives, flavonoids, phenols, peptides, and phenolic acids. They are a prolific source of novel antibiotics, anti-cancer, antiviral, antioxidant, insecticide, anti-diabetic, and immunosuppressant compounds (Mayank *et al*., 2015). The pharmacological repertoire of endophytic fungi comprises diverse bioactive

compounds such as antimycotic steroid 22-triene-3b-ol, anticancer cajanol, podophyllotoxin, and kaempferol, anti-inflammatory ergo flavin, antioxidant lectin, insecticidal heptelidic acid, immune-suppressive sydoxanthone A, B and cytotoxic radicicol (Sharma *et al*., 2016).

While residing and reproducing inside the healthy tissue in an intimate mutualistic manner, presumable gene recombination/or the precursor molecule interaction with the host indicates enhanced biosynthetic capabilities in the endophytes. This may be the reason why over 80% of the endophytes exhibit positive activity for antimicrobial and biological control (Li *et al*., 2005; Schulz *et al*., 2002). The involvement of active gene regulation molecules plays an essential role in producing secondary metabolites (Bok and Keller, 2004). Gene expression studies revealed that it is difficult to identify the exact pathway of secondary metabolite production by fungal endophytes due to the non-expression of the genes under laboratory conditions (Szewczyk *et al*., 2008).

The production of secondary metabolites by the fungal endophytes is a part of the adaptive mechanism wherein the metabolites produced by endophytes are exclusive to those of host plants. Novel metabolites isolated from endophytes indicated their active applications in medicine and agriculture (Aly *et al*., 2011).

It has been stated that the isolation and identification of endophytic mycobiota are crucial to the ethnobotanical profile of the plant since the medicinal properties of a plant can be due to the endophytes harboured within (Dastogeer *et al*., 2017). This was proved in the case of the anti-cancer drug taxol by isolating taxol-producing endophyte *Taxomyces andreanae* (Kusari *et al*., 2013), which had previously been thought to occur only in the genus *Taxus* (yew). Taxol has been demonstrated in many fungal endophytes, such as *Alternaria, Fusarium, Monochaetia, Pestalotia, Pestalotiopsis, Pithomyces,* and *Taxomyces* (Strobel *et al*., 1996).

The mutual symbiosis between the host and endophyte has benefited both living partners (Tan and Zou, 2001). Fungal endophytes are ubiquitous and do not show host specificity, whereas specific fungal endophytes frequently appear in a particular host, thus showing host preferences (Cannon and Simmons, 2002, Arnold, 2007). The research on endophytes is in focus due to the production of diverse groups of secondary metabolites of therapeutic importance. Approximately 140 novel potent drugs of natural origin were isolated from endophytic fungi between 1987 till 2000 (Tan and Zou, 2001).

1.7: Role of endophytes in host plant growth

Endophytic fungi have a potential role in benefiting the host plant, including growth enhancement and resistance to host species towards various biotic and abiotic stresses (Schulz and Boyle, 2005). Endophytic strains are reported to affect the germination rate (Hubbard *et al*., 2014) and have benefited plants in adapting to various environmental changes.

1.8: Role of endophytic fungi in phosphate (P) solubilization

Endophytic fungal micro-community showed their remarkable role in host-pathogen interactions. Adaptive mechanisms involve synthesizing diverse groups of chemically active metabolites, having beneficial roles such as P solubilization (Gimenez *et al*., 2007).

P is an essential macronutrient for biochemical and physiological processes in plants. The P solubilization method involves converting insoluble P into a soluble form, making them available to plants (Sahoo and Gupta, 2018). However, approximately 95-99% of P is available in the insoluble state in the soil (Singh *et al*., 2017) and hence is not available to the plants. This results in P deficiency, as seen in tropical soils. Recent reports suggest that many soil and plant symbionts like fungi, bacteria, and other soil microorganisms play a vital role in P solubilization (Gyaneshwar *et al*., 2002) and mobilization (Scervino *et al*., 2011). High P solubilization by fungi compared to bacteria occurs through the production of more acids reported in earlier studies (Vazquez *et al*., 2000). Fungi from the genus *Aspergillus* species are known to solubilize a high amount of tri-calcium phosphate (Gyaneshwar *et al*., 2002).

1.9: Ecological role of endophytic fungi in the nutrient recycling process

The degradation of plant material is accompanied by the action of certain microorganisms, like endophytic fungi, bacteria, litter-degrading organisms, *etc*., that exist within the plants and soil. After the senescence stage, the inner plant tissue containing endophytes switches from the oblique endophytic to the facultative saprophytic stage, which triggers nutrient recycling (Griffith, 1994). Despite knowing the importance of this micro-biota, the ecology of litter degraders has not been adequately studied (Muller *et al*., 2001).

Endophytes alter the chemical composition and thus enhance litter degradation (Andrews, 1991). It is known that Basidiomycetes and Ascomycetes fungi have high efficacy in litter degradation as they typically encode genes for laccase and cellobiohydrolase (Yuan and Chen, 2014). Few Xylariaceous endophytes possess the ability to decompose lignin and cellulose from litter (Koide *et al*., 2005).

Endophytes possess a potential activity in the field of biology and deserve exploration and detailed study. The present study was carried out to understand mangroves and their associated endophytic fungi with the following objectives.

1. To study the diversity of mangrove plant species from the selected study site.
2. To study the fungal endophytes from the selected study site.
3. To study the associative role of endophytic fungi in selected mangrove species.

Chapter 2: Review of Literature

2.1: Mangrove ecosystem and its distribution

Mangroves are forest ecotones between the land and the Sea (Kathiresan and Bingham, 2001), protecting land from natural calamities, land erosion, and high wave action (Furkawa and Wolanski, 1996). Mangroves provide a habitat for various aquatic and avian floras, helping maintain the food web. Additionally, they help in nutrient recycling and carbon (C) sequestration and stabilize coastal regimes (Kathiresan, 2008). Aquatic organisms such as fishes, crabs, birds, reptiles, and prawns are critically dependent on the mangrove ecosystem for survival.

Fig.1: World map showing the distribution of mangroves.
(Source: Florida museum; image: jim mcmahon ® mapman)

Worldwide, mangroves cover an area of approximately 15.2 million hectares of coast distributed over 118 countries. The most dominant patch of mangroves is seen in Asia, followed by Africa and North and Central America (FAO, 2007). One hundred twenty-five mangrove species have been recorded in India (Singh *et al*., 2012). Of these, 16 species are recorded in Goa, out of which 12 (true and mangrove associates) species are found at Chorao Island (Walke *et al*., 2018).

Fig. 2: Mangrove distribution in India.
(Source: MS Swaminathan Research Foundation)

2.2: Historical aspects of the mangrove ecosystem

Chapman (1975) termed mangroves as those plants which grew in the intertidal forests and referred to them as 'mangal.' The term 'mangal' was used by the Portuguese and French to refer to a particular forest community and also to an individual plant (Kathiresan and Bingham, 2001).

The mangrove species can be classified as tropical trees or shrubs found along the coastal regions with expanded roots above the ground since 1613 (http://www.niobioinformatics.in/mangroves/MANGCD/what.htm). Such halophytic species are typically known to withstand high salinity levels and low soil aeration forming a dense homologous or heterogeneous patch of mangroves in the intertidal zones (Ruth and Catherine, 2015). Based on their occurrence, habitat, and abundance, Tomlinson (1986) categorized these halophytic plant communities into major, minor, and associate mangroves.

Major mangroves or true mangroves: Comprise of mangrove species that have complete fidelity to a saline environment.

Minor mangroves: Species that prefer mangrove habitats along with the peripheral area of the saline ecosystem.

Associate mangroves: Herbaceous plants sub-woody or climbers growing in the peripheral regions of the mangrove habitat.

Important characteristics of true mangroves include those species growing at different salinity levels and not extending or growing in the terrestrial regimes. Species with aerial roots show the viviparous nature of seeds and plants with unique salt exclusion properties (https://www.floridamuseum.ufl.edu/southflorida/habitats/mangroves/adaptations/).

2.3: Adaptive mechanisms in mangrove species

The most important mangrove adaptation is the aerial root system that helps exchange gases (Shrikanth *et al*., 2016). Species like *Rhizophora* grow by a stilt root system that forms the trunk and lower branches which serve in the adaptation of the species (http://www.mangrove.at/rhizophora-mangle_red-mangrove.html). In the case of *Sonneratia* and *Avicennia* root extends from the ground in the form of a subterranean system extending along the parent plant body (Purnobasuki, 2013). Whereas in *Bruguiera* and *Ceriops* formation of knee roots with pneumatophores for aeration is an adaptive mechanism (Shrikanth *et al*., 2016).

Mangrove plant species have developed several adaptive mechanisms to grow and perpetuate under high salinity conditions. Plant species of *Rhizophora*, *Sonneratia*, *Acanthus*, *Aegiceras*, *Avicennia,* and *Excoecaria* have adapted by synthesizing salt excretory glands (Dassanayake and Larkin, 2017). The accumulation of salt glands in the leaf tissue and then shedding of the leaves are noted in species like *Excoecaria agalocha* and *Bruiguiera cylindrica* (Suarez and Medina, 2008; Yuan *et al*., 2016).

Other mechanisms towards adaptation include a character like viviparous seed germination, wherein the seed germinates directly on the parent body and gets detached only at the seedling stage (Yong *et al*., 2004). The seedling acts as a propagule that separates from the parent and grows as a single seedling. This phenomenon is mostly noted in families like Rhizophoraceae, Aviceniaceae, and Primulaceae (Friedhelm and Sabine, 2006). The vivipary in the above families can be differentiated into two types:

1. **Vivipary:** In this type, the embryo has no dormancy period. The seed grows first to break the seed coat and then cut the fruit wall while not detaching from the parent body (Joshi *et al*., 1972).
2. **Cryptovivipary:** In this type, species emerge from the seed without cutting the fruit (Bobda *et al*., 2014).

Mangroves adapt to different salinity levels by osmotic adjustment (Liang *et al*., 2008). In this mechanism, the plant synthesizes low molecular mass compounds which do not interfere with the normal biochemical processes (Khare *et al*., 2018). They produce enzymes like proline by P5C5 gene (Guan *et al*., 2018), Ferritin 1 by Fer 1 gene (Jithesh *et al*., 2006), High-affinity K^+ transporter by the HKT gene (Gupta and Huang, 2014), which are part of acclimatization. Salt overly sensitive activation by the SOS gene (Very and Sentenac, 2003) is also another mechanism for salt adaptations.

2.4: Mangrove diversity along the habitat

Avicennia marina grows in different types of soil, pH, and salinity gradients due to aerenchyma to maintain its root ventilation (Naidoo *et al*., 1997) and grows abundantly in almost all parts of India (Ragavan *et al*., 2016). Another species *Avicennia officinalis* is less diverse, probably owing to lesser tolerance toward floods and salt stress (Satyanarayana *et al*., 2009).

Acanthus ilicifolius, a highly diverse associative mangrove species, is growing at the shoreline and the edges of creeks. It is reported to be highly useful in traditional medicine to cure arthritis and other skin inflammations (Singh and Aeri, 2013).

2.5: Importance of mangroves

Mangroves are the most important productive ecosystem that provides services to all coastal, terrestrial, and avian biota (Spalding *et al*., 2010). Goa, part of the coastal belt, depends on the mangroves for wood, medicine, honey, and fish. Products from mangroves are used for leatherwork and dyeing fish nets (http://www.fao.org/forestry/mangrove/3649/en/). Mangrove areas are used for the aquaculture of crabs, fishes, prawns, *etc*. Mangrove timber is used extensively as firewood for the construction of houses and manufacturing paper (Kusmanaa and Sukristijionob, 2016). Mangrove foliage is used as fodder for cattle (Sathe *et al*., 2015). Local practitioners use the various parts of mangrove plant species to cure various human ailments (Mondal *et al*., 2016).

Blankespoor *et al*. (2017) reported that mangroves control soil erosion and protect against cyclones and tsunamis entering the land. Mangroves protect and conserve wildlife by providing a microhabitat, bird nesting sites, and breeding sites for fish, snakes, and crocodiles (Jayson, 2001).

Mangrove species require high rates of photosynthesis for their growth and development. Alongi *et al*. (2005) reported the diverse assemblage of microbiota involved in mediating

CO_2, CH_4, N_2, and N_2O gases triggering mangroves in adaptation. In the process, various soil microbes act as a driving force in acclimatization. Hence interactions among plants and microorganisms play a crucial role.

2.6: Endophytic community within plant biota

The ecosystem comprises diverse groups of microbial communities that invade different plant parts worldwide (Haruna *et al*., 2018). Entire plant communities surveyed to date harbour groups of fungi that reside inside the plant communities (Arnold and Lutzoni, 2007). They are often reported to have the potential to shift within the plant and act as biotrophic, mutualistic organisms inside the host (Mega *et al*., 2010). However, studies have proved that the influence of these symbionts has a positive role against herbivory, abiotic stress, *etc*. (Arnold *et al*., 2003). These endophytes are known to reside as asymptomatic partners in the host, as commensals (Dodd, 1980), and as per evolutionary history, may have evolved due to the loss of traits that trigger pathogenicity (Saunders and Kohn, 2009).

Individual plants can harbour hundreds of fungal endophytes, potentially benefiting the host species (Schulz and Boyle, 2005). The diversity of endophytes is dependent on host traits that mediate colonization, although the exact mechanism is still unknown (Saunders and Kohn, 2009). Plant trigger traits and tissue lignifications greatly influence endophytic fungal diversity within the host species (VanEtten *et al*., 2001).

2.7: Evolutionary aspects of fungal endophytes

The fossil evidence suggests the presence of endophytes within the host plant over 400 million years ago (Khiralla *et al*., 2017). The plant and endophytes are said to be co-evolved from gymnosperms and angiosperms around 300 million years ago (Schneider *et al*., 2004). The prominent existence of endophytes (Diaporthales and Helotiales) showed their co-evolutionary similarities within angiosperms and Gymnosperms (Zhou *et al*., 2022). The literature revealed the influence of environmental conditions as a part of evolution, where the genetic recombination of both symbiotic partners has a major role. A similar phenomenon was noted in *Neotyphodium* species and its host that showed novel genetic recombination triggered due to environmental factors leading to the evolution of both symbionts (Moricca and Ragazzi, 2008).

Conn *et al*. (2008) reported the first detection of endophyte as a pathogen that, over a period of time, resulted in the shift from pathogenic to the mutual symbiont, forming a positive host-endophyte association. Besides, Kusari *et al*. (2011) reported endophytes to attain

resistance by altering the amino acid in the catalytic domain of topoisomerase I. Endophyte site-specificity among the diverse hosts results from selective specificity among the endophytic species (Jin *et al*., 2013), which can significantly influence the host phylogeny.

2.8: Classification, colonization, and transmission of endophytic fungi

Endophytes can be classified into two major groups, clavicipitaceous and non-clavicipitaceous endophytes, colonizing various host plants. The occurrence of clavicipitaceous endophytes was seen in grasses, whereas non-clavicipitaceous endophytes colonize non-vascular and vascular plants such as angiosperms and conifers (Rodriguez *et al*., 2009; Bamisile *et al*., 2018).

In later years, the endophytes were categorized into four different classes: class 1, 2, 3, and 4. Class 1, 2, and 3 comprise clavicipitaceous endophytes, and class 4 includes non-clavicipitaceous endophytes. Class 1 and 2 endophytes colonize roots, shoots, and leaves, whereas class 3 endophytes colonize only stem tissue (aboveground parts), and class 4 endophytes colonize root parts (Rodriguez *et al*., 2009).

Recently endophytes have been classified into two broad categories; Systemic and non-systemic (transient) endophytes, based on their transmission (Wani *et al*., 2015). The systemic endophytes usually occur as a mutualistic association without any host defense response. Endophytes in a systematic group lack visible symptoms to maintain the plant's defense mechanism to regulate host endophyte association without any symptoms (Schulz and Boyle, 2006). Whereas in non-systemic endophytes, the fungus alters from a mutualistic to a parasitic stage depending on the environmental attributes. However, it provides complete virulence to the host in defense mechanisms.

The symbiotic relationship between a systemic group of fungi and its host has triggered both partners to rely on each other. The study indicates that few endophytes lose vitality once grown without their host. However, the non-systematic group of endophytes can either act as pathogenic or parasitic depending upon stressed conditions, resulting in cell death. As suggested, these endophytes might have evolved from pathogens (Hiruma *et al*., 2018).

Systemic endophytes get transmitted to another generation *via* vertical and horizontal transmission, whereas non-systemic endophytes get transmitted to the next generation only through horizontal transmission. In the case of vertical transmission, the associated endophytic genotype transmits through the seed. Thus, at each plant reproductive stage, the same fungal genotype is distributed in seeds. Khan *et al.* (2010) reported that the vertical

transmission of endophytes (seed-borne) is a potential product accompanied by different enzymes, phytohormones, antimicrobial compounds, and other secondary metabolites that can improve plants in various biotic and abiotic stresses.

Endophytic interaction exhibits mutualism or antagonism that depends totally on the environmental conditions. These interactions are mainly based on chemical signals within the host that trigger colonization (Schulz and Boyle, 2006). The most important host-endophyte interaction is to form a biotrophic lifestyle of endophytes with a host, which is an important feature for colonization and host-endophyte interaction (Wani *et al*., 2015).

Schardl *et al*. (2014) reported the fungus *Epichloe* to show vertical transmission, *i.e*., *via* hyphae growing within seeds or sexually *via* the production of stromata. Hence it becomes important for fungus to continuously modify and alter genetic combinations whenever the plant undergoes outcrossing. Genetic mismatches result in loss of infection.

2.9: Distribution and application of fungal endophytes in various host species

The distribution of fungal endophytes within plants varies per region and climatic change. As reported by Chareprasert *et al*. (2006), the assemblage of endophytes was found more in mature leaves of *Tectona grandis* L. and *Samanea saman* Merr. isolated during the rainy Season. Their distribution mainly depends on endophytic host specificity that differs within a season (Thongsandee *et al*., 2012).

In some endophytic strains, host specificity was noticed, though a host might be colonized by different endophytic species harbour within (Bamisile *et al*., 2018). Literature indicates the high degree of host specificity of fungal endophytes while colonizing the host, which has systemic applications in plant growth and development (Skipp and Christensen, 1989). On the other hand, plants growing at different geographical locations with different environmental conditions contain different endophytes. The roots of host plants in the same environment comprise diverse groups of endophytes because of the involvement of different molecular signals (Lebeis, 2014). However, altitude and site greatly influence diversity within the endophytic population (Philippe, 2016).

Endophytic fungus, upon colonization, provides various beneficial roles to the host plant. The role of each endophyte in a particular host varies per species (Qawasmeh *et al*., 2012). Few endophytic strains protect host species against different stresses, while another application involves the increased uptake of essential chemical elements for the growth and development of host species (Nair and Padmavathy, 2014).

2.10: Secondary metabolites production by endophytes

Secondary metabolites produced by endophytes are of great application in the defense mechanism of the host plant. These compounds are diverse groups of chemically active metabolites that may act as toxins, enzymes to regulate various functions, volatile organic compounds, phytohormones, *etc*. They persist in plant parts that stimulate root growth and inhibition against pathogens to provide tolerance against various stress (Gallardo-Cerda *et al*., 2018). Host endophyte recognition triggers signal transduction as a response to changing the metabolic state of the host (Joseph and Priya, 2011).

These metabolites can be classified as alkaloids, steroids, saponins, tannins, *etc*., with various bioactive potentials (Mujeeb *et al*., 2014). However, in the tissues of the higher plants, the endophytes produce active metabolites similar to plants (Gouda *et al*., 2016). Shridhar (2004) reported mangrove endophytes as key components of novel metabolites, forming the second-largest ecological group of marine fungi. These microbes adapt to extreme conditions that, make the fungus a novel source of drug discovery (Shukla *et al*., 2014). Selim *et al*. (2012) reported the biological potential of endophytes in pharmaceutical, agriculture, and environmental areas. Discoveries concerning metabolites have received much attention from researchers because endophytes have a shorter life cycle than host plants.

Some endophyte strains are an excellent source of metabolites that can improve nutrient acquisition and plant growth-promoting activities (Kaul *et al*., 2016). Endophytes help suppress the phytopathogens *via* the production of antagonistic activities within the host by inducing ISR against pathogens (Gunatilaka, 2006). Endophytes play an important role in environmental stress conditions. As reported by Khare *et al*. (2016), endophytic fungi help in the activation of salicylic and jasmonic acid during plant stress against phytopathogens. Kavroulakis *et al*. (2007) reported *Fusarium solani* (endophyte in tomato plant) induces pathogenesis-related genes in the root system against *Septoria lycopersici* pathogen.

Literature reveals the adaptative mechanism in plants is a result of phenotypic plasticity, which is the capacity of an individual genotype to produce and modify behaviour, morphology, and physiology in different environmental conditions (Price *et al*., 2003). Thus, individual genotype helps maintain function and further enhances reproductive ability under different environmental niches (Sultan, 1995). In this aspect, the role of endophytes is assumed to be crucial as endophytes could greatly influence host morphology and physiology (Faeth and Sullivan, 2003).

Pirttila *et al*. (2008) reported the application of endophytes in the browning of the leaf and the release of tannins in the tissues. The significance of such colonization within the host involves primary and secondary metabolomics (Rasmussen *et al*., 2008). These metabolites play specific interactions and communications within the host (Brader *et al*., 2014). Endophytic fungi produce a similar secondary compound that increases the host survival rate by sensing the chemical signals and pathogenesis-related genes (Howitz and Sinclair, 2008).

As noted in many grass species, the symbiotic association has helped land-dwelling plants successfully grow and multiply. Genus *Epichloe*, a grass-specific endophyte, produces a dense aerial hyphal network that transmits through the vertical mode of transmission, which helps to maintain the healthy growth of grasses. Endophyte produces alkaloids that are lethal to insects, herbivores, and nematodes, thereby increasing the host's healthy growth, seed production, and nutrient uptake (Shukla *et al*., 2015).

Endophytes are the precursor organisms involved in producing volatile organic compounds that get activated once herbivores attack the plant. König *et al*. (2018) reported that endophytes synthesize a specific scent to attract as a signal for predatory insects, which phenomenally control aphid (hoverfly) attacks in plants. This directly affects the seed number, weight, and offspring fitness which might vary depending on the host genotype (Gundel *et al*., 2012).

Endophytic colonization has triggered plants that grow in high-heat climatic conditions, although the plant has its mechanisms to grow against such stresses. The involvement of endophytic associations has additional resistance to the host (Khan *et al*., 2016). Likewise, species of *Thermomyces* are involved in habitat adaptation by inducing ubiquitin degradation, histone acetylation/deacetylation, and poly ADP-ribosylation pathways (Mchunu *et al*., 2013). In high heat-sensitive areas, the plants like *Cucumis sativus* colonization have enhanced photosynthetic rates, increased root length, and high metabolic activity (Sachdev *et al*., 2021). Some species of endophytes overlap in their distribution in the host plant, which could influence the host to adapt to biotic and abiotic factors (Jumpponen and Jones, 2009). Other factors responsible for endophyte distribution can result from natural selection, wherein the most promising symbionts will be selected, and others will get eliminated due to natural selection (Rasmussen *et al*., 2009).

2.11: Diversity and characterization of endophytic fungi within host species

Isabella *et al.* (2012) examined the occurrence of 25 species of endophytes within the leaves of mangrove plants from Northern Brazil. They reported *Guignardia* sp. and *Colletotrichum* sp. to be dominant among the endophyte assemblage. Rajamani *et al*. (2018) recorded culturable fungal endophytes (*Phomopsis*, *Xylaria*, and *Colletotrichum*) from 20 mangrove hosts of South Andaman Island. Wei-Chiung *et al*. (2019) reported 203 isolates from surface-sterilized leaves representing 47 different genera.

Jia *et al*. (2016) reported the presence of *Phomopsis* and *Pestalopsis* in the leaves of mangroves from Southern China by using the molecular sequence method. Norphanphoun *et al*. (2019) investigated 12 novel species of endophytes from mangroves based on ITS, beta-tubulin, and translation elongation factor 10 alpha coupled with morphological keys.

Yao *et al*. (2019) conducted a study revealing the relationship between plants and fungi, encountering a total of 635 fungal isolates, which were characterized by Illumina Miseq sequencing of ITS2 sequence.

2.12: Associative role of endophytes with mangrove plant species

a. Role of endophytic fungi in litter degradation

Some endophytic fungi are known as pioneers of degradation. Within the endophytic micro-community, it is revealed that ascomycetes contain genes that encode for laccase and cellobiohydrolase, responsible for degradation. DNA and RNA detection methods also indicate that litter degradation is accompanied by the rapid action of microorganisms associated with litter (Yuan and Chen, 2014) by the production of extracellular enzymes responsible for degradation. However, endophytic fungi (*Pestalotiopsis* sp. and *Glomerella* sp.) are known to utilize most of the substrate present on the host cell wall (*R. apiculata* leaf), particularly laccase (lignin-modifying enzyme) for complete leaf degradation (Kumaresan and Suryanarayanan, 2002).

b. Role of endophytic fungi in petroleum hydrocarbon degradation

Petroleum is a viscous mixture of thousands of hydrocarbons, mainly carbon and hydrogen (Mehdi and Simone, 2013). Prolonged accumulation of these hydrocarbons adds pollutants and may cause mutation or even death in the plant system (Das and Chandran, 2011). Biodegradation is a complex process that depends upon bacteria, yeast, and fungi, which provides a basis for the degradation of hydrocarbons. Fungi like *Aspergillus*, *Cladosporium*, *Corollasporium*, *Fusarium*, and *Penicillium* are reported to possess a beneficiary role in the

degradation of hydrocarbons, which is mediated by the action of certain enzymes system like oxygenases and hydroxylases (Deshmukh *et al*., 2016).

Filamentous fungi, as per reports, play a major role in hydrocarbon degradation because of their fast growth and extensive hyphal network (Olicon-Hernandez *et al*., 2017). Polyaromatic hydrocarbons (PAHs) are reported as ubiquitous xenobiotic environmental pollutants (Bisht *et al*., 2015). Hence a diverse group of fungi such as Zygomycetes (*Cunninghamella elegans*), Ascomycetes (*Aspergillus niger* and *Penicillium* sp.), and white-rot Basidiomycetes (*Trametes versicolor, Pleurotus ostreatus*) are known to oxidize and degrade PAH's (Olicon-Hernandez *et al*., 2017).

The analysis revealed the oil degradation capability of endophytic fungi through the production of extracellular enzymes by liberating a higher amount of carbon dioxide. Marin *et al*. (2018) reported the potential role of *Verticillium* and *Xylaria* species in degrading petroleum hydrocarbon from the tropical ecosystem.

c. Role of endophytic fungi in phosphate solubilization

Endophytic fungi reside as asymptomatic living partners within the host, providing various beneficial functions to plants. Phosphate solubilization is one of the major mechanisms where endophytic microorganisms help the host to solubilize the insoluble P. Renata *et al*. (2018) illustrated the presence of 115 fungal isolates in three mangrove species *viz*., *Rhizophora mangle, Laguncularia racemosa,* and *Avicennia nitida*. The isolates reported had the ability to fix N and solubilize P, that induced plant growth. Adhikari *et al*., (2018), revealed the role of potential endophytic fungi in solubilizing insoluble phosphates in the presence of tricalcium, aluminium, and iron phosphate at different temperatures through the production of phosphatases, phytases, and organic acids.

Gupta and Das (2008) isolated 106 fungi from the rhizosphere and phyllosphere of mangrove plants. Testing the fungal isolates on a solid Pikovaskaya medium plate indicated the potentially positive role of endophytes in solubilizing phosphate under different cultural conditions. A total of 36 fungi out of 106 showed a halo zone when grown under different pH and temperature. The highest zone was formed by *Aspergillus* PF8 (63 mm) and *Aspergillus* PF127 (46.5 mm), followed by *Paecilomyces, Cladobotrytis, Helminthosporium*.

2.13: Pharmacological studies of endophytic fungi

a. Anti-microbial property of endophytic fungi

Endophytic fungi have proven to be a novel source of secondary metabolites comprising diverse groups of chemical groups and bioactivity. The research indicated that endophytic fungi have a potential role in antimicrobial, antifungal, immunosuppressant, and anticancer activities (Strobel, 2002; Strobel and Daisy, 2003). Literature indicates mangroves harbour groups of endophytic fungi having diverse roles in the ecosystem and human healthcare. Handayani *et al*. (2017) isolated 12 endophytic fungi from the leaf, bark, and root of mangrove *Sonneratia grifithii* Kurz, collected from Bungus, West Sumatra.

Chi *et al*. (2019) reported the presence of 168 endophytic fungal associated within mangrove associate *Acanthus illicifolius*. A total of 28 culture extracts out of 168 showed positive antimicrobial properties against human pathogenic bacteria [*Bacillus subtilis*, *Staphylococcus aureus* (Gram-positive), and *Escherichia coli* (Gram-negative)] and fungi (*Candida albicans* and *Cryptococcus neoformans*).

Prihanto *et al*. (2011) isolated five endophytic fungi from *Rhizopora mucronata*, showing antimicrobial activity against *Staphylococcus aureus* ATCC 9144 and *Escherichia coli* ATCC 8739. The results indicated that out of five isolates, three showed positive inhibitory activity against *S. aureus* ATCC 9144, whereas two showed inhibitory activity against *E. coli* ATCC 8739. Thus, the results revealed the potency of endophytic isolates in treating diseases spread by foodborne pathogens.

Maria *et al*. (2005) studied the antimicrobial potency of 14 endophytic fungi isolated from *Acanthus ilicifolius* and *Acrostichum aureum* against bacteria (*Bacillus subtilis*, *Enterococcus* sp., *Klebsiella pneumoniae*, *Pseudomonas aerugionsa*, *Salmonella typhi* and *Staphylococcus aureus*) and fungi (*Candida albicans* and *Trichophyton metagrophytes*). The results indicated that sterile isolate MSI 1 showed high inhibitory activity against all the tested pathogenic bacteria. *Cumulospora marina* and *Pestalotiopsis* sp. revealed inhibitory activity against Gram-positive and Gram-negative bacteria. *Aspergillus* sp. 3 and *Pestalotiopsis* sp. showed inhibitory activity against bacteria and *Candida albicans*, while *Aspergillus* sp. 2 and MSI 1 showed inhibitory activity against pathogenic bacteria.

b. Anti-cancer properties of endophytic fungRRRi

Annually the number of cancer reports is increasing, a major cause of death worldwide. Hence there is a constant need to search for a drug of a natural origin of high potency. Natural

sources like endophytes are the key producers of chemically active metabolites of therapeutic importance. Therefore, there is a need to identify mangrove endophytes that will help to provide natural products with unique bioactivity for cancer therapy. Clinically endophytes proved to be an excellent source of anticancer drugs such as taxol, podophyllotoxin, camptothecin, and vinca alkaloids (Uzma *et al*., 2018).

In the current era, a tremendous demand exists for the discovery of potent medicines with minimal side effects, which could be an alternative to conventional medicines to control drug resistance issues (Kusari *et al*., 2009; Kusari *et al*., 2012). Zhou *et al*. (2022) reported *Pestalotiopsis* spp isolated from *Rhizophora mucronata* to have cytotoxicity against human cancer cell lines HeLa A549 and HepG. Hemphill *et al*. (2016) isolated endophytic fungi from the petiole of *Rhizophora harrisonii*, and reported synthesizing new compound pestalpolyol I. *Penicillium* sp. isolated from *Avicennia marina* exhibited anti-proliferative activity against Tca8113 and MG-63 (Deshmukh *et al*., 2018).

Chapter 3: To study the diversity of mangrove plant species from the selected study site

3.1: INTRODUCTION

The term mangroves refer to a heterogeneous group of halophytic woody plant species growing in tropical, sub-tropical, and temperate regions at various estuaries, tidal creeks, and marshy areas (Ragavan *et al*., 2016). In India, mangroves distributed along the coastal areas cover approximately an area of 4740 Km^2. Of these, 29% of the mangrove cover is seen growing along the Arabian Sea, 58% along the Bay of Bengal, and 13% of diverse species of plants in Andaman and Nicobar Island (Forest Survey of India, 2015). These mangrove plant species being salinity tolerant plants, activate higher affinity K^+ transporter and SOS gene as a mechanism towards adaptation (Gupta and Huang, 2014; Very and Sentenac, 2003). Depending upon the mangrove habitat (level of salinity tolerance), the ecosystem is categorized into two types, namely, true (exclusive mangrove) and associate (non-exclusive) mangroves (Giesen *et al*., 2007). The characteristic features of true or exclusive mangroves include aerial root formation, viviparous or crypto-viviparous nature of seed development, and mechanism of nutrient retention (Panda *et al*., 2017). Mangroves determine a large amount of C sequestration through their prop roots helping in nutrient recycling. They are an ecological niche that adapts to varying tidal fluctuations, salinity levels, and the influx of freshwater in the intertidal bodies (Alongi, 2008).

The genera *Rhizophora, Bruguiera,* and *Ceriops* belonging to the family Rhizophoraceae are known to withstand salt stress through a salt excludation system. In contrast, species belonging to genera *Acanthus*, *Aegiceras,* and *Avicennia* have salt-secretary glands on the leaf surface. The genera *Sonneratia*, *Xylocarpus*, and *Excoecaria* have salt accumulative properties to survive the changing salinity levels. Other mechanisms include the presence of different root adaptations to overcome salt stress. Species belonging to the genus *Bruguiera* comprise lenticels and knee roots. *Sonneratia* and *Avicennia* species have pneumatophores and cable roots, whereas stilt roots are noticed in *Rhizophora* (Panda *et al*., 2017).

Mangrove forests are considered the essential component of a wetland ecosystem that maintains the ecological balance of biodiversity. This marine ecosystem harbour groups of organisms that include animals, birds, and microorganisms and are known as an ecological hotspot for marine fungi (Rashid *et al*., 2008). They have several applications, including

food, fodder, medicine, and timber (Hendy *et al*., 2014). Traditionally, these plant species are used by local practitioners to cure various ailments (Vinoth *et al*., 2019). However, this knowledge is slowly depleted due to the non-availability of written documents. Hence, the knowledge of folklore medicine would help researchers identify novel compounds that could solve drug resistance problems (Ravindran *et al*., 2005). Despite knowing the ecological and economic significance, it is estimated that annually 0.16 to 0.39% of global mangroves are lost due to coastal development (Hamilton and Casey, 2016).

Goa is the smallest state situated along the southwestern coast of the Indian Peninsula, commonly known as 'Konkan'. The state is bounded by the Terekhol River (North), Karnataka state (South and East), and Arabian Sea (West), forming a reservoir of different flora and fauna across the state. A total of eleven rivers sustain Goa, *viz*., Terekhol, Mandovi, Baga, Zuari, Chapora, Saleri, Mandre, Harmal, Sal, Talpona, and Galjibag, that flows across the state. Nine of these 11 rivers flow from the Western Ghats to the Arabian Sea. Further, these flowing water bodies' get into intricate systems like wetlands, tidal marshes, agriculture fields, canals, lakes, *etc*. Mandovi and Zuari are the dominant rivers in the Goan Riverine landscape that drain an area of about 2553 sq. km. Among the two rivers, the Mandovi (also known as 'Mahadayi') flows through Karnataka State and into Goa (1,580 sq. km) *via* Sattari Taluka and drains into the Arabian Sea. The river has great ecological significance for the state as it forms the largest basin in Goa and is extensively used for drinking and agriculture purposes along with its other ecological and commercial importance.

Chorao Island in Mandovi River is one of the mangrove forests and houses most of the mangrove species found in Goa. The open-cast mining operations in Goa strongly affect the chemistry of the mangrove ecosystem (De Souza *et al*., 1999). Untawale *et al*. (1992) described the impact of urbanization on the coastal environment, which is responsible for drastic changes in the coastal configuration. Also, deforestation in the catchment areas and mining activity has increased sedimentation and pollution load on the rivers and estuaries (Mascarenhas and Jayakumar, 2008). Such degradation leads to biodiversity loss and severely impacts the mangrove ecosystem.

The mangrove ecosystem is a significant constituent of the Indian forest area. Therefore, various taxonomists from the field of biology have worked extensively to identify the diversity and distribution of mangroves. Goa, part of the coastal belt, is highly dominated by

mangroves. A recent checklist by Walke *et al*. (2018) reports the presence of 16 mangrove species distributed along the coastal estuaries, while earlier Saddhe *et al*. (2016) reported the presence of 14 species. However, possibly due to the addition of mangrove associates and their hybrids, there is a constant change in the total number of species in a given area (Mandal and Naskar, 2008). A review of the literature suggests that there are limited reports on the distribution of mangroves.

The present chapter reports the diversity of mangrove species at Chorao Island. Besides, an attempt has been made to document the traditional knowledge of mangrove plant species in the preparation of herbal medicine by local practitioners.

3.2: MATERIALS AND METHODS

3.2.1: Study site

The present study was undertaken at Chorao (also known as Chodna), an island along the Mandovi river (**Fig. 3.1**).

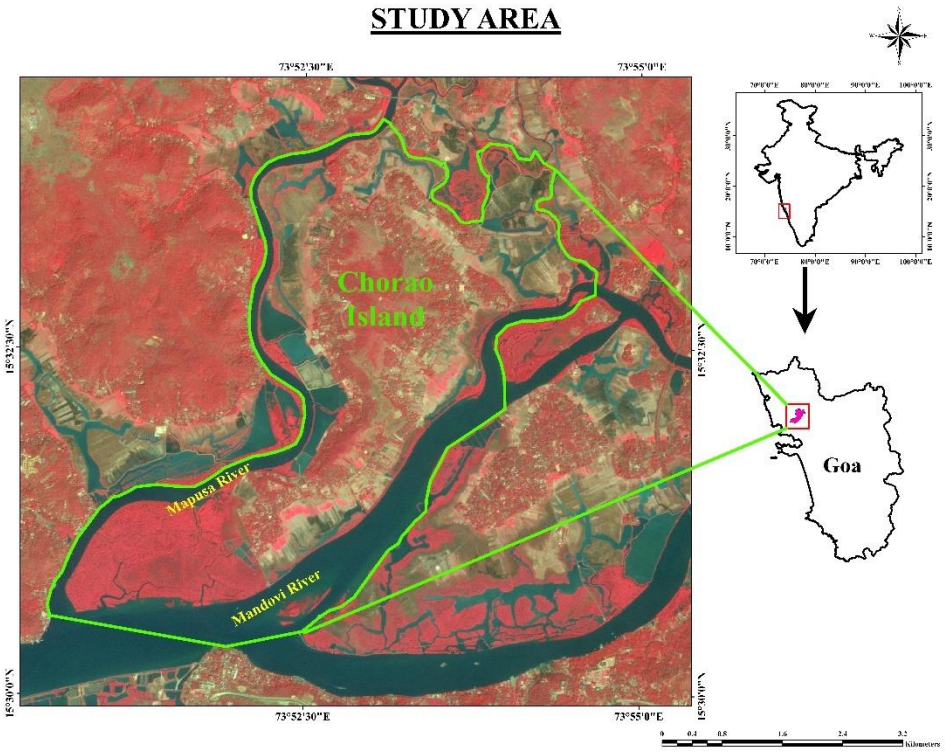

Fig. 3.1: Overview of the study area (Chorao Island) used to plot different quadrates.

A small part of the Island, covering 178 hectares of the area, was declared a Reserved Forest under the Indian Forest Act (1927) to protect and conserve mangrove forests. The reserve area is Dr. Salim Ali Bird Sanctuary, extending from $73^{0}5$' latitude to $15^{0}3$' longitude. The

sanctuary comprises thick mangrove vegetation protecting the coast from heavy tidal fluctuations. This thickly populated site provides a nesting site for various aquatic and avian species.

3.2.2: Structural distribution and diversity in mangrove plants

Mangrove studies were carried out during high and low tides through intensive field visits and sampling throughout the study period. Bitterlich variable plot method (1948) was used to lay 19 quadrates (**Fig. 3.2**) of 20x20m covering major mangrove locations to measure mangrove structural attributes (**Fig. 3.3)**.

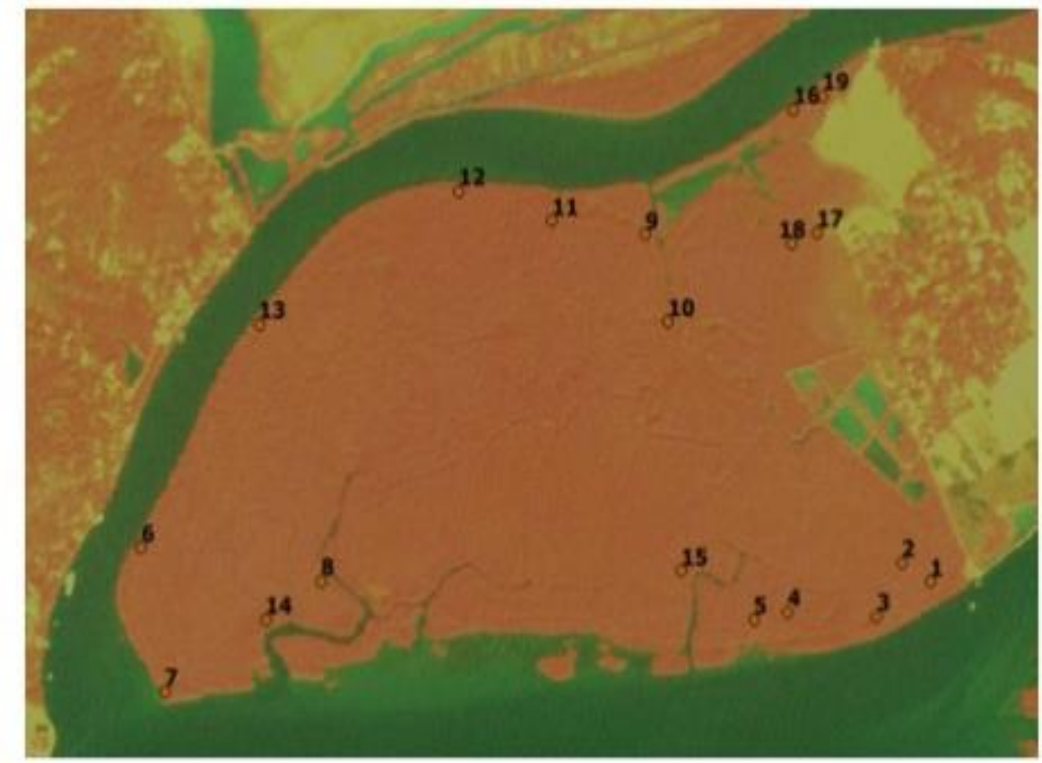

Fig. 3.2: Location of 19 quadrates at the study site.

Plants located at the center of the quadrate were tagged and labeled **(Fig. 3.4**), and counting the number of species present in each site. Geographical coordinates were recorded to locate the exact location of the study site using GPS tracker. Nondestructive *in-situ* analysis of the study area was performed for phytosociological and phytochemical analysis. The tagged, labeled mangrove flora was counted, noted, and photographed for further identification. These mangrove species were identified using standard references, floras, and identification keys (Dhargalkar *et al*., 2014).

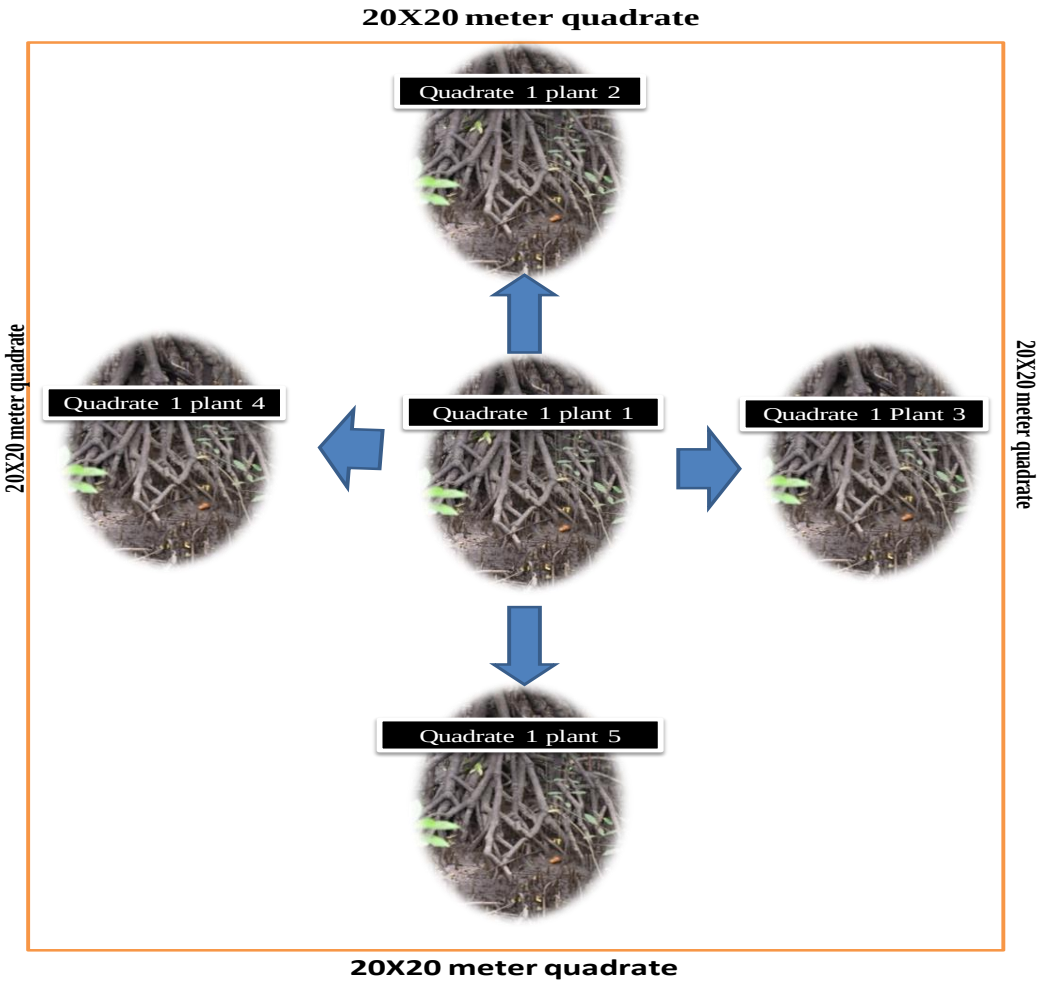

Fig. 3.3: Study of mangroves using the Bitterlich variable plot method (20x20m).

Fig. 3.4: Tagging mangrove plants.

The parameters undertaken for the study in each quadrate include:

- Tree/ plant basal area and diameter at breast height;
- Calculation of species dominance and relative abundance;
- Tree height measurement using a hypsometer, tapes, and ropes;
- Canopy diameter measurements using measuring tapes and still photographs.

The frequency of the mangroves was calculated by using Raunkiaer (1934), which directs the number of sampling units (quadrates) in which the species occur, thus, indicating the distribution of species in a particular community. The species abundance and density represent the numerical strength of the species in the community, wherein the abundance of species gives an idea of species distribution patterns at a particular place. Further Important Value Index (IVI) was developed to determine the species dominance and ecological success of species in a specific location/area. The index was calculated using relative density and relative dominance of species as follows:

- Relative Density % = Total Relative density (Number of individuals of a species ÷ Total number of individuals) x 100
- Relative Dominance % = (Total basal area of a species ÷ Basal area of all species) x 100
- Relative frequency% = (Number of the particular species ÷ Number of occurrence of all species) x100
- IVI% = Relative Density (%) + Relative Dominance (%) + Relative frequency (%)

The diversity indices were used to obtain a quantitative estimation of biological variability that compares biological entities. Therefore, Shannon Weiner diversity index (H) and Simpson's diversity index (D) was used using the following formulae:

- Shannon Weiner diversity index $H= -\Sigma (pi \ln pi)$
- Simpson's diversity index $(D)= 1- [\Sigma n(n-1)/N(N-1)]$

where pi is the proportion of individual species that contributes to the total number of individuals, n is the number of individuals of a given species and N is the total number of individuals in the community.

Species evenness was estimated using following formula:

- Species Evenness as $(\Sigma(H)= H'/H'max)$

Where $H'max=\ln S$, S=total number of species in the community.

- Canonical Correspondence Analysis (CCA) was performed using multivariable Statistical Package (MVSP) v3.1 to analyze the relationship between mangrove plant species and soil parameters in different quadrates.

3.2.3: Mangrove soil collection and analysis

Soil samples of dominant mangrove plant species present in each quadrate were collected by digging 15cm rhizosphere soil in triplicate and mixed thoroughly to obtain a composite sample. The collected composite soil samples were transferred in sterile *zip-lock* bags and brought to the laboratory for further analysis. Collected mangrove soil samples were air-dried and sieved to remove root particles, shells, and other debris. The chemical analysis of the soil samples was performed at ICAR (Central Coastal Agriculture Research Institute), Old Goa, and the Government of Goa, Agriculture Department, Soil analysis laboratory, Ela Old Goa. Analysis was performed using standard protocols. Soil pH was recorded by using a digital pH meter (Elico L1 120), and Electrical conductivity (EC) was recorded by using a conductivity meter (Elico CM 180). Soil organic carbon (%) was calculated by using Walkley and Black (1934) method, available P in soil was estimated by using Bray and Kurtz (1945) method, while potassium (K) was estimated by using ammonium acetate method from Hanway and Heidal (1952).

3.2.4: Adaptations in mangroves

Adaptation is directly proportional to species diversity and is essential to withstand changing climatic conditions in living habitats. Hence, mangroves undergo constant adaptation or modification to sustain themselves in that particular habitat. These include morphological, reproductive, and salt adaptations (Saxena *et al*., 2014). Comprehensive overviews of various morphological adaptations in mangrove species were documented using photographic evidence.

3.2.5: Ethno-botanical role of mangrove plants in herbal preparations

An ethnobotanical survey was conducted to document the information on utilizing mangrove plant species in folklore medicine. The information was gathered by interviewing local traditional healers, fishermen families, and women who specialized in treating newly born babies (locally called '*vaigins'*). The local names of the mangroves used in medicine were listed and identified using standard literature. Detailed information on the medicinal value of the plants and the mode of preparation, plant part used, and disease/s treated was documented.

3.3: RESULTS

3.3.1: Structural distribution and diversity in mangrove plants

Structural distribution is related to studying vegetation, density, and other phytosociological parameters (basal area, canopy cover, *etc*.). The present mangrove community mapping study of Chorao Island Goa includes eight dominant mangrove species belonging to five families (Aviciniaceae, Rhizophoraceae, Sonneratiaceae, Acanthaceae, and Euphorbiaceae). Altogether 1506 mangrove plants were seen growing profusely within the 19 quadrates (**Table 3.1 and 3.2**). Besides these, six mangrove plant species belonging to six different genera were seen growing on the outskirt of quadrates Island (**Table 3.3**). A well distribution of mangroves (as per site suitability) was noticed at the study site (**Fig. 3.5**) (**Plate 3.1, and Plate 3.2**).

Structural attributes such as basal area, plant height, and canopy cover were studied. The data indicated that *E. agallocha* had the highest basal area (3.11 cm), followed by *Av. marina* (0.81 cm), *S. alba* (0.59 cm), *Av. officinalis* (0.43 cm), *R. mucronata* (0.04 cm) and *B. cylindrica* (0.03 cm). Plant height in quadrates ranged from 4.73 to 10.33 meters. Maximum tree height was recorded in *Av. officinalis* (10.33 m), followed by *E. agallocha* (7 m), *Av. marina* (6.72 m), *R. mucronata* (5.07 m), *S. alba* (4.73 m) and *B. cylindrica* (3.12 m). Plant canopy diameter ranged from 3.3 cm to 18.13 cm. The largest canopy diameter was recorded in *Av. officinalis*, followed by *Av. marina, E. agallocha, R. mucronata, S. alba*, and *B. cylindrica* (**Table 3.4**).

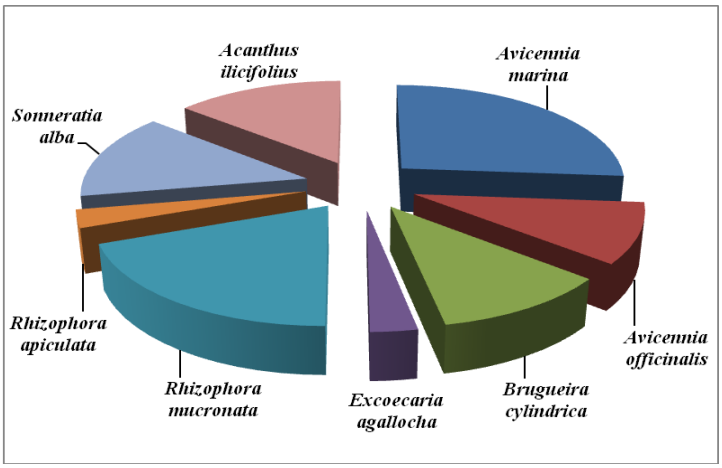

Fig. 3.5: Distribution of mangrove plant species at Chorao Island.

Table 3.1: Prevalence of mangrove plant species in quadrates at Chorao island.

Sr. No.	Plant Species	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Q15	Q16	Q17	Q18	Q19	Total no. of mangrove species
1	*Acanthus ilicifolius*	-	-	10	-	-	100	-	30	80	150	50	40	100	-	-	55	47	-	-	662
2	*Avicennia marina*	16	9	15	2	11	12	26	26	13	5	22	5	11	19	21	20	92	25	25	375
3	*Avicennia officinalis*	-	-	4	-	-		1	-	2	13	-	-	-	-	1	8	-	6	9	44
4	*Brugueira cylindrica*	-	-	-	-	-	44	19	-	8	1	-	4	18	120	2	1	-	-	-	217
5	*Excoecaria agallocha*	-	-	-	-	-	-	-	-	2	3	-	-	-	-	-	-	-	-	-	5
6	*Rhizophora mucronata*	-	-	3	6	1	-	17	2	2	1	1	1	1	15	7	1	-	1	-	59
7	*Rhizophora apiculata*	-	1	-	-	-	-		-	-	-	-	-	-	-	2	-	-	-	-	3
8	*Sonneratia alba*	7	8	2	21	4	-	-	-	-	-	-	-	-	1	11	-	67	17	3	141
	Total																				**1506**

Legend: **Q**=Quadrate**, -**: Not detected

Table 3.2: Distribution of mangrove plant species at Chorao island.

Quadrate No.	Latitude	Longitude	Plant species	Family	Mangrove type
					True/associate mangrove
1	73.52003	15.30013	*Avicennia marina* (Forssk.)Vierh. *Sonneratia alba* Sm.	Avicenniaceae Rhizophoraceae	TM TM
2	73.52002	15.30013	*Avicennia marina* (Forssk.) Vierh. *Sonneratia alba* Sm. *Rhizophora apiculata* Blume	Avicenniaceae Sonneratiaceae Rhizophoraceae	TM TM TM
3	73.52001	15.30012	*Avicennia marina* (Forssk.) Vierh. *Sonneratia alba* Sm. *Acanthus ilicifolius* L. *Rhizophora mucronata* Lam.	Avicenniaceae Sonneratiaceae Acanthaceae Rhizophoraceae	TM TM AM TM
4	73.51015	15.30012	*Sonneratia alba* Sm. *Rhizophora mucronata* Lam. *Avicennia marina* (Forssk.) Vierh.	Sonneratiaceae Rhizophoraceae Avicenniaceae	TM TM TM
5	73.51014	15.30012	*Avicennia marina* (Forssk.) Vierh. *Sonneratia alba* Sm. *Rhizophora mucronata* Lam.	Avicenniaceae Sonneratiaceae Rhizophoraceae	TM TM TM
6	73.50013	15.30014	*Acanthus ilicifolius* L. *Brugueira cylindrica* *Avicennia marina* (Forssk.) Vierh.	Acanthaceae Rhizophoraceae Avicenniaceae	AM TM TM
7	73.50014	15.3001	*Rhizophora mucronata* Lam. *Avicennia marina* (Forssk.) Vierh.	Rhizophoraceae Avicenniaceae	TM TM

8	73.51002	15.30013	*Avicennia marina* (Forssk.) Vierh. *Acanthus ilicifolius* L. *Rhizophora mucronata* Lam. *Brugueira cylindrica*	Avicenniaceae Acanthaceae Rhizophoraceae Rhizophoraceae	TM AM TM TM
9	73.51011	15.31005	*Avicennia officinalis* L. *Avicennia marina* (Forssk.) Vierh. *Acanthus ilicifolius* L. *Brugueira cylindrica* *Excoecaria agallocha* *Rhizophora mucronata* Lam.	Avicenniaceae Avicenniaceae Acanthaceae Rhizophoraceae Euphorbiaceae Rhizophoraceae	TM TM AM TM TM TM
10	73.51012	15.31003	*Excoecaria agallocha* *Avicennia officinalis* L. *Avicennia marina* (Forssk.) Vierh. *Acanthus ilicifolius* L. *Bruguiera cylindrica* (L.) Blume *Rhizophora mucronata* Lam.	Euphorbiaceae Avicenniaceae Avicenniaceae Acanthaceae Rhizophoraceae Rhizophoraceae	TM TM TM AM TM TM
11	73.51009	15.31006	*Rhizophora mucronata* Lam. *Avicennia marina* (Forssk.) Vierh. *Acanthus ilicifolius* L.	Rhizophoraceae Avicenniaceae Acanthaceae	TM TM AM
12	73.51006	15.31007	*Avicennia marina* (Forssk.) Vierh. *Rhizophora mucronata* Lam. *Bruguiera cylindrica* (L.) Blume *Acanthus ilicifolius* L.	Avicenniaceae Rhizophoraceae Rhizophoraceae Acanthaceae	TM TM TM AM
13	73.50017	15.31003	*Acanthus ilicifolius* L. *Avicennia marina (Forssk.)* Vierh. *Bruguiera cylindrica* (L.) Blume *Rhizophora mucronata* Lam.	Acanthaceae Avicenniaceae Rhizophoraceae Rhizophoraceae	AM TM TM TM TM

14	73.50017	15.30012	*Avicennia marina* (Forssk.) Vierh. *Bruguiera cylindrica* (L.) Blume *Rhizophora mucronata* Lam. *Sonneratia alba* Sm.	Avicenniaceae Rhizophoraceae Rhizophoraceae Sonneratiaceae	TM TM TM TM
15	73.51012	15.30013	*Avicennia officinalis* L. *Bruguiera cylindrica* (L.) Blume *Avicennia marina* (Forssk.) Vierh. *Rhizophora mucronata* Lam. *Rhizophora apiculata* Blume *Sonneratia alba* Sm.	Avicenniaceae Rhizophoraceae Avicenniaceae Rhizophoraceae Rhizophoraceae Sonneratiaceae	TM TM TM TM TM TM
16	73.51015	15.31009	*Avicennia officinalis* L. *Avicennia marina* (Forssk.) Vierh. *Acanthus ilicifolius* L. *Rhizophora mucronata* Lam. *Bruguiera cylindrica* (L.) Blume	Avicenniaceae Avicenniaceae Acanthaceae Rhizophoraceae Rhizophoraceae	TM TM AM TM TM
17	73.51	15.31	*Sonneratia alba* Sm. *Acanthus ilicifolius* L. *Avicennia marina* (Forssk.) Vierh.	Sonneratiaceae Acanthaceae Avicenniaceae	TM AM TM
18	73.51015	15.31005	*Avicennia marina* (Forssk.) Vierh. *Avicennia officinalis* L. *Sonneratia alba* Sm. *Rhizophora mucronata* Lam.	Avicenniaceae Avicenniaceae Sonneratiaceae Rhizophoraceae	TM TM TM TM
19	73.5106	15.31009	*Avicennia marina* (Forssk.) Vierh. *Avicennia officinalis* L. *Sonneratia alba* Sm.	Avicenniaceae Avicenniaceae Sonneratiaceae	TM TM TM

Legend: TM = True mangrove, AM= Associate mangrove.

Table 3.3: Distribution of mangrove plant species at Chorao island.

Sr. No.	Mangrove species	True / associate mangrove	Habitat	Family
1	*Clerodendron inermis*	AM	Climber	Lamiaceae
2	*Ceriops tagal*	TM	Shrub	Rhizophoraceae
3	*Aegiceras corniculatum*	TM	Shrub	Primulaceae
4	*Thespesia populnea*	AM	Tree	Malvaceae
5	*Acrosticum aurum*	AM	Fern	Pteridaceae
6	*Derris heterophylla*	AM	Climber	Fabaceae

Legend: **AM**= Associate mangrove, **TM**= True mangrove.

Table 3.4: Phytosociological analysis of mangrove plant species at Chorao island.

Plant species	BC (cm)	BA (cm)	CD (cm)	PH (m)
Avicennia marina	1.22	0.81	12.10	6.72
Sonneratia alba	0.96	0.59	9.55	4.73
Avicennia officinalis	0.96	0.43	18.13	10.33
Rhizophora mucronata	0.25	0.04	10.54	5.07
Bruguiera cylindrica	0.34	0.03	3.38	3.12
Excoecaria agallocha	2.85	3.11	16.2	7

Legend: BC=Basal circumference; BA=Basal area; CD= Canopy Diameter; PH=Plant Height

Species dominance revealed that *Ac. illicifolius* (associate mangrove) was the frequently identified species growing luxuriantly on the Island. Among true mangroves, *A. marina* and *B. cylindrica* were the most dominant true mangrove species, whereas *E.agalocha* and *R. apiculata* were distributed scarcely. At Chorao, species abundance (ha^{-1}) varied from 38 to 1655. *R. apiculata* revealed a species abundance of 38, while *Ac. illicifolius* recorded an abundance of 1655. *Av. marina* recorded the highest frequency, but abundance along the island was less than *B. cylindrica* (678). When compared to frequency, density represents the number of individual species at a particular location/area. The density (ha^{-1}) of mangrove species varied between 4 (*R. apiculata*) and 871 (*Ac. ilicifolius*). On analyzing the Important Value Index (IVI) among species, it was observed that *Ac. ilicifolius* indicated the highest IVI of 62, followed by *Av. marina* (59). In contrast, *R. apiculata* showed the least IVI (4), followed by *E. agallocha*. Among these mangroves, *Av. marina* was frequently observed species with the highest frequency (100%). *E. agallocha* and *R. apiculata* recorded the lowest frequency of 11%. Other mangrove species recorded a frequency between 37 to 74% (**Table 3.5** and **Fig. 3.6**).

Table 3.5: Abundance of mangrove plants at Chorao island.

Sr. No.	Plant Species	Frequency (%)	Abundance (ha^{-1})	Density (ha^{-1})	Relative density (%)	Relative frequency (%)	Relative dominance (%)	IVI (%)
1	*Avicennia marina*	100	493	493	25	26	7	59
2	*Avicennia officinalis*	37	157	58	3	10	1	14
3	*Brugueira cylindrica*	42	678	286	14	11	1	26
4	*Excoecaria agallocha*	11	63	7	0	3	1	4
5	*Rhizophora mucronata*	74	105	78	4	19	0	23
6	*Rhizophora apiculata*	11	38	4	0	3	0	3
7	*Sonneratia alba*	53	353	186	9	14	3	26
8	*Acanthus ilicifolius*	53	1655	871	44	14	5	63

Legend: IVI= Important Value Index

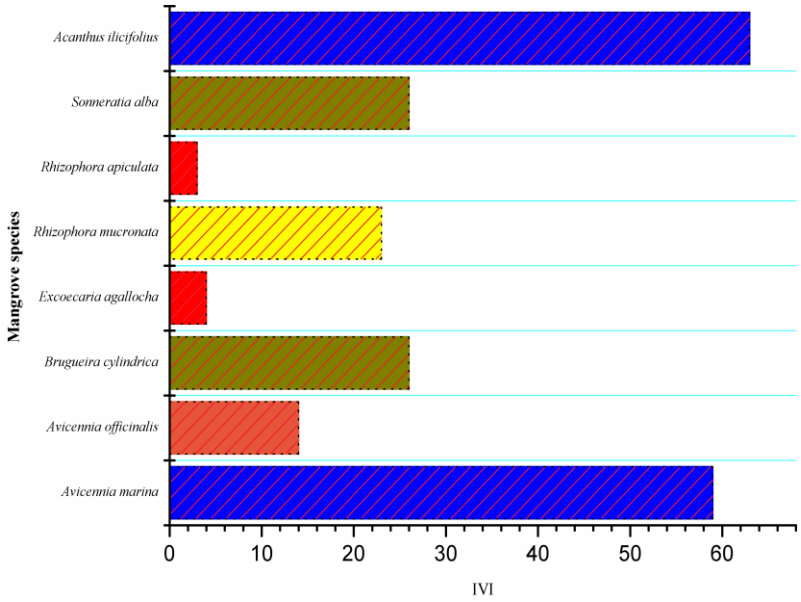

Fig. 3.6: Species-wise IVI for mangroves at Chorao island.

Quadrate-wise diversity indices Shannon-Weiner (H), Simpson diversity index (D), and Species evenness index were used to determine the diversity index of Chorao Island. The study indicated that, among 19 quadrates, quadrate no. 10 recorded the lowest H index while quadrate no. 15 indicated the highest H index of 1.63. In terms of Dominance (D), quadrate 10 showed the lowest D value, whereas quadrate 10 revealed the highest dominance value of 0.71. Species evenness index, quadrate no. 3 was seen evenly placed in terms of species dominance compared to quadrate no. 10 (**Fig. 3.7**).

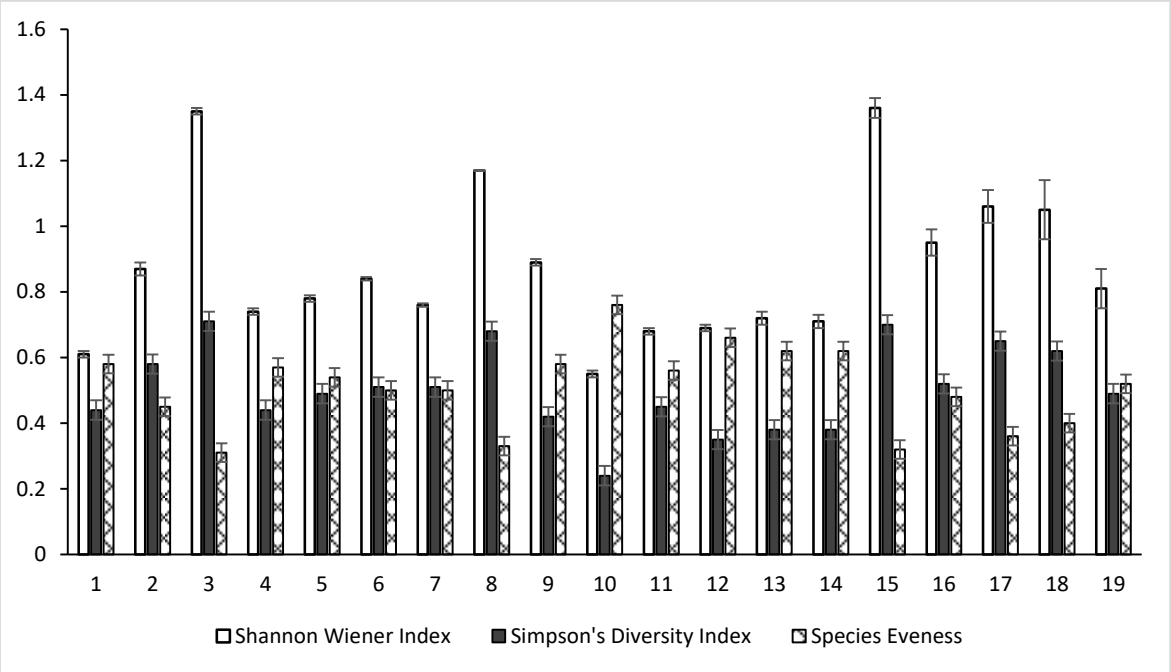

Fig. 3.7: Mangrove vegetation diversity at Chorao island.

3.3.2: Soil analysis

Physico-chemical analysis of mangrove soil revealed that the mangrove species at Chorao grow in highly acidic to neutral (3.8 to 7) pH levels. Salinity levels for different mangrove plant species ranged from 14 to 31 ppm. Organic carbon (OC %) ranged between 1.4 to 2.6 %, low P content ranged from 8.1 to 70 Kg ha^{-1}, while available K in soil ranged from 1200 to 3698 Kg ha^{-1}. CCA plot indicates the relative significance affecting the mangrove plant community, whereas the angle between variables indicates the degree of correlation. The distribution of *R. apiculata* in quadrates 6, 13, 12, and 9 mainly depend on soil variables such as pH and P. However, no soil parameters were involved in the distribution of *B. cylindrica*, *R. mucronata*, *A. marina*, *S. alba*, and *A. officinalis* in the quadrates Q14, 7, 8, 3, 19, 17, 15, 5, 1, 2, and 18. A strong correlation was observed in plants *A. illicifollius*, *E. agallocha* in quadrate 10, 11, and 16 with the levels of EC as compared to OC and K (**Fig 3.8**). The CCA variable score and its biplot score in tabulated in **Table 3.6, Table 3.7 and Table 3.8.**

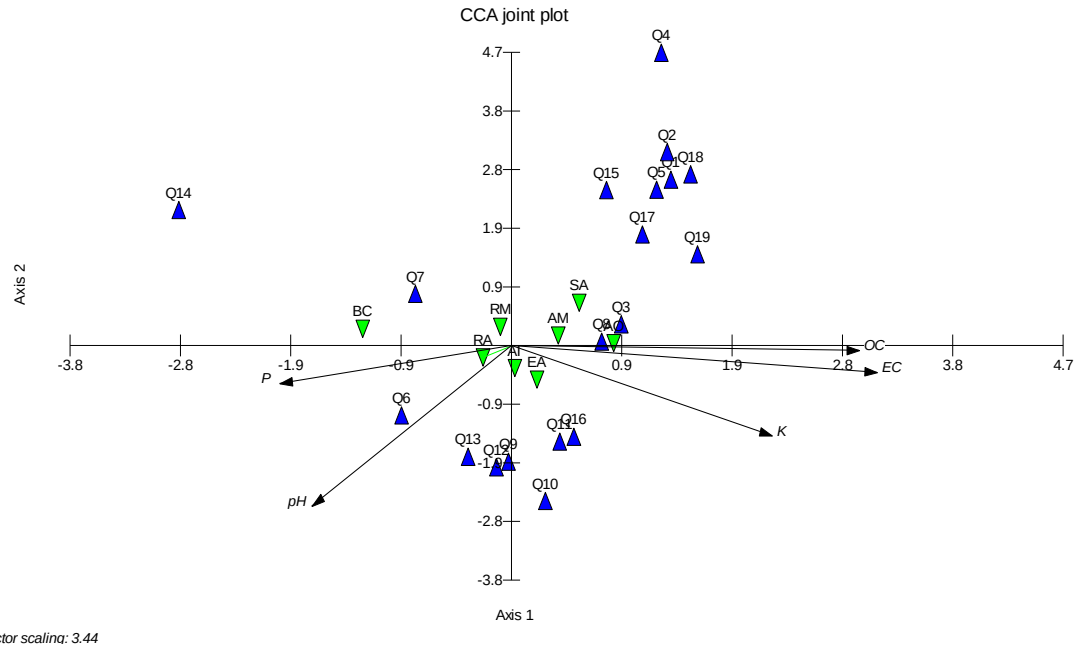

Fig. 3.8: Canonical Correspondence Analysis (CCA) of the relationship between mangrove plant species (BC= *B. cylindrica*, RM= *R. mucronata*, RA= *R. apiculata*, AI= *Ac. illicifolius*, EA= *E. agallocha*, AM= *Av. marina*, AO= *Av. officinalis*, SA= *S. alba*,) and soil analysis (pH=, K=, P=) from different quadrates (Q1= Quadrate no.1, Q2= Quadrate no. 2, Q3= Quadrate no. 3, Q4= Quadrate no. 4, Q5= Quadrate no. 5, Q6= Quadrate no. 6, Q7= Quadrate no. 7, Q8= Quadrate no. 8, Q9= Quadrate no. 9, Q10= Quadrate no.10, Q11= Quadrate no. 11, Q12= Quadrate no.12, Q13= Quadrate no.13, Q14= Quadrate no.14, Q15= Quadrate no.15, Q16= Quadrate no.16, Q17= Quadrate no.17, Q18= Quadrate no.18, and Q19= Quadrate no.19).

Table 3.6: CCA variable score of diversity of mangrove plants at study site.

Biological variables	Axis 1	Axis 2
AM	0.396	0.167
SA	0.575	0.695
AO	0.871	0.046
EA	0.215	-0.541
BC	-1.275	0.276
AI	0.026	-0.355
RM	-0.099	0.306
RA	-0.246	-0.185
Eigen value	0.332	0.123
Variation %	22.229	8.220
Cumulative %	22.229	30.449

Legend: AM= *Av. marina,* SA= *S. alba*, AO= *Av. officinalis*, EA= *E. agallocha,* AI= *Ac. illicifolius*, RM= *R. mucronata*, RA= *R. apiculata.*

Table 3.7: Biplot score of soil variables for the mangrove plant diversity at study site.

Soil variables	Axis 1	Axis 2
pH	-0.497	-0.750
EC	0.908	-0.125
OC	0.864	-0.022
P	-0.576	-0.177
K	0.647	-0.421

3.3.3: Mangrove adaptations

Salinity adaptation: Mangroves adapt in highly anaerobic soil conditions by modifications in the aerial root system. The formation of dense coiled root structures that allow atmospheric gaseous exchange is an important mechanism to withstand salt stress (Gupta and Huang, 2014). At Chorao, various root modifications were noticed. In *Rhizophora* species, stilt roots or prop roots were seen arising from the base of the stem to absorb water and nutrients, which is in accordance with the earlier observation by Mendez-Alonzo *et al.* (2014). *Avicennia* and *Sonneratia* species showed the presence of pneumatophores (**Plate 3.3**) that allow diffusion of oxygen (Curran *et al.,* 1986). At Chorao, salt exclusion was observed in the *Rhizophora*, *Acanthus*, and *Excoecaria* species (**Plate 3.4**).

Table 3.8: CCA variable score of different quadrates from study area.

Variables	Axis 1	Axis 2
Q1	1.360	2.675
Q2	1.328	3.117
Q3	0.935	0.348
Q4	1.277	4.715
Q5	1.237	2.511
Q6	-0.943	-1.117
Q7	-0.825	0.839
Q8	0.768	0.069
Q9	-0.029	-1.860
Q10	0.286	-2.492
Q11	0.409	-1.538
Q12	-0.132	-1.950
Q13	-0.374	-1.782
Q14	-2.847	2.185
Q15	0.808	2.508
Q16	0.530	-1.461
Q17	1.116	1.792
Q18	1.527	2.760
Q19	1.587	1.473

Reproductive adaptations: Seeds are the gene pool reservoir that helps transfer the genetic material to another generation. Different reproductive methods are seen within water-dispersed propagules to maintain their gene pool. Species belonging to the family Rhizophoraceae showed a viviparous type of seed germination, whereas species belonging to the family Avicenniaceae and Myrsinaceae show incipient vivipary (**Plate 3.5**).

3.3.4: Ethnobotanical applications of mangrove plant species at Chorao Island

Twelve mangrove plants belonging to eight different families having medicinal uses were documented. The details of medicinal plants and the preparation method are depicted in **Table 3.9**. The local practitioners use various plant parts to cure various human ailments, *viz*., body aches, wounds, varicose veins, jaundice, bone injury, *etc*. Therefore, ethnobotanical knowledge becomes vital in identifying medicinally useful plants for various human ailments.

Table 3.9: Medicinal uses of mangrove plants in preparation of various herbal medicines

Sr. No.	Plant species	Local Name	Part Used	Medicinal Use
1	*Acanthus ilicifolius*	Mharani	Leaf	**Body ache**: 10-20 leaves added in a bucketful of hot water used for bathing, once a day, for a period of 2-3 days.
			Root	**Wounds**: A thick paste of the roots is prepared by using little quantity of water and is applied externally on affected part twice a day till cured.
			Leaf	**Varicose veins**: leaves and stems are used to cure varicose veins; thick paste of leaves (along with spines) and stem is prepared by using parboiled water. The resultant paste is tied to the affected part.
2	*Avicennia marina*	Hipli	Stem and leaf	**Mosquito repellent**: Fresh stems and leaves are burnt along with the coconut husk around the house to repel mosquitoes
3	*Rhizophora mucronata*	Ballo	Bark	**Jaundice**: Mature bark is dried, stored and powdered. One teaspoon of powder is mixed with a glass of water and boiled for 15 minutes and orally consumed.
4	*Sonneratia alba*	chipa	Leaf	**Bone injury**: Traditionally the plant leaves are used to cure bone injury, wherein, dried and powdered and mixed with coconut feni. The resultant paste is applied to the affected part till cured.

5	*Aegiceras corniculatum*	Kanla	Root	**Growth of fetus**: Roots are tied in the hair bun of pregnant ladies for the good health of growing fetus.
6	*Thespesia populnea*	Bheniyeche Jhad	Leaf	**High blood pressure**: Fresh leaves (midrib removed) are dried in sunlight and decoction prepared using water is orally consumed.
			Fruit	**Skin infection**: Dried ash of ripe fruit is applied to the affected portion till cured.
			Leaf	**Deworming**: Decoction prepared from 2-3 leaves along with little cumin (jeera) seeds is consumed.
			Leaf	**Jaundice**: Fresh leaf (midrib removed) is boiled along with cumin (jeera) seeds in one glass water and is allowed to cool in mud vessel overnight. The decoction is consumed early in the morning before breakfast.
7	*Clerodendron innermis*	Vagati	Leaf	**Deworming**: Paste prepared from leaves using grinding stone is applied on the stomach and forehead
8	*Derris heterophylla*	Zharkoni	Seed	**Fever**: Dried seeds are ground in water resultant paste is used to apply on child's forehead.
9	*Acrosticum aureum*	Aakur	Leaf	**Healthy food source**: Tender leaves are used to prepare prawns curry.

3.4: DISCUSSION

Av. marina is the dominant true mangrove species that grows up to 3-12 m in height. It is locally known as 'Hipli,' which covers 24% of the Island area. This species is reported to grow luxuriantly in almost all parts of India (Ragavan *et al.,* 2016). *Avicennia officinalis*, occupies 3% of the area and is less diverse than *Av. marina*. It is known to have lesser tolerance to floods and salt stress (Satyanarayana *et al.,* 2009). This may be a limiting factor for its growth at Chorao. Based on the structural attributes, it is interpreted that the plant height is directly proportional to the plant canopy diameter. This can be related to species competition, especially for sunlight to produce glucose during photosynthesis (Vancea and Nevai, 2007).

Acanthus ilicifolius, locally known as 'Mharani' occupies 45% of the island and is a highly diverse associate mangrove. The plant is reported to be highly useful in traditional medicine to cure arthritis and other skin inflammations (Singh and Aeri, 2013). The diversity of mangroves at the study site indicates the restricted ability of the species to propagate at a particular location. This is due to the fluctuations in pH and salinity, which may reduce the regeneration of the adapted species. Chowdhury *et al.* (2019) suggested salinity is an important factor in mangrove growth and development. The present study also indicates the limitations of the species to germinate and adapt at varied pH ranges (4.0 to 6.2) and EC levels. The present study revealed that the mangrove species at Chorao grow in acidic pH. Seedo *et al.* (2018) observed that the survival of *Av. marina* in low salinity levels is by regulating leaf turgidity at different pH levels.

3.5: CONCLUSION

It can be concluded that the mangrove ecosystem, with its constant changes in environmental conditions, gets acclimatized by its self-adaptive mechanisms and hence contribute as a very important part of biodiversity. The mangroves are the store house of potent drugs used extensively in traditional herbal preparations to cure various ailments. Therefore, documentation of the medicinal properties of mangrove plants would help researchers to develop novel drug formulations using pharmacological and chemical analysis. This, in turn, would help device strategies for conserving native mangrove species. Additionally, studying the microsymbionts such as endophytes would provide insight into the field of pharmacology.

Chapter 4: To study the fungal endophytes from the selected study site

4.1: INTRODUCTION

Endophytes are microorganisms associated with various plant species and can invade interior plant tissues without causing noticeable disease symptoms (Xiang *et al.*, 2012). Endophytes occur in leaves, bark, stem, root, and xylem tissues of all plant species (Petrini, 1986). These associations within the plant species significantly provide a positive role in maintaining the steady growth and development of the host plant. In mangroves, an assemblage of micro-biota is involved in mediating CO_2, CH_4, N_2, and N_2O gases that trigger adaptive mechanisms (Alongi *et al*., 2005). These symbionts grow in the internal plant tissues of host plants by modulating the secondary metabolite production that helps both the living partners to grow and multiply *via* chemical signaling (Kusari *et al.,* 2012). Such metabolites, in turn, trigger the host survival rates (Waqas *et al.,* 2012).

Research on endophytic fungi (EF) has gained the scientific community's attention in recent years because of their vast diversity, particularly for potential secondary metabolites. Fungal endophytes are ubiquitous and do not show any host specificity. However, specific fungal endophytes appear frequently in a particular host, thus showing host preferences (Arnold and Lutzoni, 2007). Therefore, interactions among plants and microorganisms play a crucial role in the adaptation and growth of symbionts.

Mangroves are constantly subjected to varying climatic changes; under such environmental conditions, endophytes produce chemical compounds like the host to secure colonization (Zhao *et al.,* 2012). Despite distinct changes in the environmental conditions, these mangrove plant species harbour a high diversity of endophytic microflora (Suryanarayanan and Kumaresan, 2000). Endophytes are present in almost all plant parts (Stone, 1987) and contribute to nutrient uptake and fitness of the host (Shah *et al.,* 2018).

Among endophytes, dark septate endophyte (DSE) is another group of septate, hyaline, or darkly pigmented fungal endophytes within the plant species found under stress conditions (Mandyam and Jumpponen, 2014). This group of endophytes mostly colonizes living roots and protects plants from heavy metal stress (Regvar *et al.,* 2010).

So far, no work related to endophyte diversity has been attempted at the study site. In the present study, the occurrence of DSE in mangrove roots was examined. Attempts were made to isolate and identify the diversity of endophytes in different parts, *viz*., leaf, stem, and root

of mangrove plants. Studies related to the seasonal variation of fungal endophytes in mangrove parts were also attempted.

4.2: MATERIALS AND METHODS

4.2.1: Sample Collection

Fresh and healthy leaves, stems, and roots were collected from 13 mangrove plants (including nine true- and four associate-mangroves) species, *viz*., *Av. marina, Av. officinalis, R. mucronata, R. apiculata, E. agallocha, B. cylindrica, C. tagal, A. corniculatum, S. alba, Ac. illicifolius, D. heterophylla, C. inermis,* and *Ar. aurum*. Three collections were undertaken, *i.e.,* monsoon (June to September), winter (October to January), and summer (February to May), from pre-decided stations in triplicate. The sample collection in *Kandelia candel* was attempted only during the summer due to practical difficulties. These samples were subsequently brought to the laboratory and processed within five hours of collection to avoid saprophytic fungi growth.

4.2.2: Localization of DSE in mangrove plant roots

All 14 mangrove plant species were used for the localization of DSE. The colonization was assessed by using the Trypan blue staining method (Phillips and Hayman, 1970). Roots were thoroughly washed with running tap water to remove all the surface detritus and soil particles. The clean root samples were cut into smaller pieces, followed by heating the sample with 10% KOH at 90^0C for 1 hour to clear cytoplasm and nuclei. The sample was later rinsed with several changes of tap water to remove traces of KOH. This was followed by 5N HCl treatment for 3 - 4 minutes and then overnight staining the sample with 0.05% Trypan blue stain. The stained roots were monitored by placing them on a clean, dry glass slide using a bright-field Olympus BX 41 and Nikon Eclipse E200 research microscope to observe the presence of a fungal septate hyphal network within the roots. Micrographs of the dark septate hyphae were done using Olympus DP 12-2 and Nikon Digital Sight DS-U3 digital camera.

4.2.3: Seasonal distribution of endophytic fungi in mangroves

4.2.3.1: Sterilization of plant material

Sterilization protocols of Arnold *et al.* (2000), Suryanayanan *et al.* (1998), and Bayman *et al.* (1997) were employed in the present study. The sterilization time varied from species to species as each plant material was different, and the main aim was to eliminate epiphytic

fungal/bacterial flora from the explant. The protocol for surface sterilization involved a series of steps that included washing the plant material with 75% ethanol, followed by 4% NaOCl and finally rinsing the material with 75% ethanol. Later the plant samples were thoroughly washed with distilled water to remove all traces of chemicals. Finally, the sterilized samples were air-dried under aseptic conditions. The efficacy of sterilization was assessed using the imprint method (Schulz *et al.*, 1993).

The surface-sterilized dry sample was later cut into 0.5 cm pieces using a sterile blade and was placed on Potato Dextrose Agar (PDA) medium (nine/Petri plate), amended with streptomycin sulfate and penicillin G (150 mg L^{-1}) to inhibit bacterial growth (**Fig. 4.1)**. The inoculated plates containing the sterilized sample were incubated in 12 h dark and 12 h light conditions at room temperature (28±1^{0}C) in a tissue culture laboratory. The plates were monitored every day to check for any hyphal growth. Subsequent sub-culturing was done, and later, pure colonies were transferred to the PDA slants (glass vials) for further identification (Bills, 1996). The purified cultures were coded with isolation code and subsequently preserved at 4^{0}C for further use.

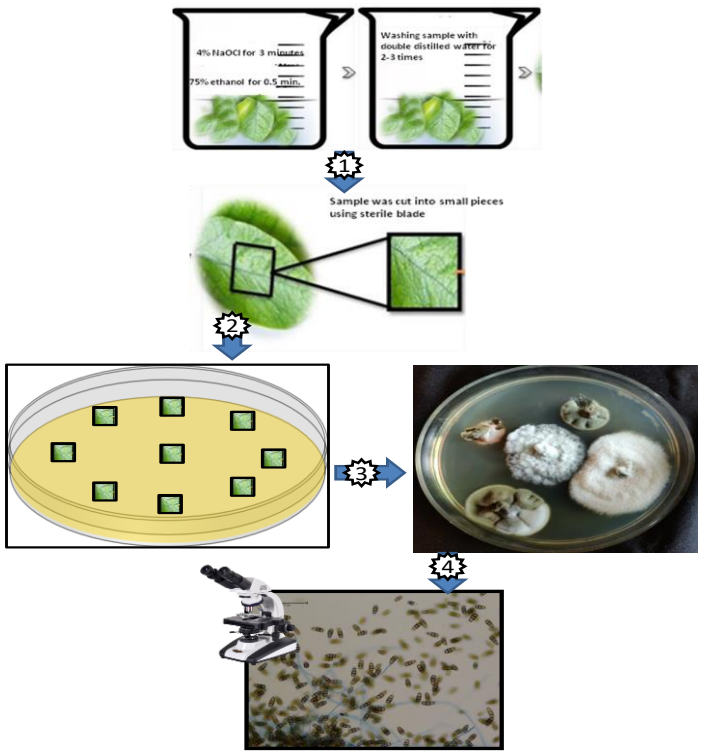

Fig. 4.1: Isolation of manglicolous fungi using three-step sterilization protocol.

4.2.4: Distribution of endophytic fungi in mangrove leaves

Five mangrove species, *viz.*, *B. cylindrica*, *Av. marina*, *C. tagal*, *E. agallocha*, and *S. alba* were selected to study the diversity of fungal endophytes appearing at different stages of leaf growth. The leaf material was collected at four different growth stages *viz.*, Stage 1: tender leaf (3^{rd} week), Stage 2: mature leaf (5^{th} week), Stage 3: senescence leaf (7^{th} week), and Stage 4: litter or the dead leaf (9^{th} week) (**Fig. 4.2**). The sample was then processed by using a three-step sterilization method and incubating fragments in PDA medium amended with streptomycin sulfate and penicillin G (150 mg/l) at 28 ± 2^0 C.

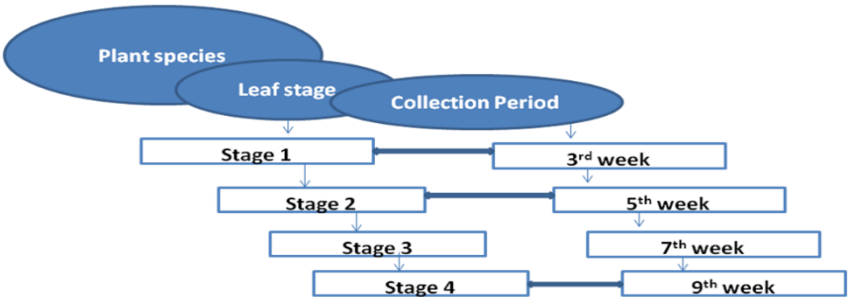

Fig. 4.2: Experimental design to study the distribution of fungal endophytes.

4.2.5: Identification of endophytic fungi

4.2.5.1: Morphological identification

The fungal endophytes were identified based on morphological characters, *viz.*, colony characteristics, pigmentation, spore type, and hyphal growth (Bills, 1996; Tibpromma *et al*., 2018). The microscopic examination was done by mounting sporulating fungal conidiophore on a clean and dry glass slide using lactophenol for coloured conidia and lactophenol cotton blue for hyaline conidia. The slides were observed under a bright-field Olympus BX41 microscope. The sporulating colonies were later identified using standard monographs and also by referring to Index Fungorum (Anonymous, 2012), and the Mycobank website. The colonies that failed to sporulate were designated sterile isolates and subsequently coded with an isolation code. Attempts were also made to induce sporulation in some sterile isolates, which failed to sporulate on the PDA medium. Non-sporulating isolates were grown on Malt agar, Oatmeal agar, Czapek, and Sabouraud, nutrient agar. Cultures were sometimes raised along with host plant extract to induce sporulation.

4.2.5.2: Molecular identification

Molecular identification was used to identify sterile endophytic isolates showing potent anticancer activity. The analysis was carried out at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerela, India. The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems), and sequence alignment was edited using Geneious Pro v5.1 (Drummond *et al*., 2010). The sequence was viewed using Chromas software, and the FASTA format of each isolate was acquired using the NCBI database. The sequence was later compared with existing DNA sequences available at NCBI GenBank (http://www. ncbi.nlm.nih.gov.blast). The phylogenetic tree was constructed using MEGA 7.0 software by the Maximum Likelihood method to identify the respective fungi.

4.2.6: Statistical data analysis

The diversity of endophytic fungi at Chorao Island was estimated by using the following statistical indices, and data were computed using SPSS and Origin software.

4.2.6.1: Relative abundance (RA)= No. of species / Total no. of species in all samples x 100

- **Isolation frequency (Fr)** = (ns/N) x100, where ns is the number of fungi recovered from a plant; N is the total number of plant segments used (Castilo *et al.,* 2003).
- Diversity indices were calculated using formulae given in chapter 3.

4.2.6.2: Sorensen coefficient

- **Cs (Sorenson's coefficient) =** 2j/(a+b), where j is the number of endophytic fungi recovered from two plant species; a and b are the total number of species from both the plants (Magurran, 2004).

4.2.6.3: Estimation of root colonization percentage

- **Colonization frequency (%)** = Number of segments colonized by fungi/Total number of segments observed x100 (Suryanarayanan *et al.,* 2003; Photita *et al.,* 2001)

4.3: RESULTS

4.3.1: DSE colonization in mangrove roots

The present study revealed the presence of DSE colonization in all 14 mangrove plants (**Plate 4.1**). Melanized septate hyphal colonization was recorded in mangrove plant roots. Hyphae of *B. cylindrica*, *C. tagal, Av. marina, Av. officinalis*, and *Ac. illicifolius* with densely

coiled structures forming microsclerotia were observed. Colonization ranged from 70% to 100% (**Fig.4.3**).

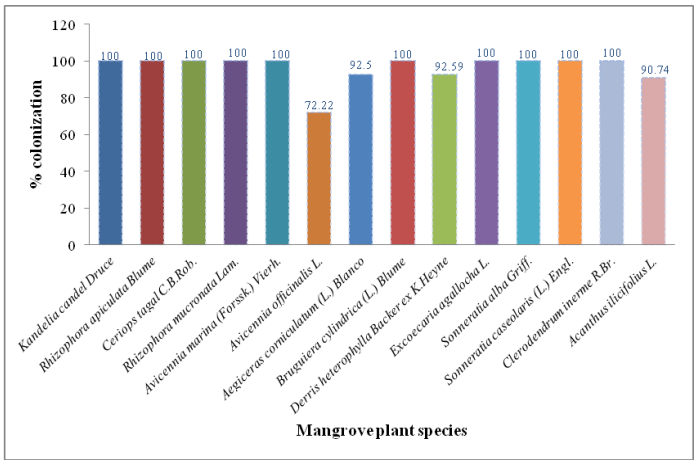

Fig. 4.3: Localization of DSE in mangrove roots.

4.3.2: Seasonal distribution of endophytic fungi

4.3.2.1: Distribution of endophytic fungi in mangrove plants

Plant-wise distribution of fungal endophytes indicated the presence of different fungal isolates in mangrove plants. During monsoon, *R. apiculata* recorded a higher number of fungal isolates (186), followed by *Ac. aurum* (185), *B. cylindrica* and *C. tagal* (177), *Ac.ilicifolius* (176), *R. mucronata* (172), *Ae. corniculatum* (169), *Av. officinalis* (169), *Av. marina* (167), *D. heterophylla* (165), *Cl. inermis* (162), and *E. agallocha* (159). During winter, *Avicennia marina* recorded highest endophytic isolates (209), followed by *Rhizophora apiculata* (185), *Avicennia officinalis* (182), *Acanthus ilicifolius* (181), *Derris heterophylla* (168), *Rhizophora mucronata* (165), *Clerodendron inermis* (162), *Excoecaria agallocha* (157), *Brugueira cylindrica* (151), *Ceriops tagal* (147), *Acrosticum aurum* (141), and *Aegiceras corniculatum* (116). Similarly, during summer highest number of isolates were recorded in *Avicennia marina* (217), followed by *Acanthus ilicifolius* (162), *Avicennia officinalis* (161), *Rhizophora apiculata* (149), *Clerodendron inermis* (141), *Derris heterophylla* (139), *Rhizophora mucronata* (136), *Brugueira cylindrica* (125), *Excoecaria agallocha* (121), *Ceriops tagal* (110), *Aegiceras corniculatum* (97), and *Acrosticum aurum* (91) (**Fig 4.4**).

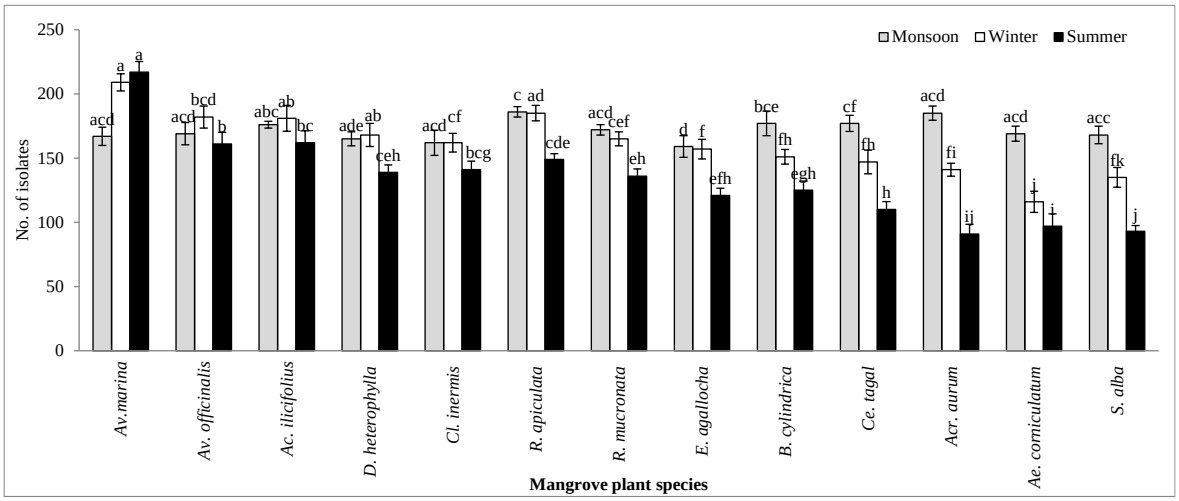

Fig. 4.4: Seasonal diversity of endophytic fungi in different mangrove species. (Values presented in each column with lowercase and uppercase letters are significantly different at $p<0.05$).

Calculation of Colonization Frequency (CF) percentage in different seasons indicated the significant difference in the occurrence of fungal endophytes within the mangrove plants. During summer, the highest endophytic colonization was recorded in *Av. marina* (13%) and the lowest in *S. alba* and *Ce.tagal* (6%). However, *D. heterophylla*, *R. mucronata*, and *B. cylindrica* recorded a colonization percentage between 8% to 7% (**Fig. 4.5**). In contrast, during monsoon CF% in plants ranged from 7 to 8% (**Fig. 4.6**). During winter, highest colonization percentage was observed in *Av. marina* (10%), and lowest in *Ae. corniculatum* (5%) (**Fig. 4.7**).

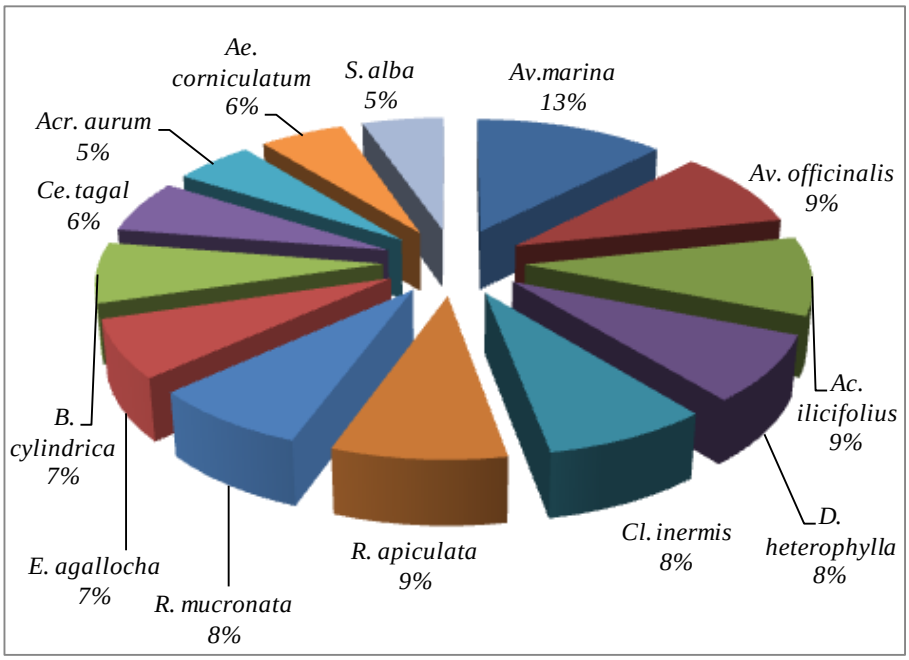

Fig. 4.5: Distribution of fungal endophytes in mangrove species in the summer season.

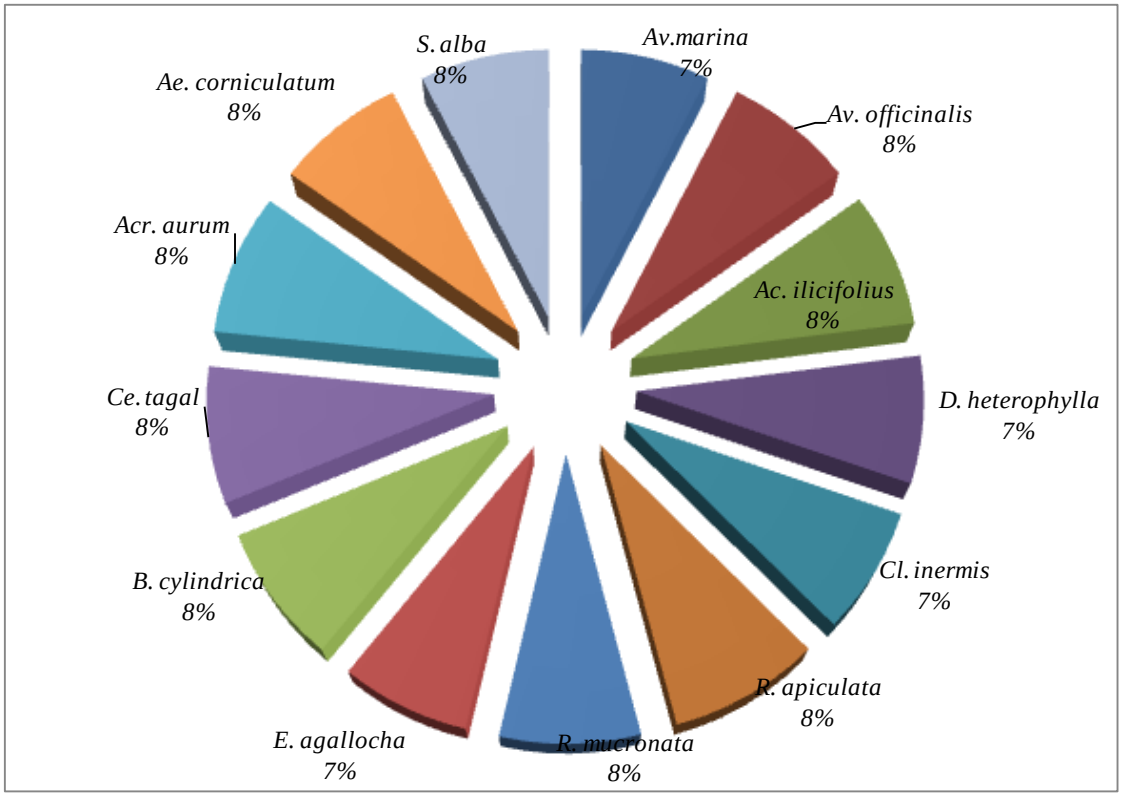

Fig. 4.6: Distribution of fungal endophytes in mangrove plant species in monsoon season.

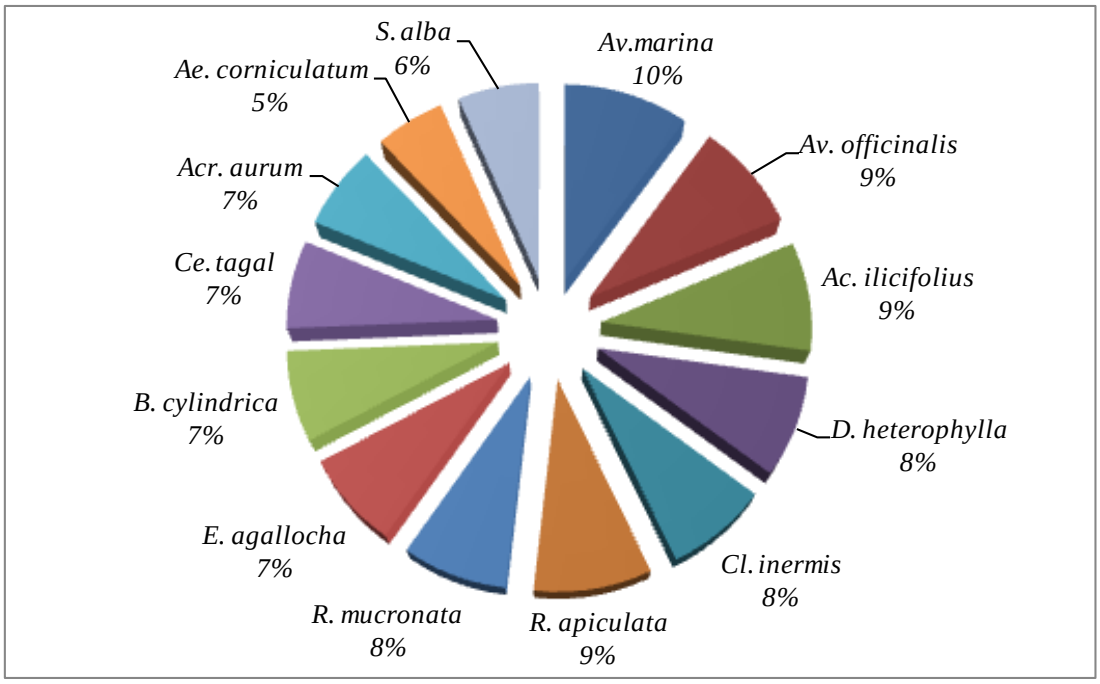

Fig. 4.7: Distribution of fungal endophytes in mangrove plant species in the winter season.

Data indicated a significant difference in the number of colonies in different plant parts. Shannon diversity index ranged from 2.43 - 2.42 during all the seasons, indicating moderate diversity of the endophyte community at the study site. Similarly, Simpson's diversity index revealed moderate diversity of 0.9 - 0.8 at the study site.

During summer maximum number of isolates were recorded in leaves, followed by stem and root. While during winter, the maximum number was recorded in leaves, followed by root and stem. Overall, the maximum number of fungal endophytes was recorded in the leaf, followed by the stem and root (**Fig. 4.8).**

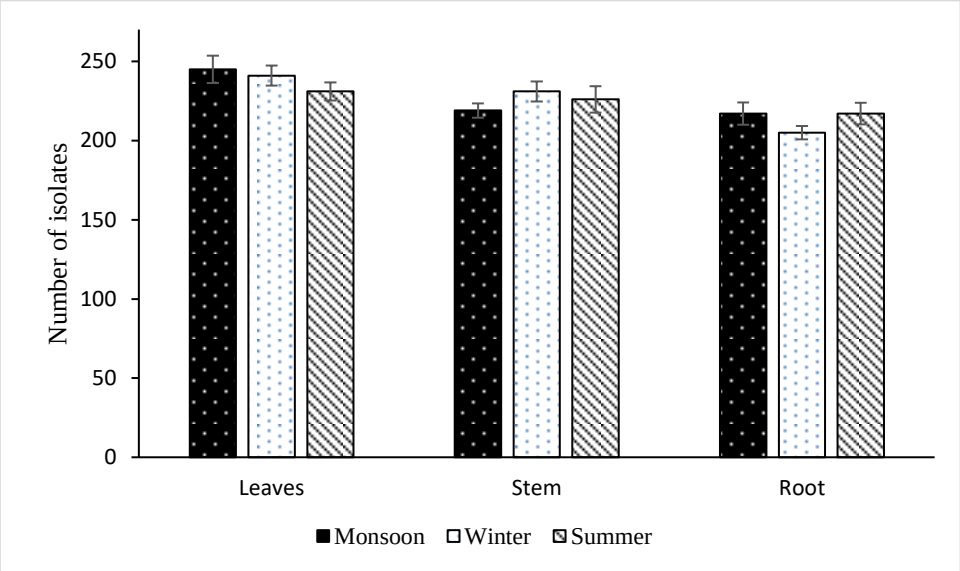

Fig. 4.8: Seasonal distribution of endophytic fungi in mangrove plant parts.

The study also revealed the prevalence of similar isolates in two different plant species. Sorenson's similarity coefficient was calculated to understand the occurrence of similar endophytic isolates within two plant species. The results indicate the presence of similar endophytic isolates within two mangrove plant species. *Av. marina* and *Av. officinalis* recorded the highest similarity coefficient of 0.7% during the winter and summer seasons, with 0.5% similar isolates identified during the monsoon season. Similarly, *Av. officinalis* and *Ac. ilicifolius* recorded 0.6% similarity during winter and summer and 0.52% similarity during the monsoon season. Mangrove associates *Ac. ilicifolius* and *D. heterophylla* recorded 0.6% similarity in all three seasons.

Similarly, *D. heterophylla* and *Cl. inermis* reported 0.5% similar isolates during summer and monsoon. *Clerodendron inermis* and *R. apiculata* recorded 0.6% similarity in all three

seasons. *Rhizophora apiculata* and *R. mucronata* recorded a similarity percentage between 0.6% to 0.65%. *R. mucronata* and *E. agallocha* recorded 0.5% similarity during winter and monsoon. *Excoecaria agallocha* and *B. cylindrica* revealed a similarity percentage of 0.4% during all three seasons. *Brugueira cylindrica* and *Ce. tagal* recorded a similarity of 0.5% during winter and monsoon seasons. *C. tagal* and *Acr. aurum* recorded a similarity of 0.5%. Similarly, 0.5% similarity was observed between *Acr. aurum* and *Ae. corniculatum*. True mangroves *viz.*, *Ae. corniculatum* and *Av. marina* recorded 0.48% similarity during winter and summer. The highest recorded similarity percentage was observed between *Ae. corniculatum* and *A. marina* during the monsoon season (**Fig. 4.9**). The results indicate that the mangrove ecosystem has some common microflora that resides within the biota and may benefit the plant system.

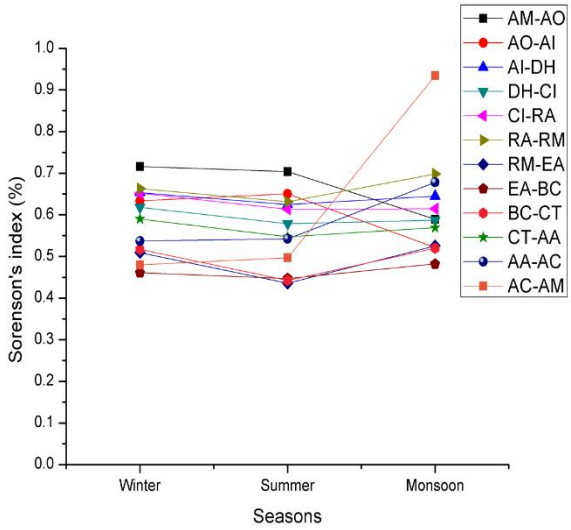

Fig. 4.9: Seasonal variation in endophytic fungal community.

4.3.3: Age-wise distribution of the endophytic fungi

In all, 35 fungal isolates were recovered from different stages of the leaves. *Ceriops tagal* and *Av. marina* hosted the highest number of colony-forming units. *Phoma* sp. and *Pestalotiopsis* spp. were the most frequently isolated, while only *Fusarium* spp. was found in association with the two host plants. The results indicated that the colonization was highest at stage 4. Besides, the fungal colonies which appeared at the early leaf stages were also recovered from the litter or degrading leaves, indicating that these fungi assist in litter degradation (**Table 4.1**).

4.3.4: Characterization of endophytic fungi

Incubation of the mangrove plant parts on PDA media resulted in the isolation of different groups of endophytic fungi. From the 13 mangrove plant species, 284 fungal colonies belonging to 33 genera were recovered (**Table 4.2, 4.3, and 4.4**). The characterization of the fungal endophytes recorded the dominance of Ascomycetes. Of the 284 fungal isolates, 31 isolates failed to sporulate even after prolonged incubation on different media. *Aspergillus* sp. was the most dominant fungal endophyte forming 831 CFU. Species belonging to *Pestalotiopsis*, *Fusarium*, *Cladosporium*, and *Drechslera* were the most representative genera (**Table 4.5**). Few isolates were labeled as unidentified species as it was difficult to characterize them taxonomically. Non-sporulating sterile isolates were categorized as sterile mycelia and distinguished based on colony characters. Sterile mycelial cultures were subsequently sub cultured and stored in sterile glass vials.

Table 4.1: Density of colonization of endophytes isolated from different leaf stages of mangrove plant species.

Plant species	Leaf stages/fungal species				Total No. of fungal isolates	Dominant species
	Stage 1	Stage 2	Stage 3	Stage 4		
Ce. tagal	0	2	3	4	9	*Phoma* sp.
Av. marina	2	2	4	1	9	*Aspergillus* sp.
Br. cylindrica	1	1	3	3	8	*Cladosporium* sp.
E. agallocha	1	1	1	2	5	*Fusarium* sp.
S. alba	1	1	1	1	4	*Phoma* sp. *Pestalotiopsis* sp.

Legend: Stage 1: tender leaf stage; stage 2: mature leaf stage; stage 3: senescence stage; stage 4: litter/dead leaf.

The photomicrographs of the sporulating fungal species are depicted in **Plate 4.2, Plate 4.3, Plate 4.4 and Plate 4.5**. Among the sterile mycelia cultures, seven cultures were frequently recovered. These non-sporulating sterile isolates were characterized based on the nucleotide sequencing technique at its rDNA region using ITS1 and ITS4 primers. The fungal rDNA sequence was matched with the existing NCBI database using BLAST search. The sequence length (base pairs) and identity (%) along the closest blast match is depicted in **Table 4.6**. The phylogenetic tree was constructed by using the maximum likelihood method. The MEGA 7.0 sequence alignment tool was used to align the sequences (**Fig. 4.10, 4.11, 4.12, 4.13, 4.14, and 4.15**).

Table 4.2: Seasonal variation of endophytic fungal colonies isolated from mangrove plants during monsoon season.

Fungal species	Isolate Code	Host plant													Total Isolates
		AM	AO	AI	DH	CI	RA	RM	EA	BC	CT	AA	AC	SA	
Setosphaeria monoceras	MEn 01	1^{a}	-	-	-	-	1^{b}	-	-	1^{c}	-	-	1^{a}	1^{a}	5^{abc}
Aspergillus sp.1	MEn 02	1^{a}	1^{a}	-	-	1^{b}	-	1^{b}	-	1^{b}	-	1^{a}	1^{b}	-	7^{ab}
Scolecobasidium sp.1	MEn 03	1^{b}	1^{a}	1^{b}	-	1^{b}	1^{c}	1^{c}	-	1^{b}	-	1^{b}	1^{c}	-	9^{abc}
Drechslera sp.1	MEn 04	1^{a}	-	1^{b}	-	1^{a}	-	1^{a}	-	1^{a}	-	1^{a}	1^{b}	-	7^{ab}
Fusarium sp.1	MEn 05	1^{c}	-	1^{c}	1^{b}	-	1^{c}	-	-	-	1^{b}	1^{b}	1^{c}	-	7^{bc}
Pestalotiopsis sp.1	MEn 06	1^{a}	-	1^{c}	-	1^{a}	-	1^{c}	1^{c}	-	1^{a}	-	1^{a}	1^{a}	8^{ac}
Scytalidium lignicola	MEn 07	-	1^{a}	-	-	-	-	-	-	1^{a}	-	-	-	1^{a}	3^{a}
Penicillium sp.1	MEn 08	1^{a}	-	-	-	1^{b}	-	-	-	-	-	1^{c}	1^{a}	1^{a}	5^{abc}
Fusarium sacchari	MEn 09	-	1^{b}	1^{a}	1^{a}	-	1^{a}	1^{c}	-	1^{b}	1^{a}	1^{a}	-	1^{a}	9^{abc}
Aspergillus sp.2	MEn 10	1^{b}	1^{c}	-	-	-	-	-	1^{a}	1^{a}	-	-	1^{a}	1^{a}	6^{abc}
Penicillium sp. 2	MEn 11	1^{b}	1^{b}	1^{b}	1^{a}	-	1^{a}	1^{a}	-	1^{b}	1^{b}	1^{a}	1^{a}	1^{a}	11^{ab}
Corynesporina elegans	MEn 12	1^{a}	1^{a}	1^{b}	1^{a}	1^{b}	-	1^{c}	1^{c}	1^{a}	1^{c}	-	1^{c}	-	10^{abc}
Drechslera sp.2	MEn 13	1^{a}	1^{c}	1^{c}	1^{c}	-	1^{b}	1^{b}	1^{c}	1^{c}	1^{c}	1^{b}	1^{a}	-	11^{abc}
Scytalidium lignicola	MEn 14	1^{b}	-	1^{b}	1^{b}	1^{c}	1^{c}	1^{b}	1^{c}	-	1^{c}	1^{c}	1^{b}	-	10^{bc}
Sterile mycelia 1	MEn 15	-	1^{c}	1^{c}	1^{c}	1^{c}	1^{c}	1^{a}	1^{a}	1^{c}	1^{a}	1^{a}	-	1^{ac}	11^{ac}
Corynespora sp.1	MEn 16	1^{a}	-	-	-	1^{a}	1^{b}	-	1^{b}	-	-	1^{a}	1^{a}	1^{b}	7^{ab}
Gilmaniella sp.1	MEn 17	-	1^{c}	1^{c}	1^{b}	1^{c}	1^{c}	1^{b}	-	1^{b}	1^{c}	1^{b}	-	-	9^{bc}
Fusarium sp.2	MEn 18	1^{a}	1^{b}	-	-	1^{a}	-	-	1^{c}	1^{a}	-	-	1^{c}	-	6^{ac}
Drechslera sp. 3	MEn 19	1^{b}	1^{c}	1^{c}	1^{c}	-	1^{b}	1^{a}	-	1^{a}	1^{a}	1^{b}	1^{c}	-	10^{abc}
Drechslera sp. 4	MEn 20	1^{b}	1^{c}	1^{b}	1^{b}	1^{b}	-	1^{c}	1^{c}	1^{c}	1^{b}	-	1^{c}	-	10^{bc}
Pestalotiopsis sp. 2	MEn 21	1^{b}	-	1^{a}	1^{a}	-	1^{c}	1^{b}	1^{a}	-	1^{a}	1^{b}	1^{a}	-	9^{abc}
Sterile mycelia 2	MEn 22	-	1^{a}	1^{b}	1^{a}	1^{a}	1^{a}	1^{b}	1^{c}	1^{b}	1^{a}	1^{a}	-	1^{a}	11^{abc}
Junctospora pulchra	MEn 23	1^{b}	-	-	-	1^{b}	1^{c}	-	1^{c}	-	-	1^{b}	1^{c}	-	6^{bc}
Curvularia sp. 1	MEn 24	-	1^{a}	1^{b}	1^{a}	1^{a}	1^{c}	1^{a}	-	1^{b}	1^{c}	1^{a}	-	-	9^{abc}
Scytalidium sp.1	MEn 25	1^{a}	1^{b}	-	-	1^{c}	-	-	1^{a}	1^{b}	1^{a}	-	1^{c}	-	7^{abv}
Setosphaeria sp.1	MEn 26	-	1^{a}	-	1^{a}	-	-	-	-	1^{c}	1^{a}	1^{a}	-	1^{c}	6^{ac}

Aspergillus niger	MEn 27	1^{a}	1^{b}	1^{c}	-	1^{c}	1^{a}	1^{b}	1^{a}	1^{b}	1^{c}	-	1^{a}	-	10^{abc}
Drechslera sp.4	MEn 28	-	1^{a}	-	1^{b}	-	-	-	-	1^{a}	1^{b}	1^{a}	-	-	5^{ab}
Scolecobasidium sp.2	MEn 29	1^{a}	-	1^{c}	1^{c}	-	1^{a}	1^{a}	1^{a}	-	-	-	1^{c}	-	7^{ac}
Junctospora pulchra	MEn 30	-	1^{b}	1^{b}	1^{c}	-	1^{b}	1^{c}	1^{b}	1^{c}	1^{b}	1^{b}	-	-	9^{bc}
Fusarium sp.3	MEn 31	-	-	1^{b}	1^{b}	-	1^{c}	1^{b}	1^{b}	-	-	1^{c}	-	-	6^{bc}
Cladosporium sp.1	MEn 32	-	1^{b}	1^{a}	-	-	1^{c}	1^{a}	1^{a}	1^{a}	1^{a}	1^{a}	-	1^{c}	9^{abc}
Myceliophthora sp.1	MEn 33	1^{b}	1^{b}	1^{c}	1^{c}	-	1^{b}	1^{b}	1^{b}	1^{c}	1^{a}	1^{a}	1^{a}	-	11^{abc}
Penicillium sp.3	MEn 34	-	1^{c}	-	-	-	-	-	-	1^{c}	1^{c}	1^{c}	-	1^{c}	5^{c}
Sterile Mycelia 3	MEn 35	1^{a}	1^{b}	1^{c}	1^{c}	1^{a}	1^{a}	1^{b}	1^{b}	1^{c}	1^{a}	-	1^{c}	1^{bc}	12^{abc}
Nigrospora sphaerica	MEn 36	1^{a}	1^{c}	1^{a}	1^{b}	1^{c}	1^{c}	1^{a}	1^{a}	1^{b}	1^{b}	1^{a}	1^{a}	1^{c}	13^{abc}
Cladosporium sp.2	MEn 37	1^{b}	1^{a}	1^{a}	1^{a}	1^{b}	1^{b}	1^{b}	1^{a}	1^{a}	1^{b}	-	1^{a}	1^{ac}	12^{ab}
Seatospheria monoceras	MEn 38	1^{a}	-	1^{b}	1^{b}	1^{a}	1^{a}	1^{a}	1^{a}	-	-	1^{b}	1^{b}	1^{b}	10^{ab}
Scolecobasidium sp.3	MEn 39	1^{c}	1^{a}	1^{c}	1^{c}	1^{b}	1^{a}	1^{b}	1^{a}	-	1^{b}	1^{c}	1^{a}	1^{c}	12^{abc}
Penicillium sp.4	MEn 40	1^{a}	-	1^{c}	-	1^{c}	1^{c}	1^{c}	1^{a}	-	1^{a}	1^{a}	1^{c}	1^{c}	10^{ac}
Aspergillus niger	MEn 41	-	1^{c}	-	1^{c}	1^{c}	-	-	-	1^{a}	1^{b}	1^{a}	-	1^{b}	7^{abc}
Pestalotiopsis sp.3	MEn 42	1^{a}	1^{c}	1^{a}	-	-	1^{a}	1^{a}	1^{c}	-	1^{a}	-	1^{b}	1^{b}	9^{abc}
Gonatobotryum sp.1	MEn 43	-	1^{b}	1^{b}	1^{c}	1^{b}	1^{c}	1^{c}	-	1^{b}	1^{b}	1^{c}	-	1^{c}	10^{bc}
Aspergillus fumigates	MEn 44	1^{a}	1^{a}	1^{b}	1^{a}	-	1^{a}	1^{a}	1^{a}	1^{b}	-	-	1^{a}	1^{a}	10^{ab}
Pestalotiopsis microspore	MEn 45	-	-	-	1^{c}	1^{a}	1^{a}	1^{a}	-	1^{c}	1^{a}	1^{a}	-	1^{c}	8^{ac}
Pestalotiopsis sp.4	MEn 46	1^{b}	1^{a}	1^{a}	1^{b}	1^{a}	-	1^{a}	1^{b}	1^{a}	-	1^{b}	1^{a}	1^{ac}	11^{ab}
Aspergillus flavus	MEn 47	1^{a}	-	-	-	1^{b}	1^{a}	-	1^{a}	1^{b}	1^{c}	1^{a}	1^{b}	1^{c}	9^{abc}
Fusarium incarnatum	MEn 48	1^{b}	1^{a}	1^{c}	1^{b}	1^{a}	-	1^{a}	1^{a}	-	1^{b}	1^{c}	1^{b}	1^{c}	11^{abc}
Unidentified 1	MEn 49	1^{a}	1^{a}	1^{c}	-	-	1^{b}	-	1^{a}	1^{a}	1^{a}	1^{a}	1^{a}	1^{bc}	10^{abc}
Aspergillus sp.3	MEn 50	-	1^{a}	1^{b}	1^{b}	-	1^{c}	1^{a}	1^{a}	-	1^{a}	-	-	1^{b}	8^{abc}
Unidentified 2	MEn 51	1^{a}	1^{b}	1^{c}	1^{a}	-	1^{c}	1^{c}	-	1^{b}	-	1^{a}	1^{c}	1^{a}	10^{abc}
Fungal endophyte	MEn 52	-	1^{c}	1^{a}	1^{a}	-	1^{a}	1^{c}	1^{a}	1^{a}	1^{c}	-	-	1^{c}	9^{ac}
Thermomyces sp.1	MEn 53	1^{a}	-	-	1^{c}	-	-	1^{c}	-	1^{a}	-	1^{c}	1^{c}	1^{c}	7^{ac}
Colletotrichum sp1.	MEn 54	1^{c}	1^{c}	1^{a}	1^{a}	1^{b}	1^{b}	1^{a}	1^{b}	1^{a}	1^{a}	1^{a}	1^{b}	1^{c}	13^{abc}
Unidentified 3	MEn 55	1^{a}	-	-	-	-	-	-	1^{a}	-	1^{a}	1^{a}	1^{a}	1^{a}	6^{a}
Aspergillus sp.4	MEn 56	-	1^{c}	1^{c}	1^{c}	1^{a}	1^{c}	1^{c}	1^{a}	-	1^{c}	1^{c}	-	-	9^{ac}

Acremonium sp.1	MEn 57	1^{b}	1^{a}	1^{a}	-	1^{a}	1^{a}	-	1^{a}	1^{c}	1^{b}	-	1^{a}	-	9^{abc}
Colletotrichum gloeosporioides	MEn 58	1^{b}	1^{a}	1^{c}	1^{c}	1^{b}	1^{b}	1^{a}	-	-	1^{a}	1^{c}	1^{b}	-	10^{abc}
Sordariomycetes sp.1	MEn 59	1^{b}	1^{b}	1^{c}	1^{b}	1^{b}	1^{b}	1^{c}	1^{b}	1^{b}	1^{c}	-	1^{b}	-	11^{bc}
Rhizopus sp.1	MEn 60	1^{b}	-	-	1^{b}	1^{b}	1^{b}	1^{a}	1^{b}	1^{b}	1^{a}	1^{a}	1^{a}	-	10^{ab}
Unidentified 4	MEn 61	1^{c}	-	1^{c}	1^{c}	1^{c}	-	1^{a}	1^{c}	1^{b}	-	1^{c}	1^{c}	-	9^{abc}
Unidentified 5	MEn 62	1^{c}	-	1^{c}	-	1^{c}	1^{a}	-	-	1^{b}	1^{c}	1^{b}	1^{a}	-	8^{abc}
Pestalotiopsis sp.5	MEn 63	1^{c}	1^{b}	1^{a}	1^{a}	-	-	1^{c}	1^{b}	1^{b}	1^{c}	1^{b}	1^{a}	-	10^{abc}
Colletotrichum sp.2	MEn 64	1^{b}	-	1^{b}	-	1^{c}	1^{c}	1^{c}	-	-	1^{a}	1^{a}	1^{a}	-	8^{abc}
Aspergillus flavus	MEn 65	1^{b}	1^{b}	1^{a}	1^{a}	1^{a}	1^{a}	1^{c}	1^{c}	1^{b}	1^{a}	-	1^{b}	1^{b}	12^{abc}
Fusarium equiseti	MEn 66	1^{c}	1^{a}	-	1^{a}	1^{a}	1^{c}	1^{c}	1^{c}	-	-	1^{a}	1^{c}	1^{b}	10^{ac}
Curvularia sp.2	MEn 67	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}	1^{a}	-	1^{b}	-	11^{ab}
Scytalidium lignicola	MEn 68	1^{c}	1^{b}	-	1^{a}	-	-	-	1^{c}	1^{b}	-	1^{a}	1^{a}	1^{b}	8^{abc}
Drechslera sp.5	MEn 69	1^{a}	1^{a}	1^{a}	1^{c}	1^{c}	1^{b}	1^{b}	-	1^{c}	1^{a}	1^{a}	1^{a}	-	11^{abc}
Pestalotiopsis sp.6	MEn 70	1^{a}	-	1^{c}	-	-	-	-	1^{a}	1^{a}	1^{c}	1^{c}	1^{a}	1^{b}	8^{ac}
Pestalotiopsis sp.7	MEn 71	1^{c}	1^{a}	1^{a}	1^{b}	1^{c}	1^{b}	1^{a}	-	-	1^{c}	1^{b}	1^{a}	1^{b}	11^{abc}
Ceratocystis sp.1	MEn 72	1^{b}	-	1^{a}	-	1^{a}	1^{a}	1^{a}	1^{b}	1^{a}	1^{b}	-	1^{a}	-	9^{ab}
Fusarium sp.4	MEn 73	1^{b}	1^{b}	1^{c}	1^{c}	1^{c}	1^{c}	1^{c}	1^{b}	-	1^{b}	1^{c}	1^{c}	1^{c}	12^{bc}
Fusarium sp.5	MEn 74	1^{c}	1^{b}	-	1^{a}	1^{a}	1^{b}	1^{b}	1^{b}	1^{a}	-	-	1^{a}	-	9^{abc}
Aspergillus sp.5	MEn 75	1^{a}	1^{a}	1^{a}	1^{c}	1^{a}	1^{b}	1^{c}	1^{c}	1^{b}	1^{a}	1^{b}	1^{c}	1^{a}	13^{abc}
Dreschera sp.6	MEn 76	-	1^{a}	-	1^{c}	-	-	-	1^{b}	1^{b}	-	1^{a}	-	-	5^{abc}
Alternaria alternata	MEn 77	-	-	1^{b}	-	1^{a}	1^{c}	1^{a}	-	1^{a}	1^{a}	1^{b}	-	1^{b}	8^{abc}
Bipolaris sp.1	MEn 78	-	1^{c}	1^{a}	1^{a}	-	-	-	1^{c}	1^{b}	1^{a}	1^{a}	-	-	7^{abc}
Alternaria sp.1	MEn 79	-	-	1^{b}	-	1^{b}	1^{c}	1^{c}	-	-	1^{b}	1^{b}	-	1^{b}	7^{bc}
Corynespora sp.2	MEn 80	-	1^{a}	1^{a}	1^{b}	1^{a}	1^{a}	1^{c}	1^{b}	1^{a}	1^{a}	-	-	-	9^{abc}
Alternaria sp.2	MEn 81	1^{a}	1^{a}	-	1^{a}	1^{a}	1^{a}	1^{a}	1^{a}	-	-	1^{a}	1^{a}	1^{a}	10^{a}
Sterile Mycelia 4	MEn 82	1^{b}	1^{b}	1^{c}	1^{c}	1^{c}	1^{c}	1^{b}	1^{b}	1^{b}	1^{c}	-	1^{b}		11^{bc}
Pestalotiopsis sp.8	MEn 83	1^{c}	1^{a}	-	1^{a}	-	-	-	1^{a}	1^{c}	-	1^{a}	1^{a}		7^{ac}
Alternaria sp.3	MEn 84	1^{a}	1^{b}	1^{b}	1^{b}	1^{b}	-	-	-	1^{b}	1^{a}	1^{a}	1^{a}		9^{ab}
Nigrospora sp.1	MEn 85	1^{c}	-	1^{c}	-	-	1^{c}	-	-	1^{c}	1^{c}	1^{c}	1^{c}		7^{c}
Nigrosopra sp.2	MEn 86	1^{a}	1^{a}	1^{a}	1^{b}	1^{a}	-	1^{b}	-	-	1^{c}	1^{c}	1^{a}		9^{abc}

Alternaria alternata	MEn 87	-	-	1[c]	-	1[b]	1[a]	-	-	-	1[a]	-	-	1[b]	5[abc]
Cladosporium sp.3	MEn 88	-	1[b]	1[a]	1[a]	1[b]	1[b]	1[b]	1[b]	-	-	-	-	1[b]	8[ab]
Cladosporium sp.4	MEn 89	-	1[c]	-	1[c]	1[c]	1[a]	1[a]	-	1[c]	-	-	-	-	6[ac]
Sterile Mycelia 5	MEn 90	1[c]	1[c]	1[c]	1[c]	1[a]	1[b]	1[b]	1[a]	-	-	-	1[a]	1[b]	10[abc]
Aspergillus ulvus	MEn 91	-	1[c]	-	1[b]	-	1[b]	1[a]	1[b]	1[a]	-	-	-	-	6[abc]
Cladosporium sp.5	MEn 92	1[b]	-	1[b]	-	1[b]	-	1[b]	1[b]	1[b]	-	-	1[b]	1[b]	8[b]
Dreschera sp.7	MEn 93	-	-	1[a]	-	-	1[a]	-	1[a]	1[c]	-	-	-	1[b]	5[ac]
Gonatobotryum sp.2	MEn 94	1[c]	-	1[b]	-	1[b]	-	1[b]	1[c]	1[c]	-	-	1[b]	-	7[bc]
Cladosporium sp.6	MEn 95	-	1[c]	1[b]	1[a]	1[a]	1[b]	-	-	1[a]	-	1[c]	-	1[c]	8[abc]
Fusarium sp. 6	MEn 96	1[b]	-	-	-	1[a]	1[b]	1[b]	1[b]	-	1[a]	-	1[b]	-	7[ab]
Fusarium semitectum	MEn 97	-	1[b]	1[b]	1[b]	1[b]	1[b]	1[b]	-	1[b]	-	1[b]	-	1[a]	9[b]
Penicillium sp.5	MEn 98	1[a]	1[c]	-	1[c]	-	1[c]	1[c]	1[a]	-	1[a]	1[a]	1[a]	-	9[ac]
Pestalotiopsis sp.9	MEn 99	-	1[a]	1[a]	1[b]	1[c]	-	1[a]	1[c]	1[b]	1[a]	1[b]	-	1[b]	10[abc]
Cladosporium sp.7	MEn 100	1[a]	1[c]	1[a]	1[c]	-	-	-	1[a]	1[c]	1[a]	1[a]	1[c]	-	9[ac]
Marieltiottia sp.1	MEn 101	-	1[c]	1[a]	1[a]	1[a]	-	-	1[c]	1[c]	1[a]	1[a]	-	1[b]	9[ac]
Pestalotiopsis sp.10	MEn 102	1[a]	-	1[a]	-	1[c]	-	-	-	1[b]	1[a]	-	1[a]	-	6[abc]
Nigrospora sp.3	MEn 103	-	1[a]	1[c]	1[b]	1[c]	1[b]	-	-	-	-	1a	-	1[a]	7[abc]
Fusarium sp.7	MEn 104	1[a]	-	-	-	1[b]	-	1[a]	-	1[a]	1[a]	-	1[b]		6[ab]
Aspergillus sp.6	MEn 105	1[a]	1[a]	1[a]	1[a]	1[c]	1[c]	-	-	-	-	1[a]	1[c]	1[b]	9[ac]
Nigrospora sp.4	MEn 106	-	1[c]	-	1[a]	-	1[a]	1[a]	1[a]	1[a]	1[b]	1[a]	-	1[b]	9a[bc]
Fusarium sp.8	MEn 107	1[a]	1[c]	1[c]	1[c]	1[b]	1[a]	1[a]	-	1[a]	1[a]	1[b]	1[a]	-	11[abc]
Pestalotiopsis sp.11	MEn 108	1[c]	1[a]	1[a]	1[a]	-	1[c]	1[b]	1[a]	1[a]	1[c]	1[b]	1[b]	1[b]	12[abc]
Phoma sp.1	MEn 109	-	-	1[a]	-	1[b]	1[a]	1[c]	1[a]	1[a]	1[a]	-	-	-	7[abc]
Aspergillus sp.7	MEn 110	1[a]	1[a]	1[a]	1[a]	1[a]	-	1[a]	1[a]	1[a]	-	-	1[a]	1[b]	10[a]
Pestalotiopsis sp.12	MEn 111	1[a]	-	-	-	1[b]	1[a]	-	1[a]	-	-	-	1[b]	1[b]	6[ab]
Gliocladium sp.1	MEn 112	-	1[a]	1[b]	1[a]	1[b]	-	1[b]	1[b]	1[b]	1[b]	-	-	-	8[ab]
Bipolaris sp.7	MEn 113	1[c]	1[a]	-	1[c]	-	1[c]	-	-	-	-	-	1[a]	1[c]	6[ac]
Aspergillus sp.8	MEn 114	1[a]	1[a]	1[a]	1[a]	-	1[a]	1[a]	1[a]	1[a]	1[a]		1[a]	-	10[a]
Penicillium sp.6	MEn 115	-	1[c]	1[c]	1[a]	-	1[c]	1[a]	-	1[a]	1[b]	-	-	1[a]	8[abc]
Sterile Mycelia 6	MEn 116	-	1[b]	1[b]	1[c]	-	1[c]	1[b]	1[c]	1[c]	1[a]	1[a]	-	-	9[abc]
Fusarium sp. 9	MEn 117	-	-	1[a]	-	-	-	1[a]	1[c]	1[c]	1[a]	-	-	1[b]	6[ac]

Thickened chlamydospores 1	MEn 118	-	1^{b}	1^{b}	1^{b}	-	1^{b}	-	1^{b}	-	1^{b}	1^{b}	-	-	7^{b}
Nigrospora sp.5	MEn 119	1^{a}	-	-	-	-	-	1^{a}	1^{c}	1^{b}	1^{a}	1^{c}	1^{a}	1^{b}	8^{abc}
Nigrospora sp.6	MEn 120	1^{a}	1^{a}	1^{a}	1^{b}	-	1^{b}	-	-	-	1^{b}	1^{b}	1^{b}	-	8^{ab}
Scolecobasidium sp.4	MEn 121	-	1^{c}	-	1^{c}	1^{b}	1^{a}	1^{b}	1^{b}	1^{a}	-	1^{a}	-	1^{a}	9^{abc}
Sterile Mycelia 7	MEn 122	1^{c}	1^{c}	1^{c}	1^{a}	-	1^{a}	1^{a}	-	1^{c}	1^{a}	1^{a}	1^{b}		10^{abc}
Pestalotiopsis sp.13	MEn 123	-	1^{b}	1^{b}	1^{a}	1^{a}	1^{a}	1^{c}	1^{c}	1^{c}	1^{b}	-	-		9^{abc}
Scytalidium sp.1	MEn 124	1^{a}	-	1^{a}		1^{b}	1^{a}	1^{b}	1^{c}	1^{c}	1^{a}	1^{a}	1^{a}	1^{c}	11^{abc}
Gilmaniella sp.2	MEn 125	-	-	1^{c}	-	1^{c}	-	1^{a}	1^{a}	1^{c}	1^{a}	-	-	1^{a}	7^{ac}
Rhizopus sp.2	MEn 126	1^{b}	-	-	-	1^{b}	1^{b}	-	1^{c}	-	-	1^{b}	1^{b}	1^{c}	7^{bc}
Sterile Mycelia 8	MEn 127	-	-	1^{c}	-	1^{c}	-	1^{c}	1^{c}	1^{b}	1^{c}	1^{c}	-	1^{c}	8^{bc}
Aspergillus flavus	MEn 128	1^{a}	-	-	-	-	1^{a}	-	-	-	-	1^{a}	1^{a}	-	4^{a}
Penicillium sp.7	MEn 129	-	1^{a}	1^{c}	1^{a}	1^{a}	1^{c}	1^{c}	1^{b}	1^{b}	1^{b}	1^{b}	-	1^{b}	11^{abc}
Pestalotiopsis sp.14	MEn 130	1^{b}	-	1^{b}	-	-	1^{b}	1^{b}	-	1^{b}	1^{b}	-	1^{b}	-	7^{b}
Humicola sp.1	MEn 131	-	1^{b}	1^{a}	1^{a}	1^{b}	1^{c}	1^{c}	1^{c}	1^{c}	1^{a}	1^{a}	-	1^{c}	11^{abc}
Linkosia sp.1	MEn 132	1^{a}	1^{a}	1^{c}	1^{a}	1^{b}	-	1^{b}	1^{a}	1^{a}	1^{a}	-	1^{b}	1^{a}	11^{abc}
Aspergillus niger	MEn 133	1^{b}	1^{a}	1^{c}	1^{a}	1^{a}	1^{c}	-	1^{a}	-	1^{a}	1^{b}	1^{b}	1^{a}	11^{abc}
Juctospora sp.1	MEn 134	-	1^{c}	-	1^{c}	1^{c}	-	1^{a}	1^{b}	1^{a}	-	1^{a}	-	1^{b}	8^{abc}
Cladosporium sp.8	MEn 135	1^{a}	1^{c}	1^{b}	1^{a}	-	1^{c}	1^{b}	-	1^{a}	1^{a}	1^{b}	1^{b}	1^{b}	11^{abc}
Rhizopus sp.3	MEn 136	-	-	-	-	-	-	1^{a}	1^{a}	-	-	1^{b}	-	1^{a}	4^{ab}
Cladosporium sp.9	MEn 137	1^{a}	1^{b}	^{1a}	1^{b}	-	1^{b}	1^{b}	-	1^{b}	1^{a}	1^{a}	1^{b}	1^{b}	11^{ab}
Pestalotiopsis sp.15	MEn 138	1^{b}	-	1^{a}	-	-	1^{c}	1^{c}	-	1^{b}	1^{a}	-	1^{b}	1^{b}	8^{abc}
Pestalotiopsis sp.16	MEn 139	-	1^{b}	1^{b}	1^{c}	1^{a}	1^{a}	-	1^{a}	1^{c}	1^{b}	1^{a}	-	1^{a}	10^{abc}
Aspergillus niger	MEn 140	1^{a}	1^{a}	1^{a}	1^{a}	-	1^{a}	1^{c}	-	1^{a}	1^{c}	-	1^{c}	1^{b}	10^{ac}
Cladosporium sp.10	MEn 141	-	1^{b}	-	1^{c}	1^{c}	1^{c}	-	1^{b}	1^{c}	-	1^{b}	-	1^{a}	10^{bc}
Rhizopus sp.4	MEn 142	1^{c}	1^{c}	1^{a}	1^{b}	1^{b}	-	1^{a}	-	-	1^{a}	1^{c}	1^{a}	1^{b}	10^{abc}
Dreschera sp.8	MEn 143	-	-	-	-	1^{a}	1^{b}	1^{b}	-	1^{c}	-	1^{b}	-	1^{b}	6^{abc}
Bipolaris sp.2	MEn 144	1^{a}	1^{a}	1^{c}	1^{c}	1^{c}	-	1^{a}	1^{b}	-	1^{a}	1^{a}	1^{b}	1^{a}	11^{abc}
Pestalotiopsis sp.17	MEn 145	-	-	-	-	1^{a}	1^{a}	1^{a}	-	1^{a}	1^{a}	-	-	1^{b}	6^{a}
Sterile Mycelia 9	MEn 146	1^{b}	1^{a}	1^{a}	1^{b}	-	1^{b}	-	1^{b}	1^{b}	1^{b}	1^{a}	1^{b}	1^{b}	11^{ab}
Dreschera sp.9	MEn 147	-	-	1^{a}	-	1^{c}	1^{b}	-	1^{c}	1^{c}	1^{a}	1^{c}	-	1^{b}	8^{abc}

Aspergillus sp.9	MEn 148	1^{a}	-	1^{c}	-	-	1^{a}	1^{a}	-	1^{b}	1^{b}	-	1^{a}	-	7^{abc}
Curvularia sp.3	MEn 149	-	1^{a}	1^{b}	1^{a}	1^{a}	-	-	1^{b}	-	-	1^{b}	-	1^{b}	7^{ab}
Penicillium sp.8	MEn 150	1^{a}	-	-	-	1^{b}	1^{a}	1^{c}	-	1^{b}	1^{a}	1^{a}	1^{a}	-	8^{abc}
Curvularia sp.4	MEn 151	-	1^{a}	1^{a}	1^{b}	1^{a}	-	-	1^{a}	-	-	1^{c}	-	1^{b}	7^{abc}
Cladosporium sp.11	MEn 152	1^{a}	1^{c}	-	1^{a}	1^{a}	1^{a}	-	-	1^{a}	1^{b}	1^{a}	1^{a}	1^{b}	10^{abc}
Pestalotiopsis sp.18	MEn 153	-	-	1^{a}	-	-	-	1^{c}	1^{a}	-	1^{a}	-	-	-	4^{ac}
Nigrospora sp.7	MEn 154	1^{b}	1^{c}	1^{a}	1^{b}	1^{a}	1^{c}	-	-	-	1^{b}	1^{a}	1^{b}	1^{c}	10^{abc}
Fusarium sp. 10	MEn 155	-	-	-	-	-	1^{a}	1^{a}	1^{a}	1^{b}	1^{a}	-	-	-	5^{ab}
Aspergillus sp.10	MEn 156	1^{a}	1^{c}	1^{a}	1^{a}	1^{c}	1^{c}	1^{c}	-	-	-	1^{a}	1^{c}	1^{a}	10^{ac}
Corynospora sp.4	MEn 157	-	-	-	-	-	1^{a}	-	1^{a}	1^{a}	1^{a}	1^{a}	-	-	5^{a}
Sordariomycetes sp.2	MEn 158	1^{a}	1^{a}	1^{a}	1^{b}	-	1^{c}	1^{b}	-	1^{a}	1^{a}	-	1^{b}	1^{b}	10^{abc}
Penicillium sp.9	MEn 159	-	-	1^{c}	-	1^{c}	-	-	1^{c}	-	1^{c}	1^{c}	-	-	5^{c}
Nigrospora sp.8	MEn 160	1^{b}	1^{b}	-	1^{b}	-	1^{a}	1^{a}	-	1^{b}	-	-	1^{a}	1^{b}	8^{ab}
Aureobasidium pullulans	MEn 161	-	-	1^{a}	-	1^{c}	-	-	1^{a}	-	1^{a}	1^{a}	-	-	5^{ac}
Pestalotiopsis sp.19	MEn 162	1^{a}	1^{a}	-	1^{a}	1^{b}	1^{b}	1^{b}	-	1^{a}	-	1^{a}	1^{b}	1^{a}	10^{ab}
Aspergillus niger	MEn 163	-	-	1^{b}	-	-	1^{a}	-	1^{c}	-	1^{c}	-	-		4^{abc}
Penicillium sp.10	MEn 164	1^{c}	1^{a}	-	1^{a}	1^{a}	1^{a}	1^{a}	-	1^{b}	-	1^{a}	1^{a}		9^{abc}
Sterile Mycelia 10	MEn 165	-	-	1^{a}	-	-	1^{c}	-	1^{a}	-	1^{c}	-	-	1^{a}	5^{ac}
Aspergillus sp.11	MEn 166	1^{a}	1^{a}	-	1^{b}	1^{c}	-	1^{a}	-	1^{a}	-	1^{a}	1^{b}	1^{b}	9^{abc}
Sterile Mycelia 11	MEn 167	-	-	1^{a}	-	-	1^{a}	-	1^{a}	-	1^{a}	-	-	-	4^{a}
Cladosporium sp.12	MEn 168	1^{b}	1^{a}	-	1^{a}	1^{a}	-	1^{c}	-	1^{a}	-	1^{b}	1^{c}	1^{c}	9^{abc}
Penicillium sp.11	MEn 169	-	-	1^{a}	-	-	1^{a}	-	1^{b}	-	1^{a}	-	-	-	4^{ab}
Sterile Mycelia 12	MEn 170	1^{a}	1^{a}	-	1^{a}	1^{a}	-	1^{c}	-	1^{a}	-	1^{a}	1^{c}	1^{a}	9^{ac}
Scolecobasidium sp.5	MEn 171	-	-	1^{c}	-	-	1^{a}	-	1^{a}	-	1^{b}	-	-	1^{c}	5^{abc}
Gliocladium sp.2	MEn 172	1^{a}	1^{a}	-	1^{b}	1^{a}	1^{b}	1^{c}	-	1^{c}	-	1^{a}	1^{b}	1^{c}	10^{abc}
Dreschera sp.10	MEn 173	-	-	1^{b}	-	-	1^{a}	-	1^{a}	-	1^{a}	-	-	1^{b}	5^{ab}
Phoma sp.2	MEn 174	1^{a}	1^{a}	-	1^{a}	1^{a}	1^{a}	1^{b}	-	1^{b}	-	1^{b}	1^{a}	1^{c}	10^{ab}
Cladosporium sp.13	MEn 175	-	-	1^{b}	-	-	1^{c}	-	1^{b}	-	1^{b}	-	-	-	4^{bc}
Ascospore 1	MEn 176	1^{a}	1^{a}	-	1^{a}	1^{a}	-	1^{a}	-	1^{a}	-	1^{c}	1^{a}	1^{b}	9^{ac}
Alternaria sp.4	MEn 177	-	-	1^{c}	-	-	1^{a}	-	1^{a}	-	1^{b}	-	-	-	4^{abc}
Scytalydium sp.2	MEn 178	1^{b}	1^{a}	-	1^{a}	1^{a}	-	1^{c}	-	1^{b}	-	1^{a}	1^{a}	1^{b}	9^{abc}

Corynospora sp.4	MEn 179	-	-	1^{b}	-	-	1^{b}	-	1^{a}	-	1^{a}	-	-	1^{a}	5^{ab}
Aspergillus sp.11	MEn 180	1^{b}	1^{b}	-	1^{b}	1^{b}	1^{b}	1^{b}	-	1^{b}	-	1^{b}	1^{a}	1^{b}	10^{ab}
Pestalotiopsis sp.18	MEn 181	-	-	1^{a}	-	-	1^{a}	-	1^{a}	-	1^{a}	-	-	1^{b}	5^{a}
Alternaria sp.5	MEn 182	1^{b}	1^{b}	-	1^{a}	1^{a}	1^{b}	1^{b}	-	1^{b}	-	1^{c}	1^{b}	-	9^{abc}
Sterile Mycelia 13	MEn 183	-	-	1^{c}	-	-	-	-	1^{c}	-	1^{c}	-	-	-	3^{c}
Nigrospora sp.9	MEn 184	1^{b}	1^{a}	-	1^{a}	1^{a}	1^{a}	1^{b}	-	1^{b}	-	1^{a}	1^{b}	-	9^{ab}
Sterile Mycelia 14	MEn 185	-	-	1^{a}	-	-	-	-	1^{c}	-	1^{a}	-	-	-	3^{ac}
Dreschera sp.11	MEn 186	1^{a}	1^{a}	-	1^{a}	1^{c}	1^{c}	1^{a}	-	1^{a}	-	1^{a}	1^{a}	-	9^{ac}
Limposoma sp.1	MEn 187	-	-	1^{b}	-	-	-	-	1^{b}	-	1^{b}	-	-	-	3^{b}
Sterile Mycelia 15	MEn 188	1^{b}	1^{b}	-	1^{c}	1^{c}	1^{c}	1^{c}	-	1^{b}	-	1^{c}	1^{b}	-	9^{bc}
Ascospores 2	MEn 189	-	-	1^{c}	-	-	1^{a}	-	1^{b}	-	1^{a}	-	-	1^{a}	4^{abc}
Pestalotiopsis sp.20	MEn 190	1^{a}	1^{c}	-	1^{a}	1^{a}	1^{a}	1^{b}	-	1^{c}	-	1^{c}	1^{c}	1^{b}	10^{abc}
Gilmaniella sp.2	MEn 191	-	-	1^{a}	-	-	1^{b}	-	1^{a}	-	1^{a}	-	-	-	4^{ab}
Nigrospora sp.10	MEn 192	1^{b}	1^{b}	-	1^{b}	1^{c}	1^{c}	1^{b}	-	1^{b}	-	1^{b}	1^{b}	1^{c}	10^{bc}
Juctospora pulchura	MEn 193	-	-	1^{c}	-	-	-	-	1^{b}	-	1^{a}	-	-	-	3^{abc}
Bipolaris sp.3	MEn 194	1^{a}	1^{c}	-	1^{a}	1^{a}	1^{c}	1^{a}	-	1^{c}	-	1^{a}	1^{a}	1^{a}	10^{ac}
Fusarium sp. 11	MEn 195	-	-	1^{a}	-	-	-	-	1^{b}	-	1^{a}	-	-	1^{c}	4^{ab}
Gilberella sp.2	MEn 196	1^{a}	1^{b}	-	1^{a}	1^{c}	1^{a}	1^{a}	-	1^{a}	-	1^{a}	1^{c}	1^{c}	10^{abc}
Phomopsis sp.1	MEn 197	-	-	1^{c}	-	-	1^{c}	-	-	-	1^{a}	-	-	1^{b}	4^{ac}
Pestalotiopsis sp.21	MEn 198	1^{b}	1^{a}	-	1^{a}	1^{a}	1^{c}	1^{b}	1^{a}	1^{a}	-	1^{a}	1^{b}	1^{c}	11^{abc}
Cladosporium sp.14	MEn 199	-	-	1^{a}	-	-	1^{a}	-	-	-	1^{a}	-	-	-	3^{a}
Aspergillus niger	MEn 200	1^{c}	1^{c}	-	1^{c}	1^{a}	-	1^{c}	1^{a}	1^{a}	-	1^{a}	1^{c}	1^{b}	10^{ac}
Penicillium sp.12	MEn 201	-	-	1^{c}	-	-	1^{b}	-	1^{a}	-	1^{a}	-	-	-	4^{abc}
Catenate spores 1	MEn 202	1^{c}	-	-	-	1^{a}	1^{b}	1^{b}	-	1^{a}	-	1^{c}	1^{a}	1^{b}	8^{abc}
Curvularia sp.5	MEn 203	-	1^{c}	1^{c}	-	-	1^{b}	-	1^{b}	-	1^{a}	-	-	1^{a}	6^{abc}
Curvularia sp.6	MEn 204	1^{c}	-	-	-	1^{b}	-	1^{a}	-	1^{b}	-	1^{a}	1^{a}	1^{b}	7^{abc}
Sterile Mycelia 16	MEn 205	-	1^{c}	1^{c}	-	-	1^{c}	-	1^{c}	-	1^{c}	-	-	1^{b}	6^{c}
Nigrospora sp.11	MEn 206	1^{b}	1^{c}	-	1^{a}	1^{a}	-	-	1^{a}	1^{b}	-	1^{a}	1^{a}	-	8^{abc}
Aspergillus sp.12	MEn 207	-	-	1^{c}	-	-	1^{a}	1^{b}	-	-	1^{a}	-	-	-	4^{abc}
Dreschera sp.12	MEn 208	1^{b}	1^{a}	-	1^{a}	1^{a}	-	-	1^{b}	-	-	1^{a}	1^{a}	-	7^{ab}
Phoma sp.3	MEn 209	-	-	-	1^{b}	-	1^{c}	1^{b}	-	1^{b}	-	-	-	-	4^{bc}

Colletotrichum sp.5	MEn 210	1^{a}	1^{b}	1^{b}	-	1^{b}	-	1^{a}	1^{b}	-	1^{a}	1^{a}	1^{a}	-	9^{ab}
Aspergillus sp.13	MEn 211	-	1^{c}	-	1^{c}	-	1^{a}	-	-	1^{c}	-	-	-	-	4^{ac}
Aspergillus sp.14	MEn 212	1^{b}	-	1^{a}	-	-	-	1^{c}	1a	1^{b}	1^{a}	-	1^{a}	-	7^{abc}
Nigrospora sp.12	MEn 213	1^{b}	1^{b}	1^{a}	1^{c}	1^{c}	1^{c}	-	-	-	1^{a}	1^{a}	1^{b}		9^{abc}
Sterile Mycelia 17	MEn 214	1^{a}	-	-	1^{c}	-	-	1^{c}	1^{a}	1^{a}	-	-	1^{a}		6^{ac}
Fusarium sp.12	MEn 215	1^{b}	1^{a}	1^{a}	-	1^{a}	1^{a}	1^{b}	-	-	1^{a}	1^{b}	1^{b}		9^{ab}
Pestalotiopsis sp.22	MEn 216	1^{c}	-	-	1^{a}	1^{b}	-	-	1^{b}	1^{c}	-	1^{a}	1^{c}		7^{abc}
Penicillium sp.13	MEn 217	1^{c}	1^{c}	1^{b}	-	-	1^{a}	1^{a}	-	1^{b}	1^{a}	1^{a}	1^{a}		9^{abc}
Pestalotiopsis sp.23	MEn 218	1^{a}	-	1^{a}	1^{a}	1^{a}	-	-	1^{a}	-	1^{a}	1^{a}	1^{a}	1^{a}	9^{a}
Corynospora sp.5	MEn 219	1^{b}	1^{b}	-	-	-	1^{b}	1^{a}	-	1^{a}	-	1^{a}	1^{b}	-	7^{ab}
Sterile Mycelia 18	MEn 220	1^{a}	-	1^{c}	1^{c}	1^{c}	-	-	1^{a}	-	1^{c}	1^{a}	1^{a}	1^{a}	9^{ac}
Sterile Mycelia 19	MEn 221	1^{c}	1^{c}	-	-	1^{b}	1^{c}	1^{c}	-	1^{c}	-	1^{c}	1^{b}	-	8^{bc}
Fusarium sp.13	MEn 222	1^{c}	-	1^{c}	1^{a}	-	-	-	1^{b}	-	1^{c}	-	1^{a}	1^{b}	7^{abc}
Scytalidium sp.3	MEn 223	1^{c}	1^{c}	-	-	1^{b}	1^{b}	1^{a}	-	1^{a}	-	1^{b}	1^{a}	-	8^{abc}
Dreschera sp.13	MEn 224	1^{a}	-	1^{b}	1^{c}	-	-	-	1^{c}	-	1^{b}	1^{a}	1^{b}	-	7^{abc}
Aspergillus sp.15	MEn 225	1^{b}	1^{b}	-	-	1^{a}	1^{c}	1^{c}	-	1^{c}	-	1^{a}	1^{b}	1^{c}	9^{abc}
Nigrospora sp.13	MEn 226	1^{a}	-	1^{b}	1^{b}	-	-	-	1^{a}	-	1^{c}	1^{a}	1^{a}	-	7^{abc}
Sterile Mycelia 20	MEn 227	1^{a}	1^{a}	-	-	1^{c}	1^{b}	1^{b}	-	1^{a}	-	-	1^{a}	1^{a}	8^{abc}
Sterile Mycelia 21	MEn 228	1^{a}	-	1^{b}	1^{b}	-	-	-	1^{c}	-	1^{a}	1^{b}	1^{b}	1^{b}	8^{abc}
Aspergillus sp.16	MEn 229	1^{a}	1^{c}	-	-	1^{c}	1^{b}	1^{b}	-	1^{a}	-	-	1^{c}	-	7^{abc}
Cladosporium sp.15	MEn 230	1^{c}	-	1^{b}	1^{b}	-	-	-	1^{b}	-	1^{a}	1^{a}	1^{b}	1^{a}	8^{abc}
Pestalotiopsis sp.24	MEn 231	1^{c}	1^{a}	-	-	1^{c}	1^{b}	1^{b}	-	1^{a}	-	-	1^{b}	-	7^{abc}
Curvularia sp.7	MEn 232	1^{c}	-	1^{c}	1^{a}	-	-	-	1^{b}	-	1^{a}	1^{a}	1^{a}	1^{c}	8^{abc}
Penicillium sp.14	MEn 233	1^{a}	1^{a}	-	-	1^{a}	1^{c}	1^{c}	-	1^{a}	-	1^{a}	1^{c}	1^{b}	9^{ac}
Nigrospora sp.14	MEn 234	1^{a}	-	1^{a}	1^{b}	-	-	-	1^{a}	-	1^{b}	1^{b}	1^{b}	-	7^{ab}
Aspergillus sp.16	MEn 235	1^{b}	1^{a}	-	-	1^{a}	1^{b}	1^{b}	-	1^{a}	-	1^{c}	1^{a}	1^{b}	9^{abc}
Penicillium sp.15	MEn 236	1^{a}	-	1^{c}	1^{a}	-	-	-	1^{c}	-	1^{a}	1^{b}	1^{b}	-	7^{abc}
Penicillium sp.16	MEn 237	1^{c}	1^{c}	-	-	1^{c}	1^{c}	1^{c}	-	1^{c}	-	-	1^{c}	1^{a}	8^{c}
Fusarium sp. 14	MEn 238	1^{c}	-	1^{c}	1^{c}	-	-	-	1^{c}	-	1^{c}	1^{c}	1^{c}	-	7^{c}
Fusarium sp. 15	MEn 239	-	1^{a}	-	-	1^{b}	1^{c}	1^{a}	-	1^{a}	-	-	-	1^{a}	6^{abc}
Phoma sp.4	MEn 240	1^{b}	-	1^{a}	1^{c}	-	-	-	1^{a}	-	1^{c}	1^{a}	1^{a}	1^{a}	8^{abc}

Phoma sp.5	MEn 241	1^{a}	1^{b}	-	-	1^{b}	1^{c}	1^{c}	-	1^{a}	-	1^{b}	1^{a}	1^{a}	9^{abc}
Aspergillus sp.17	MEn 242	1^{a}	-	^{1a}	1^{c}	-	-	-	1^{c}	-	1^{a}	1^{a}	1^{a}	1^{a}	8^{ac}
Fusarium sp.16	MEn 243	1^{b}	1^{a}	-	-	1^{c}	1^{c}	1^{a}	-	1^{b}	-	1^{a}	1^{a}	1^{a}	9^{abc}
Curvularia sp.8	MEn 244	1^{a}	-	1^{a}	1^{a}	-	-	-	1^{a}	-	1^{b}	-	1^{a}	1^{a}	7^{ab}
Fusarium sp.17	MEn 245	1^{c}	1^{c}	-	-	1^{c}	1^{c}	1^{a}	-	1^{b}	-	1^{a}	1^{a}	1^{a}	9^{abc}
Phoma sp.6	MEn 246	1^{a}	-	1^{a}	1^{c}	-	-	-	1^{a}	-	1^{a}	-	1^{a}	1^{a}	7^{ac}
Cladosporium sp.16	MEn 247	1^{a}	1^{a}	-	-	1^{a}	1^{a}	1^{b}	-	1^{a}	-	1^{b}	1^{a}	1^{a}	9^{ab}
Scolecobasidium sp.6	MEn 248	1^{a}	-	1^{b}	1^{c}	-	-	-	1^{a}	-	1^{a}	-	1^{a}	1^{b}	7^{abc}
Aspergillus sp.18	MEn 249	1^{b}	1^{b}	-	-	1^{b}	1^{a}	1^{a}	-	1^{c}	-	1^{a}	1^{b}	1^{b}	9^{abc}
Pestalotiopsis sp.25	MEn 250	1^{b}	-	1^{a}	1^{c}	-	-	-	1^{a}	-	1^{b}	1^{b}	1^{b}	1^{b}	8^{abc}
Fusarium sp.18	MEn 251	1^{c}	1^{c}	-	-	1^{a}	1^{b}	1^{a}	-	1^{a}	-	1^{c}	1^{b}	-	8^{abc}
Phomopsis sp.2	MEn 252	-	-	1^{b}	1^{a}	-	-	-	1^{c}	-	1^{c}	1^{b}	-	-	5^{abc}
Alternaria sp.6	MEn 253	-	1^{a}	-	-	1^{a}	-	1^{a}	-	1^{a}	-	1^{a}	-	-	5^{a}
Setosphaeria monoceras	MEn 254	-	-	1^{b}	1^{c}	-	1^{c}	-	1^{c}	-	1^{b}	-	-	-	5^{bc}
Nigrospora sp.15	MEn 255	-	1^{c}	-	-	1^{c}	-	1^{a}	-	1^{b}	-	1^{a}	-	-	5^{abc}
Ascospore 4	MEn 256	-	-	1^{b}	1^{c}	-	1^{a}	-	-	-	1^{a}	-	-	-	4^{abc}
Sterile Mycelia 22	MEn 257	-	1^{a}	-	-	1^{b}	1^{a}	1^{b}	-	1^{c}	-	1^{c}	-	-	6^{abc}
Sterile Mycelia 23	MEn 258	-	-	1^{a}	1^{c}	-	1^{c}	-	-	-	1^{a}	1^{a}	-	-	5^{ac}
Curvularia sp.9	MEn 259	-	1^{b}	-	-	1^{b}	1^{a}	1^{b}	-	1^{b}	-	1^{b}	-	-	6^{ab}
Aspergillus niger	MEn 260	-	-	1^{a}	1^{a}	-	1^{b}	-	-	-	1^{a}	1^{c}	-	-	5^{abc}
Curvularia sp.10	MEn 261	-	-	-	-	1^{c}	-	1^{b}	-	1^{c}	-	-	-	-	3^{bc}
Dreschera sp.16	MEn 262	-	1^{b}	1^{a}	1^{a}	-	1^{a}	-	-	-	1^{c}	1^{c}	-	-	6^{abc}
Aspergillus flavus	MEn 263	-	-	-	-	1^{b}	-	1^{b}	-	1^{b}	-	-	-	-	3^{b}
Aspergillus terrus	MEn 264	-	1^{b}	1^{b}	-	-	1^{c}	-	-	-	1^{b}	1^{b}	-	-	5^{bc}
Penicillium sp.17	MEn 265	-	1^{a}	-	1^{a}	1^{c}	1^{c}	-	-	1^{a}	-	-	-	-	5^{ac}
Penicillium sp.18	MEn 266	-	-	1^{b}	-	-	1^{b}	1^{b}	1^{b}	-	1^{b}	1^{b}	-	-	6^{b}
Non sporulating basidiomycete 1	MEn 267	-	1^{c}	-	1^{b}	1^{a}	1^{a}	-	-	-	-	1^{b}	-	-	5^{abc}
Aspergillus sp.19	MEn 268	-	-	-	1^{a}	-	-	1^{b}	1^{b}	1^{a}	-	1^{c}	-	-	5^{abc}
Dreschera sp.14	MEn 269	-	1^{c}	1^{b}	-	1^{a}	1^{a}	-	-	-	1^{c}	1^{c}	-	1^{b}	7^{abc}
Sterile Mycelia 24	MEn 270	-	1^{a}	-	1^{c}	-	-	-	-	1^{b}	-	1^{a}	1^{b}	1^{c}	6^{abc}

Nigrospora sp.16	MEn 271	-	-	1^{b}	-	-	1^{c}	1^{c}	1^{c}	1^{c}	1^{b}	-	1^{c}	-	7^{bc}
Penicillium sp.19	MEn 272	-	1^{b}	-	1^{a}	1^{a}	1^{b}	-	-	1^{a}	1^{b}	1^{b}	-	-	7^{ab}
Dreschera sp.15	MEn 273	-	-	-	1^{a}	-	-	1^{b}	1^{a}	1^{c}	1^{a}	-	-	1^{a}	7^{abc}
Curvularia sp.11	MEn 274	-	1^{b}	1^{c}	-	1^{a}	1^{a}	1^{a}	1^{b}	1^{c}	1^{c}	1^{c}	-	1^{a}	11^{abc}
Cladosporium sp.17	MEn 275	1^{a}	-	-	1^{c}	-	-	-	-	-	1^{a}	1^{b}	1^{b}	1^{a}	7^{abc}
Lignicola sp.2	MEn 276	-	1^{a}	1^{a}	-	-	1^{c}	1^{b}	1^{b}	1^{b}	-	1^{a}	-	1^{a}	9^{abc}
Sterile Mycelia 25	MEn 277	-	-	1^{a}	1^{a}	1^{b}	-	-	-	-	1^{a}	1^{a}	-	1^{a}	7^{ab}
Phoma sp.7	MEn 278	-	1^{a}	-	-	-	1^{c}	1^{c}	1^{a}	1^{b}	-	-	-	1^{a}	7^{abc}
Aspergillus sp.20	MEn 279	-	-	1^{b}	1^{b}	1^{a}	-	-	-	1^{c}	1^{c}	1^{a}	-	1^{a}	8^{abc}
Curvularia sp.12	MEn 280	-	1^{a}	-	-	1^{a}	1^{b}	1^{b}	1^{a}	1^{a}	1^{c}	1^{b}	-	1^{a}	10^{abc}
Pestalotiopsis sp.26	MEn 281	-	-	1^{b}	1^{b}	-	-	-	-	1^{a}	1^{b}	1^{a}	-	1^{a}	7^{ab}
Pestalotiopsis sp.27	MEn 282	-	1^{a}	-	-	1^{b}	1^{c}	1^{c}	1^{c}	-	1^{b}	1^{a}	-	1^{b}	9^{abc}
Nigrospora sp.17	MEn 283	1^{c}	-	1^{b}	1^{a}	-	-	-	-	1^{c}	-	1^{b}	1^{a}	1^{b}	8^{abc}
Pestalotiopsis sp.28	MEn 284	-	1^{b}	-	-	1^{a}	1^{a}	1^{a}	1^{c}	-	1^{b}	1^{a}	-	1^{b}	9^{abc}
Total		167	169	176	165	162	186	172	159	177	177	185	169		

Legend: MEn=Culture coded with isolation code M denotes mangroves and En denotes endophytes; ^{a}= Fungal endophyte in leaf; ^{b}= Fungal endophyte in stem; ^{c}=Fungal endophyte in root. ^{ab}=Fungal endophyte in leaf and stem; ^{ac}= fungal endophyte in leaf and root; ^{bc}=Fungal endophyte in stem and root; ^{abc}=Fungal endophyte in leaf, stem and root.

Table 4.3: Endophytic fungal colonies isolated from mangrove plants during winter season.

Fungal species	Isolate Code	Host plant													Total Isolates
		AM	AO	AI	DH	CI	RA	RM	EA	BC	CT	AA	AC	SA	
Setosphaeria monoceras	MEn 01	1^{b}	-	-	-	-	-	-	-	-	-	-	1^{b}	-	2^{b}
Aspergillus sp.1	MEn 02	1^{b}	1^{a}	-	-	1^{a}	-	1^{c}	-	-	-	-	1^{a}	-	5^{abc}
Scolecobasidium sp.1	MEn 03	1^{c}	1^{a}	1^{b}	-	1^{c}	1^{a}	1^{a}	-	1^{a}	-	1^{a}	1^{b}	-	9^{abc}
Drechslera sp.1	MEn 04	1^{c}	1^{c}	1^{c}	1^{b}	-	-	-	1^{b}	1^{b}	-	1^{a}	-	-	7^{abc}
Fusarium sp.1	MEn 05	-	1^{c}	-	-	1^{b}	1^{b}	1^{b}	-	-	1^{c}	1^{b}	-	-	6^{bc}
Pestalotiopsis sp.1	MEn 06	1^{b}	1^{a}	1^{a}	-	-	-	1^{b}	1^{a}	-	-	-	-	1^{c}	5^{ab}
Scytalidium lignicola	MEn 07	-	1^{b}	-	-	-	-	-	-	1^{c}	-	-	-	-	2^{bc}
Penicillium sp.1	MEn 08	1^{c}	-	-	-	1^{a}	1^{c}	-		-	-	1^{a}	1^{a}	-	5^{ac}
Fusarium sacchari	MEn 09	1^{b}	-	1^{b}	-	-	1^{b}	1^{a}	1^{a}	1^{a}	-	1^{b}	-	-	7^{ab}
Aspergillus sp.2	MEn 10	1^{b}	1^{a}	1^{a}	-	1^{b}	-	1^{c}	1^{c}	1^{a}	1^{a}	-	1^{b}	1^{a}	9^{abc}
Penicillium sp.2	MEn 11	1^{b}	1^{c}	1^{c}	1^{c}	-	1^{a}	1^{b}	-	1^{a}	1^{b}	-	-	-	8^{abc}
Corynesporina elegans	MEn 12	1^{c}	-	1^{a}	1^{c}	1^{c}	-	1^{a}	1^{c}	1^{a}	1^{a}	1^{a}	1^{c}	1^{c}	10^{ac}
Drechslera sp.2	MEn 13	1^{b}	-	1^{b}	1^{b}	1^{a}	1^{b}	1^{a}	1^{a}	1^{a}	1^{a}	1^{a}	1^{b}	1^{c}	11^{ab}
Scytalidium lignicola	MEn 14	1^{a}	-	1^{c}	1^{b}	1^{a}	1^{b}	1^{b}	-	-	-	-	-	1^{c}	6^{abc}
Sterile mycelia 1	MEn 15	-	1^{b}	-	-	1^{a}	1^{a}	1^{c}	1^{a}	1^{a}	1^{a}	-	1^{b}	1^{a}	8^{abc}
Corynespora sp.1	MEn 16	1^{a}	-	1^{a}	-	1^{a}	1^{c}	-	-	-	1^{b}	1^{a}	-	1^{b}	6^{abc}
Gilmaniella sp.1	MEn 17	1^{c}	-	-	1^{a}	1^{b}	1^{b}	1^{a}	1^{a}	-	1^{c}	-	1^{c}	-	8^{abc}
Fusarium sp.2	MEn 18	1^{a}	-	1^{a}	1^{a}	1^{a}	-	-	1^{a}	1^{a}	-	-	1^{a}	1^{c}	7^{a}
Drechslera sp.3	MEn 19	1^{b}	1^{b}	1^{a}	-	1^{c}	1^{a}	1^{a}	-	1^{c}	1^{c}	1^{b}	-	-	9^{abc}
Drechslera sp.4	MEn 20	1^{a}	-	1^{a}	1^{a}	1^{c}	1^{b}	1^{b}	-	1^{a}	1^{a}	-	1^{b}	1^{c}	9^{abc}
Pestalotiopsis sp.2	MEn 21	1^{a}	1^{c}	-	1^{b}	-	1^{c}	1^{a}	1^{a}	-	1^{a}	1^{b}	1^{a}	1^{a}	9^{abc}
Sterile mycelia 2	MEn 22	1^{a}	1^{c}	-	1^{a}	1^{b}	-	-	1^{a}	1^{a}	1^{c}	1^{a}	-	1^{c}	8^{abc}
Junctospora pulchra	MEn 23	1^{c}	-	-	-	1^{a}	1^{b}	-	1^{b}	-	-	-	1^{c}	1^{c}	5^{abc}
Curvularia sp.1	MEn 24	1^{a}	1^{c}	-	1^{b}	1^{a}	1^{a}	1^{a}	-	1^{c}	1^{c}	-	1^{b}	-	9^{abc}
Scytalidium sp.1	MEn 25	1^{a}	1^{a}	1^{c}	-	1^{c}	-	-	1^{c}	1^{a}	-	-	-	1^{a}	6^{ac}
Setosphaeria sp.1	MEn 26	-	1^{c}	-	1^{a}	-	-	-	-	-	1^{c}	1^{c}	-	-	4^{ac}
Aspergillus niger	MEn 27	1^{a}	1^{a}	-	-	1^{a}	1^{b}	1^{b}	1^{a}	1^{b}	-	-	1^{b}	1^{a}	8^{ab}

Drechslera sp.4	MEn 28	-	1^{a}	1^{b}	1^{a}	-	-	-	-	1^{a}	-	-	-	-	4^{ab}
Scolecobasidium sp.2	MEn 29	1^{b}	1^{b}	-	1^{b}	1^{b}	1^{a}	1^{b}	1^{c}	-	-	-	-	1^{a}	7^{abc}
Junctospora pulchra	MEn 30	1^{a}	1^{c}	1^{c}	1^{a}	1^{a}	1^{c}	1^{c}	-	-	-	1^{a}	-	1^{b}	8^{ac}
Fusarium sp.3	MEn 31	-	-	1^{a}	1^{a}	-	1^{b}	-	1^{a}	-	-	1^{c}	-	1^{b}	5^{abc}
Cladosporium sp.1	MEn 32	-	1^{a}	1^{a}	-	1^{b}	1^{a}	1^{a}	-	1^{c}	1^{c}	-	1^{c}	1^{a}	8^{abc}
Myceliophthora sp.1	MEn 33	1^{a}	-	1^{c}	1^{b}	-	1^{b}	1^{b}	1^{a}	1^{b}	-	1^{c}	1^{a}	1^{b}	9^{abc}
Penicillium sp.3	MEn 34	-	1^{a}	-	-	-	-	-	-	1^{c}	1^{b}	1^{a}	-	-	4^{abc}
Sterile Mycelia 3	MEn 35	-	1^{c}	1^{a}	1^{a}	1^{b}	1^{a}	1^{a}	1^{c}	1^{a}	1^{a}	-	1^{a}	1^{b}	10^{abc}
Nigrospora sphaerica	MEn 36	1^{b}	1^{b}	1^{c}	1^{a}	1^{b}	-	1^{b}	1^{b}	1^{a}	1^{b}	1^{c}	1^{c}	1^{a}	11^{abc}
Cladosporium sp.2	MEn 37	1^{a}	1^{c}	1^{a}	1^{a}	1^{b}	1^{a}	1^{a}	1^{b}	-	1^{a}	-	1^{a}	1^{a}	10^{abc}
Seatospheria monoceras	MEn 38	-	1^{c}	1^{a}	1^{a}	1^{b}	1^{a}	1^{c}	1^{a}	-	-	1^{a}	1^{b}	1^{a}	9^{abc}
Scolecobasidium sp.3	MEn 39	1^{c}	1^{b}	1^{b}	1^{a}	1^{c}	1^{c}	1^{a}	1^{a}	-	1^{a}	1^{b}	-	1^{a}	10^{abc}
Penicillium sp.4	MEn 40	1^{a}	1^{a}	-	-	1^{b}	1^{a}	1^{c}	1^{a}	-	1^{b}	-	1^{a}	1^{a}	8^{abc}
Aspergillus niger	MEn 41	-	1^{c}	1^{a}	1^{a}	1^{b}	-	-	-	1^{a}	-	-	-	-	5^{abc}
Pestalotiopsis sp.3	MEn 42	1^{b}	-	1^{c}	-	-	1^{c}	1^{b}	1^{b}	-	1^{c}	-	1^{b}	1^{c}	7^{bc}
Gonatobotryum sp.1	MEn 43	-	1^{c}	1^{b}	1^{a}	1^{a}	1^{c}	1^{c}	-	1^{b}	1^{a}	1^{a}	-	-	9^{abc}
Aspergillus fumigates	MEn 44	1^{a}	1^{c}	1^{c}	1^{a}	-	1^{a}	1^{a}	1^{c}	1^{a}	-	-	1^{a}	1^{a}	9^{ac}
Pestalotiopsis microspore	MEn 45	-	-	-	1^{b}	1^{b}	1^{a}	1^{b}	-	1^{a}	-	1^{a}	-	-	6^{ab}
Pestalotiopsis sp.4	MEn 46	1^{c}	1^{a}	-	-	1^{c}	-	1^{a}	1^{a}	1^{c}	-	1^{b}	1^{a}	1^{b}	8^{abc}
Aspergillus flavus	MEn 47	1^{a}	1^{b}	-	-	1^{a}	1^{b}	-	1^{a}	1^{a}	1^{a}	1^{a}	-	1^{a}	8^{ab}
Fusarium incarnatum	MEn 48	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}	-	1^{b}	-	1^{b}	-	-	1^{a}	8^{b}
Unidentified 1	MEn 49	-	1^{b}	1^{a}	-	-	1^{c}	1^{b}	1^{a}	1^{a}	1^{a}	1^{c}	1^{a}	1^{a}	9^{abc}
Aspergillus sp.3	MEn 50	1^{b}	1^{c}	1^{c}	-	-	1^{a}	1^{a}	1^{b}	-	-	-	-	1^{a}	6^{abc}
Unidentified 2	MEn 51	1^{c}	-	1^{b}	1^{a}	1^{c}	1^{b}	-	-	1^{a}	-	1^{c}	1^{b}	-	8^{abc}
Fungal endophyte	MEn 52	-	1^{a}	1^{b}	1^{c}	-	1^{c}	1^{c}	1^{c}	1^{a}	1^{b}	-	1^{b}	1^{a}	9^{abc}
Thermomyces sp.1	MEn 53	1^{c}	-	1^{b}	1^{a}	-	-	1^{b}	1^{b}	1^{b}	-	1^{a}	1^{b}	-	8^{abc}
Colletotrichum sp1.	MEn 54	-	1^{a}	1^{a}	1^{a}	1^{c}	1^{b}	1^{a}	1^{b}	1^{b}	1^{b}	1^{c}	1^{b}	1^{b}	11^{abc}
Unidentified 3	MEn 55	1^{c}	1^{c}	1^{a}	-	-	-	-	1^{c}	-	-	-	-	1^{a}	4^{ac}
Aspergillus sp.4	MEn 56	1^{b}	1^{a}	1^{a}	1^{b}	1^{b}	1^{c}	1^{a}	1^{c}	-	1^{b}	1^{a}	1^{b}	1^{a}	11^{abc}

Acremonium sp.1	MEn 57	1^{b}	1^{c}	1^{c}	-	1^{c}	1^{b}	-	1^{c}	1^{b}	1^{b}	-	-	1^{a}	8^{bc}
Colletotrichum gloeosporioides	MEn 58	-	1^{a}	1^{a}	1^{a}	1^{c}	1^{c}	1^{a}	-	-	1^{a}	1^{c}	1^{c}	-	9^{ac}
Sordariomycetes sp.1	MEn 59	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}	-	-	-	-	-	-	-	1^{b}	5^{b}
Rhizopus sp.1	MEn 60	1^{c}	-	-	1^{b}	1^{c}	1^{b}	1^{c}	1^{c}	-	-	-	-	1^{b}	6^{bc}
Unidentified 4	MEn 61	1^{a}	1^{c}	1^{b}	1^{a}	1^{a}	1^{c}	-	-	-	-	1^{b}	1^{a}	1^{c}	8^{abc}
Unidentified 5	MEn 62	1^{a}	-	1^{a}	-	1^{a}	1^{a}	1^{a}	1^{a}	1^{a}	-	-	-	-	7^{a}
Pestalotiopsis sp.5	MEn 63	-	1^{c}	1^{c}	1^{c}	-	1^{c}	1^{c}	1^{c}	1^{c}	1^{c}	1^{a}	-	1^{b}	9^{ac}
Colletotrichum sp.2	MEn 64	1^{c}	1^{c}	1^{a}	-	1^{c}	1^{c}	1^{a}	1^{c}	-	1^{a}	-	-	-	8^{ac}
Aspergillus flavus	MEn 65	1^{b}	1^{a}	1^{a}	1^{c}	-	1^{a}	1^{c}	1^{c}	1^{b}	1^{b}	1^{a}	-	1^{c}	10^{abc}
Fusarium equiseti	MEn 66	-	-	-	1^{a}	1^{a}	1^{b}	1^{a}	1^{b}	-	1^{a}	1^{a}	1^{b}	1^{c}	8^{ab}
Curvularia sp.2	MEn 67	1^{a}	-	1^{a}	1^{c}	1^{b}	1^{b}	1^{a}	1^{a}	1^{a}	1^{c}	1^{a}	1^{b}	1^{b}	11^{abc}
Scytalidium lignicola	MEn 68	1^{c}	1^{c}	-	1^{c}	-	-	-	1^{c}	1^{c}	-	1^{c}	-	1^{c}	6^{c}
Drechslera sp.5	MEn 69	1^{b}	-	1^{c}	-	1^{b}	1^{a}	1^{c}	-	1^{a}	1^{a}	1^{b}	1^{a}	-	9^{abc}
Pestalotiopsis sp.6	MEn 70	1^{c}	-	1^{a}	-	-	1^{c}	-	1^{a}	1^{b}	-	-	-	1^{a}	5^{abc}
Pestalotiopsis sp.7	MEn 71	-	1^{a}	1^{a}	1^{c}	1^{a}	1^{b}	1^{c}	-	1^{a}	1^{c}	1^{a}	-	-	9^{abc}
Ceratocystis sp.1	MEn 72	1^{b}	1^{b}	-	-	1^{a}	1^{a}	1^{b}	1^{a}	-	1^{c}	1^{a}	1^{b}	1^{b}	9^{abc}
Fusarium sp.4	MEn 73	1^{a}	1^{b}	1^{c}	1^{c}	-	-	1^{c}	1^{b}	1^{b}	1^{a}	1^{b}	1^{b}	1^{b}	10^{abc}
Fusarium sp.5	MEn 74	1^{c}	-	1^{a}	1^{b}	1^{c}	1^{a}	1^{a}	1^{b}	1^{c}	-	-	-	1^{b}	8^{abc}
Aspergillus sp.5	MEn 75	1^{a}	1^{a}	1^{a}	1^{c}	1^{b}	1^{a}	1^{a}	1^{c}	1^{a}	1^{b}	1^{a}	-	1^{c}	11^{abc}
Dreschera sp.6	MEn 76	1^{a}	1^{a}	1^{a}	-	-	-	-	-	-	-	1^{a}	-	1^{b}	4^{a}
Alternaria alternata	MEn 77	1^{c}	-	1^{c}	-	1^{b}	1^{b}	1^{c}	1^{b}	-	-	-	-	-	6^{bc}
Bipolaris sp.1	MEn 78	1^{a}	1^{b}	1^{a}	1^{b}	1^{b}	-	-	1^{a}	1^{a}	-	-	-	1^{c}	7^{ab}
Alternaria sp.1	MEn 79	-	-	1^{a}	-	1^{a}	1^{a}	-	-	1^{a}	1^{a}	-	-	-	5^{a}
Corynespora sp.2	MEn 80	-	-	-	1^{b}	-	1^{a}	1^{c}	1^{c}	1^{c}	1^{b}	1^{a}	1^{a}	1^{b}	8^{abc}
Alternaria sp.2	MEn 81	1^{a}	1^{a}	-	1^{b}	1^{a}	-	1^{a}	1^{a}	-	1^{b}	1^{a}	-	1^{a}	8^{ab}
Sterile Mycelia 4	MEn 82	1^{a}	1^{b}	1^{a}	1^{c}	1^{a}	1^{a}	1^{c}	-	1^{a}	1^{b}	-	1^{a}	1^{b}	10^{abc}
Pestalotiopsis sp.8	MEn 83	1^{a}	1a	-	-	-	1^{a}	-	1^{a}	1^{a}	-	1^{a}	1^{a}	1^{a}	7^{a}
Alternaria sp.3	MEn 84	1^{a}	1^{c}	1^{c}	1^{c}	1^{a}	-	1^{c}	-	1^{a}	1^{a}	-	-	-	8^{ac}
Nigrospora sp.1	MEn 85	1^{a}	1^{c}	-	-	-	1^{c}	1^{c}	-	1^{c}	1^{a}	1^{a}	-	-	7^{ac}
Nigrosopra sp.2	MEn 86	-	1^{c}	1^{b}	1^{c}	1^{c}	-	1^{b}	1^{b}	1^{b}	-	1^{c}	-	-	8^{bc}

Alternaria alternata	MEn 87	1^{a}	-	1^{b}	-	1^{a}	1^{c}	-	-	1^{b}	1^{a}	1^{a}	-	-	7^{abc}
Cladosporium sp.3	MEn 88	1^{a}	1^{c}	1^{c}	1^{a}	1^{c}	1^{a}	1^{a}	1^{a}	-	-	-	1^{c}	1^{b}	9^{abc}
Cladosporium sp.4	MEn 89	-	1^{b}	-	1^{a}	1^{a}	1^{b}	1^{c}	-	-	-	-	-	-	5^{abc}
Sterile Mycelia 5	MEn 90	-	-	1^{a}	1^{c}	1^{a}	1^{b}	1^{a}	1^{c}	-	-	1^{b}	1^{a}	1^{a}	8^{abc}
Aspergillus ulvus	MEn 91	1^{a}	1^{b}	-	1^{c}	-	1^{a}	-	-	-	-	-	-	1^{b}	4^{abc}
Cladosporium sp.5	MEn 92	1^{a}	1^{c}	1^{c}	-	1^{b}	1^{a}	-	-	-	-	-	-	1^{b}	5^{abc}
Dreschera sp.7	MEn 93	-	-	1^{b}	-	-	-	-	1a	-	1^{a}	1^{c}	1^{a}	1^{a}	5^{abc}
Gonatobotryum sp.2	MEn 94	1^{a}	-	1^{c}	-	1^{c}	-	1^{a}	1a	-	-	-	-	1^{c}	5^{ac}
Cladosporium sp.6	MEn 95	-	-	-	-	-	1^{b}	-	-	1^{b}	-	1^{b}	1^{b}	-	4^{b}
Fusarium sp. 6	MEn 96	-	-	-	-	1^{c}	1^{b}	1^{c}	1^{c}	-	1^{b}	-	1^{b}	1^{b}	6^{bc}
Fusarium semitectum	MEn 97	1^{a}	1^{a}	1^{a}	1^{a}	1^{a}	1^{b}	1^{a}	-	-	-	-	-	-	7^{ab}
Penicillium sp.5	MEn 98	1^{c}	1^{c}	-	1^{c}	-	1^{c}	-	1^{c}	1^{c}	1^{c}	1^{c}	1^{c}	1^{a}	9^{c}
Pestalotiopsis sp.9	MEn 99	-	1^{b}	1^{c}	1^{b}	1^{a}	-	1^{a}	1^{b}	1^{b}	1^{b}	1^{b}	-	1^{c}	9^{abc}
Cladosporium sp.7	MEn 100	1^{a}	-	1^{c}	-	-	-	1^{b}	1^{c}	1^{a}	1^{a}	1^{c}	1^{b}	1^{a}	8^{abc}
Marieltiottia sp.1	MEn 101	-	1^{b}	1^{c}	1^{a}	1^{a}	1^{a}	1^{b}	1^{c}	1^{a}	-	-	-	1^{c}	8^{abc}
Pestalotiopsis sp.10	MEn 102	1^{c}	1^{b}	1^{a}	-	1^{b}	-	1^{a}	1^{c}	-	-	-	-	-	6^{abc}
Nigrospora sp.3	MEn 103	-	1^{a}	1^{c}	1^{b}	1^{a}	1^{b}	-	-	-	-	1^{b}	-	-	6^{abc}
Fusarium sp.7	MEn 104	1^{a}	-	-	-	1^{c}	-	1^{c}	-	1^{c}	1^{b}	-	1^{c}	-	6^{abc}
Aspergillus sp.6	MEn 105	-	-	-	1^{a}	1^{a}	1^{a}	-	1^{a}	-	1^{a}	1^{a}	1^{a}	-	7^{a}
Nigrospora sp.4	MEn 106	-	1^{b}	1^{c}	1^{b}	1^{b}	-	-	-	-	-	-	-	1^{a}	4^{bc}
Fusarium sp.8	MEn 107	-	1^{a}	1^{a}	1^{b}	1^{c}	1^{b}	1^{c}	1^{b}	1^{a}	1^{c}	1^{a}	-	-	10^{abc}
Pestalotiopsis sp.11	MEn 108	1^{a}	1^{c}	1^{a}	1^{a}	-	1^{a}	1^{c}	1^{c}	1^{b}	1^{c}	1^{b}	1^{a}	1^{a}	11^{abc}
Phoma sp.1	MEn 109	-	-	-	-	1^{c}	1^{c}	1^{c}	1^{a}	1^{c}	-	1^{a}	-	1^{a}	6^{ac}
Aspergillus sp.7	MEn 110	1^{b}	1^{b}	1^{a}	1^{c}	1^{b}	-	1^{b}	1^{b}	-	-	-	-	1^{a}	7^{abc}
Pestalotiopsis sp.12	MEn 111	1^{b}	-	-	-	1^{b}	1^{b}	-	1^{b}	-	-	-	1^{b}	1^{a}	5^{b}
Gliocladium sp.1	MEn 112	1^{c}	1^{c}	1^{b}	1^{a}	1^{b}	-	1^{c}	1^{c}	1^{a}	-	-	-	1^{b}	8^{abc}
Bipolaris sp.7	MEn 113	1^{c}	1^{c}	-	1^{b}	-	1^{c}	-	-	-	-	1^{b}	-	-	5^{bc}
Aspergillus sp.8	MEn 114	1^{a}	1^{c}	1^{b}	1^{a}	-	1^{c}	1^{b}	1^{a}	1^{c}	1^{a}	-	1^{b}	1^{a}	10^{abc}
Penicillium sp.6	MEn 115	1^{c}	1^{c}	1^{c}	1^{c}	-	1^{c}	1^{c}	-	-	1^{c}	-	-	-	7^{c}
Sterile Mycelia 6	MEn 116	-	1^{a}	1^{a}	1^{c}	-	1^{b}	1^{a}	1^{b}	1^{c}	1^{c}	1^{b}	-	1^{c}	9^{abc}
Fusarium sp. 9	MEn 117	-	-	1^{b}	1^{a}	1^{b}	-	1^{b}	1^{b}	-	-	-	-	1^{c}	5^{ab}

Thickened chlamydospores 1	MEn 118	1^{c}	1^{a}	1^{a}	1^{b}	-	1^{b}	-	1^{c}	-	1^{b}	-	-	1^{b}	7^{abc}
Nigrospora sp.5	MEn 119	1^{b}	-	-	-	1^{c}	-	1^{b}	1^{c}	1^{b}	1^{c}	-	-	1^{c}	6^{bc}
Nigrospora sp.6	MEn 120	1^{b}	1^{b}	1^{b}	1^{a}	-	1^{b}	-	-	-	1^{c}	1^{b}	1^{a}	-	8^{abc}
Scolecobasidium sp.4	MEn 121	1^{b}	-	-	1^{a}	1^{c}	1^{b}	1^{a}	1^{c}	1^{b}	-	1^{a}	-	1^{b}	8^{abc}
Sterile Mycelia 7	MEn 122	1^{a}	1^{c}	1^{c}	1^{c}	-	1^{b}	1^{a}	-	1^{c}	1^{a}	1^{b}	1^{a}	-	10^{abc}
Pestalotiopsis sp.13	MEn 123	1^{c}	1^{a}	1^{a}	1^{b}	1^{b}	1^{b}	1^{a}	1^{b}	1^{c}	-	-	-	1^{c}	9^{abc}
Scytalidium sp.1	MEn 124	1^{b}	1^{c}	1^{a}	1^{a}	-	-	1^{b}	1^{a}	1^{c}	1^{a}	1^{b}	-	1^{c}	9^{abc}
Gilmaniella sp.2	MEn 125	-	1^{b}	1^{a}	1^{a}	1^{c}	-	-	-	-	1^{a}	1^{b}	-	1^{a}	6^{abc}
Rhizopus sp.2	MEn 126	1^{b}	1^{c}	-	1^{b}	1^{b}	1^{c}	-	1^{a}	-	-	-	-	1^{c}	6^{abc}
Sterile Mycelia 8	MEn 127	-	-	1^{c}	-	1^{a}	-	1^{a}	1^{c}	1^{a}	1^{a}	1^{b}	-	1^{c}	7^{abc}
Aspergillus flavus	MEn 128	1^{b}	-	-	-	1^{b}	1^{b}	-	1^{c}	-	-	-	-	-	4^{bc}
Penicillium sp.7	MEn 129	-	1^{a}	1^{c}	1^{a}	1^{a}	1^{c}	1^{c}	-	1^{c}	1^{c}	1^{a}	1^{a}	1^{b}	10^{ac}
Pestalotiopsis sp.14	MEn 130	-	-	1^{a}	-	-	1^{a}	1^{a}	1^{a}	1^{a}	1^{b}	-	1^{a}	-	7^{ab}
Humicola sp.1	MEn 131	1^{a}	1^{b}	1^{a}	1^{c}	-	1^{c}	1^{a}	-	1^{b}	1^{a}	1^{a}	1^{a}	1^{c}	10^{abc}
Linkosia sp.1	MEn 132	1^{b}	1^{a}	-	1^{c}	1^{b}	-	1^{a}	1^{c}	1^{a}	1^{b}	1^{a}	1^{a}	1^{a}	10^{abc}
Aspergillus niger	MEn 133	1^{a}	1^{a}	1^{a}	-	1^{a}	1^{a}	-	1^{a}	1^{a}	1^{a}	1^{a}	1^{a}	1^{a}	10^{a}
Juctospora sp.1	MEn 134	-	1^{b}	1^{b}	1^{c}	1^{c}	1^{b}	1^{b}	1^{b}	-	-	-	-	1^{b}	7^{bc}
Cladosporium sp.8	MEn 135	1^{c}	1^{b}	1^{a}	1^{a}	-	1^{b}	1^{c}	-	1^{b}	1^{a}	1^{a}	1^{a}	-	10^{abc}
Rhizopus sp.3	MEn 136	-	-	-	-	-	-	1^{a}	1^{c}	-	-	1^{a}	-	1^{a}	3^{ac}
Cladosporium sp.9	MEn 137	-	1^{a}	1^{c}	1^{a}	1^{b}	1^{a}	1^{a}	-	1^{b}	1^{c}	1^{a}	1^{b}	-	10^{abc}
Pestalotiopsis sp.15	MEn 138	1^{b}	-	1^{a}	1^{c}	-	1^{a}	1^{b}	-	1^{a}	-	-	-	-	6^{abc}
Pestalotiopsis sp.16	MEn 139	-	1^{c}	1^{b}	1^{b}	1^{b}	1^{c}	-	1^{a}	1^{b}	1^{a}	-	1^{b}	1^{a}	9^{abc}
Aspergillus niger	MEn 140	1^{c}	1^{c}	-	1^{c}	-	1^{c}	1^{a}	-	1^{c}	1^{a}	-	1^{a}	-	8^{ac}
Cladosporium sp.10	MEn 141	1^{b}	-	1^{b}	1^{b}	1^{b}	-	-	1^{b}	1^{b}	-	1^{b}	-	1^{b}	7^{b}
Rhizopus sp.4	MEn 142	1^{b}	1^{c}	1^{b}	1^{a}	1^{b}	-	1^{a}	-	-	1^{c}	1^{b}	-	-	8^{abc}
Dreschera sp.8	MEn 143	-	-	1^{b}	-	1^{c}	1^{c}	1^{b}	-	1^{b}	-	-	-	-	5^{bc}
Bipolaris sp.2	MEn 144	1^{b}	1^{b}	1^{c}	1^{a}	1^{b}	-	1^{a}	1^{c}	-	1^{b}	1^{c}	1^{b}	1^{b}	10^{abc}
Pestalotiopsis sp.17	MEn 145	-	-	-	-	1^{c}	1^{c}	1^{c}	-	1^{c}	-	-	1^{c}	-	5^{c}
Sterile Mycelia 9	MEn 146	1^{b}	1^{b}	1^{c}	1^{b}	-	1^{a}	1^{b}	-	1^{a}	1^{a}	1^{b}	1^{b}	1^{b}	10^{abc}
Dreschera sp.9	MEn 147	1^{c}	-	1^{b}	-	1^{a}	1^{c}	-	1^{a}	1^{a}	1^{b}	-	-	1^{c}	7^{abc}

Aspergillus sp.9	MEn 148	1^{b}	-	1^{c}	-	-	1^{a}	1^{b}	-	1^{a}	1^{c}	1^{b}	-	-	7^{abc}
Curvularia sp.3	MEn 149	1^{a}	1^{c}	1^{c}	1^{c}	1^{a}	-	-	1^{a}	-	1^{c}	1^{a}	-	1^{b}	8^{ac}
Penicillium sp.8	MEn 150	1^{a}	1^{a}	-	-	1^{a}	1^{a}	1^{a}	-	1^{a}	1^{a}	1^{c}	-	-	8^{ac}
Curvularia sp.4	MEn 151	-	1^{b}	1^{c}	-	1^{a}	-	-	1^{b}	-	-	1^{b}	1^{c}	1^{a}	6^{abc}
Cladosporium sp.11	MEn 152	1^{b}	1^{c}	-	1^{c}	1^{b}	1^{c}	-	-	1^{b}	1^{c}	1^{b}	-	-	8^{bc}
Pestalotiopsis sp.18	MEn 153	1^{c}	1^{c}	1^{c}	-	-	-	1^{c}	-	-	-	-	-	1^{a}	4^{c}
Nigrospora sp.7	MEn 154	1^{b}	1^{a}	1^{c}	1^{b}	1^{c}	1^{b}	-	-	1^{a}	-	1^{a}	1^{a}	-	9^{abc}
Fusarium sp. 10	MEn 155	1^{c}	-	-	-	-	1^{a}	-	1^{c}	1^{c}	1^{c}	-	-	1^{a}	5^{ac}
Aspergillus sp.10	MEn 156	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}	-	-	-	1^{b}	-	-	8^{b}
Corynospora sp.4	MEn 157	1^{a}	-	-	-	-	1^{a}	-	1^{b}	-	1^{c}	-	1^{a}	1^{a}	5^{abc}
Sordariomycetes sp.2	MEn 158	1^{c}	1^{b}	1^{c}	1^{a}	-	1^{b}	-	-	1^{a}	1^{c}	1^{a}	1^{b}	-	9^{abc}
Penicillium sp.9	MEn 159	-	-	1^{a}	-	1^{b}	1^{a}	-	1^{a}	-	1^{a}	1^{b}	1^{a}	1^{c}	7^{ab}
Nigrospora sp.8	MEn 160	1^{b}	1^{b}	-	1^{b}	1^{c}	1^{a}	1^{a}	-	1^{b}	-	-	-	-	7^{abc}
Aureobasidium pullulans	MEn 161	-	-	1^{c}	-	-	-	-	1^{b}	-	1^{c}	1^{c}	1^{c}	1^{a}	5^{bc}
Pestalotiopsis sp.19	MEn 162	1^{b}	1^{a}	1^{b}	1^{b}	1^{b}	1^{a}	1a	-	1^{a}	-	1^{b}	-	-	9^{ab}
Aspergillus niger	MEn 163	-	-	1^{a}	-	-	1^{c}	-	1^{a}	-	-	1^{b}	-	1^{c}	4^{abc}
Penicillium sp.10	MEn 164	1^{a}	1^{b}	-	1^{c}	1^{a}	-	1^{c}	-	-	-	-	-	-	5^{abc}
Sterile Mycelia 10	MEn 165	1^{c}	-	1^{b}	-	-	1^{c}	-	1^{b}	-	-	-	-	1^{a}	4^{bc}
Aspergillus sp.11	MEn 166	1^{c}	1^{b}	-	1^{b}	1^{a}	-	1^{c}	-	1^{a}	-	1^{a}	1^{b}	-	8^{abc}
Sterile Mycelia 11	MEn 167	-	-	1^{b}	-	-	-	-	-	-	1^{a}	1^{c}	1^{b}	1^{a}	4^{abc}
Cladosporium sp.12	MEn 168	1^{a}	1^{b}	1^{a}	1^{c}	1^{a}	-	1^{a}	-	1^{c}	-	1^{b}	-	-	8^{abc}
Penicillium sp.11	MEn 169	-	-	-	-	-	1^{a}	-	1^{c}	-	1^{b}	-	1^{a}	1^{b}	4^{abc}
Sterile Mycelia 12	MEn 170	1^{c}	1^{c}	1^{a}	1^{b}	1^{a}	-	1^{a}	-	1^{b}	-	1^{c}	-	-	8^{abc}
Scolecobasidium sp.5	MEn 171	1^{a}	-	1^{b}	-	-	1^{a}	-	1^{b}	-	-	-	-	1^{a}	4^{abc}
Gliocladium sp.2	MEn 172	1^{b}	1^{b}	1^{a}	1^{a}	1^{a}	1^{b}	1^{a}	-	1^{a}	-	-	-	-	8^{ab}
Dreschera sp.10	MEn 173	-	-	1^{c}	-	-	1^{c}	1^{c}	1^{c}	-	-	-	-	1^{a}	4^{c}
Phoma sp.2	MEn 174	1^{b}	1^{a}	1^{a}	1^{a}	1^{a}	1^{b}	1^{b}	-	1^{b}	-	1^{a}	-	-	9^{ab}
Cladosporium sp.13	MEn 175	1^{a}	1^{a}	1^{c}	1^{a}	-	1^{c}	-	1^{c}	-	1^{c}	-	1^{a}	1^{b}	8^{ac}
Ascospore 1	MEn 176	1^{b}	1^{b}	-	1^{a}	1^{a}	-	1^{b}	-	1^{a}	-	1^{b}	1^{b}	-	8^{ab}
Alternaria sp.4	MEn 177	1^{c}	-	-	-	-	1^{c}	-	1^{b}	-	1^{a}	-	-	1^{a}	4^{abc}
Scytalydium sp.2	MEn 178	1^{b}	1^{c}	-	1^{a}	1^{a}	-	1^{c}	-	1^{b}	-	1^{c}	1^{a}	-	8^{abc}

Corynospora sp.4	MEn 179	1[a]	-	1[c]	-	-	1[a]	-	-	-	1[b]	-	-	1[a]	4[abc]
Aspergillus sp.11	MEn 180	1[a]	1[a]	1[a]	1[a]	1[a]	1[a]	1[a]	-	1[a]	-	1[a]	-	-	9[a]
Pestalotiopsis sp.18	MEn 181	1[c]	-	1[b]	-	-	1[b]	-	1[c]	-	1[b]	-	-	1[a]	5[bc]
Alternaria sp.5	MEn 182	1[b]	1[c]	1[c]	1[b]	1[a]	1[a]	1[a]	-	1[b]	-	1[c]	-	-	9[abc]
Sterile Mycelia 13	MEn 183	1[a]	-	1[b]	-	-	-	-	1[a]	-	-	-	-	1[c]	3[ab]
Nigrospora sp.9	MEn 184	1[b]	1[b]	-	1[b]	1[c]	1[c]	1[c]	-	1[a]	1[a]	-	1[b]	-	9[abc]
Sterile Mycelia 14	MEn 185	1[a]	-	1[c]	-	-	-	-	1[a]	-	1[a]	-	1[b]	1[c]	5[abc]
Dreschera sp.11	MEn 186	1[c]	1[c]	1[c]	1[c]	1[c]	1[c]	1[a]	-	-	-	1[b]	1[b]	-	9[abc]
Limposoma sp.1	MEn 187	1[a]	-	1[a]	-	-	-	-	1[a]	-	-	-	-	1[b]	3[a]
Sterile Mycelia 15	MEn 188	1[c]	1[a]	-	1[b]	1[a]	1[a]	1[c]	-	1[c]	-	1[b]	1[a]	-	9[abc]
Ascospores 2	MEn 189	1[b]	-	1[b]	-	-	1[c]	-	1[a]	-	-	-	-	1[b]	4[abc]
Pestalotiopsis sp.20	MEn 190	1[b]	1[c]	-	1[c]	1[c]	1[b]	-	-	-	-	-	-	-	5[bc]
Gilmaniella sp.2	MEn 191	-	-	1[c]	-	-	1[a]	-	-	-	1[a]	-	1[a]	1[a]	4[ac]
Nigrospora sp.10	MEn 192	1[b]	1[b]	1[b]	1[b]	1[b]	1[b]	1[b]	-	1[b]	-	1[b]	-	-	9[b]
Juctospora pulchura	MEn 193	-	-	1[c]	-	-	-	-	1[a]	-	1[b]	-	-	1[b]	3[abc]
Bipolaris sp.3	MEn 194	1[c]	1[c]	1[c]	1[c]	1[b]	1[a]	1[c]	-	1[a]	-	1[b]	-	-	9[abc]
Fusarium sp. 11	MEn 195	-	-	1[c]	-	-	-	-	1[c]	-	1[c]	-	-	1[b]	3[c]
Gilberella sp.2	MEn 196	1[b]	1[b]	-	1[a]	1[b]	1[a]	-	-	-	-	-	-	-	5[ab]
Phomopsis sp.1	MEn 197	1[b]	-	-	-	-	1[c]	-	-	-	1[a]	-	-	-	3[abc]
Pestalotiopsis sp.21	MEn 198	1[a]	1[b]	-	1[c]	1[a]	1[a]	-	1[b]	1[c]	1[a]	1[a]	1[b]	1[a]	10[abc]
Cladosporium sp.14	MEn 199	1[c]	-	-	-	-	1[c]	-	-	-	1[a]	-	-	-	3[ac]
Aspergillus niger	MEn 200	1[a]	1[b]	-	1[a]	1[c]	-	1[a]	-	-	-	-	-	1[a]	5[abc]
Penicillium sp.12	MEn 201	-	1[a]	1[c]	-	-	-	-	1[b]	-	1[a]	-	1[b]	1[a]	5[abc]
Catenate spores 1	MEn 202	1[a]	1[b]	-	-	1[c]	1[c]	1[c]	-	1[c]	-	1[c]	-	-	7[abc]
Curvularia sp.5	MEn 203	-	1[a]	1[c]	-	-	1[b]	-	-	-	1[a]	-	1[b]	1[b]	5[abc]
Curvularia sp.6	MEn 204	-	-	-	1[a]	1[b]	-	1[c]	-	1[a]	-	1[b]	1[b]	-	6[abc]
Sterile Mycelia 16	MEn 205	1[b]	1[a]	1[c]	-	-	1[a]	-	-	-	1[c]	-	-	1[c]	5[abc]
Nigrospora sp.11	MEn 206	1[a]	-	-	1[c]	1[c]	-	-	1[a]	1[c]	1[a]	1[a]	1[b]	1[a]	8[abc]
Aspergillus sp.12	MEn 207	1[b]	-	1[c]	-	-	1[a]	1[b]	-	-	-	-	-	-	4[abc]
Dreschera sp.12	MEn 208	1[a]	1[c]	-	1[b]	1[a]	-	-	1[c]	-	1[b]	1[a]	-	1[b]	7[abc]
Phoma sp.3	MEn 209	-	-	-	1[b]	-	1[a]	1[c]	-	1[c]	-	-	-	-	4[abc]

Colletotrichum sp.5	MEn 210	1[a]	1[a]	1[a]	-	1[a]	-	1[b]	1[c]	-	1[a]	1[c]	1[a]	1[b]	9[abc]
Aspergillus sp.13	MEn 211	-	1[c]	-	1[a]	-	1[c]	1[b]	-	-	-	-	-	-	4[abc]
Aspergillus sp.14	MEn 212	1[c]	1[b]	1[a]	-	-	-	1[a]	1[c]	1[b]	1[b]	-	1[b]	1a	8[abc]
Nigrospora sp.12	MEn 213	1[a]	1[a]	-	1[c]	1[a]	1[b]	-	1[b]	1[b]	1[b]	1[b]	-	-	9[abc]
Sterile Mycelia 17	MEn 214	1[c]	-	1[b]	1[a]	-	-	-	1[c]	1[b]	-	-	1[a]	1[a]	6[abc]
Fusarium sp.12	MEn 215	1[b]	1[a]	1[a]	1[a]	1[c]	1[c]	1[a]	-	-	1[b]	1[c]	-	-	9[abc]
Pestalotiopsis sp.22	MEn 216	1[c]	-	-	1[b]	1[a]	1[c]	-	1[b]	-	1[a]	1[a]	1[a]	1[b]	8[abc]
Penicillium sp.13	MEn 217	1[a]	1[b]	-	1[b]	-	1[c]	1[a]	-	1[b]	1[a]	1[c]	1[a]	-	9[abc]
Pestalotiopsis sp.23	MEn 218	1[b]	-	1[a]	1[c]	1[a]	1[b]	-	1[c]	-	1[a]	1[b]	-	1[a]	8[abc]
Corynospora sp.5	MEn 219	1[a]	1[a]	-	-	-	1[c]	1[b]	1[b]	1[a]	-	-	1[c]	-	7[abc]
Sterile Mycelia 18	MEn 220	1[c]	-	1[a]	1[b]	-	-	-	1[a]	1[a]	1[b]	1[c]	1[a]	1[a]	8[abc]
Sterile Mycelia 19	MEn 221	1[c]	1[b]	-	-		1[a]	1[c]	1[b]	1[a]	-	1[a]	-	-	8[abc]
Fusarium sp.13	MEn 222	1[a]	-	1[a]	1[b]	-	-	-	1[b]	-	1[a]	-	1[a]	1[b]	6[ab]
Scytalidium sp.3	MEn 223	1[c]	1[c]	1[b]	-	1[b]	1[b]	1[c]	-	1[c]	-	1[b]	-	-	8[bc]
Dreschera sp.13	MEn 224	1[b]	1[b]	1[a]	1[b]	1[b]	-	-	1[c]	-	1[a]	-	-	1[c]	7[abc]
Aspergillus sp.15	MEn 225	1[c]	1[a]	-	-	1[a]	1[a]	1[a]	-	1[a]	-	1[a]	-	-	7[ac]
Nigrospora sp.13	MEn 226	1[c]	-	1[c]	1[c]	-	1[c]	-	1[c]	-	1[c]	1[c]	-	1[a]	7[c]
Sterile Mycelia 20	MEn 227	1[a]	1[a]	-	-	1[a]	1[a]	1[a]	-	1[c]	-	-	1[a]	-	7[ac]
Sterile Mycelia 21	MEn 228	1[a]	-	1[b]	1[c]	-	-	1[a]	1[a]	-	1[c]	1[b]	-	1[c]	7[abc]
Aspergillus sp.16	MEn 229	1[c]	1[b]	-	1[a]	-	1[c]	-	1[b]	1[a]	-	-	1[c]	-	7[abc]
Cladosporium sp.15	MEn 230	1[a]	1[a]	1[a]	1[a]	-	1[a]	-	1[a]	-	1[a]	1[a]	1[a]	1[b]	9[a]
Pestalotiopsis sp.24	MEn 231	1[c]	1[b]	-	-	1[a]	1[a]	1[b]	-	-	-	1[a]	1[c]	-	7[abc]
Curvularia sp.7	MEn 232	1[b]	-	1[c]	1[a]	-	-	-	1[b]	1[a]	-	1[c]	1[c]	1[b]	7[abc]
Penicillium sp.14	MEn 233	1[c]	1[b]	1[b]	1[b]	1[c]	1[c]	1[c]	-	-	1[b]	-	-	-	8[bc]
Nigrospora sp.14	MEn 234	1[a]	1[b]	-	-	1[b]	-	-	1[a]	-	1[a]	1[b]	1[a]	1[a]	7[ab]
Aspergillus sp.16	MEn 235	1[a]	1[b]	1[a]	1[c]	-	-	1[a]	-	1[b]	-	1[c]	1[a]	-	8[abc]
Penicillium sp.15	MEn 236	1[c]	-	1[b]	1[a]	-	-	1[c]	-	1[a]	1[a]	1[b]	-	1[c]	7[abc]
Penicillium sp.16	MEn 237	1[b]	1[a]	-	-	1[b]	1[a]	-	1[c]	1[b]	1[b]	-	-	-	7[abc]
Fusarium sp. 14	MEn 238	-	1[b]	-	1[a]	-	1[b]	-	1[c]	-	1[c]	1[c]	1[b]	1[c]	7[abc]
Fusarium sp. 15	MEn 239	1[b]	1[b]	1[b]	-	-	-	1[b]	-	-	-	1[a]	-	-	5[ab]
Phoma sp.4	MEn 240	-	1[c]	1[a]	1[a]	1[c]	-	-	1[b]	-	-	1[a]	1[c]	1[a]	7[abc]

Phoma sp.5	MEn 241	1^{a}	1^{b}	1^{c}	1^{a}	-	-	1^{c}	-	1^{c}	-	1^{c}	1^{c}	-	8^{abc}
Aspergillus sp.17	MEn 242	1^{c}	-	1^{b}	1^{a}	-	1^{b}	-	1^{c}	-	1^{a}	1^{b}	1^{b}	1^{c}	8^{abc}
Fusarium sp.16	MEn 243	1^{a}	1^{a}	-	-	1^{b}	1^{a}	1^{c}	-	1^{b}	1^{c}	-	1^{a}	-	8^{abc}
Curvularia sp.8	MEn 244	1^{b}	1^{c}	1^{a}	1^{c}	-	1^{a}	-	1^{b}	-	-	-	-	1^{a}	6^{abc}
Fusarium sp.17	MEn 245	1^{a}	1^{a}	-	-	1^{a}	1^{a}	1^{a}	1^{a}	1^{a}	1^{a}	-	-	-	8^{a}
Phoma sp.6	MEn 246	1^{b}	1^{b}	-	1^{a}	-	-	-	1^{c}	-	1^{b}	-	1^{a}	1^{a}	6^{abc}
Cladosporium sp.16	MEn 247	1^{a}	1^{c}	1^{b}	1^{c}	-	-	-	1^{a}	1^{a}	1^{a}	1^{c}	-	-	8^{abc}
Scolecobasidium sp.6	MEn 248	1^{b}	1^{a}	1^{c}	1^{a}	1^{b}	1^{c}	-	1^{a}	-	1^{b}	-	-	1^{a}	8^{abc}
Aspergillus sp.18	MEn 249	1^{a}	1^{a}	1^{c}	1^{b}	-	-	1^{a}	-	1^{a}	-	1^{c}	1^{a}	-	8^{abc}
Pestalotiopsis sp.25	MEn 250	1^{a}	1^{b}	-	1^{b}	1^{b}	-	1^{c}	1^{a}	-	1^{b}	1^{a}	-	1^{a}	8^{abc}
Fusarium sp.18	MEn 251	1^{a}	1^{c}	1^{c}	-	1^{c}	1^{a}	1^{a}	1^{b}	1^{a}	-	-	-	-	8^{abc}
Phomopsis sp.2	MEn 252	1^{a}	-	1^{c}	1^{c}	-	-	-	1^{c}	1^{a}	1^{c}	1^{c}	-	1^{c}	7^{ac}
Alternaria sp.6	MEn 253	1^{b}	1^{b}	-	-	1^{c}	-	1^{b}	-	1^{b}	-	-	-	-	5^{bc}
Setosphaeria monoceras	MEn 254	1^{c}	1^{c}	-	1^{b}	-	1^{a}	-	1^{a}	-	-	-	-	1^{c}	5^{abc}
Nigrospora sp.15	MEn 255	1^{b}	1^{b}	1^{b}	-	1^{b}	-	1^{b}	-	-	-	-	-	-	5^{b}
Ascospore 4	MEn 256	-	-	1^{c}	1^{a}	-	1^{b}	-	-	-	-	-	1^{a}	-	4^{abc}
Sterile Mycelia 22	MEn 257	1^{b}	1^{b}	-	-	-	-	1^{a}	-	1^{c}	-	-	-	-	4^{abc}
Sterile Mycelia 23	MEn 258	-	-	1^{b}	1^{a}	-	1^{c}	-	1^{b}	-	1^{c}	1^{c}	1^{a}	-	7^{abc}
Curvularia sp.9	MEn 259	-	1^{c}	1^{a}	-	1^{b}	1^{b}	1^{a}	-	1^{c}	-	-	-	-	6^{abc}
Aspergillus niger	MEn 260	-	-	1^{b}	1^{c}	-	1^{c}	-	-	-	1^{b}	1^{c}	-	-	5^{bc}
Curvularia sp.10	MEn 261	-	-	-	-	1^{b}	1^{b}	1^{b}	-	-	-	-	-	-	3^{b}
Dreschera sp.16	MEn 262	1^{b}	1^{c}	1^{b}	1^{b}	-	1^{a}	-	-	-	1^{c}	-	-	-	6^{abc}
Aspergillus flavus	MEn 263	1^{c}	-	-	-	-	-	1^{c}	-	1^{a}	-	-	-	-	3^{ac}
Aspergillus terrus	MEn 264	-	1^{a}	1^{b}	-	-	1^{b}	-	1^{c}	-	1^{a}	1^{a}	1^{b}	-	7^{abc}
Penicillium sp.17	MEn 265	-	1^{a}	-	1^{b}	1^{c}	1^{c}	-	-	1^{a}	-	-	-	-	5^{abc}
Penicillium sp.18	MEn 266	1^{c}	-	-	-	-	1^{a}	1^{c}	1^{b}	-	1^{a}	1^{a}	-	1^{b}	6^{abc}
Non sporulating basidiomycete 1	MEn 267	1^{a}	1^{b}	-	1^{a}	1^{b}	1^{b}	-	-	-	-	-	-	-	5^{ab}
Aspergillus sp.19	MEn 268	1^{a}	-	-	1^{a}	-	-	1^{c}	1^{c}	1^{a}	-	-	-	1^{b}	5^{ac}
Dreschera sp.14	MEn 269	-	1^{b}	1^{b}	-	-	-	-	-	-	-	-	-	-	2^{b}
Sterile Mycelia 24	MEn 270	1^{c}	1^{b}	-	1^{a}	-	-	-	-	1^{c}	-	1^{b}	1^{c}	-	6^{abc}

Nigrospora sp.16	MEn 271	1^{c}	-	1^{c}	-	-	1^{c}	1^{a}	1^{b}	1^{b}	1^{b}	-	-	1^{c}	7^{abc}
Penicillium sp.19	MEn 272	1^{c}	1^{c}	-	1^{b}	1^{c}	1^{a}	-	-	1^{a}	1^{b}	-	-	-	7^{abc}
Dreschera sp.15	MEn 273	1^{a}	-	-	1^{c}	-	-	1^{a}	1^{b}	1^{b}	-	-	-	1^{a}	5^{abc}
Curvularia sp.11	MEn 274	1^{a}	1^{a}	1^{a}	-	1^{a}	1^{a}	1^{a}	1^{a}	1^{a}	1^{a}	-	-	1^{b}	9^{a}
Cladosporium sp.17	MEn 275	1^{a}	-	-	1^{a}	-	-	-	-	-	-	-	-	-	2^{a}
Lignicola sp.2	MEn 276	1^{b}	1^{a}	1^{b}	-	-	1^{b}	1^{b}	1^{a}	1^{b}	-	-	-	1^{b}	7^{ab}
Sterile Mycelia 25	MEn 277	-	-	1^{a}	1^{c}	1^{a}	-	-	-	-	1^{b}	1^{b}	-	-	$5a^{bc}$
Phoma sp.7	MEn 278	1^{c}	1^{c}	-	-	-	1^{c}	1^{a}	1^{a}	-	-	-	-	1^{a}	5^{ac}
Aspergillus sp.20	MEn 279	1^{b}	-	1^{c}	1^{a}	1^{a}	-	-	-	1^{b}	1^{a}	-	-	-	6^{abc}
Curvularia sp.12	MEn 280	-	1^{a}	-	-	1^{b}	1^{b}	1^{a}	1^{a}		1^{c}	-		1^{a}	8^{abc}
Pestalotiopsis sp.26	MEn 281	1^{a}	-	1^{b}	1^{c}	-	-	-	-	1^{a}	1^{c}	-	-	-	5^{abc}
Pestalotiopsis sp.27	MEn 282	-	-	-	-	1^{c}	1^{b}	1^{a}	1^{a}	-	1^{a}	-	-	1^{c}	5^{abc}
Nigrospora sp.17	MEn 283	1^{b}	1^{a}	1^{a}	1^{c}	-	-	-	-	1^{a}	-	1^{b}	-	-	6^{abc}
Pestalotiopsis sp.28	MEn 284	1^{a}	1^{c}	-	-	1^{b}	1^{a}	1^{a}	-	-	-	-	-	1^{c}	5^{abc}
Total		209	182	181	168	162	185	165	157	151	147	141	116		

Legend: MEn=Culture coded with isolation code M denotes mangroves and En denotes endophytes; ^{a}= Fungal endophyte in leaf; ^{b}= Fungal endophyte in stem; ^{c}=Fungal endophyte in root. ^{ab}=Fungal endophyte in leaf and stem; ^{ac}= fungal endophyte in leaf and root; ^{bc}=Fungal endophyte in stem and root; ^{abc}=Fungal endophyte in leaf, stem and roots.

Table 4.4: Endophytic fungal colonies isolated from mangrove plants during summer season.

Fungal species	Isolate Code	Host plant													Total Isolates
		AM	AO	AI	DH	CI	RA	RM	EA	BC	CT	AA	AC	SA	
Setosphaeria monoceras	MEn 01	1^{c}	-	-	-	-	-	-	-	1^{c}	-	-	1^{c}	1^{c}	4^{c}
Aspergillus sp.1	MEn 02	1^{b}	1^{a}	-	-	1^{a}	-	1^{b}	-	1^{b}	-	-	-	1^{b}	6^{ab}
Scolecobasidium sp.1	MEn 03	1^{c}	1^{b}	1^{c}	-	1^{a}	-	-	-	-	-	-	-	1^{c}	4^{abc}
Drechslera sp.1	MEn 04	1^{b}	-	1^{a}	-	1^{c}	-	-	-	-	-	-	-	1^{b}	3^{abc}
Fusarium sp.1	MEn 05	1^{c}	-	1^{c}	1^{c}	-	1^{c}	1^{c}	-	-	-	-	^{1a}	1^{c}	6^{ac}
Pestalotiopsis sp.1	MEn 06	1^{b}	-	1^{b}	-	1^{b}	-	1^{b}	1^{b}	-	-	-	-	1^{b}	5^{b}
Scytalidium lignicola	MEn 07	1^{c}	-	-	-	-	-	-	-	1^{c}	-	-	-	1^{c}	2^{c}
Penicillium sp.1	MEn 08	1^{a}	1^{c}	1^{b}	-	1^{b}	1^{a}	-	-	-	-	-	-	1^{a}	5^{abc}
Fusarium sacchari	MEn 09	-	1^{b}	1^{b}	1^{a}	1^{a}	1^{c}	1^{a}	-	1^{b}	-	-	-	-	7^{abc}
Aspergillus sp.2	MEn 10	1^{b}	1^{a}	-	-	1^{b}	-	-	1^{c}	1^{b}	-	-	-	1^{b}	5^{abc}
Penicillium sp.2	MEn 11	1^{c}	1^{c}	1^{b}	1^{a}	1^{a}	-	1^{a}	-	1^{c}	1^{a}	1^{b}	-	1^{c}	9^{abc}
Corynesporina elegans	MEn 12	1^{b}	1^{b}	1^{a}	1^{a}	1^{c}	-	1^{c}	1^{a}	1^{b}	1^{a}	-	-	1^{b}	9^{abc}
Drechslera sp.2	MEn 13	-	-	-	-	-	1^{a}	1^{a}	1^{a}	1^{c}	1^{b}	1^{a}	1^{a}	-	7^{abc}
Scytalidium lignicola	MEn 14	1^{a}	-	1^{c}	1^{c}	1^{b}	1^{b}	1^{b}	1^{a}	-	-	-	-	1^{a}	7^{abc}
Sterile mycelia 1	MEn 15	-	1^{a}	1^{c}	1^{c}	1^{c}	1^{b}	1^{a}	1^{a}	1^{c}	-	-	-	-	8^{abc}
Corynespora sp.1	MEn 16	1^{a}	1^{b}	-	1^{c}	1^{a}	1^{a}	-	-	-	-	-	-	1^{a}	5^{abc}
Gilmaniella sp.1	MEn 17	-	1^{a}	1^{a}	1^{b}	1^{b}	1^{a}	-	-	-	-	-	-	-	5^{ab}
Fusarium sp.2	MEn 18	1^{a}	1^{b}	-	-	1^{a}	-	-	1^{a}	1^{a}	-	-	-	1^{a}	5^{ab}
Drechslera sp.3	MEn 19	1^{c}	1^{c}	1^{b}	1^{b}	1^{c}	1^{a}	1^{a}	-	1^{c}	-	-	-	1^{c}	8^{abc}
Drechslera sp.4	MEn 20	1^{a}	1^{a}	1^{c}	1^{a}	1^{c}	-	1^{c}	1^{c}	1^{a}	-	-	-	1^{a}	8^{ac}
Pestalotiopsis sp.2	MEn 21	1^{a}	1^{c}	1^{b}	1^{b}	-	1^{c}	1^{a}	1^{a}	-	-	-	-	1^{a}	7^{abc}
Sterile mycelia 2	MEn 22	-	1^{b}	1^{a}	1^{c}	1^{a}	1^{b}	-	-	-	-	-	-	-	5^{abc}
Junctospora pulchra	MEn 23	1^{a}	-	-	-	1^{a}	1^{b}	-	1^{c}	-	-	-	-	1^{a}	4^{abc}
Curvularia sp.1	MEn 24	-	1^{a}	1^{a}	1^{b}	1^{c}	1^{c}	1^{a}	-	1^{b}	1^{a}	-	-	-	8^{abc}
Scytalidium sp.1	MEn 25	1^{b}	1^{a}	-	-	1^{a}	-	-	1^{c}	1^{c}	1^{c}	-	1^{b}	1^{b}	7^{abc}
Setosphaeria sp.1	MEn 26	-	1^{c}	-	1^{b}	-	-	-	-	1^{a}	1^{a}	-	-	-	4^{abc}
Aspergillus niger	MEn 27	1^{c}	1^{a}	1^{b}	-	1^{a}	1^{b}	1^{c}	1^{a}	1^{a}	-	-	-	1^{c}	8^{abc}

Drechslera sp.4	MEn 28	-	-	-	-	-	-	1^{b}	-	1^{b}	1^{b}	1^{c}	-	-	4^{bc}
Scolecobasidium sp.2	MEn 29	1^{a}	-	1^{c}	-	-	-	-	-	1^{a}	1^{b}	1^{a}	1^{a}	1^{a}	6^{abc}
Junctospora pulchra	MEn 30	1^{c}	1^{c}	1^{b}	1^{a}	-	1^{a}	1^{c}	1^{a}	1^{c}	-	-	-	1^{c}	8^{abc}
Fusarium sp.3	MEn 31	1^{b}	-	1^{a}	1^{c}	-	1^{a}	1^{b}	-	-	-	-	-	1^{b}	5^{abc}
Cladosporium sp.1	MEn 32	-	1^{b}	1^{b}	-	-	1^{b}	1^{a}	1^{b}	1^{c}	1^{a}	-	-	-	7^{abc}
Myceliophthora sp.1	MEn 33	1^{a}	1^{b}	-	1^{c}	1^{b}	1^{a}	-	1^{a}	1^{c}	1^{b}	1^{c}	1^{b}	1^{a}	10^{abc}
Penicillium sp.3	MEn 34	-	-	-	-	-	-	-	-	1^{c}	1^{b}	1^{a}	-	-	3^{abc}
Sterile Mycelia 3	MEn 35	1^{a}	-	-	-	-	-	-	-	-	-	-	-	1^{a}	1^{a}
Nigrospora sphaerica	MEn 36	1^{a}	1^{b}	-	-	-	-	-	-	-	-	-	-	1^{a}	2^{ab}
Cladosporium sp.2	MEn 37	-	-	-	-	-	-	-	-	-	-	-	1^{a}	-	1^{a}
Seatospheria monoceras	MEn 38	1^{c}	-	1^{c}	-	-	-	-	-	-	-	1^{b}	1^{c}	1^{c}	4^{bc}
Scolecobasidium sp.3	MEn 39	1^{a}	1^{b}	1^{c}	-	-	-	-	1^{c}	-	1^{a}	1^{c}	1^{b}	1^{a}	7^{abc}
Penicillium sp.4	MEn 40	-	-	1^{c}	-	1^{b}	1^{a}	1^{a}	1^{c}	-	1^{b}	1^{a}	1^{c}	-	8^{abc}
Aspergillus niger	MEn 41	-	1^{b}	-	1^{c}	1^{a}	-	-	-	1^{c}	1^{b}	-	-	-	5^{abc}
Pestalotiopsis sp.3	MEn 42	-	-	-	-	-	1^{a}	1^{c}	1^{c}	-	1^{b}	-	1^{a}	-	5^{abc}
Gonatobotryum sp.1	MEn 43	-	-	-	-	1^{b}	1^{a}	1^{c}	-	1^{a}	1^{b}	1^{a}	-	-	6^{abc}
Aspergillus fumigates	MEn 44	-	-	-	-	-	-	1^{b}	1^{c}	1^{a}	-	-	1^{b}	-	4^{abc}
Pestalotiopsis microspore	MEn 45	1^{b}	-	-	-	-	-	1^{c}	-	1^{b}	1^{a}	1^{a}	-	1^{b}	5^{abc}
Pestalotiopsis sp.4	MEn 46	1^{c}	1^{b}	1^{a}	1^{c}	-	-	-	-	-	-	-	1^{b}	1^{c}	5^{abc}
Aspergillus flavus	MEn 47	1^{a}	-	-	-	-	-	-	-	-	1^{a}	1^{b}	1^{c}	1^{a}	4^{abc}
Fusarium incarnatum	MEn 48	1^{c}	1^{c}	1^{a}	-	-	-	1^{b}	1^{b}	-	1^{b}	1^{c}	1^{a}	1^{c}	8^{abc}
Unidentified 1	MEn 49	1^{a}	1^{a}	-	-	-	-	-	-	-	-	-	-	1^{a}	2^{a}
Aspergillus sp.3	MEn 50	-	1^{c}	1^{b}	1^{a}	-	1^{b}	1^{b}	-	-	-	-	-	-	5^{abc}
Unidentified 2	MEn 51	1^{b}	1^{a}	1^{a}	1^{c}	-	-	-	-	-	-	-	-	1^{b}	4^{abc}
Fungal endophyte	MEn 52	-	-	-	-	-	1^{b}	1^{c}	1^{a}	1^{a}	1^{a}	-	-	-	5^{abc}
Thermomyces sp.1	MEn 53	-	-	-	-	-	-	1^{b}	-	1^{a}	-	1^{c}	1^{c}	-	4^{abc}
Colletotrichum sp1.	MEn 54	1^{b}	1^{b}	1^{c}	-	-	-	-	-	-	-	-	1^{a}	1^{b}	4^{abc}
Unidentified 3	MEn 55	1^{a}	-	-	-	-	-	-	1^{a}	-	1^{c}	1^{b}	-	1^{a}	4^{abc}
Aspergillus sp.4	MEn 56	-	1^{a}	1^{a}	1^{b}	1^{a}	1^{c}	-	-	-	-	-	-	-	5^{abc}

Acremonium sp.1	MEn 57	1^{b}	1^{a}	1^{b}	-	1^{b}	1^{a}	-	-	-	-	-	-	1^{b}	5^{ab}
Colletotrichum gloeosporioides	MEn 58	1^{a}	1^{a}	1^{a}	1^{a}	1^{a}	1^{b}	1^{a}	-	-	1^{a}	-	-	1^{a}	8^{ab}
Sordariomycetes sp.1	MEn 59	-	-	-	-	-	1^{a}	1^{c}	1^{a}	1^{a}	1^{a}	-	1^{b}	-	6^{abc}
Rhizopus sp.1	MEn 60	1^{b}	-	-	1^{b}	1^{b}	1^{b}	1^{b}	-	-	-	-	-	1^{b}	5^{b}
Unidentified 4	MEn 61	1^{b}	-	1^{b}	1^{c}	1^{c}	-	1^{b}	1^{b}	-	-	-	-	1^{b}	6^{bc}
Unidentified 5	MEn 62	-	-	-	-	-	-	-	-	1^{c}	1^{c}	1^{c}	1^{c}	-	4^{c}
Pestalotiopsis sp.5	MEn 63	1^{b}	1^{a}	1^{a}	1^{a}	-	-	1^{c}	1^{b}	1^{a}	1^{a}	-	-	1^{b}	8^{abc}
Colletotrichum sp.2	MEn 64	1^{a}	-	1^{a}	-	1^{b}	1^{a}	1^{a}	-	-	1^{a}	1^{b}	1^{a}	1^{a}	8^{ab}
Aspergillus flavus	MEn 65	1^{b}	-	-	-	-	-	-	-	-	-	-	-	1^{b}	1^{b}
Fusarium equiseti	MEn 66	1^{c}	1^{c}	-	1^{a}	1^{a}	1^{c}	-	-	-	-	-	-	1^{c}	5^{ac}
Curvularia sp.2	MEn 67	-	-	-	-	-	-	1^{c}	1^{c}	1^{b}	1^{a}	-	1^{b}	-	5^{abc}
Scytalidium lignicola	MEn 68	1^{a}	1^{b}	-	1^{c}	-	-	-	1^{a}	1^{b}	-	1^{b}	-	1^{a}	6^{abc}
Drechslera sp.5	MEn 69	1^{c}	1^{c}	1^{c}	1^{c}	1^{c}	1^{c}	-	-	-	-	-	-	1^{c}	6^{c}
Pestalotiopsis sp.6	MEn 70	1^{b}	1^{b}	1^{b}	1^{b}	-	-	-	1^{b}	1^{b}	1^{a}	-	-	1^{b}	7^{ab}
Pestalotiopsis sp.7	MEn 71	-	1^{b}	1^{c}	1^{b}	1^{b}	1^{b}	1^{c}	-	-	1^{b}	1^{b}	1^{c}	-	9^{bc}
Ceratocystis sp.1	MEn 72	1^{a}	1^{c}	1^{c}	-	1^{a}	1^{c}	1^{c}	1^{c}	1^{a}	-	-	-	1^{a}	8^{ac}
Fusarium sp.4	MEn 73	1^{a}	-	-	1^{b}	1^{a}	1^{b}	1^{a}	1^{c}	-	1^{a}	1^{b}	1^{b}	1^{a}	9^{abc}
Fusarium sp.5	MEn 74	-	-	-	1^{b}	1^{a}	1^{b}	1^{c}	1^{c}	1^{c}	-	-	1^{a}	-	7^{abc}
Aspergillus sp.5	MEn 75	-	-	-	-	-	-	-	-	-	-	1^{b}	1^{b}	-	2^{b}
Dreschera sp.6	MEn 76	-	1^{a}	-	1^{a}	-	-	-	-	-	-	1^{a}	1^{a}	-	4^{a}
Alternaria alternata	MEn 77	1^{a}	1^{b}	1^{b}	-	1^{a}	1^{c}	1^{a}	1^{b}	1^{c}	1^{a}	1^{a}	1^{a}	1^{a}	11^{abc}
Bipolaris sp.1	MEn 78	1^{b}	1^{a}	1^{c}	1^{a}	-	-	-	1^{b}	1^{a}	-	-	-	1^{b}	6^{abc}
Alternaria sp.1	MEn 79	-	-	1^{a}	-	1^{a}	1^{a}	1^{a}	-	-	1^{a}	-	-	-	5^{a}
Corynespora sp.2	MEn 80	1^{a}	1^{b}	1^{b}	1^{a}	1^{b}	-	-	-	-	-	-	-	1^{a}	5^{ab}
Alternaria sp.2	MEn 81	1^{c}	1^{c}	-	1^{a}	1^{a}	1^{a}	1^{c}	1^{c}	-	-	1^{a}	-	1^{c}	8^{ac}
Sterile Mycelia 4	MEn 82	1^{c}	1^{b}	-	1^{a}	1^{a}	1^{a}	1^{c}	1^{b}	1^{a}	-	-	-	1^{c}	8^{abc}
Pestalotiopsis sp.8	MEn 83	1^{c}	1^{c}	-	1^{a}	-	-	-	1^{b}	1^{c}	-	1^{c}	-	1^{c}	6^{abc}
Alternaria sp.3	MEn 84	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}	-	-	-	1^{c}	1^{c}	1^{c}	-	1^{b}	8^{bc}
Nigrospora sp.1	MEn 85	1^{a}	-	1^{b}	-	-	1^{c}	-	1^{a}	1^{a}	-	-	-	1^{a}	5^{abc}
Nigrosopra sp.2	MEn 86	1^{b}	1^{b}	1^{a}	1^{b}	1^{c}	-	1^{c}	-	-	1^{c}	1^{a}	-	1^{b}	8^{abc}

Alternaria alternata	MEn 87	-	-	1[a]	-	1[a]	1[a]	-	-	-	1[a]	-	-	-	4[a]
Cladosporium sp.3	MEn 88	-	1[a]	1[b]	1[b]	1[b]	1[b]	1[a]	-	-	-	-	-	-	6[ab]
Cladosporium sp.4	MEn 89	-	1[b]	-	1[a]	1[a]	1[c]	1[b]	-	1[a]	-	-	-	-	6[abc]
Sterile Mycelia 5	MEn 90	1[a]	1[b]	1[a]	1[c]	1[c]	1[a]	1[a]	1[b]	-	-	-	-	1[a]	8[abc]
Aspergillus ulvus	MEn 91	-	-	-	-	-	-	1[c]	1[c]	1[c]	-	-	-	-	3[c]
Cladosporium sp.5	MEn 92	1[c]	-	-	-	1[a]	-	1[a]	1[c]	1[a]	1[a]	1[a]	1[a]	1[c]	8[ac]
Dreschera sp.7	MEn 93	1[c]	-	-	-	-	-	-	1[c]	1[c]	-	-	-	1[c]	3[c]
Gonatobotryum sp.2	MEn 94	1[a]	-	1[a]	-	1[c]	-	1[a]	-	-	-	-	-	1[a]	4[ac]
Cladosporium sp.6	MEn 95	1[b]	1[b]	1[b]	1[a]	1[b]	-	-	-	-	-	-	-	1[b]	5[ab]
Fusarium sp. 6	MEn 96	1[c]	1[b]	-	-	1[b]	1[c]	1[b]	1[b]	-	-	-	-	1[c]	6[bc]
Fusarium semitectum	MEn 97	1[a]	1[b]	1[c]	1[a]	1[b]	1[b]	1[b]	-	-	-	-	-	1[a]	7[abc]
Penicillium sp.5	MEn 98	-	-	-	-	-	-	1[a]	1[a]	-	1[a]	1[a]	1[a]	-	5[a]
Pestalotiopsis sp.9	MEn 99	1[b]	1[a]	1[c]	1[c]	1[b]	-	1[a]	1[a]	1[a]	-	-	-	1[b]	8[abc]
Cladosporium sp.7	MEn 100	1[b]	1[a]	1[a]	1[c]	-	1[a]	1[b]	1[c]	1[a]	-	-	-	1[b]	8[abc]
Marieltiottia sp.1	MEn 101	1[a]	1[a]	1[b]	1[a]	1[c]	-	-	1[a]	1[b]	1[a]	-	-	1[a]	8[abc]
Pestalotiopsis sp.10	MEn 102	1[a]	-	1[a]	-	-	-	-	-	-	-	-	1[a]	1[a]	3[a]
Nigrospora sp.3	MEn 103	-	-	1[b]	1[b]	1[a]	1[c]	-	1[b]	-	-	1[a]	-	-	6[abc]
Fusarium sp.7	MEn 104	-	-	-	-	1[c]	-	-	-	1[c]	1[c]	1[c]	1[c]	-	5[c]
Aspergillus sp.6	MEn 105	1[b]	-	1[b]	1[c]	1[b]	1[b]	-	-	-	-	1[c]	1[b]	1[b]	7[bc]
Nigrospora sp.4	MEn 106	1[b]	-	-	1[a]	-	1[b]	1[b]	1[c]	1[b]	1[b]	1[a]	-	1[b]	8[abc]
Fusarium sp.8	MEn 107	1[b]	1[a]	1[a]	1[c]	1[c]	1[a]	1[b]	-	1[a]	1[c]	-	-	1[b]	9[abc]
Pestalotiopsis sp.11	MEn 108	1[a]	1[a]	1[c]	1[b]	-	1[b]	1[c]	1[c]	1[b]	1[b]	-	-	1[a]	9[abc]
Phoma sp.1	MEn 109	-	-	1[a]	-	1[a]	1[a]	1[b]	1[a]	-	-	-	-	-	5[ab]
Aspergillus sp.7	MEn 110	1[a]	1[c]	1[b]	1[b]	1[a]	-	1[c]	1[b]	-	-	-	1[a]	1[a]	8[abc]
Pestalotiopsis sp.12	MEn 111	1[b]	-	-	-	1[b]	1[b]	-	-	-	-	-	1[b]	1[b]	4[b]
Gliocladium sp.1	MEn 112	1[c]	1[b]	1[b]	1[a]	1[c]	-	1[b]	-	-	-	-	-	1[c]	6[abc]
Bipolaris sp.7	MEn 113	1[a]	1[c]	-	1[a]	-	1[b]	-	-	-	-	-	-	1[a]	4[abc]
Aspergillus sp.8	MEn 114	1[a]	1[a]	1[a]	1[a]	-	1[a]	-	-	-	-	-	-	1[a]	5[a]
Penicillium sp.6	MEn 115	-	1[b]	1[b]	1[b]	-	1[b]	-	-	1[a]	1[a]	-	-	-	6[ab]
Sterile Mycelia 6	MEn 116	-	1[c]	1[b]	1[b]	-	1[a]	1[c]	1[a]	1[a]	1[b]	-	-	-	8[abc]
Fusarium sp. 9	MEn 117	-	-	1[c]	-	1[c]	-	1[c]	1[c]	1[c]	-	-	-	-	5[c]

Thickened chlamydospores 1	MEn 118	1^{b}	1^{c}	1^{a}	1^{b}	-	-	-	-	-	-	-	-	1^{b}	4^{abc}
Nigrospora sp.5	MEn 119	1^{b}	-	-	-	1^{b}	-	1^{b}	1^{c}	1^{b}	-	-	-	1^{b}	5^{bc}
Nigrospora sp.6	MEn 120	1^{b}	-	-	-	-	-	-	-	-	-	-	1^{b}	1^{b}	2^{b}
Scolecobasidium sp.4	MEn 121	-	1^{c}	-	1^{b}	1^{a}	-	-	-	-	-	1^{b}	-	-	4^{abc}
Sterile Mycelia 7	MEn 122	1^{a}	1^{c}	1^{b}	-	-	-	-	-	-	-	1^{a}	1^{b}	1^{a}	5^{abc}
Pestalotiopsis sp.13	MEn 123	1^{b}	-	-	1^{b}	-	-	1^{a}	-	-	1^{b}	-	-	1^{b}	4^{ab}
Scytalidium sp.1	MEn 124	1^{a}	-	-	-	-	-	-	-	-	-	-	-	1^{a}	1^{a}
Gilmaniella sp.2	MEn 125	-	1^{a}	1^{a}	1^{c}	-	-	-	1^{a}	-	-	-	-	-	4^{ac}
Rhizopus sp.2	MEn 126	1^{c}	-	-	-	1^{a}	1^{b}	-	1^{a}	-	-	-	1^{b}	1^{c}	5^{abc}
Sterile Mycelia 8	MEn 127	-	-	-	-	-	-	1^{b}	1^{b}	1^{b}	1^{b}	-	-	-	4^{b}
Aspergillus flavus	MEn 128	1^{a}	-	-	-	-	1^{c}	1^{b}	1^{b}	1^{a}	1^{a}	-	-	1^{a}	6^{abc}
Penicillium sp.7	MEn 129	1^{b}	1^{a}	-	-	-	-	-	-	1^{c}	1^{b}	-	-	1^{b}	4^{abc}
Pestalotiopsis sp.14	MEn 130	1^{c}	1^{b}	1^{b}	-	-	1^{c}	1^{b}	-	1^{b}	-	-	-	1^{c}	6^{bc}
Humicola sp.1	MEn 131	1^{a}	-	1^{b}	-	-	-	-	1^{c}	1^{b}	1^{a}	1^{b}	-	1^{a}	6^{abc}
Linkosia sp.1	MEn 132	1^{c}	1^{c}	1^{c}	-	-	-	-	-	-	-	-	1^{c}	1^{c}	4^{c}
Aspergillus niger	MEn 133	1^{a}	1^{a}	1^{a}	1^{a}	1^{a}	-	-	-	-	-	-	-	1^{a}	5^{a}
Juctospora sp.1	MEn 134	-	1^{c}	-	1^{b}	1^{b}	-	1^{a}	1^{c}	1^{a}	-	-	-	-	6^{abc}
Cladosporium sp.8	MEn 135	1^{b}	1^{b}	1^{b}	1^{b}	-	1^{b}	-	-	-	-	-	-	1^{b}	5^{b}
Rhizopus sp.3	MEn 136	-	-	-	-	-	-	1^{b}	1^{c}	-	-	1^{b}	-	-	3^{bc}
Cladosporium sp.9	MEn 137	-	1^{c}	1^{a}	1^{b}	1^{a}	1^{a}	1^{a}	-	1^{c}	1^{b}	1^{b}	1^{b}	-	10^{abc}
Pestalotiopsis sp.15	MEn 138	1^{b}	1^{b}	^{1b}	-	-	1^{a}	1^{b}	-	1^{a}	1^{a}	-	-	1^{b}	7^{ab}
Pestalotiopsis sp.16	MEn 139	1^{a}	1^{b}	1^{a}	1^{b}	1^{c}	-	-	-	-	-	-	-	1^{a}	5^{abc}
Aspergillus niger	MEn 140	^{1b}	1^{a}	1^{c}	1^{a}	-	1^{a}	1^{b}	-	-	-	-	1^{a}	^{1b}	7^{abc}
Cladosporium sp.10	MEn 141	1^{b}	1^{b}	-	1^{c}	1^{b}	1^{b}	-	1^{c}	1^{b}	-	-	-	1^{b}	7^{bc}
Rhizopus sp.4	MEn 142	1^{b}	1^{a}	1^{a}	1^{c}	1^{c}	1^{a}	1^{a}	-	-	1^{b}	-	-	1^{b}	8^{abc}
Dreschera sp.8	MEn 143	-	-	-	-	1^{b}	1^{b}	1^{b}	-	1^{b}	-	-	-	-	4^{b}
Bipolaris sp.2	MEn 144	1^{c}	1^{b}	1^{a}	1^{a}	1^{c}	-	1^{a}	1^{a}	-	1^{b}	-	-	1^{c}	8^{abc}
Pestalotiopsis sp.17	MEn 145	1^{b}	-	-	-	1^{b}	1^{a}	1^{c}	-	-	-	-	1^{b}	1^{b}	5^{abc}
Sterile Mycelia 9	MEn 146	1^{a}	1^{c}	1^{b}	1^{a}	1^{b}	1^{b}	-	-	-	-	1^{c}	1^{a}	1^{a}	8^{abc}
Dreschera sp.9	MEn 147	1^{a}	-	1^{c}	1^{a}	1^{a}	1^{a}	-	1^{a}	1^{c}	-	-	1^{a}	1^{a}	8^{ac}

Aspergillus sp.9	MEn 148	1^{a}	1^{a}	1^{b}	1^{c}	-	1^{a}	1^{b}	-	-	-	-	1^{c}	1^{a}	7^{abc}
Curvularia sp.3	MEn 149	1^{b}	1^{c}	1^{a}	1^{a}	1^{a}	1^{c}	1^{b}	-	-	-	-	1^{a}	1^{b}	8^{abc}
Penicillium sp.8	MEn 150	1^{a}	-	-	-	1^{a}	1^{c}	1^{b}	-	1^{a}	1^{b}	1^{a}	1^{a}	1^{a}	8^{abc}
Curvularia sp.4	MEn 151	1^{c}	-	-	1^{c}	1^{c}	-	-	1^{c}	-	-	1^{c}	-	1^{c}	5^{c}
Cladosporium sp.11	MEn 152	1^{c}	1^{b}	1^{a}	1^{a}	1^{a}	-	-	-	1^{a}	1^{a}	1^{c}	1^{b}	1^{c}	9^{abc}
Pestalotiopsis sp.18	MEn 153	1^{c}	-	1^{a}	1^{b}	-	1^{b}	1^{a}	-	-	-	-	-	1^{c}	5^{abc}
Nigrospora sp.7	MEn 154	1^{b}	1^{c}	1^{b}	1^{b}	1^{b}	1^{c}	1^{a}	-	-	1^{b}	-	1^{c}	1^{b}	9^{abc}
Fusarium sp. 10	MEn 155	1^{a}	1^{a}	-	-	-	1^{a}	-	1^{c}	1^{a}	-	-	-	1^{a}	5^{ac}
Aspergillus sp.10	MEn 156	1^{a}	1^{c}	1^{b}	-	1^{a}	-	-	-	-	1^{c}	-	1^{b}	1^{a}	6^{abc}
Corynospora sp.4	MEn 157	1^{b}	1^{b}	-	-	-	1^{b}	1^{c}	1^{b}	-	-	-	-	1^{b}	5^{bc}
Sordariomycetes sp.2	MEn 158	1^{c}	-	-	1^{a}	1^{b}	-	-	-	-	-	-	1^{a}	1^{c}	4^{abc}
Penicillium sp.9	MEn 159	-	-	-	-	-	1^{a}	-	1^{a}	-	1^{a}	1^{a}	-	-	4^{a}
Nigrospora sp.8	MEn 160	1^{a}	1^{a}	1^{a}	-	-	-	-	-	1^{b}	-	-	1^{a}	1^{a}	5^{ab}
Aureobasidium pullulans	MEn 161	1^{c}	1^{b}	-	-	-	-	1^{b}	1^{c}	-	1^{b}	-	-	1^{c}	5^{bc}
Pestalotiopsis sp.19	MEn 162	1^{a}	1^{c}	1^{b}	1^{a}	1^{c}	1^{c}	1^{b}	-	1^{a}	-	1^{b}	1^{a}	1^{a}	10^{abc}
Aspergillus niger	MEn 163	-	-	-	-	-	1^{c}	-	1^{b}	-	1^{a}	-	-	-	3^{abc}
Penicillium sp.10	MEn 164	1^{a}	1^{b}	1^{a}	1^{a}	1^{a}	1^{a}	1^{a}	-	1^{b}	-	1^{b}	-	1^{a}	9^{ab}
Sterile Mycelia 10	MEn 165	1^{c}	1^{c}	-	-	-	1^{c}	-	1^{c}	-	-	-	-	1^{c}	4^{c}
Aspergillus sp.11	MEn 166	1^{c}	1^{b}	1^{a}	1^{b}	1^{a}	-	1^{c}	-	1^{a}	-	1^{b}	-	1^{c}	8^{abc}
Sterile Mycelia 11	MEn 167	1^{c}	1^{b}	1^{a}	-	-	-	-	1^{c}	-	1^{b}	-	-	1^{c}	5^{abc}
Cladosporium sp.12	MEn 168	1^{b}	1^{b}	1^{a}	1^{a}	1^{c}	-	1^{b}	-	1^{b}	-	-	1^{a}	1^{b}	8^{abc}
Penicillium sp.11	MEn 169	1^{b}	-	1^{b}	-	-	1^{c}	-	1^{b}	-	-	-	-	1^{b}	4^{bc}
Sterile Mycelia 12	MEn 170	1^{a}	1^{a}	-	-	1^{a}	-	1^{c}	-	1^{b}	1^{a}	1^{b}	1^{a}	1^{a}	8^{abc}
Scolecobasidium sp.5	MEn 171	1^{b}	-	1^{b}	-	-	1^{b}	-	1^{b}	-	-	-	-	1^{b}	4^{b}
Gliocladium sp.2	MEn 172	1^{a}	1^{c}	1^{c}	1^{a}	1^{b}	1^{a}	1^{c}	-	1^{b}	-	1^{a}	-	1^{a}	9^{abc}
Dreschera sp.10	MEn 173	-	-	-	-	-	1^{c}	-	1^{b}	-	1^{a}	-	1^{b}	-	4^{abc}
Phoma sp.2	MEn 174	1^{b}	-	-	-	-	-	-	-	-	-	-	1^{a}	1^{b}	2^{ab}
Cladosporium sp.13	MEn 175	1^{a}	-	1^{c}	-	-	1^{b}	-	1^{a}	-	-	-	-	1^{a}	4^{abc}
Ascospore 1	MEn 176	1^{c}	1^{c}	-	1^{a}	1^{b}	1^{a}	1^{c}	-	1^{a}	-	1^{b}	-	1^{c}	8^{abc}
Alternaria sp.4	MEn 177	1^{a}	-	1^{c}	-	-	1^{c}	-	1^{a}	-	-	-	-	1^{a}	4^{ac}
Scytalydium sp.2	MEn 178	1^{b}	1^{a}	1^{c}	1^{b}	1^{a}	-	1^{c}	-	1^{b}	-	1^{a}	-	1^{b}	8^{abc}

Corynospora sp.4	MEn 179	1^{a}	-	1^{a}	-	-	1^{c}	-	1^{a}	-	1^{a}	1^{a}	1^{a}	1^{a}	7^{ac}
Aspergillus sp.11	MEn 180	1^{b}	1^{a}	1^{b}	1^{b}	1^{a}	1^{a}	1^{b}	-	1^{a}	-	-	-	1^{b}	8^{ab}
Pestalotiopsis sp.18	MEn 181	1^{c}	1^{c}	1^{b}	-	-	1^{a}	-	-	-	-	-	-	1^{c}	4^{abc}
Alternaria sp.5	MEn 182	1^{a}	1^{a}	1^{a}	1^{a}	1^{a}	1^{a}	1^{a}	-	1^{a}	-	-	1^{a}	1^{a}	9^{a}
Sterile Mycelia 13	MEn 183	-	-	-	-	-	-	-	1^{b}	-	1^{b}	-	1^{b}	-	3^{b}
Nigrospora sp.9	MEn 184	1^{c}	1^{b}	-	1^{a}	1^{a}	1^{c}	1^{b}	-	1^{b}	-	-	1^{a}	1^{c}	8^{abc}
Sterile Mycelia 14	MEn 185	1^{a}	-	-	-	-	-	-	1^{a}	-	1^{a}	-	-	1^{a}	3^{a}
Dreschera sp.11	MEn 186	1^{a}	1^{b}	1^{c}	1^{a}	-	-	-	-	-	-	-	-	1^{a}	4^{abc}
Limposoma sp.1	MEn 187	-	1^{c}	1^{c}	-	-	-	-	1^{c}	-	-	-	-	-	3^{c}
Sterile Mycelia 15	MEn 188	1^{a}	1^{b}	1^{c}	1^{b}	-	-	-	-	-	-	-	-	1^{a}	4^{abc}
Ascospores 2	MEn 189	1^{a}	1^{c}	1^{b}	-	-	1^{b}	-	-	-	-	-	-	1^{a}	4^{abc}
Pestalotiopsis sp.20	MEn 190	1^{c}	1^{b}	1^{a}	1^{c}	1^{b}	1^{a}	1^{c}	-	1^{b}	-	1^{a}	-	1^{c}	9^{abc}
Gilmaniella sp.2	MEn 191	1^{a}	-	1^{b}	-	-	1^{c}	-	1^{a}	-	-	-	-	1^{a}	4^{abc}
Nigrospora sp.10	MEn 192	1^{c}	1^{b}	1^{a}	1^{c}	1^{b}	1^{a}	1^{c}	-	1^{b}	-	-	1^{a}	1^{c}	9^{abc}
Juctospora pulchura	MEn 193	1^{b}	-	1^{a}	-	-	-	-	1^{a}	-	-	-	-	1^{b}	3^{ab}
Bipolaris sp.3	MEn 194	1^{c}	1^{a}	-	1^{c}	1^{b}	1^{b}	-	-	-	-	-	-	1^{c}	5^{abc}
Fusarium sp. 11	MEn 195	1^{b}	-	1^{b}	-	-	-	-	1^{a}	-	-	-	-	1^{b}	3^{ab}
Gilberella sp.2	MEn 196	1^{a}	1^{a}	1^{b}	1^{a}	1^{a}	1^{a}	1^{b}	-	1^{a}	-	1^{a}	-	1^{a}	9^{ab}
Phomopsis sp.1	MEn 197	1^{c}	-	1^{c}	-	-	1^{a}	-	-	-	-	-	-	1^{c}	3^{ac}
Pestalotiopsis sp.21	MEn 198	1^{a}	^{1a}	1^{b}	1^{b}	1^{a}	1^{a}	1^{b}	-	-	-	-	1^{b}	1^{a}	8^{ab}
Cladosporium sp.14	MEn 199	-	-	1^{c}	-	-	1^{a}	-	-	-	1^{c}	-	-	-	3^{ac}
Aspergillus niger	MEn 200	1^{c}	1^{a}	1^{b}	1^{b}	1^{b}	-	1^{a}	1^{a}	1^{c}	-	-	1^{b}	1^{c}	9^{abc}
Penicillium sp.12	MEn 201	1^{a}	-	1^{a}	-	-	1^{a}	-	-	-	1^{b}	-	-	1^{a}	4^{ab}
Catenate spores 1	MEn 202	1^{b}	-	-	1^{b}	1^{b}	1^{a}	1^{a}	1^{a}	-	-	-	1^{b}	1^{b}	7^{ab}
Curvularia sp.5	MEn 203	-	1^{c}	1^{b}	-	1^{b}	1^{a}	-	-	-	-	-	-	-	4^{abc}
Curvularia sp.6	MEn 204	1^{b}	-	-	1^{b}	1^{b}	-	1^{a}	-	1^{a}	-	1^{a}	-	1^{b}	6^{ab}
Sterile Mycelia 16	MEn 205	1^{a}	1^{b}	1^{a}	-	-	1^{a}	-	-	-	1^{c}	-	-	1^{a}	5^{abc}
Nigrospora sp.11	MEn 206	1^{b}	-	-	1^{a}	1^{a}	-	-	1^{a}	1^{b}	1^{c}	1^{b}	1^{b}	1^{b}	8^{abc}
Aspergillus sp.12	MEn 207	-	-	1^{c}	-	-	1^{a}	1^{b}	-	-	1^{a}	-	-	-	4^{abc}
Dreschera sp.12	MEn 208	1^{a}	1^{b}	1^{a}	1^{b}	1^{b}	-	-	1^{b}	-	1^{a}	-	1^{c}	1^{a}	8^{abc}
Phoma sp.3	MEn 209	1^{c}	-	-	1^{b}	-	1^{c}	1^{a}	-	-	-	-	-	1^{c}	4^{abc}

Colletotrichum sp.5	MEn 210	1^{b}	-	-	-	-	-	-	-	-	-	-	1^{c}	1^{b}	2^{bc}
Aspergillus sp.13	MEn 211	1^{c}	1^{b}	-	1^{a}	-	1^{a}	-	-	-	-	-	-	1^{c}	4^{abc}
Aspergillus sp.14	MEn 212	1^{c}	1^{b}	1^{a}	-	-	-	1^{c}	1^{a}	-	1^{b}	-	1^{b}	1^{c}	7^{abc}
Nigrospora sp.12	MEn 213	1^{a}	1^{c}	-	-	1^{c}	1^{c}	-	1^{a}	1^{b}	1^{a}	1^{a}	1^{a}	1^{a}	9^{abc}
Sterile Mycelia 17	MEn 214	1^{a}	1^{a}	-	1^{b}	-	-	1^{a}	1^{b}	1^{a}	-	-	-	1^{a}	6^{ab}
Fusarium sp.12	MEn 215	1^{b}	-	1^{c}	-	1^{b}	1^{b}	1^{a}	-	-	1^{c}	1^{a}	1^{b}	1^{b}	8^{abc}
Pestalotiopsis sp.22	MEn 216	1^{b}	-	-	1^{b}	-	1^{c}	-	1^{b}	-	1^{c}	1^{b}	1^{b}	1^{b}	7^{bc}
Penicillium sp.13	MEn 217	1^{c}	1^{c}	-	1^{c}	-	1^{c}	1^{c}	-	1^{c}	1^{c}	1^{c}	1^{c}	1^{c}	9^{c}
Pestalotiopsis sp.23	MEn 218	1^{a}	-	1^{b}	1^{a}	1^{b}	1^{a}	-	1^{c}	-	1^{c}	1^{c}	-	1^{a}	8^{abc}
Corynospora sp.5	MEn 219	-	1^{a}	-	-	-	1^{a}	1^{a}	-	1^{b}	1^{b}	1^{b}	1^{a}	-	7^{ab}
Sterile Mycelia 18	MEn 220	1^{c}	-	1^{c}	1^{b}	1^{c}	-	-	1^{c}	-	1^{c}	1^{b}	1^{b}	1^{c}	8^{bc}
Sterile Mycelia 19	MEn 221	1^{a}	1^{b}	1^{c}	-	1^{b}	1^{b}	1^{b}	-	1^{b}	-	1^{c}	-	1^{a}	8^{abc}
Fusarium sp.13	MEn 222	1^{b}	-	1^{c}	1^{a}	-	-	-	1^{c}	-	1^{b}	-	1^{a}	1^{b}	6^{abc}
Scytalidium sp.3	MEn 223	1^{a}	1^{c}	1^{c}	-	1^{c}	1^{c}	1^{c}	-	1^{a}	-	-	1^{c}	1^{a}	8^{ac}
Dreschera sp.13	MEn 224	1^{a}	1^{a}	-	1^{c}	-	-	-	1^{a}	-	1^{c}	1^{b}	1^{a}	1^{a}	7^{abc}
Aspergillus sp.15	MEn 225	1^{c}	1^{c}	-	-	1^{c}	-	-	-	-	-	-	1^{c}	1^{c}	4^{c}
Nigrospora sp.13	MEn 226	1^{a}	-	1^{a}	1^{c}	1^{b}	-	-	1^{a}	-	1^{b}	-	1^{c}	1^{a}	7^{abc}
Sterile Mycelia 20	MEn 227	1^{b}	1^{a}	1^{a}	-	1^{c}	1^{a}	1^{b}	-	-	-	-	1^{a}	1^{b}	7^{abc}
Sterile Mycelia 21	MEn 228	1^{c}	1^{b}	-	1^{a}	-	-	-	1^{c}	-	1^{b}	1^{b}	1^{a}	1^{c}	7^{abc}
Aspergillus sp.16	MEn 229	1^{a}	-	1^{a}	-	1^{c}	1^{a}	1^{c}	-	1^{a}	-	-	1^{a}	1^{a}	7^{ac}
Cladosporium sp.15	MEn 230	-	-	1^{c}	1^{c}	-	-	-	1^{a}	1^{c}	1^{a}	1^{c}	1^{a}	-	7^{ac}
Pestalotiopsis sp.24	MEn 231	1^{c}	1^{a}	-	-	1^{b}	1^{b}	1^{a}	-	-	1^{b}	-	1^{c}	1^{c}	7^{abc}
Curvularia sp.7	MEn 232	1^{b}	1^{a}	1^{a}	-	-	-	-	1^{a}	-	1^{b}	1^{b}	1^{b}	1^{b}	$7a^{b}$
Penicillium sp.14	MEn 233	1^{a}	-	-	1^{c}	-	-	-	-	-	-	1^{a}	1^{a}	1^{a}	4^{ac}
Nigrospora sp.14	MEn 234	1^{c}	-	-	1^{c}	-	-	-	1^{a}	1^{a}	1^{a}	1^{c}	1^{c}	1^{c}	7^{ac}
Aspergillus sp.16	MEn 235	1^{a}	1^{b}	1^{b}	-	1^{a}	1^{a}	1^{a}	-	1^{a}	-	-	1^{a}	1^{a}	8^{ab}
Penicillium sp.15	MEn 236	1^{b}	-	1^{b}	1^{c}	-	1^{c}	-	-	-	1^{b}	1^{c}	1^{c}	1^{b}	7^{bc}
Penicillium sp.16	MEn 237	1^{a}	1^{b}	-	-	1^{c}	1^{b}	1^{b}	-	1^{a}	-	-	-	1^{a}	6^{abc}
Fusarium sp. 14	MEn 238	1^{c}	-	1^{c}	1^{c}	-	-	-	1^{b}	1^{c}	-	1^{b}	1^{b}	1^{c}	7^{bc}
Fusarium sp. 15	MEn 239	-	1^{a}	-	-	1^{a}	1^{a}	1^{a}	-	1^{a}	-	-	-	-	5^{a}
Phoma sp.4	MEn 240	1^{b}	-	1^{a}	1^{b}	-	-	-	1^{a}	-	1^{a}	1^{c}	1^{a}	1^{b}	7^{abc}

Phoma sp.5	MEn 241	1^{a}	1^{c}	1^{c}	-	1^{b}	1^{a}	1^{a}	-	1^{b}	-	1^{a}	-	1^{a}	8^{abc}
Aspergillus sp.17	MEn 242	1^{c}	1^{a}	1^{a}	1^{b}	-	-	-	1^{a}	-	1^{a}	-	1^{c}	1^{c}	7^{abc}
Fusarium sp.16	MEn 243	1^{a}	1^{a}	1^{b}	-	1^{b}	1^{b}	1^{a}	-	1^{a}	-	-	-	1^{a}	7^{ab}
Curvularia sp.8	MEn 244	1^{a}	1^{a}	1^{b}	-	-	-	-	1^{a}	1^{c}	1^{c}	-	-	1^{a}	6^{abc}
Fusarium sp.17	MEn 245	1^{c}	1^{c}	-	-	1^{b}	1^{a}	1^{b}	-	1^{a}	-	1^{a}	1^{a}	1^{c}	8^{abc}
Phoma sp.6	MEn 246	-	-	1^{b}	1^{b}	-	-	1^{a}	1^{a}	-	1^{b}	-	1^{a}	-	6^{ab}
Cladosporium sp.16	MEn 247	1^{b}	1^{a}	-	-	-	-	-	-	1^{a}	-	1^{b}	1^{a}	1^{b}	5^{ab}
Scolecobasidium sp.6	MEn 248	1^{a}	-	1^{a}	1^{c}	-	-	-	1^{a}	-	1^{a}	-	1^{c}	1^{a}	6^{ac}
Aspergillus sp.18	MEn 249	1^{a}	1^{a}	1^{b}	-	1^{a}	1^{a}	1^{b}	-	1^{c}	-	1^{a}	-	1^{a}	8^{abc}
Pestalotiopsis sp.25	MEn 250	1^{c}	1^{a}	1^{a}	1^{c}	-	-	-	1^{c}	-	1^{b}	1^{b}	-	1^{c}	7^{abc}
Fusarium sp.18	MEn 251	1^{b}	1^{a}	1^{c}	-	1^{a}	1^{a}	1^{c}	-	1^{c}	-	1^{a}	-	1^{b}	8^{abc}
Phomopsis sp.2	MEn 252	1^{c}	-	1^{a}	1^{c}	-	-	-	1^{c}	-	1^{c}	-	-	1^{c}	5^{ac}
Alternaria sp.6	MEn 253	1^{a}	1^{b}	-	-	1^{c}	-	1^{c}	-	1^{b}	-	-	-	1^{a}	5^{abc}
Setosphaeria monoceras	MEn 254	1^{b}	-	1^{b}	1^{b}	-	1^{c}	-	1^{b}	-	-	-	-	1^{b}	5^{bc}
Nigrospora sp.15	MEn 255	1^{a}	1^{a}	-	-	1^{a}	-	1^{b}	-	1^{b}	-	-	-	1^{a}	5^{ab}
Ascospore 4	MEn 256	1^{b}	-	1^{b}	1^{c}	-	1^{b}	-	1^{b}	-	-	-	-	1^{b}	5^{bc}
Sterile Mycelia 22	MEn 257	1^{a}	1^{c}	-	-	1^{a}	1^{a}	1^{a}	-	1^{c}	-	-	-	1^{a}	6^{ac}
Sterile Mycelia 23	MEn 258	1^{c}	-	1^{c}	1^{c}	-	1^{b}	-	-	-	1^{b}	-	-	1^{c}	5^{bc}
Curvularia sp.9	MEn 259	1^{b}	1^{a}	-	-	1^{a}	1^{c}	1^{a}	-	1^{b}	-	-	-	1^{b}	6^{abc}
Aspergillus niger	MEn 260	1^{a}	-	1^{c}	1^{c}	-	1^{c}	-	1^{a}	-	-	-	-	1^{a}	5^{ac}
Curvularia sp.10	MEn 261	-	-	-	-	1^{b}	-	1^{b}	1^{b}	-	-	-	-	-	3^{b}
Dreschera sp.16	MEn 262	1^{c}	1^{c}	1^{a}	1^{b}	-	1^{b}	-	-	-	1^{a}	-	-	1^{c}	6^{abc}
Aspergillus flavus	MEn 263	-	-	-	-	-	-	1^{a}		1^{a}	-	1^{a}	-	-	3^{a}
Aspergillus terrus	MEn 264	-	1^{c}	-	-	1^{a}	1^{c}	-		-	1^{a}	1^{c}	-	-	5^{ac}
Penicillium sp.17	MEn 265	-	-	-	1^{b}	1^{c}	1^{c}	-	-	1^{b}	-	1^{b}	-	-	5^{bc}
Penicillium sp.18	MEn 266	1^{a}	-	1^{a}	-	-	1^{b}	1^{a}	1^{b}	-	1^{b}	-	-	1^{a}	6^{ab}
Non sporulating basidiomycete 1	MEn 267	1^{c}	1^{a}	-	1^{c}	1^{b}	1^{a}	-	-	-	-	-	-	1^{c}	5^{abc}
Aspergillus sp.19	MEn 268	1^{a}	-	-	-	-	-	1^{a}	1^{c}	1^{a}	-	1^{c}	-	1^{a}	5^{ac}
Dreschera sp.14	MEn 269	-	-	1^{c}	-	1^{b}	1^{a}	-	-	-	1^{b}	1^{a}	1^{c}	-	6^{abc}
Sterile Mycelia 24	MEn 270	-	1^{a}	-	1^{b}	-	-	-	-	-	-	1^{a}	1^{b}	-	4^{ab}

Nigrospora sp.16	MEn 271	1^{c}	-	1^{c}	-	-	1^{c}	1^{a}	1^{b}	1^{b}	1^{a}	-	-	1^{c}	7^{abc}
Penicillium sp.19	MEn 272	-	-	-	1^{b}	1^{b}	1^{a}	-	-	1^{a}	1^{b}	1^{a}	1^{a}	-	7^{ab}
Dreschera sp.15	MEn 273	1^{b}	-	-	1^{b}	-	-	1^{c}	1^{b}	1^{b}	-	-	-	1^{b}	5^{bc}
Curvularia sp.11	MEn 274	1^{b}	1^{b}	1^{c}	-	1^{b}	1^{a}	-	-	-	-	-	-	1^{b}	5^{abc}
Cladosporium sp.17	MEn 275	1^{a}	1^{c}	-	-	-	-	-	-	-	1^{a}	1^{a}	1^{c}	1^{a}	5^{ac}
Lignicola sp.2	MEn 276	1^{c}	1^{b}	1^{a}	-	-	1^{a}	1^{c}	1^{b}	1^{b}	-	-	-	1^{c}	7^{abc}
Sterile Mycelia 25	MEn 277	1^{a}	-	1^{c}	1^{a}	1^{a}	-	-	-	-	1^{b}	-	-	1^{a}	5^{abc}
Phoma sp.7	MEn 278	1^{c}	1^{c}	-	-	-	-	-	1^{a}	1^{c}	-	-	1^{a}	1^{c}	5^{ac}
Aspergillus sp.20	MEn 279	-	-	1^{b}	1^{c}	-	-	-	-	1^{c}	1^{c}	1^{b}	1^{b}	-	6^{bc}
Curvularia sp.12	MEn 280	-	-	-	-	1^{c}	1^{c}	1^{a}	1^{c}	1^{a}	1^{c}	1^{a}	1^{c}	-	8^{ac}
Pestalotiopsis sp.26	MEn 281	1^{c}	-	-	1^{a}	-	-	-	-	1^{a}	1^{b}	1^{a}	-	1^{c}	5^{abc}
Pestalotiopsis sp.27	MEn 282	1^{a}	-	-	-	1^{b}	1^{a}	1^{c}	1^{a}	-	1^{a}	1^{b}	-	1^{a}	7^{abc}
Nigrospora sp.17	MEn 283	1^{c}	1^{a}	-	-	1^{a}	-	1^{c}	-	1^{a}	1^{a}	1^{c}	1^{a}	1^{c}	8^{ac}
Pestalotiopsis sp.28	MEn 284	1^{a}	1^{b}	-	-	-	-	-	-	-	-	-	-	1^{a}	1^{b}
Total		209	182	181	168	162	185	165	157	151	147	141	116		

Legend: MEn=Culture coded with isolation code M denotes mangroves and En denotes endophytes; ^{a}= Fungal endophyte in leaf; ^{b}= Fungal endophyte in stem; ^{c}=Fungal endophyte in root. ^{ab}=Fungal endophyte in leaf and stem; ^{ac}= fungal endophyte in leaf and root; ^{bc}=Fungal endophyte in stem and root; ^{abc}=Fungal endophyte in leaf, stem and roots.

Table 4.5: Seasonal distribution of fungal endophytic colony forming units (CFU) and its frequency of occurrence.

Sr. No.	Genus/Species	Summer		Monsoon		Winter		Total CFU
		Number of isolates	Frequency (%)	Number of Isolates	Frequency (%)	Number of isolates	Frequency (%)	
1	*Setosphaeria monoceras*	14	0.78	16	0.76	12	0.60	42
2	*Aspergillus* sp	230	12.86	308	14.60	293	14.54	831
3	*Scolecobasidium* sp.	36	2.01	49	2.32	51	2.53	136
4	*Drechslera* sp.	108	6.04	132	6.26	125	6.20	365
5	*Fusarium* sp.	158	8.83	173	8.20	163	8.09	494
6	*Pestalotiopsis* sp.	205	11.46	236	11.18	217	10.77	658
7	*Scytalidium lignicola*	60	3.35	56	2.65	41	2.03	157
8	*Penicillium* sp.	122	6.82	135	6.40	133	6.60	390
9	*Corynesporina* sp.	43	2.40	43	2.04	45	2.23	131
10	*Gilmaniella* sp.	14	0.78	20	0.95	20	0.99	54
11	*Junctospora pulchra*	24	1.34	26	1.23	27	1.34	77
12	*Bipolaris* sp.	36	2.01	43	2.04	31	1.54	110
13	*Cladosporium* sp.	111	6.20	129	6.11	122	6.05	362
14	*Myceliophthora* sp.	10	0.56	11	0.52	10	0.50	31
15	*Gonatobotryum* sp.	11	0.61	10	0.47	15	0.74	36
16	*Thermomyces* sp.	4	0.22	7	0.33	8	0.40	19
17	*Colletotrichum* sp.	26	1.45	31	1.47	39	1.94	96
18	*Acremonium* sp.	6	0.34	9	0.43	9	0.45	24
19	*Rhizopus* sp.	24	1.34	24	1.14	18	0.89	66
20	*Ceratocystis* sp.	9	0.50	9	0.43	10	0.50	28
21	*Alternaria* sp.	56	3.13	44	2.09	47	2.33	147
22	*Nigropsora* sp.	126	7.04	122	5.78	118	5.86	366

23	*Marieltiottia* sp.	9	0.50	9	0.43	9	0.45	27
24	*Phoma* sp.	42	2.35	52	2.46	49	2.43	143
25	*Gliocladium* sp.	7	0.39	8	0.38	9	0.45	24
26	*Gilmaniella* sp.	7	0.39	11	0.52	11	0.55	29
27	*Humicola* sp.	5	0.28	11	0.52	11	0.55	27
28	*Curvularia* sp.	61	3.41	72	3.41	63	3.13	196
29	*Aureobasidium pullulans*	6	0.34	5	0.24	6	0.30	17
30	*Limposoma* sp.	5	0.28	3	0.14	4	0.20	12
31	*Gilberella* sp.	10	0.56	10	0.47	5	0.25	25
32	*Phomopsis* sp.	4	0.22	9	0.43	11	0.55	24
33	Thickened chlamydospores	5	0.28	7	0.33	8	0.40	20
34	Ascospore	11	0.61	15	0.71	17	0.84	43
35	Sordariomycetes sp.	3	0.17	10	0.47	9	0.45	22
36	Catenate spores	8	0.45	8	0.38	7	0.35	23
37	*Non sporulating Basidiomycete*	6	0.34	5	0.24	5	0.25	16
38	*Lignicola* sp.	8	0.45	9	0.43	8	0.40	25
39	Sterile mycelia	135	7.55	216	10.24	195	9.68	546
40	Unidentified sp.	24	1.34	17	0.81	34	1.69	75

Table 4.6: Characterization of sterile fungal taxa using GenBank sequence.

Fungal Isolate	Closest blast match in NCBI database	Similarity	Base pairs	Phylum
MEn01	*Setosphaeria monoceras*	100%	924	Ascomycota
MEn09	*Fusarium sacchari*	100%	506	Ascomycota
MEn66	*Fusarium equiseti*	100%		Ascomycota
MEn36	*Nigrospora sphaerica*	99%	873	Ascomycota
MEn52	Fungal endophyte	99%	922	-
MEn59	Sordariomyctes	98%	938	Ascomycota

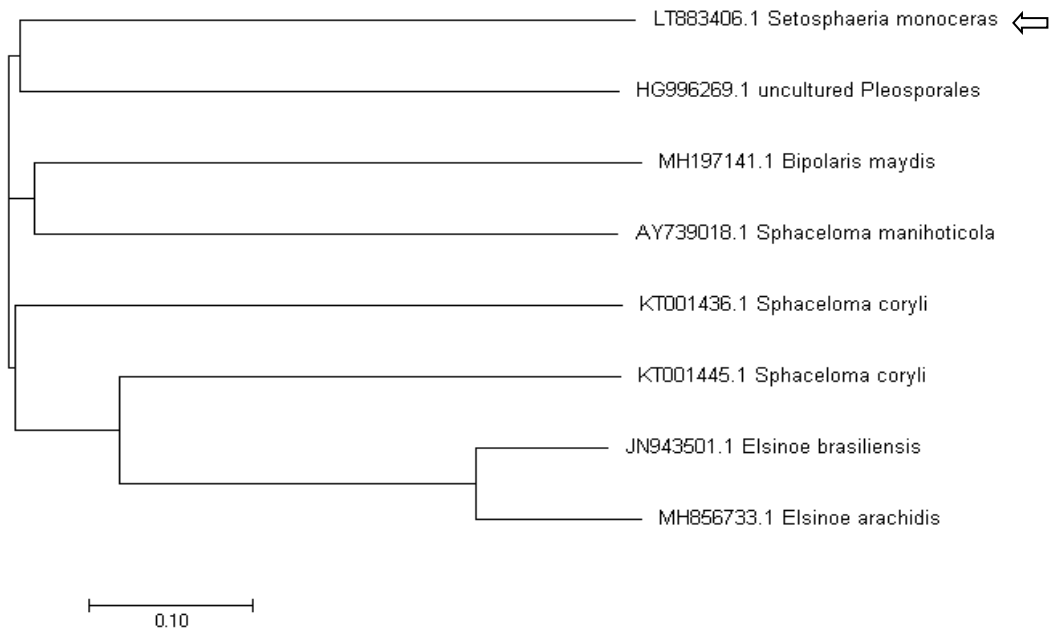

Fig. 4.10: Dendrogram showing the phylogenetic position of endophytic fungus with other fungal isolates.

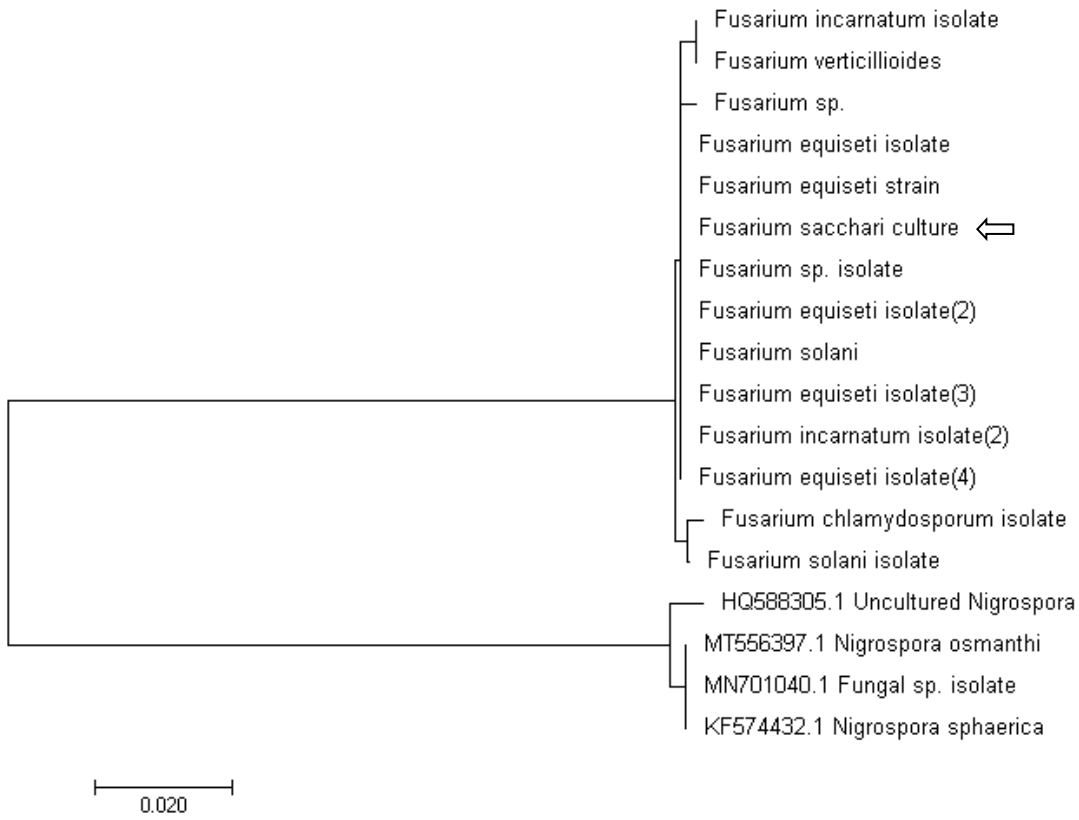

Fig. 4.11: Dendrogram showing the phylogenetic position of endophytic fungus with other fungal isolates.

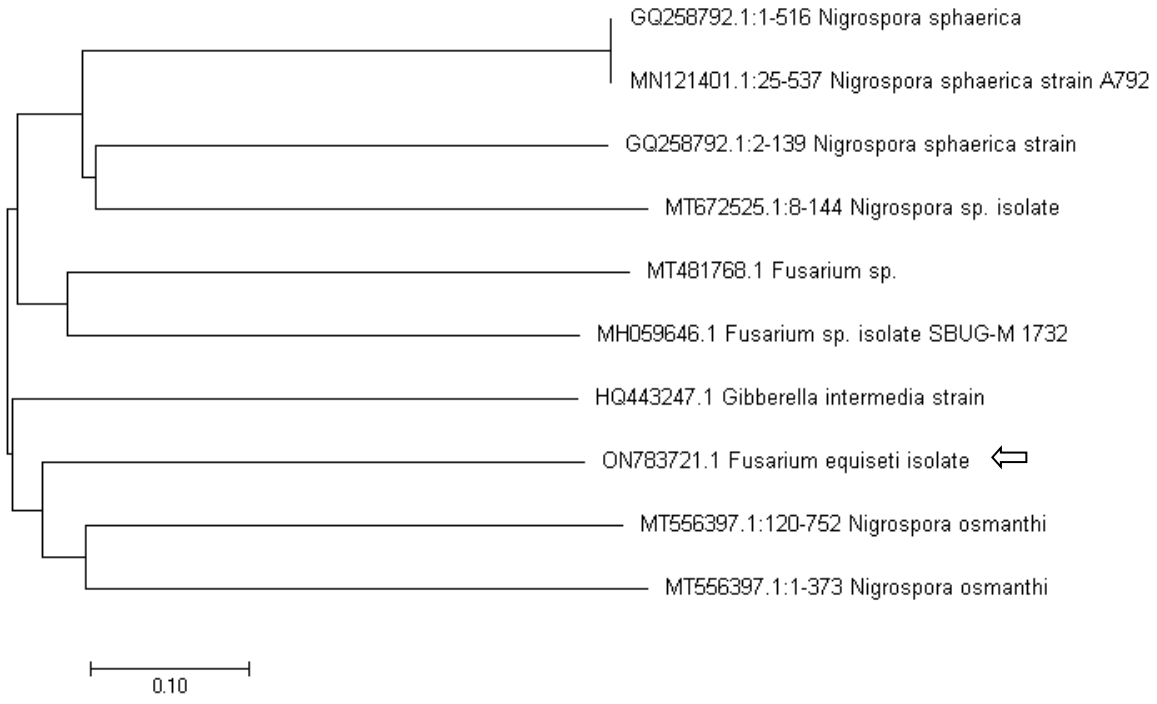

Fig. 4.12: Dendrogram showing the phylogenetic position of endophytic fungus with other fungal isolates.

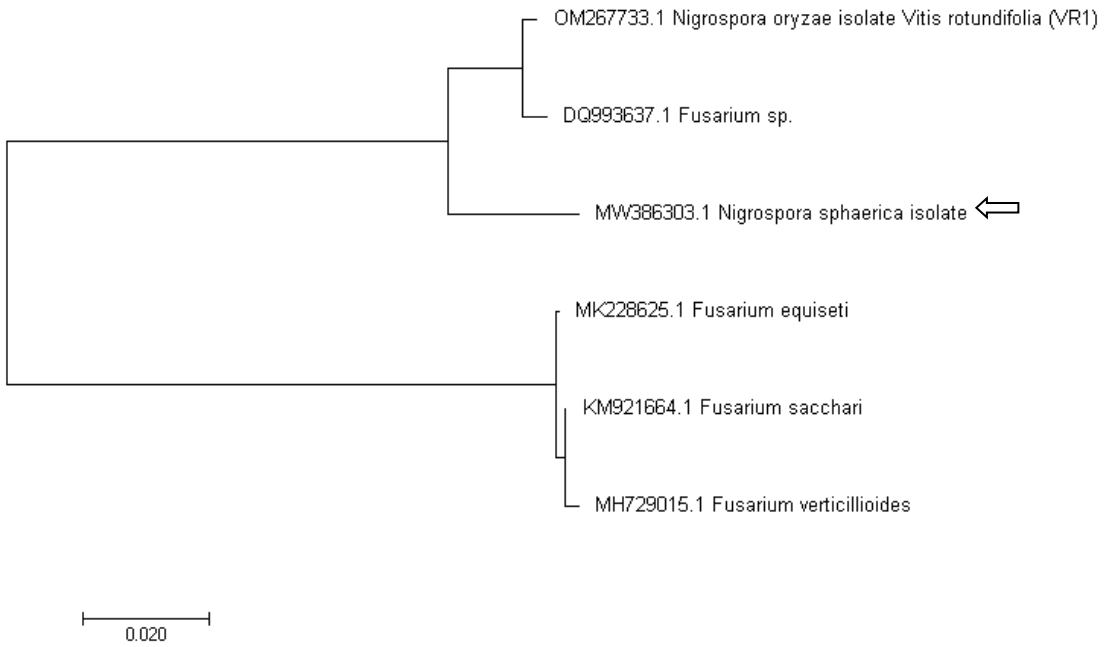

Fig. 4.13: Dendrogram showing the phylogenetic position of endophytic fungus with other fungal isolates.

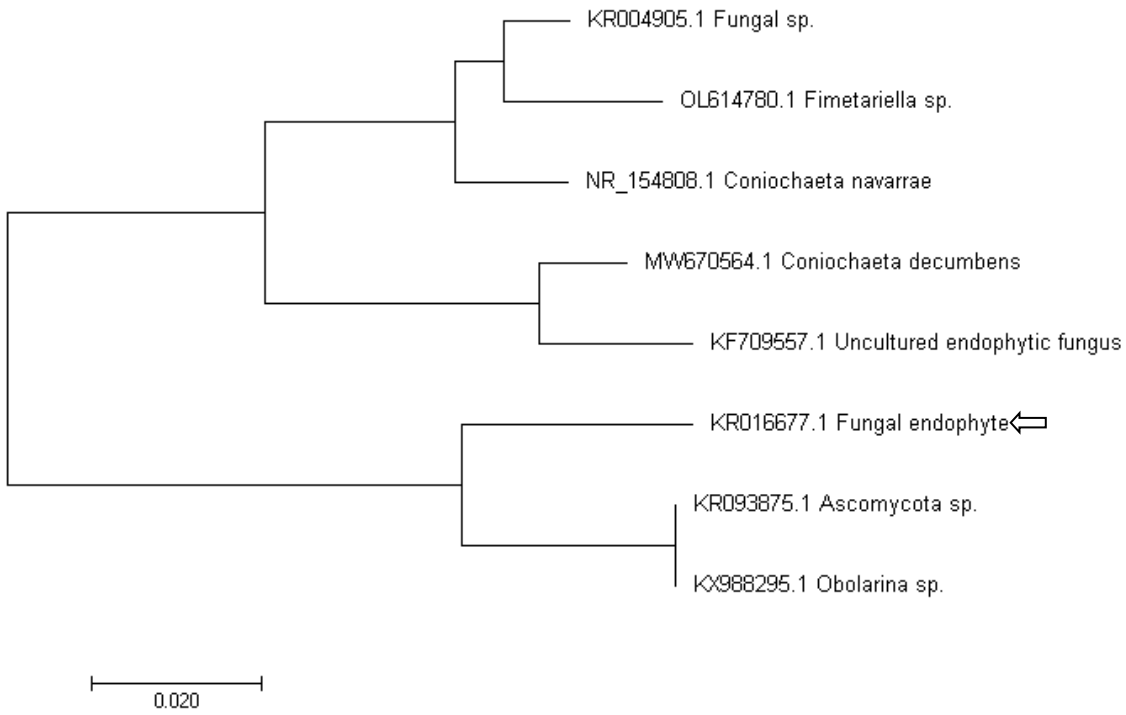

Fig. 4.14: Dendrogram showing the phylogenetic position of endophytic fungus with other fungal isolates.

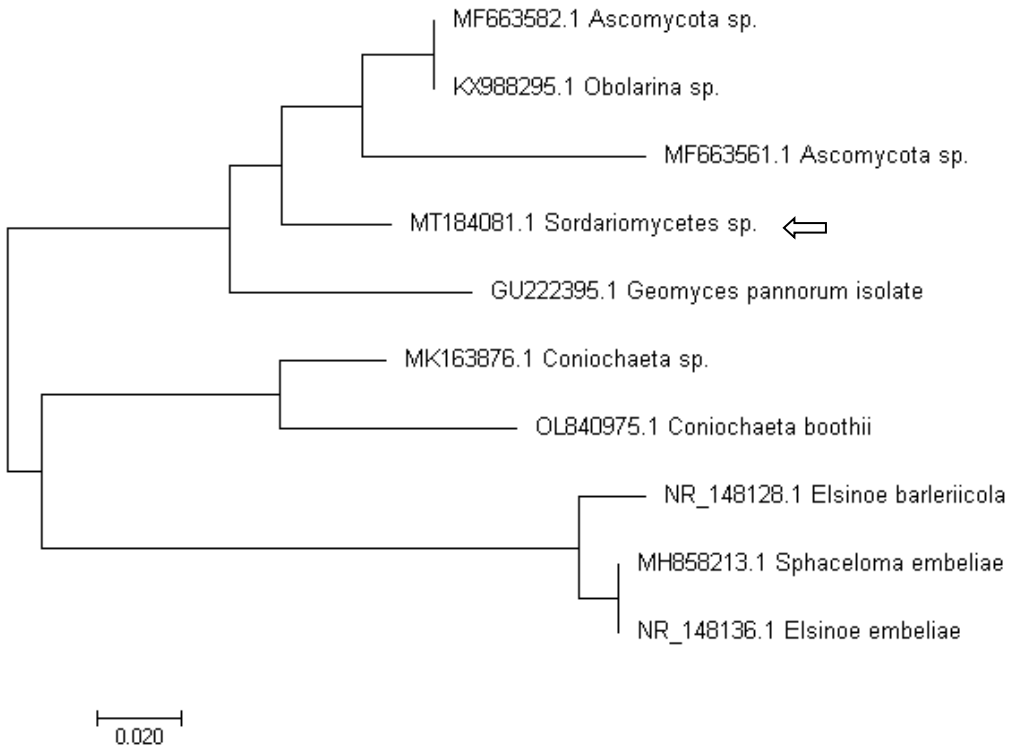

Fig. 4.15: Dendrogram showing the phylogenetic position of endophytic fungus with other fungal isolates.

4.4: DISCUSSION

The occurrence and distribution of endophytic fungi contribute to shaping different ecological niches in the mangrove ecosystem. The persistence of such microbiota within the living plant parts has immense applications in the growth of plant species and therefore deserves exploration. The present study confirms the prevalence of different fungal species in mangrove parts. Among these, morphologically varied dark septate endophytes were localized within the roots. The localization of such structures within the plant roots indicates that the plants grow under stress conditions. Due to mining activity, the Mandovi and Zuari rivers have iron (Fe) and manganese (Mn) pollution. As many of these mangroves are in the river basins, they are constantly under pressure due to heavy metals like Fe, zinc (Zn), Mn, *etc*. The association of endophytes with the mangrove plant species may help to alleviate the metal toxicity and hence deserve further exploration. Literature also indicates that these DSE assist plants in synthesizing proteolytic enzymes, having the ability to mineralize organic N into free inorganic forms (Qin *et al.*, 2017) that facilitate the N cycle and, in turn, help in the decomposition of organic matter. The presence of darkly melanized septate hyphae in the dominant mangrove roots provides a protective layer within the cell that helps

to promote host survival rate and nutrient uptake (Barrow, 2003). The colonization frequency of endophytes depends on various edaphic factors such as altitude, humidity, the density of the canopy, precipitation, and host susceptibility (Petrini and Carroll, 1981; Suryanarayanan *et al.,* 1998; Rajagopal and Suryanarayanan, 2000; Elamo *et al.,* 1999).

Sun and Guo (2012) revealed the importance of surface sterilization and sterilization time in the recovery of maximum endophytic isolates from plant tissue. In the present study, sterilization time varied for different plant materials. Tissue size also contributes significantly to the recovery of endophytes from plant segments. Gamboa *et al*. (2002) demonstrated an increased number of endophytic recoveries with decreased size of the plant segment. Therefore, to recover the maximum number of endophytes from the explants, the size of the segments was kept at approximately 0.5cm. Media constituents also influence the isolation of endophytes. Researchers across the globe are using different media constituents. These include standard PDA (Potato Dextrose Agar), MEA (Malt Extract Agar), and minimal media with plant tissue or extract (Fovo *et al.,* 2017). In the present study, PDA medium was used to isolate and culture the fungal endophytes.

During the study, 31 isolates failed to sporulate. This may be attributed to the fungal life cycle, wherein some endophytes resist the sporulation within the plant and remain inactive. An earlier study revealed that approximately 54% of the isolates did not sporulate even after prolonged incubation in different media (Kumaresan and Suryanarayanan, 2002). Therefore, these isolates cannot be assigned taxonomic characterization. Guo *et al*. (1998) reported increased sporulation percentage in sterile isolates by prolonging incubation of host tissue in media for three months. However, a few isolates remained sterile even after prolonged incubation and could not be identified.

The present study revealed no host specificity among the fungal endophytes. Cohen (2006) observed that a single endophyte could invade a variety of plant species without showing any host preference. However, Gu *et al*., (2012) reported tissue specificity in *Ceriops tagal*. In the present study, *A. marina* (Avicinaceae) and *R. mucronata* (Rhizophoraceae) reported the highest number of endophytic isolates, indicating that these two hosts have some preferences for growth endophytes within. Studies suggest that tannins and phenols in the Rhizophoraceae family and increased salinity in the leaves of Avicinaceae are responsible for high fungal diversity in these plants (Naidoo, 2006).

A recent checklist of fungi associated with mangrove plants revealed that 625 fungi exist at the global scale (278 Ascomycetes, 277 anamorphic taxa, 30 Basidiomycetes, and 14 Oomycetes). Maximum mangrove-associated fungi have been reported from South-East Asia compared to other parts of the world (Schmit and Shearer, 2003). Maria and Sridhar (2003) reported 25 endophytic fungi (3 ascomycetes, 20 mitosporic fungi, and 2 sterile isolates) from *Acanthus ilicifolius* and *Acrostichum aureum*. Ananda and Sridhar (2002) have studied the diversity of endophytic fungi in four mangrove plant species from Karnataka and reported several fungi associated with the roots. In the Pichavaram mangrove ecosystem, Gayathria *et al*. (2009) reported 24 endophytic fungal genera from the leaves of *Rhizophora apiculata, R. mucronata,* and *Aegiceras* sp. According to Jones and Vrijmoed (1997), 55 mangrove plant species (including mangrove associates) yielded about 200 different marine fungal isolates. *Rhizophora apiculata,* among the mangrove tree species, is known to harbour 63 higher marine fungi (Sarma *et al*., 2001). Studies on foliar endophytes of the mangrove community revealed the occurrence of several soil fungi (Suryanarayanan and Kumaresan, 2000; Kumaresan and Suryanarayanan, 2001). Endophytes of tropical plants constitute a species-rich ecological assemblage of fungi (Huang *et al.,* 2008). Therefore, isolating mangrove endophytes and their identification will lead to further investigation into identifying novel bioactive compounds and their applications.

4.5: CONCLUSION

The study revealed rich endophytic fungal diversity in mangrove plant species from the study site. Besides, these fungi were found to colonize different parts of the host plant. The symbiotic association of the fungal endophytes suggests their ecological role and therefore deserves attention to exlore and exploit them for human benefits.

Chapter 5: To study the associative role of endophytic fungi in selected mangrove plant species

5: INTRODUCTION

The symbiotic relationship between endophytes and host species has benefited plants in terms of growth promotion and adaptation to changing environmental conditions. While residing and reproducing inside the healthy tissue in an intimate mutualistic manner, presumable gene recombination/or the precursor molecule interaction with the host indicates enhanced biosynthetic capabilities in the endophytes (Li *et al.,* 2005). These symbionts are chemical synthesizers within plants and are supposed to be used by host plant species for chemical defense against various stresses (Wang *et al.,* 2008). Hence, it becomes crucial for endophytes to continuously modify their secondary metabolites, to penetrate the host plant *via* the chemotactic process. These adaptations have helped endophytes produce therapeutically important bioactive compounds exclusive to those of host plants with pharmacological, industrial, and agricultural significance. Considering the significance of endophytic secondary metabolites in host as well as in drug discoveries, the present objective was divided into two sections:

5A: The biological role of fungal endophytes in mangrove plant species, and

5B: Extraction and characterization of secondary metabolites from fungal endophytes having pharmacological significance.

Section 5A

5.1: The biological role of fungal endophytes in mangrove plant species

Mangrove ecosystems or *mangal* located along the tropical and subtropical regions comprise high organic matter in the form of litter (Ogbonna, 2011). The decomposition of the organic litter depends on the diversity and action of microorganisms (Hossain and Hoque, 2008). It is an important process for controlling plant nutrient flow into the soil (Chapin *et al.,* 2002) that directly affects the global carbon balance (Wardle *et al.,* 2004). It depends upon the action of decomposers that contribute to maintaining the steady flow of organic matter (Aerts, 1997) along with environmental factors such as temperature, humidity, and biomass quality (Zhang *et al.,* 2008; Austin *et al.,* 2014; Karhu *et al.,* 2014). Several microorganisms, including fungal endophytes, are known to degrade dead and decaying plant biomass (Saikkonen *et al.,* 2015). These organisms play a major role in nutrient recycling, as they live within the plant throughout

their life (Osono, 2006). Similar to other living organisms, plants also have a senescence period. Hence, it becomes essential to recycle the nutrient content present in the plant/plant parts and restore it back to the soil. After the senescence stage, the inner tissues containing endophytes switch from oblique endophytic to a facultative saprophytic stage which triggers nutrient recycling (Griffith, 1994). Despite knowing the importance of this micro-biota, the ecology of litter degraders has not been thoroughly studied (Muller *et al.,* 2001). Reports on endophytes as litter degraders suggest that few Xylariaceous endophytes possess the ability to decompose lignin and cellulose from the litter (Koide *et al.,* 2005). Besides, studies reveal that endophytes help alter the chemical composition and enhance degradation (Andrews, 1991). It is known that the members of Basidiomycetes and Ascomycetes have a high efficacy in litter degradation as they typically encode genes for laccase and cellobiohydrolase (Yuan and Chen, 2014). Endophytes possess the inherent capability to modify host C, N ratio, and P uptake, affecting litter decomposition (Gundel *et al.,* 2016). Since fungal endophytes persist in plants throughout their life span, they are called 'pioneers of decomposition' (Hirose *et al.,* 2013). Various exoenzymes produced by endophytes play a vital role in degradation (Robl *et al.,* 2013).

Phosphorus is one of the essential macronutrients for plant growth. However, in the soil, it is available in an insoluble state. The mangrove soil has a high capacity to restore N and P, but in sediments, it binds to calcium and iron, making P unavailable to plants. Endophytes are symbionts that help host plants fix environmental N and inorganic P (Santoyo *et al.,* 2016). Many soil and plant symbionts, like fungi, bacteria, and other soil microorganisms, play an important role in P solubilization (Gyaneshwar *et al.,* 2002) and mobilization (Scervino *et al.,* 2011). In the mangrove ecosystem, the soil pH varies from highly acidic to alkaline (Hossain and Nuruddin, 2016), and thus P gets precipitated due to the abundance of cations (Kpomblekou and Tabatabai, 1994). In acidic soils, iron and aluminum bind to P, while in alkaline soils, calcium precipitates P leading to P deficiency (Matos *et al.,* 2017). In such cases, the role of P solubilizers is very crucial to help plants overcome P deficiencies. Plant colonizers like endophytic fungi trigger the solubilization and mineralization of inorganic phosphates into soluble forms (Illmer and Schinner, 1995). The microbial endophytes mobilize the insoluble phosphate and enhance N accumulation within the plant system (Sharma *et al.,* 2003).

Besides, endophytic fungi have wide applications in bioremediation. As the coastal belt of Goa is constantly loaded with an influx of heavy metals and other contaminants, bioremediation is widely practiced by researchers. In such studies, the identification of microorganisms plays a

crucial role in *in-situ* biodegradation. Prolonged accumulation of these contaminants adds to pollution and may cause mutation or even plant death (Das and Chandran, 2011).

Petroleum is a viscous mixture of thousands of hydrocarbons comprising C and hydrogen (Mehdi and Simone, 2013). Petroleum degradation in the presence of hydrocarbon-degrading micro-organisms is a complex process of biodegradation that depends upon bacteria, yeast, and fungi. Fungi like *Aspergillus, Cladosporium*, *Corollasporium*, *Fusarium*, and *Penicillium* are reported to possess a beneficiary role in the degradation of hydrocarbons. This degradation is mediated by the action of certain enzymes like oxygenases and hydroxylases (Deshmukh *et al.,* 2016). These hydrocarbon-degrading micro-organisms are reported to detoxify the plant system by stimulating secondary metabolites. Without these microbes, plants would not have survived under such conditions (Baoune *et al.,* 2018). Filamentous fungi play a significant role in hydrocarbon degradation because of their fast growth and extensive hyphal network (Olicón-Hernández *et al.,* 2017). PAHs (Poly Aromatic hydrocarbons) have been reported as ubiquitous xenobiotic environmental pollutants (Bisht *et al.,* 2015). A diverse group of fungi such as Zygomycetes (*Cunninghamella elegans*), Ascomycetes (*Aspergillus niger* and *Penicillium* sp.), and white-rot Basidiomycetes (*Trametes versicolor*, *Pleurotus ostreatus*) are known to oxidize and degrade PAH's (Olicón-Hernández *et al.,* 2017).

These mangalicolous endophytes are ubiquitous. They survive as predators, competitors, or pathogens and grow and multiply in adverse conditions in the host (Kohl *et al.,* 2019). The behavioural mechanism to arrest the attack of microbial pathogens has been a positive plant endophyte interaction that helps in the growth and survival of the plant species (Ganley *et al.,* 2008). Endophytic organisms, therefore, play a major role in plant systems, benefiting the host from the deleterious effect of the pathogen through the production of antibiotics by competing for colonization sites and nutrient exchange (Ownley *et al.,* 2010). Thus, these fungi enhance plant growth through the production of phytohormones by increasing the susceptibility of the host toward pathogens. (Khan *et al.,* 2015). Phyto-pathogens are known to induce disease in the plant system; therefore, endophytes can be used as an alternative source of biological control. Hanada *et al.* (2010) reported that species of *Pestalotiopsis*, *Curvularia*, and *Fusarium* to have antagonistic activity against pathogens. Fadiji and Babalolo (2007) reported that few endophytic species, when introduced within the host, trigger the host biomass that would provide additional benefits to the agriculture industry. The present study aimed to investigate the biological role of fungal endophytes in mangrove ecosystems.

5.2: MATERIALS AND METHODS

5.2.1: Sample collection and processing

Fresh and healthy leaf samples were collected and brought to the laboratory in sterile *zip*-lock bags. The samples were processed within five hours of collection. The leaves were surface sterilized using a three-step method (Bayman *et al*., 1997) and dried at 60^0C (**Plate 5.1**). These dried and sterilized leaf samples were used as a substrate for the *in* v*ivo* and *in vitro* studies involving a modified method of Yuan and Chen (2014).

5.2.2: Selection of endophytic isolates (*In vitro* and *in vivo* mangrove leaf litter degradation)**.** Fourteen fungal endophytic isolates *viz*., MEn04, MEn06, MEn09, MEn10, MEn16, MEn24, MEn27, MEn30, MEn34, MEn36, MEn37, MEn38, MEn39, and MEn42 were used for the study.

5.2.3: *In vitro* degradation using Plate Culture Method

The dried and sterilized leaf samples were cut into pieces and placed in Petri-plates containing 20 mL of low-nutrient synthetic agar medium. The fungal inoculum was added adjacent to the sterilized litter sample. The plates were sealed with parafilm and incubated in the dark at room temperature until the media was exhausted. A sterilized dry litter sample without adding fungal inoculum served as a control.

5.2.4: *In vivo* degradation using Microcosm Method

The samples were dried, weighed, and added with fungal inoculum in *zip-lock* bags (litter bags) containing 150g of sterile sand. The bags were then sealed, incubated in the dark, and monitored every month. After 12 months of incubation, the leaf samples were separated from the sand and placed in silica gel desiccators to remove moisture content. The samples were then weighed, and leaf mass loss was calculated using the formula:

Leaf Mass Loss (g) = Dry leaf weight - Litter weight.

5.2.5: Chemical analysis of litter using CHNS analysis

Percent C, H, N, and S were analyzed from the degraded leaf litter using Elementar Variomicro Cube CHNS Analyzer to quantify the total amount of organic matter degraded by the fungal endophytes.

5.2.6: Enzymatic activity of the selected fungal endophytes

Enzymatic activity of 14 fungal isolates *viz*., MEn04, MEn06, MEn09, MEn10, MEn16, MEn24, MEn27, MEn30, MEn34, MEn36, MEn37, MEn38, MEn39, and MEn42 was studied qualitatively by using Plate Assay. The fungal endophytes were tested for the production of amylase, cellulase, laccase, and lipase using the following methods.

5.2.6.1: Amylase activity

The fungal isolates were grown on Glucose Yeast Peptone Medium (glucose 1g, 0.1g yeast extract, 0.5g peptone, 16g agar, and 0.2% soluble starch in 1L distilled water) at pH 6. At the end of 6 days, plates were flooded with iodine solution. The yellow zone around the actively growing fungal colony confirmed the amylase activity.

5.2.6.2: Cellulase activity

The fungal isolate was grown on Yeast Peptone Agar Medium (yeast 10g, peptone 20g, and agar 20g in 1L distilled water). After 3-5 days, the plates were flooded with congo red solution. The cellulase activity was confirmed by the appearance of a yellow area around the fungal colony.

5.2.6.3: Laccase activity

The fungal isolate was grown on Glucose Yeast Peptone Agar Medium amended with 0.05 g 1-naphthol L^{-1} at pH 6.0. The laccase activity was confirmed by the colour change of the medium from colourless to blue.

5.2.6.4: Lipase activity

The fungal isolate was grown on a 1L medium containing peptone (10 g) and agar (20 g) amended with NaCl (5 g), $CaCl_2.2H_20$ (0.1 g), and Tween 20 (1 mL) at pH 6.0. The formation of a halo zone around the fungal colony confirmed the lipase activity.

5.2.7: Phosphate (P) solubilization by endophytic fungi (EF)

5.2.7.1: Qualitative assay of P solubilization activity of EF

The dominant fungal cultures (approx. 3 mm) were inoculated on Pikovskaya's medium. The Petri plates were incubated in the dark for 7 - 8 days. Halo zone-forming fungi were selected to quantify the amount of P solubilization. The solubilization Index (S.I.) was calculated by using the formula:

S.I. = Colony diameter + Halo zone diameter/Colony diameter (Edi-Premono *et al.*, 1996).

5.2.7.2: Quantitative assay of P solubilization by EF

Quantitative estimation of P solubilization was carried out using the Vanadophosphomolybdate method (Jackson, 1958). The P solubilizing fungal species were separately inoculated into Pikovskaya's broth medium in triplicate and incubated at 28ºC for 14 days. 10 mL of suspension was taken from the flask and centrifuged at 10,000 rpm for 10 min. The mycelia were separated from the culture broth after ten days of incubation. Initial pH and change in pH were noted for all the samples using a digital pH meter. From this, 5 mL culture filtrate was adjusted to 50 mL with distilled water. From this, 1 mL was taken to which 2.5 mL of Barton's reagent was added, and the final volume was adjusted to 50 mL. After 10 minutes, the resultant yellow-coloured solution was estimated using a spectrophotometer at 420 nm.

5.2.8: Biodegradation of residual petroleum hydrocarbons by fungal endophytes

As the study site was under constant influx of oil spillage due to barges and nearby service stations, an experiment was designed to investigate the role of fungal endophytes (if any) bioremediation (**Fig. 5.1**).

5.2.8.1: Media preparation

Seven sets containing seven fungal endophytes *viz*., Set I (MEn10), Set II (MEn06), Set III (MEn09), Set IV (MEn37), Set V (MEn27), Set VI (MEn24), and Set VII (MEn36) were selected for the study. These isolates were than inoculated in the modified Bushnell-Hass (BH) broth medium along with oil 1 (two-wheeler) , oil 2(four-wheeler) and oil 3 (boat oil) (Borah and Yadav, 2014) (**Table 5.1**).

Fig. 5.1: Influx of petroleum hydrocarbons at the study site.

Three agar plugs containing endophytic fungal culture were inoculated into 50 mL of sterilized modified BH broth containing 1 mL residual oil and 1 mL of 2% 2,6- Dichlorophenol indophenol indicator (**Table 5.1**). Controls were maintained separately for each test. Some of

the characteristics of residual oil collected from the service station are depicted in **Table 5.2**. The inoculated flasks were incubated at 37^0C with constant shaking on the Scigenics Biotech shaker for 15 days with a 12h dark and 12h light period. The aliquots in the flasks were monitored daily for any colour change. Once the colour changed from deep blue to colourless, the sample was filtered using filter paper to separate the mycelia mat, followed by centrifugation for 15 minutes at 8000 rpm to separate the mycelia suspended in the filtrate.

Table 5.1: Composition of modified Bushnell-Hass Broth medium.

Sr. No.	Molecular Formula	Compound	Quantity
1	$MgSO_4$	Magnesium sulfate	0.2 g l^{-1}
2	$CaCl_2$	Calcium chloride	0.2 g l^{-1}
3	KH_2PO_4	Mono Potassium hydrogen phosphate	1 g l^{-1}
4	K_2HPO_4	Dipotassium hydrogen phosphate	1 g l^{-1}
5	$FeCl_2$	Ferric chloride	0.5 g l^{-1}
6	NH_4NO_3	Ammonium nitrate	1 g l^{-1}
7	Tween 80	Tween 80	0.1%
8	NaCl	Sodium chloride	0.5 g l^{-1}
9	2,6- Dichlorophenol indophenol	2,6-Dichlorophenol indophenol	2%

5.2.8.2: Separation of leftover oil from culture filtrate

A separating funnel was used to separate the leftover oil from the culture filtrate, and the former was quantified and later subjected to FT-IR analysis.

Table 5.2: Some characteristic features of residual oil collected from the motor vehicle service station.

Sr. No.	Source of sample	Residual oil color	pH	Boiling point (^{0}C)
1	Two-wheeler	Blackish brown	7.1±0.1	246
2	Four-wheeler	Dark black	7.1±0.1	230
3	Boat	Blackish grey	6.8±0.2	240

Legend: All values are means of 3 readings.

5.2.8.3: FT-IR analysis

FT-IR analysis was performed by using Vertex 80 FTIR system to record the IR spectra of the oil samples at SAIF IIT Bombay, India. The IR detection was recorded between 400 to 450

cm^{-1} with a spectral resolution of 0.2 cm^{-1}. The interpretation of the degraded hydrocarbon groups was identified using standard literature by Nakamoto (2009).

5.2.9: Effect of endophytic fungal colonization on the growth of *A. marina*

5.2.9.1: Screening of plant growth-promoting endophytic fungi

5.2.9.1.1: Sterilization of seed and preparation of pot culture

To study the plant growth-promoting activity of fungal endophytes, seeds were surface sterilized and allowed to germinate in sterile vials. The pre-germinated seeds were sown in sterilized plastic pots (15 cm) and separately inoculated with actively growing fungal isolates of MEn10 (T_1), MEn04 (T_2), MEn06 (T_3), MEn24 (T_4), MEn27 (T_5), MEn30 (T_6), MEn36 (T_7), and MEn37 (T_8) (3 agar plugs of 3 mm each). The pots were then placed in a greenhouse. In control, seeds were sown along with agar disks without fungal endophyte. The experiment was performed in triplicate. The pots were watered after every 7^{th} day using brackish water. The seedlings were allowed to grow for six months. The shoot length was recorded after every month.

5.2.9.1.2: Plant harvest and pigment analysis

After the seventh month, the plants were harvested, and the roots were thoroughly washed using running tap water to remove all the attached sand particles. Shoot, root lengths, and fresh weights were recorded. The plants of each treatment were washed separately using double distilled water and used for further analysis. One gram of leaf material was used for chlorophyll analysis. The dry weights of the shoot and root were determined after oven-drying the samples at 60^0C for three consecutive days.

5.2.9.1.3: Root colonization study

Freshly harvested roots of control and treated plants were stained using Trypan blue (Phillips and Hayman, 1970) and observed under a light microscope.

5.2.9.2: Isolation and identification of the pathogen

Infected leaf samples of *Avicennia* species were collected and brought to the laboratory in *zip-lock* bags (**Fig. 5.2**). Samples were washed using sterile distilled water to remove all the surface detritus and salt particles. After washing, each sample showing visible symptoms was cut into smaller segments (approx. 2 mm) and inoculated on sterile PDA plates. The plates were sealed

and incubated at 25±2^{0}C. After nine days of incubation, fungal outgrowths emerging from the inoculated leaf samples were purified and later identified using molecular sequencing.

Fig. 5.2: Infected leaf of *A. marina.*

5.2.9.3: Antagonistic activity using dual culture method

The antagonistic activity of the isolated endophytic fungi *viz.*, MEn04, MEn06, MEn10, MEn24, MEn30, MEn36, and MEn37 were tested against the isolated plant pathogen *viz*., *Elsinoe embeliae* using the dual culture method as described by Hamzah *et al*. (2018). In this method, a 2 mm size culture disc of endophytic fungi was placed along the plant pathogen in the PDA media. Control was maintained without the fungal endophyte. Plates were incubated at 25±2^{0}C for 12 days. The growth of both the pathogen and the endophyte was measured after 13 days of incubation.

The percent inhibition was calculated using the following formula

% inhibition= (R_1-R_2/R_1) x100

Where R_1 is the radial growth of the pathogen without endophytic fungi, and R_2 is the radial growth of the pathogen inoculated with endophytic fungi (Rabha *et al*., 2014).

5.3: RESULTS

5.3.1: *In vitro* and *in vivo* mangrove leaf degradation

5.3.1.1: *In vitro* leaf degradation

In the present study, variation in leaf degradation patterns by the inoculated endophytes was recorded. It was observed that a few of the endophytes exhibited sporulation. Bleaching of leaf material was recorded in the treated plates. In contrast, there was no degradation or bleaching

in the control plates. Seven of the 14 endophytic isolates tested, *viz*., MEn10, MEn06, MEn37, MEn27, MEn36, MEn42, and MEn30, showed significant litter degradation activity. Two sterile isolates, *viz.*, MEn37 (isolated from *A. officinalis*) and MEn38 (isolated from *B. cylindrica*), exhibited sporulation in the medium amended with host leaf litter. Based on the sporulation, the two fungal isolates were identified as species of *Cladosporium* (MEn37) and *Curvularia* (MEn38). The isolate MEn10 (*Aspergillus* sp.) recorded maximum degradation activity in the leaves of *A. marina* and *E. agalocha*. Complete degradation of *E. agallocha* leaf was achieved using MEn42 (*Pestalotiopsis* sp.). Isolates MEn27 (*Aspergillus niger*) and MEn36 (*Nigrospora* sp.) recorded maximum leaf degradation activity in *A. illicifolius* (**Plate 5.2**)

5.3.1.2: *In vivo* leaf degradation

The *in vivo* leaf degradation activity results indicated that endophytic isolates decompose the litter at a relatively higher rate compared to the control. Litter samples in inoculated bags showed more pigmentation and tissue softening with high moisture content compared to the control, where leaves were least pigmented and had low moisture content (**Plate 5.3**). Five isolates, *viz*., MEn30, MEn04, MEn34, MEn36, and MEn09, recorded higher litter degradation compared to other isolates. The amount of biomass loss is presented in **Fig. 5.3**. Therefore, the samples inoculated with these five isolates were further analyzed using a CHNS analyzer.

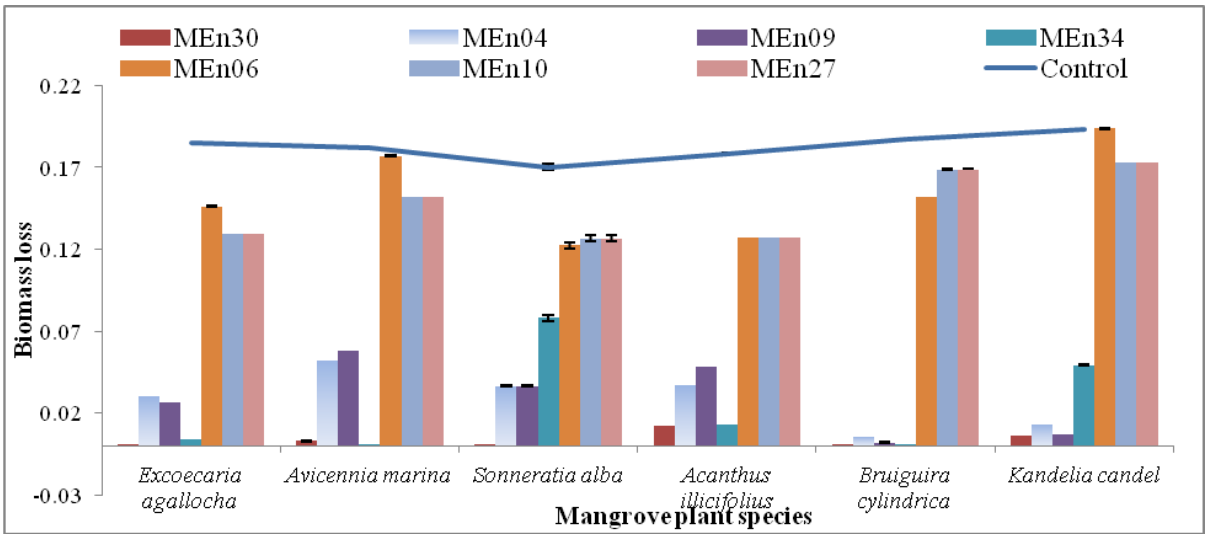

Fig. 5.3: Leaf litter degradation by endophytic fungi.

5.3.1.3: Chemical analysis of litter using CHNS analyser

The study revealed a significant reduction in the C content of the leaf litter inoculated with fungal endophytes compared to the uninoculated control (**Fig. 5.4, Fig. 5.5, and Fig. 5.6**). The endophytic degradation of the leaf litter is known to alter its chemical composition (Gundel *et al.*, 2016).

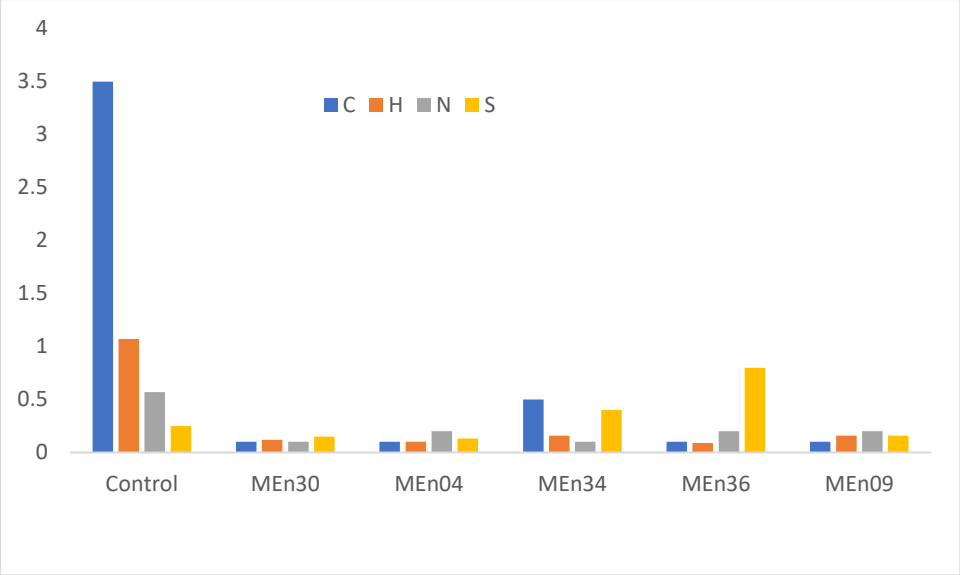

Fig. 5.4: CHNS analysis of *S. alba* leaf litter degraded by endophytic fungi.

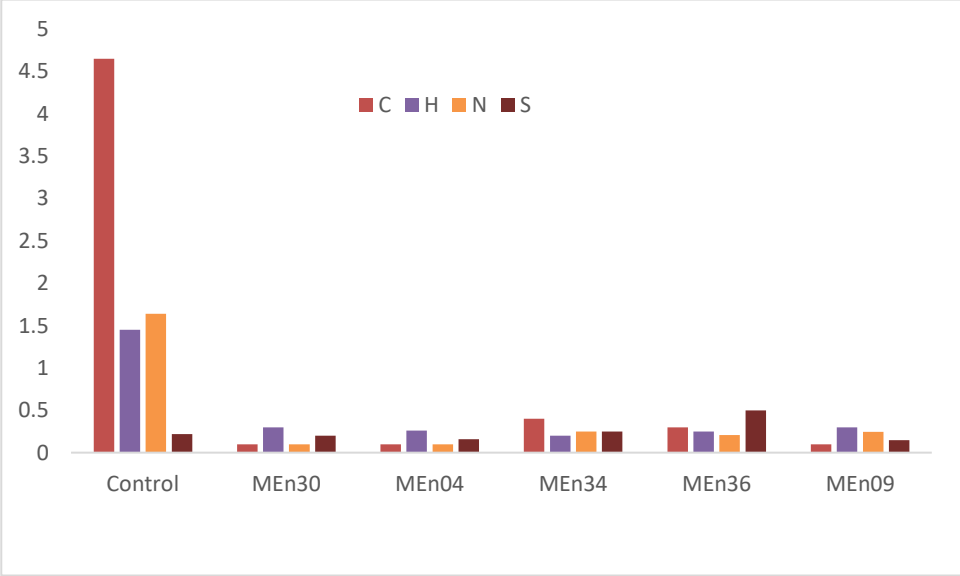

Fig. 5.5: CHNS analysis of *A. ilicifolius* leaf litter degraded by endophytic fungi.

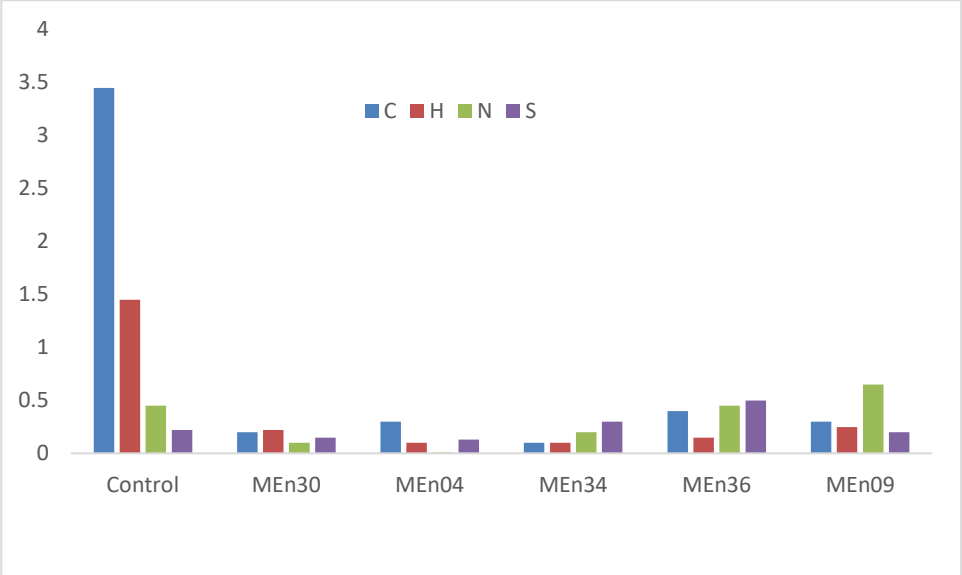

Fig. 5.6: CHNS analysis of *A. marina* leaf litter degraded by endophytic fungi.

5.3.1.4: Enzyme assay

Out of 14 endophytic isolates, nine recorded positive activity for amylase production. The maximum amylase activity was observed in *Drechslera* sp., followed by *Aspergillus* sp. Isolates MEn06 and MEn37 showed positive activity for amylase and protease, indicating their involvement in the degradation of organic matter from the litter. MEn09 and MEn42 recorded positive activity for cellulose production, indicating their role in the degradation of cellulose/glucose matrix from the plant material. MEn04, MEn10, MEn27, MEn38, and MEn42 recorded the production of amylase and cellulose, thus contributing to nutrient flow. MEn16 showed the production of amylase, cellulase, and lipase, while the isolate MEn38 was involved in the production of all four enzymes (**Table 5.3**).

Table 5.3: Qualitative estimation of extracellular enzyme production by endophytic fungi.

Sr. No.	Isolation code	Fungal species	Activity			
			Amylase	**Cellulase**	**Lipase**	**Protease**
1	MEn04	*Drechslera* sp.	+	+	-	-
2	MEn06	*Pestalotiopsis* sp.	+	-	-	+
3	MEn09	*Fusarium sp.*	-	+	-	-
4	MEn10	*Aspergillus sp.*	+	+	-	-
5	MEn16	*Corynespora sp.*	+	+	+	-
6	MEn24	*Curvularia sp.*	-	+	+	-
7	MEn27	*Aspergillus niger*	+	+	-	-
8	MEn30	*Junctospora pulchra*	-	-	-	-
9	MEn34	*Penicillium* sp.	-	-	+	-
10	MEn36	*Nigrospora* sp.	-	+	-	+
11	MEn37	*Cladosporium* sp.	+	-	-	+
12	MEn38	*Seatosheria monoceras*	+	+	+	+
13	MEn39	*Scolecobasidium* sp.	-	+	-	+
14	MEn42	*Pestalotiopsis* sp.	+	+	-	-

Legend: + = Positive activity; - = Negative activity; MEn = Mangrove Endophyte.

5.3.2 Phosphate solubilization by EF

5.3.2.1 Screening for P solubilization activity on solid medium

The results indicated that the P solubilizing index (PSI) ranged from 2 to 3.8 cm. The highest solubilizing index (3.8 cm) was recorded in MEn19 (*Drechslera* sp.) **(Fig 5.7)** (**Plate 5.4**).

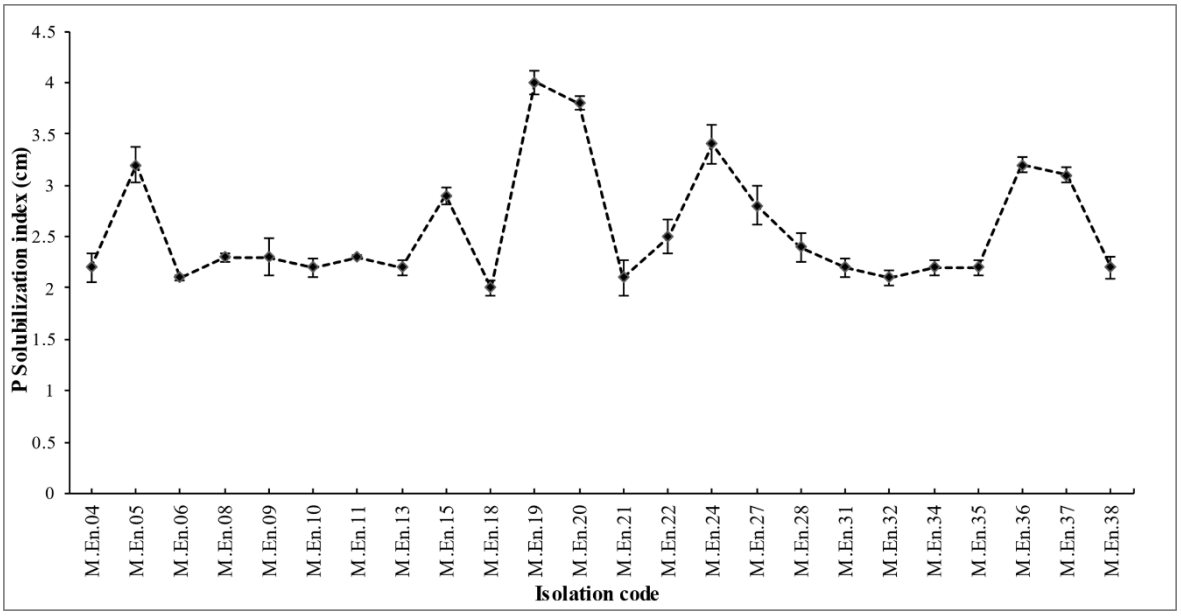

Fig. 5.7: Phosphate solubilization activity of fungal endophytes.

5.3.2.2: Quantitative estimation of P solubilization

The total P solubilized by endophytic isolates ranged from 9.70 to 0.1 µg mL^{-1}. The highest P solubilization was recorded using *Drechslera* sp. (9.70 µg mL^{-}). In a liquid medium, a drastic decrease in pH was observed from 5.7 to 3 **(Fig. 5.8).**

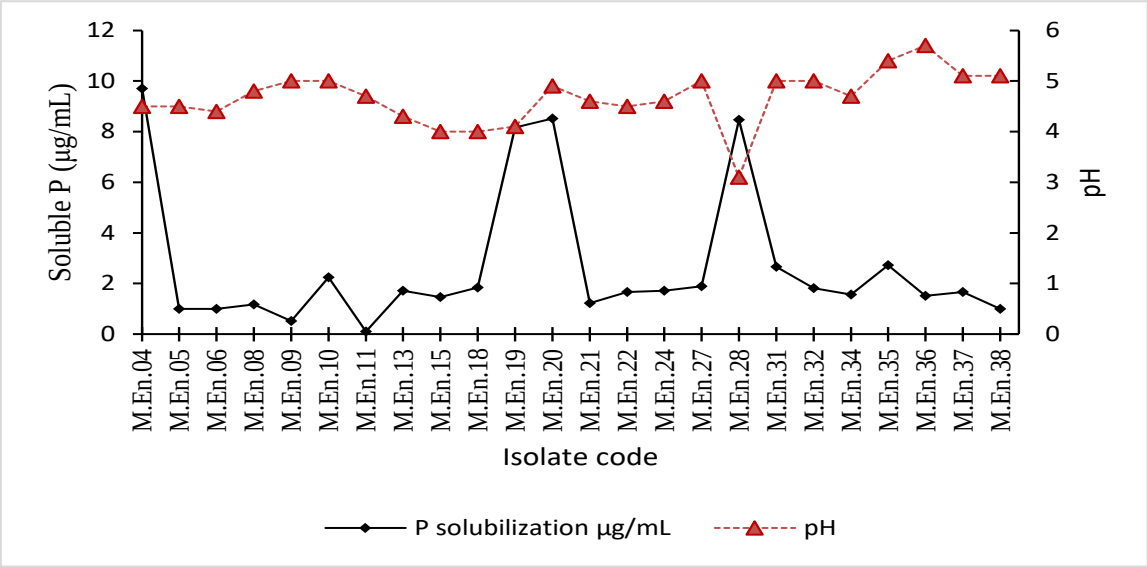

Fig. 5.8: Phosphate solubilization activity of fungal endophytes in the liquid medium.

5.3.3: Biodegradation of residual petroleum hydrocarbon by fungal endophytes

5.3.3.1: Visual -qualitative analysis

The results of the quantitative degradation of the oils are depicted in **Fig. 5.9.** The preliminary observation indicated a colour change from blackish-blue to colourless (**Plate 5.5**). A gradual decrease in the medium was observed in the flasks inoculated with fungal isolates. This decrease suggests that the growing mycelia utilize the media constituents. In the inoculated flasks, tiny drops of oil adhering to the fungal mass were noticed, suggesting the encapsulation of the oil drops by fungal endophytes for degradation.

5.3.3.2: FT-IR analysis of leftover oil

The leftover oil was subjected to FT-IR analysis along with control to understand the degradation of hydrocarbons. The results indicated the difference in the peak/band formation between each tested organism against the control. It can be interpreted that endophytic isolates utilize petroleum hydrocarbons as the only source of C. It was also noted that the different species showed variation in their ability to degrade hydrocarbons (**Fig. 5.10** and **Fig. 5.11**). Seven isolates *viz*., MEn06, MEn09, MEn10, MEn24, MEn27, MEn36, and MEn37 recorded

higher hydrocarbon degradation activity in two and four-wheeler samples. However, these isolates accounted for the partial degradation of boat oil. The FT-IR analysis revealed that the tested endophytic isolates degraded alkanes and aromatic hydrocarbons from the residual oil. Among the various isolates studied, *Nigrospora* sp. was the most promising isolate degrading maximum functional groups from two and four-wheeler oil, followed by *Aspergillus* sp. However, few isolates showed the least hydrocarbon degradation. *Fusarium* sp. recorded a maximum hydrocarbon degradation in the two-wheeler oil compared to other isolates.

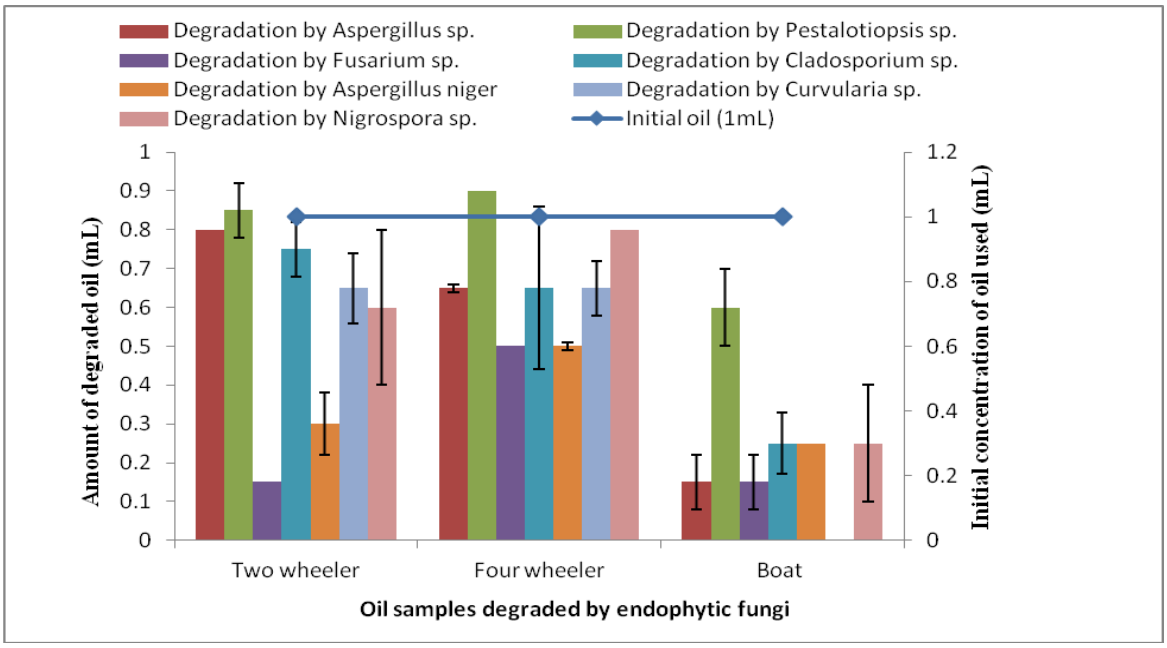

Fig. 5.9: Quantitative degradation of oil by mangrove endophytes.

5.3.3.3: Analysis of two-wheeler residual oil

The FT-IR analysis in control revealed a broad peak at 2925.35 cm^{-1} and 2852.81 cm^{-1} corresponding to -C-H stretching of the aliphatic compounds of phenols and alcohols. This is attributed to the presence of saturation due to the -C-H vibrations of phenols and alcohols. A distinct sharp band at 1714.73 cm^{-1} confirms the presence of C=O stretching vibration due to the acid. The absorption bands at 722 and 946.9 cm^{-1} are attributed to the C-H out-of-plane bending mode of the aromatic components. The C=C vibrations are observed between 1600-1400 cm^{-1}, while the symmetric and anti-symmetric vibrations of the carboxylate group for the organic acid (ν_{COO^-}) appear at 1463 and 1377 cm^{-1}, respectively.

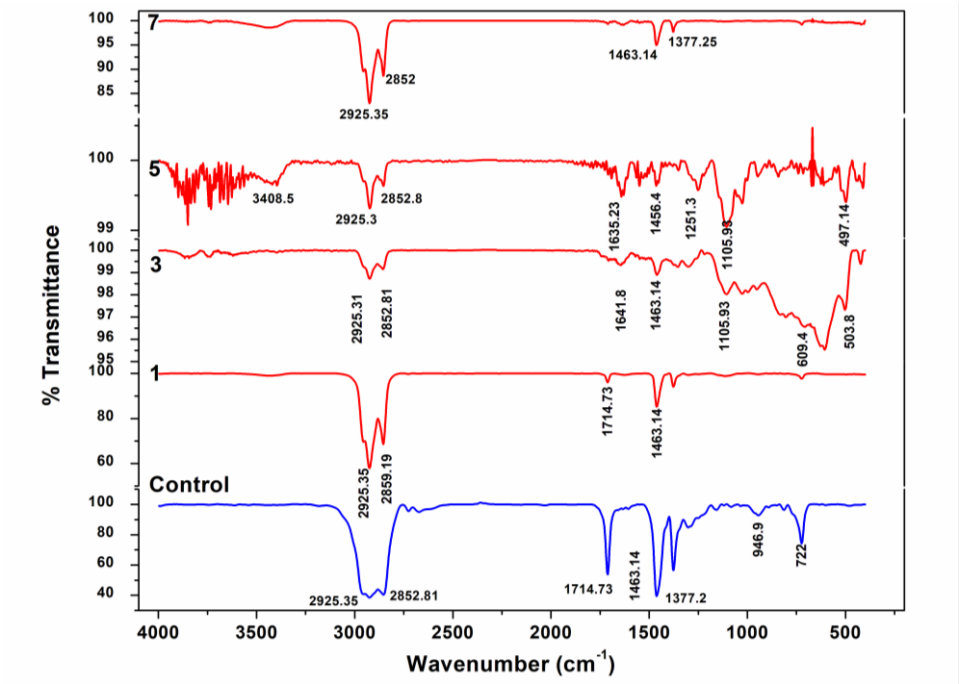

Fig. 5.10: FT-IR spectrum of two-wheeler residual oil degradation by *Aspergillus* sp. (Chromatogram 1); *Aspergillus niger* (Chromatogram 3); *Pestalotiopsis* sp (Chromatogram 5) and: *Fusarium* sp. (Chromatogram 7).

Chromatogram 1 depicts oil degradation by *Aspergillus* sp. It revealed the presence of sharp bands at 2925.35 cm^{-1} and 2859.19 cm^{-1} indicating =C-H stretching vibrations of the aliphatic compounds (phenol and alcohol). In control, the peaks showed broad spectral signatures compared to the experimental set indicating the degradation of some hydrocarbons in the presence of endophytic inoculum. A sharp peak at 1714.73 cm^{-1} was observed in the control, which was negligible in the experimental set. In control, sharp peaks at 1463.14 cm^{-1}, 1377.2 cm^{-1}, 946.9 $cm^{-1,}$ and 722 cm^{-1} were noticed, which were seen as fading peaks in the experimental set. The fading of the above absorptions in the experimental set was due to degradation by fungal endophytes.

Chromatogram 3 depicts oil degradation by *Aspergillus niger*. It revealed that absorption bands centered at 2925.31 cm^{-1} and 2852.81 cm^{-1} indicated the presence of -C-H stretching vibrations of the organic moieties (phenols and alcohols). Complete degradation of the prominent peak centered at 1714 cm^{-1} was noticed. While degrading hydrocarbons, at peak 1463.14 cm^{-1}, traces of functional groups were noticed. A small absorption peak detected at 1105.93 cm^{-1} might be involved in forming a new functional group while degrading hydrocarbons. It was also noticed that spectral peaks at 722 cm^{-1} (seen in the control) were absent in the experimental isolate. However, new bands at 609.4 cm^{-1} and 503.8 cm^{-1} were noticed, corresponding to alkyl halides.

Chromatogram 5 depicts oil degradation by *Pestalotiopsis adusta*. It revealed the presence of spectral bands at 3408.5 $cm^{-1,}$ indicating the presence of hydroxyl stretching of alcohol and phenol. In control, broadband centered at 2925 cm^{-1} and 2852 cm^{-1} corresponding to -C-H stretching of the aliphatic phenols and alcohols was replaced by a small sharp peak in the experimental set, indicating the degradation of hydrocarbons by the fungus. At 1635.23 cm^{-1} another small broadband was noticed corresponding to C=O of the carbonyl group of organic acid, which was absent in control, indicating the formation of new functional groups by the endophytes. The sharp spectral band at 1463 cm^{-1} in control was degraded by the fungal endophyte revealing the decisive role of *P. adusta* in degradation. However, at 1377.2 cm^{-1}, 946.9 cm^{-1}, and 722 cm^{-1} bands were fully degraded by the isolates, while a new functional group at 1251.3 cm^{-1}, 1105 $cm^{-1,}$ and 497.14 cm^{-1} was detected.

Chromatogram 7 depicts oil degradation by *Fusarium* sp. It indicated the degradation of broad spectral peaks observed in control at 2925.35 cm^{-1} and 2852 cm^{-1} were replaced by small sharp peaks indicating the degradation of (-C-H) aliphatic compound of phenols and alcohols. Prominent sharp bands at 1714 cm^{-1}, 946.9 cm^{-1}, and 722 cm^{-1} were fully degraded in the experimental set. A sharp long band in control at 1463.14 cm^{-1} representing the methylene group of CH_2 and CH_3 was degraded at the maximum level, whereas traces of hydrocarbons were degraded at 1377.2 cm^{-1}.

5.3.3.4: Analysis of Four wheelers residual oil

The FT-IR analysis of control (untreated) dataset showed the presence of spectral bands at 3450.54 cm^{-1} (alcohols and phenols), 29927.04 cm^{-1} and 2850 cm^{-1} (aliphatic -C-H vibrations), 1629 cm^{-1} (C=O), 1461.16 cm^{-1} (methylene group), 1378 cm^{-1} and at 722 cm^{-1} (alkyl halides).

Chromatogram 2 depicts oil degradation by *Fusarium* sp. It revealed that the isolate degraded traces of alcohol and phenol at 3450 cm^{-1}. Peaks at 2927 cm^{-1} and 2850 cm^{-1} (representing aliphatic compounds) were not fully degraded. The sharp band at 1461 cm^{-1} was replaced by a short sharp band. However, the band at 1370 cm^{-1} was absent in the experimental set. The new band at 1105.91 cm^{-1} was noticed, indicating the C-O stretch of the alcohol and the production of new functional groups by the fungus. The sharp peak at 722 cm^{-1} in the control was replaced by a small peak at 722 cm^{-1} in the experimental set, indicating the degradation of alkyl halides.

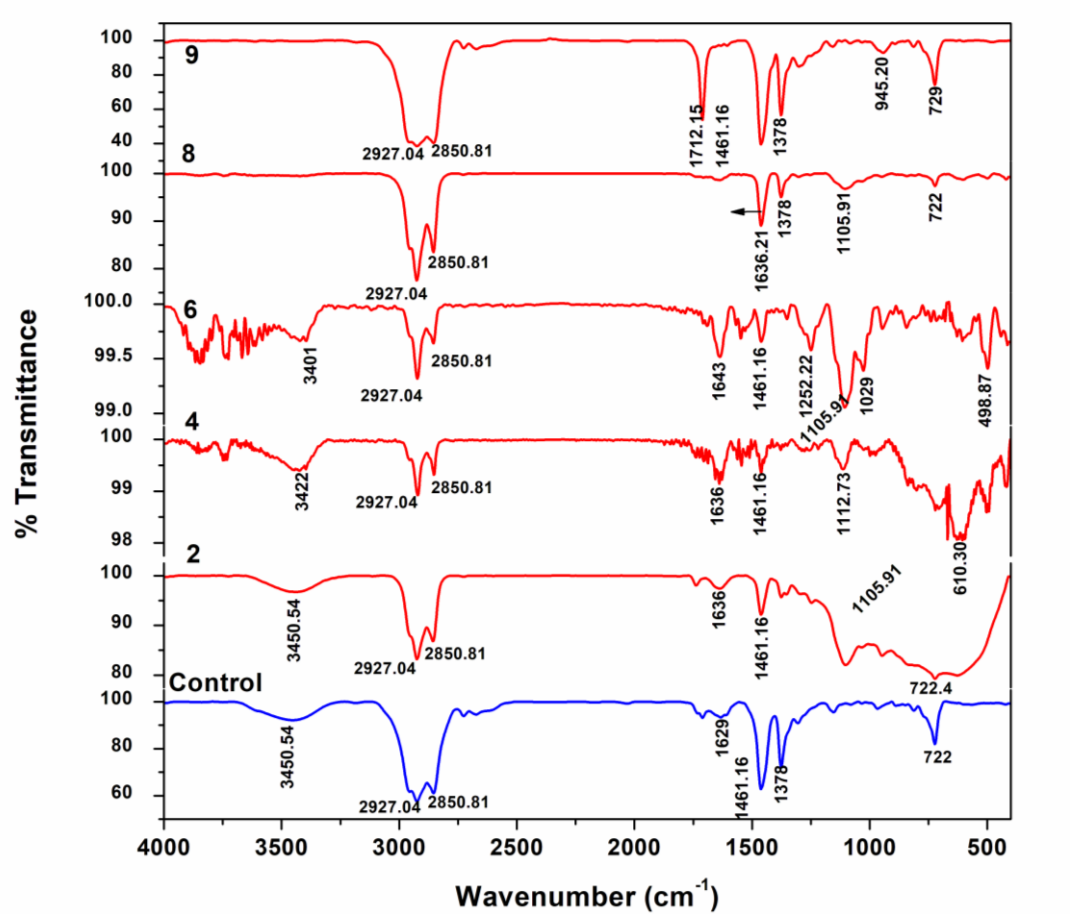

Fig. 5.11: FT-IR spectrum of four-wheeler residual oil degradation by *Fusarium* sp. (Chromatogram 2); *Aspergillus niger* (Chromatogram 4); *Curvularia* sp. (Chromatogram 6); *Aspergillus* sp. (Chromatogram 8) and *Nigrospora* sp. (Chromatogram 9).

Chromatogram 4 depicts oil degradation by *Aspergillus niger*. It indicated that bands at 2927 cm^{-1} and 2850 cm^{-1} corresponding to the -C-H stretching were degraded by the fungal isolate. The peak at 1461 cm^{-1} was degrading, while the formation of new functional groups at 1112.73 cm^{-1} comprised the C-O band. The absorption peak at 1378 cm^{-1} and 722 cm^{-1} was absent in the experimental set, while a new peak at 488.57 cm^{-1} was observed.

Chromatogram 8 depicts oil degradation by *Aspergillus* sp. Endophyte isolates indicated full degradation of hydrocarbons at 3450 cm^{-1}, whereas a sharp peak was noticed at 2927 cm^{-1} and 2850 cm^{-1} due to the -C-H functionality. The spectral band at 1461 cm^{-1} was fully degraded, while the new functional group at 1105.91 cm^{-1} was seen. Prominent bands at 1636 cm^{-1}, 1370 cm^{-1}, and 722 cm^{-1} were degraded in the experimental set.

Chromatogram 9 depicts oil degradation by *Nigrospora* sp. The results indicated clear degradation of 3450.54 cm^{-1} and 1629 cm^{-1} followed by slight degradation of the spectral bands

at 2927 cm^{-1} and 2850 cm^{-1}. Additional bands observed at 1712 and 945 cm^{-1} suggested the presence of -C=O and -C-H out-of-plane functional group modes.

5.3.4: Effect of fungal endophytes on plant growth

5.3.4.1: *In vivo* effect on plant growth by fungal endophyte

The study revealed that inoculation with eight fungal isolates showed variation in the growth pattern (**Fig. 5.12)**. Maximum shoot length was recorded in *A. marina* plants inoculated with MEn24, followed by MEn27, MEn30, MEn10, MEn37, and MEn36. In contrast, the least growth was recorded in plants inoculated with MEn06 (**Plate 5.6, Plate 5.7** and **Fig. 5.13**).

5.3.4.2. Plant biomass and pigment analysis

Plants of *Av. marina* inoculated with various fungal endophytes recorded a higher amount of chlorophyll b than chlorophyll a (**Fig. 5.14)**. The inoculated plants recorded higher biomass compared to the control. Maximum biomass was recorded in plants inoculated with T_6 (MEn30), followed by T_2 (MEn04) and T_3 (MEn06). However, the least biomass was recorded in T_5 (MEn27) (**Fig. 5.15).**

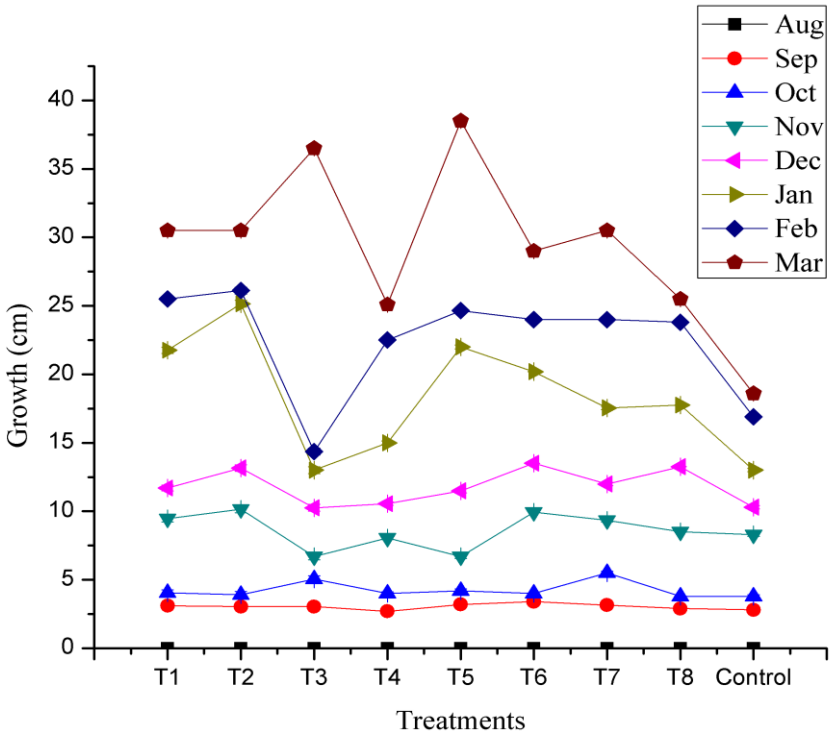

Fig. 5.12: Effect of inoculated fungal endophytes on growth of *A. marina* T_1= MEn10, T_2 = MEn04, T_3 = MEn06, T_4 = MEn24, T_5 = MEn27, T_6 = MEn30, T_7 = MEn36, and T_8 = MEn37.

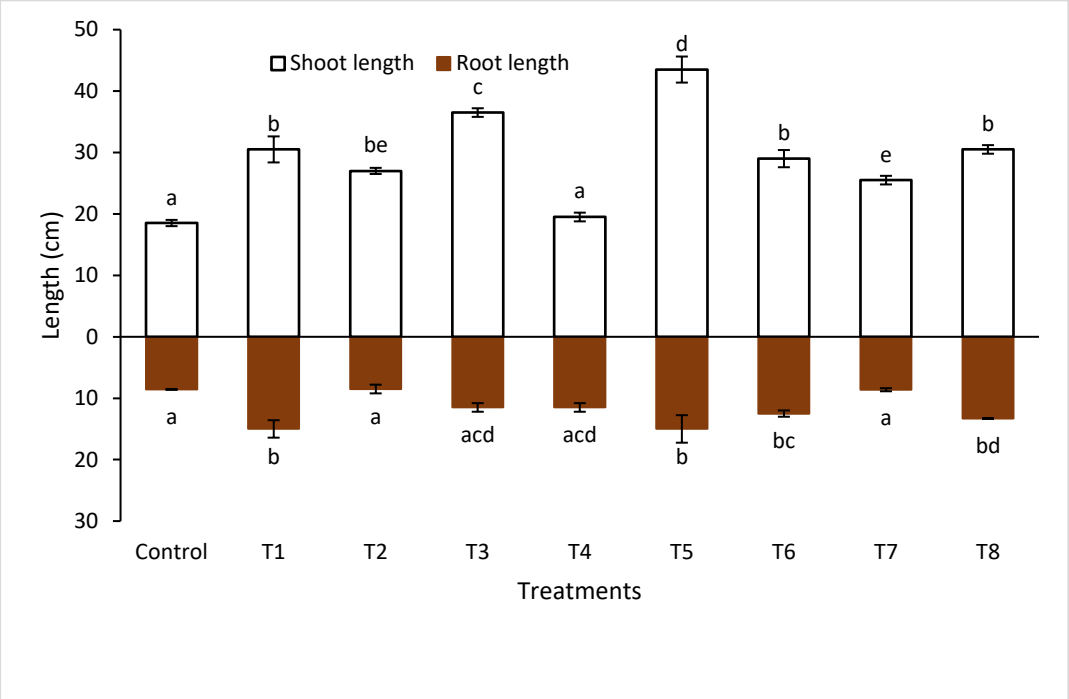

Fig. 5.13: Effect of fungal endophytes on *in vitro* growth in *A. marina* (after six months). (Values in each column presented with different lowercase and uppercase letters are significantly different at $p<0.05$). T_1= MEn10, T_2 = MEn04, T_3 = MEn06, T_4 = MEn24, T_5 = MEn27, T_6 = MEn30, T_7 = MEn36, and T_8 = MEn37.

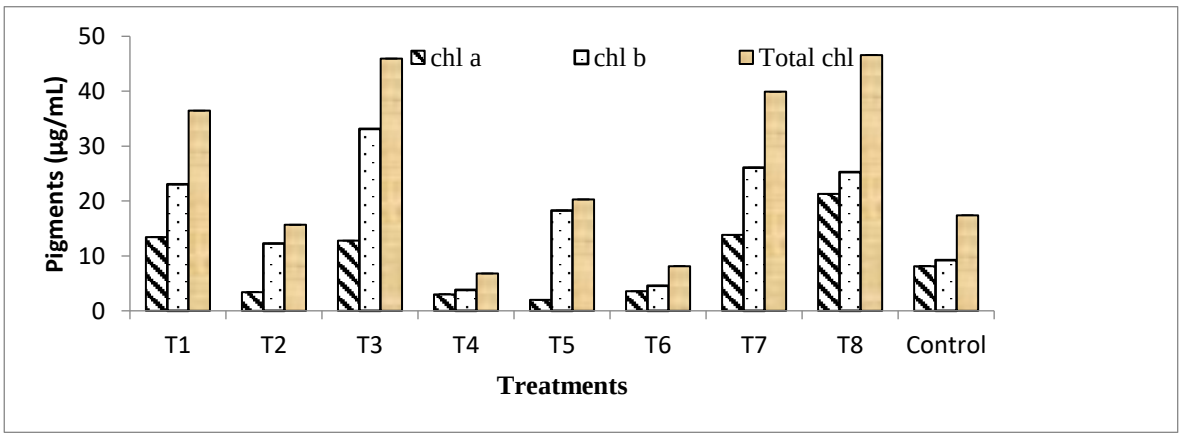

Fig. 5.14: Effect of endophytic fungal inoculation on leaf chlorophyll content.

5.3.4.3: Root morphology and colonization:

The seedling inoculated with fungal endophytes produced dense coiling of lateral as well as adventitious root hairs with dense septate colonization. The control plants, however, showed the least colonization. This may be attributed to the vertical transmission of the endophytes (**Plate 5.8** and **Plate 5.9**).

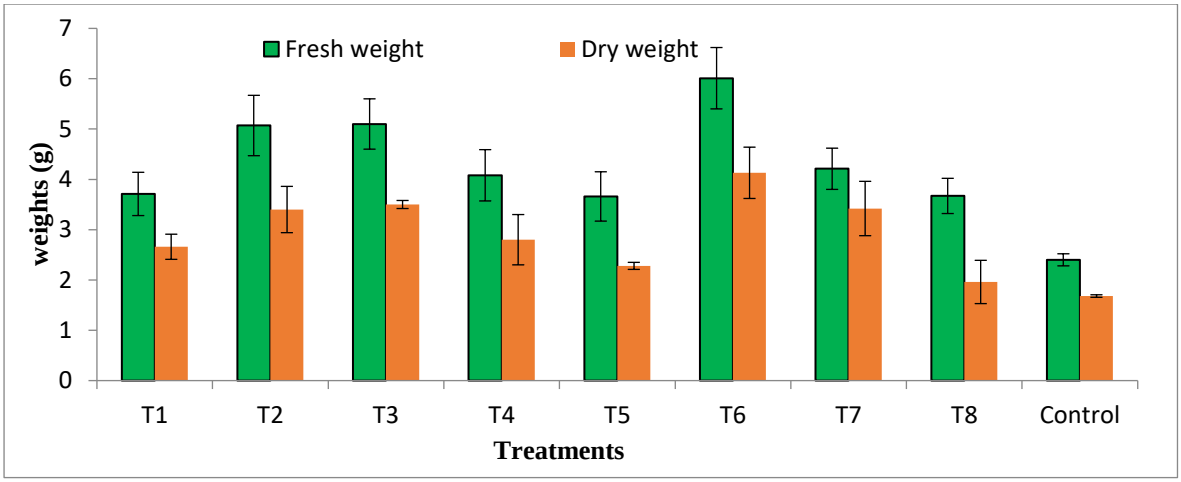

Fig. 5.15: Effect of endophytic fungal inoculation on plant biomass.

5.3.5: Screening of fungal endophytes for antagonistic activity against the pathogen

5.3.5.1: Isolation and molecular identification of the pathogen

The plant pathogen was isolated and maintained on a PDA medium (**Plate 5.10**).

5.3.5.2: Molecular identification

Molecular identification of the pathogenic culture revealed 99% similarity with *Elsinoe embeliae* (**Fig. 5.16**).

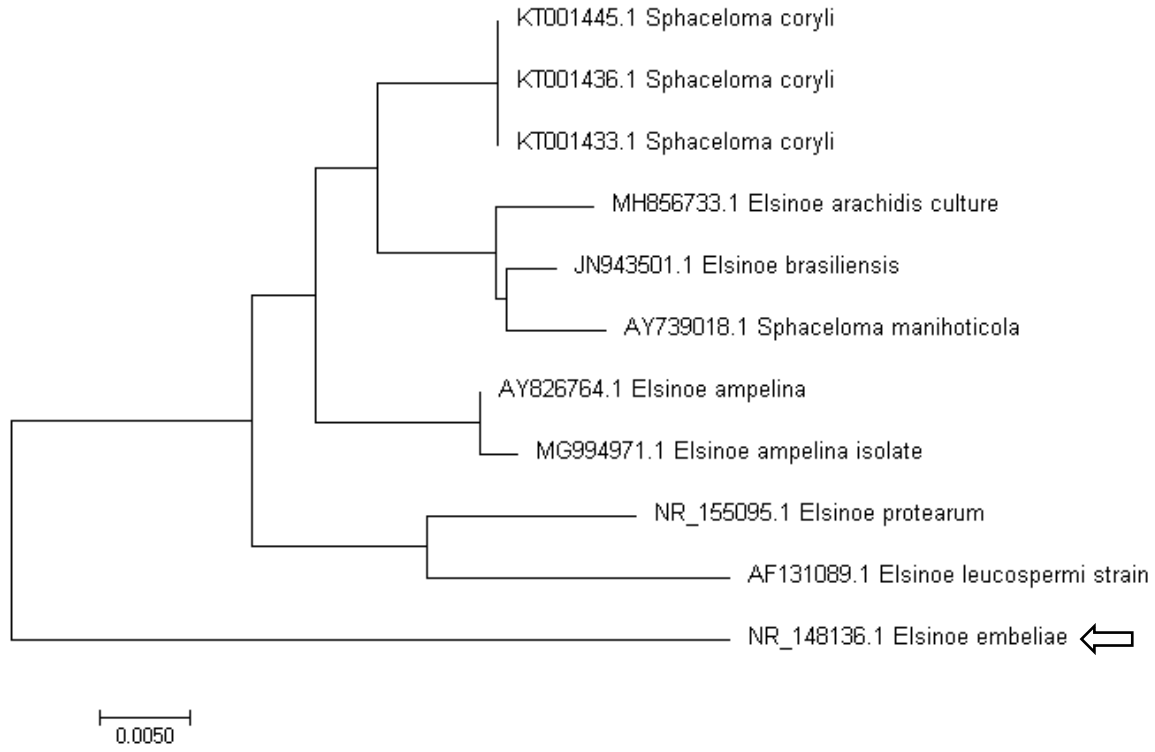

Fig. 5.16: Phylogenetic tree of *Elsinoe embeliae.*

5.3.5.3: Antagonistic activity of the isolated endophyte against *Elsinoe embeliae*

Eight endophytic fungal cultures, *viz*., MEn24 (*Curvularia* sp.*)*, MEn27 (*Aspergillus niger),* MEn10 *(Aspergillus* sp.*),* MEn30 (*Junctospora pulchra*), MEn37 *(Cladosporium* sp.*),* MEn36 (*Nigrospora* sp.), MEn04 (*Drechslera* sp.), MEn06 *(Pestalotiopsis* sp.), MEn30 (*Junctospora pulchura*) and MEn36 (*Nigrospora* sp.) were isolated and tested against the plant pathogen using dual culture plates. Three fungal isolates, *viz*., MEn06, MEn30, and MEn36, showed 70% inhibition, followed by MEn27 (61.66%) and MEn10 (53.33%). While the two isolates, *viz*., MEn04 and MEn37, showed the least inhibition (45%) (**Table 5.4).**

Table 5.4: Antagonistic activity of fungal endophytes against *Elsinoe embeliae*.

Isolation code	**Endophytic fungi**	**Radial growth of pathogen without endophyte (R_1) (cm)**	**Radial growth of pathogen inoculated with endophyte (R_2) (cm)**	**% inhibition= (R_1-R_2/R_1) x 100**
MEn04	*Drechslera* sp.	1.2±0.00	0.9±0.01	45
MEn06	*Pestalotiopsis* sp.		0.6±0.0	70
MEn10	*Aspergillus* sp.		0.8±0.02	53.33
MEn24	*Curvularia* sp.		0.9±0.01	45
MEn27	*Aspergillus niger*		0.7±0.01	61.66
MEn30	*Junctospora pulchra*		0.6±0.02	70
MEn36	*Nigrospora* sp.		0.6±0.01	70
MEn37	*Cladosporium* sp.		0.9±0.1	45

Legend: ± = Standard deviation.

5.4: DISCUSSION

Mangroves are biodiversity hotspots for marine fungi, having various applications in the nutrient recycling process (Thatoi *et al.,* 2013). These magnolicious fungi persist in plants as saprobes, degraders, and symbionts. Litter degradation is a major process for transferring mineral nutrients from vegetation into the soil by breaking organic matter into CO_2, water, and other mineral nutrients (Lambers *et al.,* 1998). The decomposition is achieved by the action of various biotic and abiotic factors involved in the degradation of vegetative material by colonizing and further degrading into organic matter (Matondkar *et al.,* 1981). The organic matter later flows in the form of energy into various forms of life and also gets mixed into the

muddy mangrove soil. Therefore, the soil nutrient flow is crucial to maintaining the soil compositions that contain proper influx and dissociation of macro- and micro-nutrients. The present study on *in vitro* and *in vivo* leaf litter degradation confirms the ability of endophytes in degradation. Species belonging to *Penicillium* and *Nigrospora* degraded litter at significantly higher levels. It was also observed that a sole endophyte did not fully degrade the leaf litter. The reason could be that degradation is the result of factors like climatic conditions of a particular location, microorganisms involved in degradation, and the chemical composition of the plant material (Singh and Gupta, 1977). Also, recent evidence suggests the degradation of litter is by the process called photodegradation, wherein decomposition is achieved by the absorption of photons found in sunlight, *i.e.,* by using infrared, visible, and ultraviolet light (Austin *et al*., 2014). Leaf Morphology (resistance to physical damage) is an important factor affecting the litter decomposition rate (Sundarapandian and Swamy, 1999). A thick wax coating on the outer cuticle layer of mangrove leaves appears to be an important factor affecting the litter decomposition rate.

Nutrient release from the leaf litter is a process that involves the leaching of leaf matter through the action of microorganisms (Seta *et al.,* 2017). In forest ecosystems, it is known that decomposition is associated with a lower C and N ratio (Swift *et al.,* 1979). In the mangrove ecosystem, the tidal variations result in the acceleration of sediment, which often buries the decaying leaves triggering the action of soil microorganisms in degradation (Gulis and Suberkropp, 2003), thus enhancing N composition in the soil (Lambers *et al.*, 1998). A similar observation has been recorded in the present study, where a significantly lowered C content was recorded in the litter that was inoculated with fungal endophytes compared to the uninoculated control.

It is also observed that only the members of Ascomycetes were involved in the litter degradation activity in the present study. However, in a study, Ukoima (1995) compared the diversity of litter-degrading fungal communities between terrestrial and mangrove ecosystems and revealed the presence of similar species belonging to Deuteromycetes, Ascomycetes, and Oomycetes. He related this observation to the probability that these fungal spores get into the mangrove ecosystem through the influx of freshwater sources.

These microsymbionts immensely contribute to the production of secondary metabolites and enzymes involved in the degradation process. The present study confirms that different enzymes like amylase, protease, lipase, and cellulase have a positive role in ecological and

biological perspectives. As such, fungi are known to degrade lingo-cellulose matrix in litter by producing various extracellular enzymes (Janusz *et al.*, 2017). The amylase activity reveals the role of endophytic fungi in degrading starch while plants undergo the senescence stage (Patil *et al*., 2015). It also indicates the breakdown of complex organic nutrients in the plant system (Fadiji and Babalolo, 2020), thus directly helping in nutrient flow.

The present study on P solubilization revealed the potency of 24 endophytic fungal isolates to solubilize P. Mangrove endophytes showed clear halo zones in the culture plates, indicating their role in P solubilization. Among these species of *Fusarium, Penicillium, Aspergillus,* and *Pestalotiopsis* have frequently been reported to be P solubilizers under laboratory conditions (Mahadevamurthy *et al*., 2016). Upon culturing in liquid Pikovskaya's media for 14 days, these species showed a drastic decrease in pH. This decrease in pH could be attributed to the acidification and mineralization of P by microbial cells (Singh *et al.*, 2017). Igual *et al*. (2001) also reported a similar activity of fungi suggesting a higher solubilizing potential for growing fungi under basic to acidic conditions. They revealed that acid production was solely responsible for P solubilization.

Bioremediation using microorganisms is a result of microbial activity involved in degrading environmental pollutants into less toxic forms (Aparna *et al*., 2010). In the present study, it was observed that the endophytic isolates degrade petroleum hydrocarbons by degrading the functional groups or forming new structural compounds. Das and Chandran (2011) suggested that the fungal endophytes degrade oil by microdroplet encapsulation at the hydrophobic microbial cell surface, which further converts the substrate into carbon dioxide and water.

It was also observed that *Aspergillus* sp. could degrade petroleum hydrocarbons from residual two- and four-wheeler oil samples. Singh (2006) reported that species belonging to *Aspergillus*, *Cephalosporium,* and *Penicillium* had the potential to degrade hydrocarbons from crude oil. In our study, maximum degradation was achieved by *Nigrospora* sp., which could degrade a large number of hydrocarbons. Biodegradation depends on various factors such as environmental temperature, oil viscosity, the inherent biodegradative capability of microbe, and available nutrient content (Brusseau, 1998). Marine water bodies typically have lower levels of N and P availability. During oil spills, the C level in the marine system increases, making it difficult for degraders to degrade hydrocarbons (Atlas, 1975). A high level of NPK decreases the amount of degradation, especially of aromatic hydrocarbons (Carmichael and Pfaender, 1997). Studies reveal that within petroleum fractions, the most preferred substrate for biodegradation

is n-alkanes and branched alkanes of length between C-10 to C-20 (Bogusławska-Was and Da¸Browski, 2001). Bhat *et al*. (2011) indicated that n-alkanes and branched alkanes (of length between C-10 to C-20) from the petroleum fractions were the most preferred substrate for degradation. The present study revealed that the tested endophytic isolates consumed alkanes and aromatic hydrocarbons of residual oil. *Nigrospora* sp. was the most promising isolate in the degradation of maximum functional groups from two- and four-wheeler oil. Besides, *Fusarium* sp. also degraded a higher amount of two-wheeler hydrocarbons than other isolates.

Endophytic fungi are useful symbionts that help to suppress the negative effect by pathogens on the host plants. In the present study, three of the fungal endophytes, *viz*., *Pestalotiopsis* sp., *J. pulchura,* and *Nigrospora* sp., recorded the highest inhibitory activity against the plant pathogen *Elsinoe embeliae.* Endophytes are known to harbour antimicrobial and antifungal properties that assist the plant system in overcoming the damage caused by phytopathogens (Gunatilaka, 2006). Other mechanisms include the secretion of enzymes that enhance the antagonistic potential of endophytes against plant pathogens (Babalola, 2007). Dai *et al*. (2008), reported that the production and release of auxins by endophytes assist in suppressing the growth of pathogens.

Chlorophyll is a major constituent of the biochemical pathway that leads to healthy plant growth. In the present study *A. marina* seedlings inoculated with the endophytic species *viz.*, MEn10, MEn04, MEn06, MEn24, MEn27, MEn30, MEn36, and MEn37 recorded higher amounts of chlorophyll b and total chlorophyll compared to uninoculated control. Chlorophyll b is associated with adaptation mechanisms for survival and acclimatization in stressed ecosystems (Dale and Causton, 1992).

Dense hyphal coiling was observed in the roots of seedlings inoculated with endophyte species in *A. marina*. Besides, the inoculated seedlings recorded significantly higher biomass production than the uninoculated control. Santoyo *et al*. (2016) observed that the application of endophytes has a positive response to plant growth and biomass. In the present study, profuse rooting was observed in inoculated plants, which may be responsible for increased growth and biomass. Shimizu (2011) observed that few endophytic species produce IAA hormonal compounds that enhance the elongation and production of adventitious roots.

5.5: CONCLUSION

The study attempted to understand the role of endophytic fungi in mangrove ecosystem. It can be concluded from the present study that these endo-symbionts are essential degrading agents that reside in the plant system. The study also confirms the effectiveness of fungal endophytes in P solubilization. The presence of such endophytes in the mangrove ecosystem, therefore, minimizes soil fertility-related problems enabling their use as biofertilizers. The study also suggests that mangrove endophytes have a potential role in the bioremediation process. Thus the commercial application of the endophytic inoculum would provide a beneficial role for plants as well as humans.

Section 5B

5.1: Extraction and characterization of secondary metabolites

Endosymbionts are gaining the scientific community's attention because of their genetic potential to produce metabolites that are beneficial to the growing pharmaceutical, agricultural, and industrial research (Vitorino and Bessa, 2017). Such mycobiota provide a wide variety of direct and indirect interactions between plants and herbivores, including increasing resistance to disease, abiotic stress, and enhancing plant growth (Rodriguez, 2009). Compounds produced by fungal endoph ytes (alkaloids, terpenoids, flavonoids, steroids, *etc.*) can be used for plant growth promotion and defense against pathogens. Endophytes can produce diverse classes of phytochemicals that have multiple medicinal and pharmaceutical applications (Min *et al*., 2016).

5.2: MATERIALS AND METHODS

5.2.1: Extraction of bioactive metabolites

Fourteen fungal endophytic isolates, MEn01, MEn04, MEn06, MEn10, MEn24, MEn27, MEn30, MEn36, MEn37, MEn85, MEn87, MEn89, MEn67, and MEn102, were used in the study. For the extraction of bioactive metabolites, the method of Radji *et al*. (2011) was employed. Each fungal isolate was inoculated in a 500 ml Erlenmeyer flask containing 300 ml PD broth (Potato Dextrose) at pH 6.5 and incubated under stationary conditions at 25^0C for 15 days. Fungal colonies from the incubated flasks were filtered using three-layered Whatman filter paper. To the filtrate, equal amounts of ethyl acetate were added and mixed well until two

immiscible layers appeared. The upper layer of ethyl acetate fraction containing the fungal metabolites was extracted using a sterile pipette under aseptic conditions. It was then kept on a rotary shaker until the solvent evaporated to obtain a concentrated extract. The resultant powder was mixed with DMSO (1 mg ml^{-1} DMSO) and kept at 4^0C (**Plate 5.11**).

5.2.2: Phytochemical screening of the fungal endophytes

Different crude extracts of fungal endophytes *viz*., MEn01, MEn04, MEn06, MEn10, MEn24, MEn27, MEn30, MEn36, MEn37, MEn85, MEn87, MEn89, MEn67, and MEn102 were tested for the presence of various secondary metabolites *viz*., alkaloids, saponins, terpenoids, flavonoids, steroids, phenols, and tannins using standard methods (Bhardwaj *et al*., 2015).

5.2.2.1: Test for alkaloids

Mayers reagent test: The addition of 2 ml Mayers reagent and 1 ml diluted HCl to endophytic fungal extract resulted in the production of a yellow precipitate indicating the presence of alkaloids.

5.2.2.2: Test for Flavonoids

Ferric chloride test

The addition of neutral ferric chloride solution to endophytic fungal extract resulted in the appearance of blackish-green colour, indicating the presence of flavonoids.

5.2.2.3: Test for phenolics

Ellagic acid test

Adding a few drops of a mixture of 5% glacial acetic acid and 5% sodium to endophytic fungal extract resulted in a chocolate colour, indicating the presence of phenolics.

5.2.2.4: Test for steroids

Salkowski test

The addition of concentrated H_2SO_4 to endophytic fungal extract resulted in the appearance of wine red colour, indicating the presence of steroids.

5.2.2.5: Test for tannin

Gelatin test

Adding a few drops of 1% gelatin solution to endophytic fungal extract resulted in the appearance of a white precipitate indicating the presence of tannins.

5.2.3: Thin layer chromatography (TLC) for the separation of compounds

5.2.3.1: Preparation of TLC plates

The crude ethyl acetate fractions of fungal isolates *viz*., MEn01, MEn04, MEn06, MEn10, MEn24, MEn27, MEn30, MEn36, MEn37, MEn85, MEn87, MEn89, MEn67, and MEn102 were subjected to thin layer chromatography (TLC) to separate the secondary metabolites.

5.2.3.2: Preparation of TLC plates

Glass plates were thoroughly washed and air-dried. Silica gel was prepared by mixing 20 g of silica powder in 40 ml of distilled water. Dried plates were then coated with approximately 0.5 mm thick silica gel. The coated plates were activated by incubating at 60^0C for 3h prior to use. The samples were loaded on to activated TLC plate 1 cm above with the help of a capillary tube and allowed to dry.

5.2.3.3: Preparation of TLC solvent

The solvents of different polarities and ratios were used to separate the compounds from the crude extract. The Retention factor (Rf) value of each band was obtained as the ratio of the distance moved by the solute to that of the solvent front.

Rf = Distance traveled by solute/ Distance traveled by solvent.

5.2.4: Characterization of bioactive metabolites by using Gas Chromatography-Mass Spectroscopy (GC-MS) analysis

The GC-MS analysis of six crude extracts of fungal endophytes *viz*., MEn01, MEn85, MEn87, MEn89, MEn67, and MEn102 showing potential anticancer activity was performed at IIT Bombay. Two µl of each sample was employed for analysis using Elegant Hp 7880 with a column of 30 m length and 0.32 thicknesses. Helium gas was used as carrier gas at a constant flow rate of 1 mL min^{-1}. The injector temperature was set at 100^0C. The oven temperature was programmed from 50^0C to 280^0C at 10^0C min^{-1} to 200^0C, then 100^0C 3 min^{-1} to 250^0C, ending with 5 minutes isothermal at 280^0C. The sample was injected in split mode as 50:1 (Devi *et al*., 2013). The metabolites were identified by considering their retention time, area percentage, molecular formula, and molecular weights.

5.2.5: Antimicrobial potential of endophytes against human pathogens

5.2.5.1: Selection of endophytic fungi

Fourteen crude extracts *viz*., MEn01, MEn04, MEn06, MEn10, MEn24, MEn27, MEn30, MEn36, MEn37, MEn85, MEn87, MEn89, MEn67, and MEn102 were selected to screen their antimicrobial potential.

5.2.5.2: Screening for antibacterial activity

The crude extracts of the above selected fungal endophytes were screened against seven human pathogens *viz*., *Klebsiella pneumonia* ATCC 700603, *Escherichia coli* ATCC 8739, *Shigella boydii* ATCC 12030, *Staphylococcus aureus* ATCC 6538, *Candida albicans* 3147 ATCC 10231, *Aspergillus brasiliensis* WLRI 034, and *Salmonella enterica serovar typhimurium* ATCC 14028 (procured from Goa Medical College, Bambolim, Goa), using Agar well diffusion method. Mueller-Hinton Agar (MHA) was used as a nutrient supplement for the test pathogens. The young bacterial suspension corresponding to 0.5 McFarland standards was mixed with 20 mL Mueller-Hinton Agar and poured into sterile Petri plates (Sardessai *et al*., 2014). The solidified plates were bored with 12 mm diameter wells. The wells were later filled with 100, 90, 80, 70, 60, 50, 40, 30, 20, and 10 µl of the crude extract. The plates were kept for pre-diffusion at 4^0C for 20 minutes, followed by 24hr incubation at 37^0C. Appropriate positive and negative controls were maintained throughout the study. Each set of experiments was carried out in triplicate.

5.2.5.3: Determination of Minimum Inhibitory Concentration (MIC)

Inoculum preparation involved two-fold serial dilutions using Mueller-Hinton broth (MHB) as the medium. Eighteen hours old bacterial/fungal suspensions were adjusted to 0.5 McFarland standards in sterile saline to achieve concentrations of 10^7 CFU mL^{-1} (Khan *et al.,* 2009). The MIC determination involved serial dilutions of varying concentrations, followed by an incubation period of 24h at 37^0C. The MIC of the culture was the lowest concentration at which growth failed to occur. MIC of the crude ethyl acetate extract was determined using the serial dilution tube method and measuring the optical density at 600 nm using Elico Calorimeter.

5.2.5.4: Minimum bactericidal concentration (MBC)

Minimum Bactericidal Concentration (MBC) was determined by culturing aliquots from the MIC tubes on Mueller-Hinton agar medium and incubating for 48h at 37^0C. The lowest concentration at which no bacterial colony was noticed in the plates was considered MBC of the culture.

5.2.6: Anticancer potential of endophytic fungi

Crude extracts of 14 endophytic isolates *viz*., MEn01, MEn04, MEn06, MEn10, MEn24, MEn27, MEn30, MEn36, MEn37, MEn85, MEn87, MEn89, MEn67, and MEn102 were screened for their anticancer potential using a colorimetric assay. Human Lung cancer cell line (A549) and mouse fibroblast cells (L929) were obtained from National Centre for Cell Sciences, Pune-India. A viable cell suspension of 50 µL was seeded in a 96-well flat bottom microplate. The final volume was up to 150 µL by adding DMEM (Dulbecco's Modified Eagle Medium). The endophytic extract was diluted in DMEM to obtain different concentrations, followed by incubation for 48h at 37^0C in 95% humidity and 5% CO_2 incubator. After the incubation period, 20 µL of MTT reagent 3- (4,5- dimethylthiazol-2-yl)-2,5-diphenyltetra-zolium bromide, 5 mg mL^{-1} in Phosphate buffer solution (PBS) was added to each well. After 4h of dark incubation, the supernatant was removed without disturbing the precipitated formazan crystals. Later the formazan crystals were dissolved in 100 µL of DMSO (di-methyl sulfoxide), and optical density (OD) was measured at 492nm. Different concentrations, *viz*., 1000, 500, 250, 125, 62.5, and 31.25 µg mL^{-1} of the samples, were used for the study following an incubation period of 24h. The cells in the well were washed twice with PBS, followed by 20 µL of the MTT staining solution (5 mg ml^{-1} in phosphate buffer solution), and were incubated at 37^0C. After 4h, 100µL of DMSO was added to each well to dissolve the formazan crystals, and absorbance was recorded at 570nm using a microplate reader.

The activity of the extract against the A549 and L929 cell was analyzed by calculating cell viability and cell inhibition percentage. Further, the IC_{50} value was calculated to analyze the extract concentration required to inhibit cancer cell growth by 50%. The analysis was calculated using Prism 8.0 software from the dose-response curve (Nagarajan and Pandian, 2018).

5.3: RESULTS

5.3.1: Extraction of bioactive metabolites

In the present study, the different endophytes recorded variations in the yield of secondary metabolites. Fermented potato dextrose broth yielded approximately 1 - 3 mg of the dried compound after 6 - 8 weeks of culturing. The results of the qualitative phytochemical analysis confirmed the presence of alkaloids, flavonoids, phenols, and steroids (**Table 5.5**).

Table 5.5: Phyto-chemical screening of fungal extracts.

Isolation code	Endophytic fungi	Alk.	Fla.	Phe.	Terp.	Ste.	Sap.
MEn04	*Drechslera* sp.	+	+	+	-	+	-
MEn06	*Pestalotiopsis* sp.	+	+	+	-	+	-
MEn10	*Aspergillus* sp.	+	+	+	-	+	-
MEn24	*Curvularia* sp.	+	+	+	-	+	-
MEn27	*Aspergillus niger*	+	+	+	-	+	-
MEn30	*Junctospora pulchra*	+	+	+	-	+	-
MEn36	*Nigrospora* sp.	+	+	+	-	+	-
MEn37	*Cladosporium* sp.	+	+	+	-	+	-

Legend: Alk. = Alkaloid; Fla = Flavonoids; Phe = Phenols, Terp = Terpenoid; Ste = Steroids, and Sap = Saponins; + = present; - = absent.

5.3.2: Separation of bioactive compounds from fungal endophytes using TLC

In the present study, two solvent systems, *viz*., acetone: chloroform and chloroform: methanol, were used, of which the latter system with chloroform: methanol in the ratio 95:5 resulted in a clear separation of compounds (**Table 5.6**). Some endophytic extracts showed well-separated spots on TLC, while others, *viz*., MEn01, MEn87, MEn102, MEn89, MEn67, and MEn85, did not show clear separation (**Table 5.7** and **Plate 5.12**).

Table 5.6: Solvent system used for separation of bioactive compounds.

Sr. No.	Solvent system	Ratio	Inference
1.	Acetone:Chloroform	50:50	No separation
2.	Chloroform:Methanol	95:5	3-4 spots

5.3.3: Characterization of secondary metabolites using GCMS analysis

The crude extracts were subjected to GC-MS analysis to characterize bioactive compounds (**Table 5.8**). The crude ethyl acetate extract of endophytic fungus *S. monoceras* (MEn01) revealed the presence of various bioactive secondary metabolites *viz*., sulfurous acid, 2-ethylhexyl isohexyl ester, heptacosane, dodecyl pentyl ester, eicosane 2- methyl, and 2-bromotetradecane (**Fig. 5.17**).

Table 5.7: Separation of bioactive compounds using TLC.

Fungal isolate	Spot	Solvent front (cm)	Solute (cm)	Rf value
MEn04 (*Drechslera* sp.)	1	7.2	0.8	0.11
	2		1.8	0.25
	3		5.7	0.80
	4		5.9	0.81
MEn06 (*Pestalotiopsis* sp.)	1	6.5	0.6	0.09
	2		0.9	0.14
	3		1.6	0.25
	4		1.9	0.29
MEn10 (*Aspergillus* sp.)	1	7.5	0.8	0.11
	2		1.2	0.16
	3		1.8	0.24
	4		3.2	0.437
	5		5.1	0.68
MEn24 (*Curvularia* sp.)	1	5.9	0.6	0.10
	2		0.9	0.15
	3		2.1	0.35
	4		3.1	0.52
MEn27 (*Aspergillus niger*)	1	7.5	0.9	0.12
	2		1.2	0.16
	3		3.1	0.41
	4		4.1	0.54
	5		5.2	0.69
MEn30 (*Junctospora pulchra)*	1	6.8	0.7	0.10
	2		0.9	0.13
	3		1.5	0.22
	4		3.2	0.47
MEn36 (*Nigrospora* sp.)	1	7.6	0.5	0.06
	2		1.3	0.17
	3		2.9	0.38
	4		4.9	0.64
MEn37 (*Cladosporium* sp.)	1	7.5	0.6	0.08
	2		1.6	0.21
	3		3.5	0.46
	4		4.2	0.56

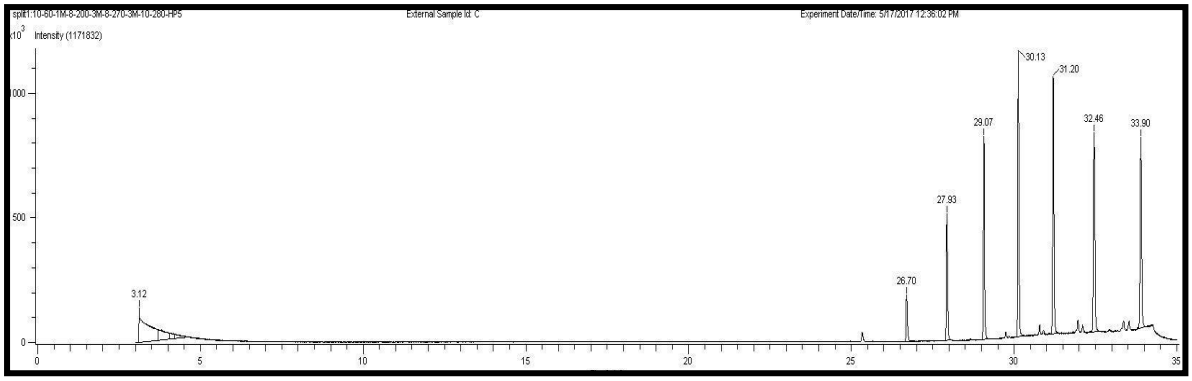

Fig. 5.17: GC-MS Chromatogram of volatile and aliphatic compounds isolated from endophytic fungi *S. monoceras* (MEn01).

The GC-MS revealed the presence of Sulfurous acid, 2-ethylhexyl isohexyl ester (at 24.44), 1-lodo-2-methylundecane (at RT 26.57), Heptacosane (at RT 27.63, 28.70 and 35.5), 1-lodo-2-methylnonane (at RT 29.96, 31.46, 33.30, 35.34, 35.49 and 35.48) in MEn87 isolate (*A. alternata*) (**Fig. 5.18**).

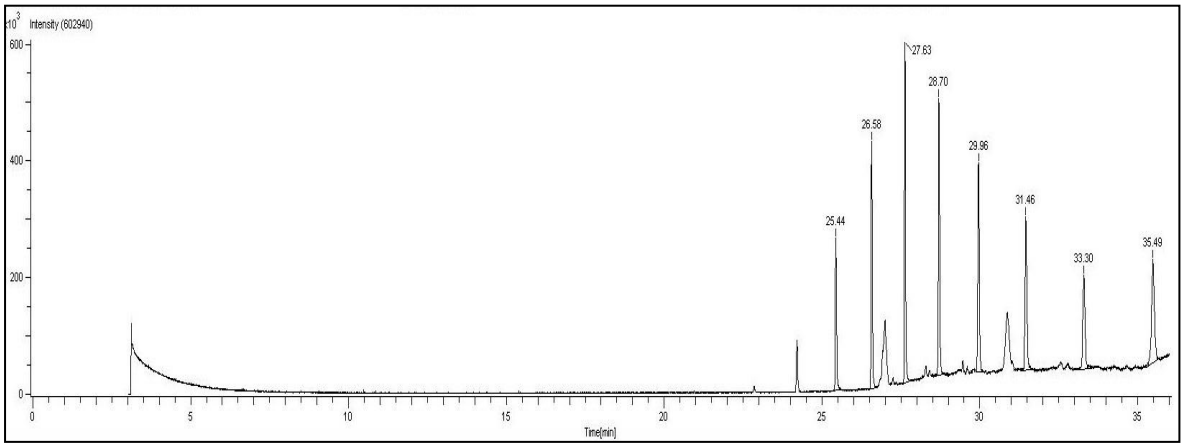

Fig. 5.18: GC-MS Chromatogram of volatile and aliphatic compounds isolated from endophytic fungi *A. alternata* (MEn87).

Metabolites isolated from endophytic isolate MEn102 (*Pestalotiopsis*) revealed the presence of Hexadecane (RT 8.73), n-Hexadecanoic acid (14.74), Octadecanoic acid (18.73), Myristic anhydride (23.15), 9- Octadecanoic acid (Z), and 2-hydroxy-3-[(1-oxohexadecyl) oxy] propyl ester (**Fig. 5.19).**

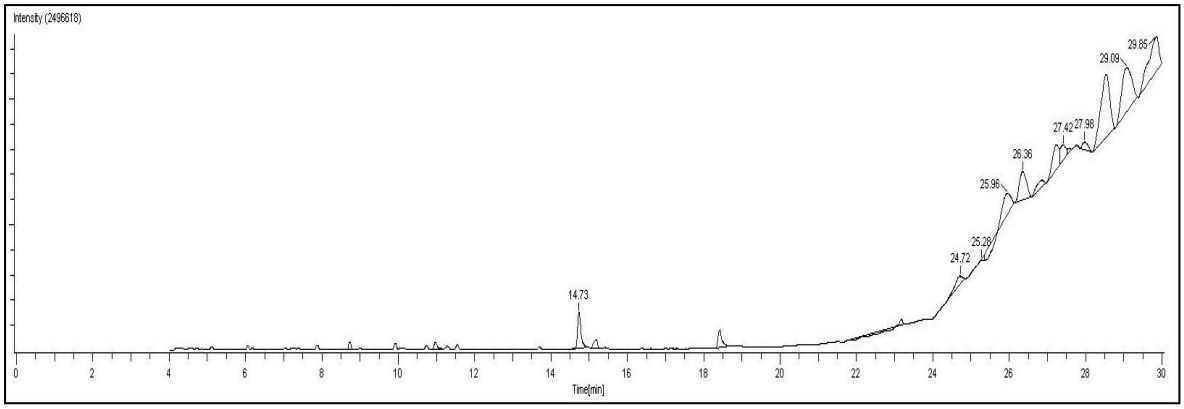

Fig. 5.19: GC-MS Chromatogram of volatile and aliphatic compounds isolated from endophytic fungi *Pestalotiopsis* sp. (MEn102).

Chromatogram of endophytic extract belonging to MEn89 revealed the presence of Octane,3-ethyl-2,7,dimethyl- ($Rt_{(min)}$7.88), Octatriacontyl trifluoroacetate (8.74), Hexadecane (10.75, 13.71), Dodecane,2,6,11-trimethyl (10.98), Tetradecanoic acid (11.56), 7,9-Di-tert-butyl-1-Ocaspiro (4,5) deca-6,9-diene-2,8-dione (14.53), n-hexadecanoic acid (14.74), Octadecanoic acid (18.43), 2-Oxepanol,5-(1,1-dimethylethyl)- (24.24), 12-Hydroxydodecanoic acid (27.26), and Oleic acid (27.79) **(Fig. 5.20).**

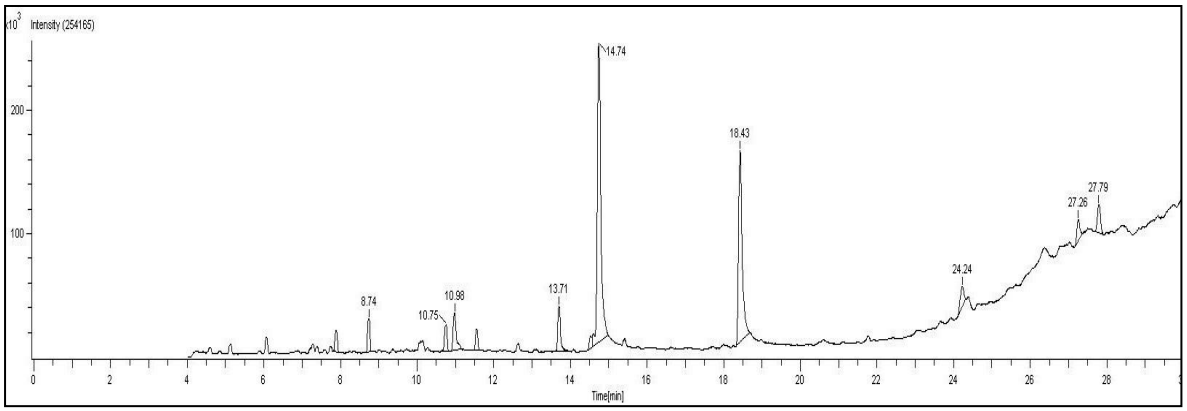

Fig. 5.20: GC-MS Chromatogram of volatile and aliphatic compounds isolated from endophytic fungi *Cladosporium* sp. (MEn89).

Isolate MEn67 (*Curvularia* species) indicated the presence of Tetradecane (6.05), Dodecane, 2,6,11-trimethyl (7.86), Hexadecane (8.73), Tetradecanoic acid (10.98), Hexadecane (11.55), n-Hexadecanoic acid (14.73), Octadecanoic acid (18.42), 10-undecanoic acid, and octyl ester (at 26.75, 26.76 and 18.41) (**Fig. 5.21**).

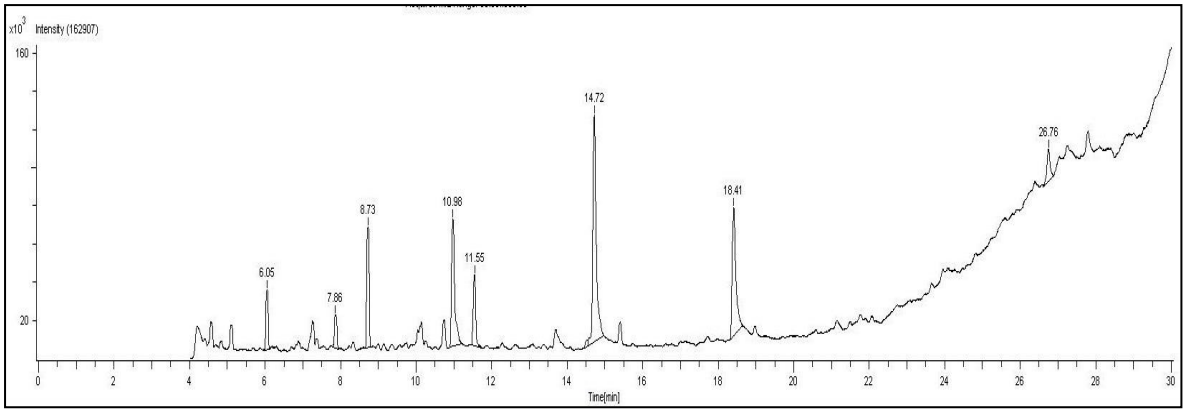

Fig. 5.21: GC-MS Chromatogram of volatile and aliphatic compounds isolated from endophytic fungi *Curvularia* sp. (MEn67).

Endophytic fungi belonging to *Nigrospora* sp. (MEn85) revealed the presence of B-D-Glucopyranose,1,6-anhydro- (11.34), Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (26.54, 26.94), Hexadecanoic acid,1-(hydroxymethyl)-1,2-ethanediyl ester (27.48), and Oleic acid (at 32.25) **(Fig. 5.22)**.

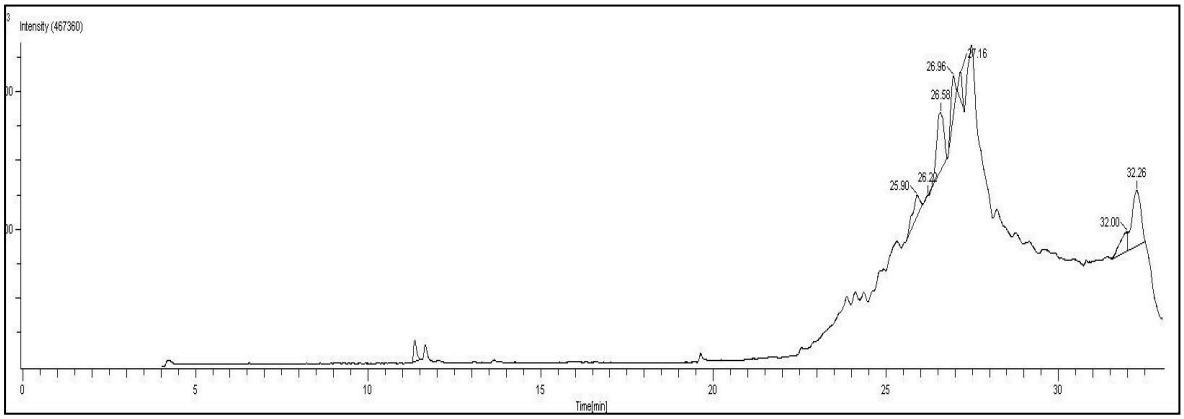

Fig. 5.22: GC-MS Chromatogram of volatile and aliphatic compounds isolated from endophytic fungi *Nigrospora* sp. (MEn85).

5.3.4: Antimicrobial activity of fungal endophytes on human pathogens

Of the 14 fungal crude extracts prepared in ethyl acetate, the extract of *S. monoceras* exhibited antibacterial activity. It inhibited all the bacterial pathogens tested in the study showing relatively large zones of inhibition. It demonstrated efficacy at low concentrations (10 µL) against the target pathogens. However, it did not exhibit inhibitory activity against *Aspergillus brasiliensis* (mold) and *Candida albicans* (yeast), indicating the absence of antifungal activity. DMSO (Dimethyl sulphoxide), used as a negative control, did not show a zone of inhibition (**Table 5.9, Table 5.10,** and **Plate 5.13**).

Table 5.8: Bioactive compounds identified from mangrove endophytic fungi by using GCMS analysis.

Sr. No.	R.T. (min)	Peak area%	Compound/s	Molecular Formula	Molecular weight	Fungal Endophyte	Isolate code
1.	26.70	3.01	Sulfurous acid, 2-ethylhexyl isohexyl ester	$C_{14}H_{30}O_3S$	278	*S.monoceras*	MEn01
2	27.93	7.35	Heptacosane	$C_{27}H_{56}$	380		
3	29.07	11.28	Heptacosane	$C_{27}H_{56}$	380		
4	30.13	13.11	Sulfurous acid, Dodecyl pentyl ester	$C_{17}H_{36}O_3S$	320		
5	33.12	15.23	Eicosane, 2-methyl	$C_{21}H_{44}$	296		
6	32.46	14.78	2-Bromotetradecane	$C_{14}H_{29}Br$	276		
1	24.44	7.30	Sulfurous acid, 2-ethylhexyl isohexyl ester	$C_{14}H_{30}O_3S$	278	*A.alternata*	MEn87
2	26.57	11.31	1-lodo-2-methylundecane	$C_{12}H_{25l}$	296		
3	27.63	14.72	Heptacosane	$C_{27}H_{56}$	380		
4	28.70	13.88	Heptacosane	$C_{27}H_{56}$	380		
5	29.96	12.13	1-lodo-2-methylnonane	$C_{12}H_{25l}$	268		
6	31.46	10.75	1-lodo-2-methylundecane	$C_{12}H_{25l}$	296		
7	33.30	10.75	1-ludo-2-methylnonane	$C_{10}H_{21l}$	268		
8	35.34	8.06	1-ludo-2-methylnonane	$C_{10}H_{21l}$	268		
9	35.49	0.11	1-ludo-2-methylnonane	$C_{10}H_{21l}$	268		
10	8.73	1.23	Hexadecane	$C_{16}H_{34}$	226	*Pestalotiopsis* sp.	MEn102
11	14.74	8.85	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256		
12	18.73	4.83	Octadecanoic acid	$C_{18}H_{36}O_2$	284		
13	23.15	4.51	Myristic anhydride	$C_{28}H_{54}O_3$	438		
14	25.83	7.76	9-Octadecanoic acid (Z), 2-hydroxy-3-[(1-oxohexadecyl)oxy] propyl ester	$C_{37}H_{70}O_5$	594		
15	26.26	14.17	9-Octadecanoic acid (Z), 2-hydroxy-3-[(1-oxohexadecyl)oxy] propyl ester	$C_{37}H_{70}O_5$	594		
16	29.1	31.47	9-Octadecanoic acid (Z), 2-hydroxy-3-[(1-oxohexadecyl)oxy] propyl ester	$C_{37}H_{70}O_5$	594		
17	29.67	27.15	9-Octadecanoic acid (Z), 2-hydroxy-3-[(1-oxohexadecyl)oxy] propyl ester	$C_{37}H_{70}O_5$	594		
18	7.88	22.17	Octane,3-ethyl-2,7,dimethyl-	$C_{12}H_{26}$	170	*Cladosporium* sp.	MEn89
19	8.74	2.27	Octatriacontyl trifluoroacetate	$C_{40}H_{77}F_{3O2}$	646		
20	10.75	21.77	Hexadecane	$C_{16}H_{34}$	226		
21	10.98	20.53	Dodecane,2,6,11-trimethyl	$C_{15}H_{32}$	212		
22	11.56	22.69	Tetradecanoic acid	$C_{14}H_{28}O_2$	228		

23	13.71	21.32	Hexadecane	$C_{16}H_{34}$	226		
24	14.53	22.17	7,9-Di-tert-butyl-1-Ocaspiro(4,5) deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276		
25	14.74	18.59	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256		
26	18.43	17.62	Octadecanoic acid	$C_{18}H_{36}O_2$	284		
27	24.24	15.28	2-Oxepanol,5-(1,1-dimethylethyl)-	$C_{10}H_{22}O_2$	172		
28	27.26	17.56	12-Hydroxydodecanoic acid	$C_{12}H_{24}O_3$	216		
29	27.79	19.46	Oleic acid	$C_{18}H_{34}O_2$	282		
30	6.05	17.35	Tetradecane	$C_{14}H_{30}$	198	*Curvularia* sp.	MEn67
31	7.86	30.29	Dodecane,2,6,11-trimethyl	$C_{15}H_{32}$	212		
32	8.73	8.55	Hexadecane	$C_{16}H_{32}$	226		
33	10.98	35.69	Tetradecanoic acid	$C_{14}H_{28}O_2$	228		
34	11.55	64.39	Hexadecane	$C_{16}H_{34}$	226		
35	14.73	24.51	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256		
36	18.42	74.38	Octadecanoic acid	$C_{18}H_{36}O_2$	284		
37	26.75	20.15	10-undecanoic acid, octyl ester	$C_{19}H_{36}O_2$	296		
38	18.41	35.60	10-undecanoic acid, octyl ester	$C_{19}H_{36}O_2$	296		
39	26.76	142.47	10-undecanoic acid, octyl ester	$C_{19}H_{36}O_2$	296		
40	11.34	1.18	B-D-Glucopyranose,1,6-anhydro-	$C_6H_{10}O_5$	162	*Nigrospora* sp.	MEn85
41	26.54	7.30	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	568		
42	26.94	2.80	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	568		
43	27.48	1.66	Hexadecanoic acid,1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	568		
44	32.25	87.04	Oleic acid	$C_{18}H_{34}O_2$	282		

Legend: MEn=Mangrove Endophyte.

5.3.5: Minimum inhibitory concentration (MIC)

The MIC results revealed a decrease in the growth of pathogenic bacterial strains, ranging from 3.1 - 6.25 µg $mL^{-1,}$ as observed by the decreasing absorbance values at 6.25 µg mL^{-1} (**Plate 5.14**). Further MBC study, the aliquots from the MIC tubes were sub-cultured on the Muller Hinton agar medium, and the plates with aliquots with concentrations in the range from 3.12 to 6.25 µg mL^{-1} did not show any bacterial colony, indicating its strong bactericidal property (MBC) (**Plate 5.15**).

Table 5.9: Antibacterial activity of fungal endophytes.

Isolation code	Endophytic fungi	Zone of inhibition (cm)				
		K. pneumonia ATCC 700603	*S. aureus* ATCC 6538	*S. boydii* ATCC 12030	*S. enterica* ATCC 14028	*E. coli* ATCC 8739
MEn04	*Drechslera* sp.	0.5±0.01	0.9±0.02	0.7±0.01	0.4±0.02	0.2±0.02
MEn06	*Pestalotiopsis* sp.	0.3±0.02	0.5±0.03	0.4±0.02	0.8±0.01	0.2±0.01
MEn10	*Aspergillus* sp.	0.5±0.1	0.4±0.02	0.6±0.01	0.8±0.01	0.5±0.01
MEn24	*Curvularia* sp.	0.1±0.03	0.6±0.01	0.5±0.01	0.4±0.03	0.4±0.03
MEn27	*Aspergillus niger*	0.4±0.02	0.5±0.01	0.8±0.02	0.4±0.01	0.6±0.03
MEn30	*Junctospora pulchra*	0.2±0.02	0.0±0.00	0.0±0.00	0.2±0.02	0.1±0.01
MEn36	*Nigrospora* sp.	0.4±0.01	0.3±0.01	0.4±0.02	0.6±0.03	0.5±0.01
MEn37	*Cladosporium* sp.	0.5±0.03	0.4±0.02	0.6±0.02	0.4±0.02	0.6±0.02
Control (–ve)	DMSO	-	-	-	-	-
Control (+ve)	Streptomycin	1.2±0.01	1.0±0.01	1.2±0.01	1.6±0.01	1.5±0.01

Legend: All values are the means of three reading. + =Positive activity; - = No activity; MEn=Mangrove Endophyte 01; K = *Klebsiella*; S= *Staphylococcus*; S= *Shigella*; S= *Salmonella*; E= *Escherichia.*

5.3.6: Anti-cancer activity

The IC_{50} values of the extracts ranged from 2.4 to 3.4 μg mL^{-1}. Results of the anticancer study revealed maximum inhibitory activity in MEn89 (*Cladosporium* sp.) at 1000 μg mL^{-1} concentration with an IC_{50} value of 2.672 μg mL^{-1}, while MEn87 (*Alternaria alternata*) had an IC_{50} value of 3.012 μg mL^{-1} (**Table 5.11; Plate 5.16**). However, the results of the biocompatibility assay using Mouse Fibroblast cell line L929 revealed a nontoxic effect as the cells exhibited normal proliferation and growth.

Although MEn67 showed promising anticancer activity against the A549 cell line. However, it recorded the least number of viable cells when tested for bio-compatibility assay against the L929 cell line. Among the other isolates, MEn102 showed moderate inhibition against the A549 cell line and showed the maximum viability percentage in the L929 cell line. Two isolates, *viz*., MEn87 and MEn85, inhibited the growth of the A549 cell line and had no lethal effect on L929 (**Table 5.12**).

Table 5.10: Antibacterial activity of *S. monoceras*.

Isolate code	Concentration of extract	Zone of inhibition (cm)				
		K. pneumonia ATCC 700603	*S. aureus* ATCC 6538	*S. boydii* ATCC 12030	*S. enterica* ATCC 14028	*E. coli* ATCC 8739
MEn01	100µl	4.35±0.02	4.12±0.05	5.05±0.02	4.52±0.02	4.31±0.01
	90µl	4.25±0.05	4.22±0.02	4.75±0.00	4.11±0.01	4.32±0.03
	80µl	3.96±0.01	4.25±0.01	4.55±0.05	4.02±0.0	3.42±0.00
	70µl	3.70±0.01	4.18±1.05	4.35±0.00	3.50±0.02	3.21±0.06
	60µl	3.60±0.06	3.55±1.02	4.20±0.09	3.41±0.01	3.21±0.02
	40µl	3.55±0.06	3.30±0.05	4.15±0.05	3.12±0.02	3.01±0.025
	30µl	3.55±.036	3.45±0.00	4.05±0.00	3.00±0.01	3.03±0.06
	20µl	3.40±0.06	3.10±0.02	4.05±0.00	2.90±0.03	3.03±0.05
	10µl	3.06±.041	3.00±0.026	3.95±0.02	2.10±0.01	2.92±0.05
Control (–ve)	DMSO	-	-	-	-	-
Control (+ve)	Streptomycin	1.2±0.01	1.0±0.01	1.2±0.01	1.6±0.01	1.5±0.01

Legend: All values are the mean of three reading. + =Positive activity; - = No activity; MEn01: Mangrove Endophyte 01; K = *Klebsiella*; S= *Staphylococcus*; S= *Shigella*; S= *Salmonella*; E= *Escherichia.*

5.4: DISCUSSION

Endophytic fungi are versatile organisms that synthesize active secondary metabolites to acclimatize to the host plant and, in the process, yield bioactive components having pharmaceutical applications. Of the total medicinal products available in the market, 30% are of fungal origin (Mishra *et al*., 2016). Hence, understanding the relationship between host and endophyte will help ideal drug production by manipulating the standard growth parameters.

In the present study, ethyl acetate was used as a solvent in the extract preparation as it is known to enhance the efficacy of the extraction. Besides, it evaporates easily and is nontoxic (Asyura *et al.,* 2017).

Endophytes produce an array of metabolites of varied structural groups such as terpenoids, steroids, xanthones, chinones, phenols, isocoumarins, benzopyranones, tetralones, cytochalasines and enniatines (Schulz *et al.,* 2002). In the present study, the presence of alkaloids, phenols, and terpenoids was confirmed from the selected endophytes. These fungal symbionts are a prolific source of novel antibiotics, anticancer, antiviral, antioxidant, insecticide, anti-diabetic and immunosuppressant compounds (Mayank *et al*., 2015).

Table 5.11: Cytotoxic activity of the endophytic extract against lung cancer cell line A549.

Isolate code	Concentration (µg/mL)	% cell viability	% cell inhibition	IC_{50} value (µg/mL)
MEn87	Control	100	0	
	1000	40.78	59.22	3.021
	500	48.46	51.54	
	250	60.01	39.99	
	125	63.16	36.84	
	62.5	74.80	25.20	
	31.25	96.33	3.67	
MEn102	Control	100	0	
	1000	53.74	46.26	2.430
	500	55.05	44.95	
	250	63.19	36.81	
	125	75.13	24.87	
	62.5	89.86	10.14	
	31.25	90.35	9.65	
MEn89	Control	100	0	
	1000	40.29	59.71	2.672
	500	45.70	54.30	
	250	53.22	46.78	
	125	54.46	45.54	
	62.5	59.74	40.26	
	31.25	67.65	32.35	
MEn67	Control	100	0	
	1000	44.23	55.77	3.428
	500	49.77	50.23	
	250	54.43	45.57	
	125	56.76	43.24	
	62.5	65.22	34.78	
	31.25	68.57	31.43	
MEn85	Control	100	0	
	1000	64.14	35.86	2.719
	500	68.14	31.96	
	250	71.33	28.67	
	125	73.43	26.57	
	62.5	80.02	19.98	
	31.25	84.28	15.72	

Legend: MEn = Mangrove Endophyte; All readings are mean of three readings; SD = Standard deviation.

Table 5.12: Biocompatibility assay of the endophytic extract against Normal Mouse Fibroblast cell line L929.

Isolate code	Concentration (µg/mL)	% cell viability	% cell inhibition
MEn87	1000	70.4	29.6
	500	83.6	16.4
	250	90.3	9.7
	125	91.3	8.7
	62.5	95.9	4.1
MEn102	1000	86.9	13.1
	500	89.9	10.2
	250	94.6	5.4
	125	93.1	6.9
	62.5	100	0
MEn89	1000	75.8	24.2
	500	83.3	16.7
	250	79.7	20.3
	125	95.8	4.2
	62.5	100	0
MEn67	1000	70.7	29.3
	500	78.5	21.5
	250	78.3	21.7
	125	85.7	14.3
	62.5	96.9	3.1
MEn85	1000	84.2	15.8
	500	86.6	11.4
	250	89.3	10.7
	125	94.6	5.4
	62.5	96.3	3.7

Legend: MEn = Mangrove Endophyte; All readings are mean of three values; SD=Standard deviation.

The pharmacological repertoire of endophytic fungi comprises diverse bioactive compounds such as antimycotic steroid 22-triene-3b-ol, anticancer cajanol, podophyllotoxin and kaempferol, anti-inflammatory ergo flavin, antioxidant lectin, insecticidal heptelidic acid, immune-suppressive sydoxanthone A, B, and cytotoxic radicicol (Sharma *et al*., 2016). In the present study, the fungal endophyte *S. monoceras* revealed the presence of strong anticancer activity against the human cancer cell line A-549 at different concentrations.

GC-MS analysis of *S. monoceras* revealed the presence of different biological compounds. Isolate MEn87 showed the presence of Sulfurous acid, 2-ethylhexyl isohexyl ester, 1-lodo-2-

methylundecane, Heptacosane, 1-lodo-2-methylnonane and Hexadecane. Sulfurous acid, 2-ethylhexyl isohexyl ester is known to have antioxidant activity (Arulkumar *et al.,* 2018), while heptacosane has antitumor activity (Poma *et al.,* 2018). Literature indicates that 1-lodo-2-methylnonane has antioxidant activity (Lalitha *et al.,* 2015). The metabolite Hexadecane is reported to have anticancer properties (Marrez *et al.,* 2019). Therefore, it is suggested that cell inhibition property in MEn87 extract is due to the presence of such potent metabolites.

GC-MS analysis of the isolate MEn102 confirmed the presence of four active compounds *viz.,* n-Hexadecanoic acid, Octadecanoic acid, Myristic anhydride, 9-Octadecanoic acid (Z), 2-hydroxy-3-[(1-oxohexadecyl)oxy], propyl ester, and Octane,3-ethyl-2,7,dimethyl-. The compound n-Hexadecanoic acid is known to have anti-inflammatory and antioxidant properties (Vasudevan, 2012). Octadecanoic acid is reported to have apoptosis-inducing properties (Yoo *et al.,* 2007). Al Bratty *et al.,* (2020) reported the 9-Octadecanoic acid (Z) and 2-hydroxy-3-[(1-oxohexadecyl) oxy] propyl ester as emulsifying agents. However, the activity of the compounds Myristic anhydride and Octane, 3-ethyl-2,7, dimethyl- is not known.

Several compounds having beneficial role in the host were identified from the isolate MEn89. The compound Octatriacontyl trifluoroacetate has insecticidal properties (Kumar *et al.,* 2010). Tetradecanoic acid has larvicidal and insect-repellent properties (Mustafa *et al*., 2004). 12-Hydroxydodecanoic acid and tetradecane are reported to have a potent role in anticancer activity (Arulkumar *et al.,* 2018). Dodecane, 2,6,11-trimethyl, and Octadecanoic acid 2-Oxepanol,5-(1,1-dimethylethyl)- is reported to have antimicrobial and antibacterial properties (Begum *et al.,* 2016). 7,9-Di-tert-butyl-1-Ocaspiro(4,5) deca-6,9-diene-2,8-dione has antiseptic properties (Chandrasekar *et al.,* 2015), n-hexadecanoic acid and oleic acid have promising antioxidant properties (Chandrasekar *et al.,* 2015; Elagbar *et al.,* 2016) while the role of Hexadecane is not known.

GC-MS chromatogram of MEn67 indicated the presence of eight dominant peaks. The role of some of the metabolites identified, *viz*., Dodecane,2,6,11-trimethyl, Hexadecane, 10-undecanoic acid, octyl ester, and tetradecanoic acid is unknown. The compound n-Hexadecanoic acid is known to have anti-inflammatory and antioxidant properties (Vasudevan *et al.,* 2012; Kumar *et al.,* 2010). Octadecanoic acid is known to have antimicrobial activity (Rahuman *et al.,* 2000), while β-D-Glucopyranose,1,6-anhydro- is reported to have antibacterial properties (Kamal *et al.,* 2015).

The Isolate MEn85 recorded the prominent peaks indicating the presence of Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester, and Oleic acid. These compounds have antioxidant, nematicidal, insecticidal, lubricant, antiandrogenic, hemolytic, hypo – cholesterolemic properties (Arora and Meena, 2017).

In MEn01, different bioactive secondary metabolites such as sulfurous acid, 2-ethylhexyl isohexyl ester, heptacosane, dodecyl pentyl ester, eicosane 2-methyl, and 2-bromotetradecane are reported. These secondary metabolites have antibacterial, antifungal, and anti-diabetic properties (Sharma *et al*., 2016; Devi and Singh, 2013).

5.5: CONCLUSION

From the present study, it can be concluded that fungal endophytes from mangroves synthesize various secondary metabolites having various applications in the pharmacological industry. The extraction of such novel compounds from stressed habitats such as mangroves will be beneficial for humans. Besides their presence in mangrove plant species will assist them to overcome the stressful environment, making them grow, survive and perpetuate under such conditions.

Chapter 6: SUMMARY

Mangroves are important plant species growing in halophytic conditions. These species harbour different symbionts including endophytes. Such symbionts immensely contribute to the production of secondary metabolites, which are beneficial to both the living partners. Additionally, there are various other applications of such associations such as increased P uptake, nutrient recycling, and providing defense against phytopathogens. Thus, understanding the diversity of mangroves can provide insights into various applications of the symbionts.

Chorao Island, situated along the Mandovi river was undertaken for the study. Mangrove diversity at the entire Island was carried out using the Bitterlich variable plot method (1948) by laying nineteen quadrates (20 x 20 m). Subsequently, the geographical coordination of these quadrates was noted. The various plant parameters, *viz*., basal area and diameter at breast height, tree height (using hypsometer, tapes, and ropes), and canopy diameter (using measuring tapes and still photographs) were measured. Soil analysis was carried out for the dominant plant species. Also, indigenous knowledge of different herbal preparations used in folklore medicines was documented.

In the present study, DSE (Dark septate endophytes) colonization was studied in 14 mangrove plant species *viz*., *Avicennia marina, Av. officinalis, Rhizophora mucronata, R. apiculata, Excocaria agallocha, Brugueira cylindrica, Ceriops tagal, Aegiceras corniculatum, Sonneratia alba, Acanthus illicifolius, Derris heterophylla, Clerodendron inermis, Acrosticum aurum* and *Kandelia candel.* With the exception of *Kandelia candel,* fungal endophytes from the remaining 13 mangrove plant species were isolated from leaves, stems, and roots. The endophytic isolates were later identified based on morphology and molecular sequencing.

Besides, distribution of the endophytic fungi in mangrove leaves from five mangrove species *viz., B. cylindrica, Av. marina, C. tagal, E. agallocha,* and *S. alba* was attempted for four different growth stages *viz.,* Stage 1: tender leaf (3^{rd} week), Stage 2: mature leaf (5^{th} week), Stage 3: senescence leaf (7^{th} week), and Stage 4: litter or the dead leaf (9^{th} week).

An attempt was also made to explore the role of 14 endophytes *viz*., MEn04, MEn06, MEn09, MEn10, MEn16, MEn24, MEn27, MEn30, MEn34, MEn36, MEn37, MEn38, MEn39, and MEn42 in leaf litter degradation by using *in vivo* and *in vitro* methods. Chemical analysis of the degraded leaf litter was done by using CHNS analysis. Enzymatic activities *viz*., amylase, protease, cellulase, and lipase of the above 14 fungal isolates were studied.

All the 24 fungal isolates were screened for their P solubilizing potential. Also, as the study area is under influx of petroleum hydrocarbons, an attempt was made to understand the potential of seven fungal endophytes *viz*., MEn06, MEn09, MEn10, MEn24, MEn27, MEn36, and MEn37 in biodegradation. Further, eight fungal endophytes *viz*., MEn10 (T_1), MEn04 (T_2), MEn06 (T_3), MEn24 (T_4), MEn27 (T_5), MEn30 (T_6), MEn36 (T_7), and MEn37 (T_8) (selected on the basis of dominance) were screened for their effect on plant growth in *A. marina.* An attempt was also made to isolate and identify phytopathogen infecting *A. marina* plant species. The antagonistic activity of the above eight isolates were tested against the isolated phytopathogen from *A. marina* .

As fungal endophytes are known to be reservoirs of secondary metabolites, an attempt was made to extract and characterize the bioactive metabolites in 14 selected fungal endophytes *viz*., MEn01, MEn04, MEn06, MEn10, MEn24, MEn27, MEn30, MEn36, MEn37, MEn85, MEn87, MEn89, MEn67, and MEn102. Based on the potent metabolites present, studies were initiated to explore theie role in antimicrobial and anticancer activities.

Eight dominant mangrove species belonging to five families *viz.*, Aviciniaceae, Rhizophoraceae, Sonneratiaceae, Acanthaceae, and Euphorbiaceae were recorded at the study site. Besides, six mangrove plants belonging to six different genera were found to occur on the Island which were outside the quadrates. In all, 1506 mangrove plants were recorded in the 19 quadrates. Structural attributes indicated that *Excoecaria agallocha* had the highest basal area (3.11 cm), with least in *Bruguiera cylindrica* (0.03 cm). Plant height in the quadrates ranged from 4.73 to 10.33 meters. The highest plant height was noted in *Avicennia officinalis* (10.33 m) and the lowest in *Bruguiera cylindrica* (3.12 m). Plant canopy diameter ranged from 3.3 to 18.13 cm. The maximum canopy diameter was recorded in *Avicennia officinalis* and minimum in *B. cylindrica.* Species dominance revealed that *A. illicifolius* (associate mangrove) was the most frequently identified species growing luxuriantly on the Island. Among true mangroves, *Av. marina* and *B. cylindrica* were dominant, whereas *E. agalocha* and *R. apiculata* were scarce in distribution. The density (ha^{-1}) of mangrove species varied between 4 (*R. apiculata*) and 871 (*A. ilicifolius*). Quadratwise diversity indices *viz.*, Shannon-Weiner (H'), Simpson diversity index (D), and Simpson evenness index (1-D) were used to determine the diversity index at Chorao Island. The study revealed that, among the 19 quadrates, quadrate no.10 recorded the lowest H' index while quadrate no. 15 indicated the highest H' index. In Dominance (D), quadrate no. 10 showed the lowest D value, whereas quadrate no. 10 recorded the highest dominance value. For Simpson evenness index, quadrate no. 3 was seen evenly placed in terms

of species dominance compared to quadrate no. 10. Physico-chemical analysis of mangrove soil revealed a pH range from highly acidic (3.8) to neutral (7). Salinity levels for different mangrove species ranged from 14 to 31 ppt. Organic carbon (OC) ranged between 1.4 to 2.6 %, P content ranged from 8.1 to 70 kg ha^{-1}, while available potassium (K) in soil ranged from 1200 to 3698 kg ha^{-1}. The local indigenous medicinal uses of nine mangrove plants belonging to eight different families have been documented.

The present study revealed the presence of DSE colonization in all 14 mangrove plant species. Melanized septate hyphal colonization was noticed in mangrove plant roots. *Brugueira cylindrica, C. tagal, Av. marina, Av. officinalis,* and *A. illicifolius* showed hyphae with densely coiled structures forming microsclerotia. Colonization ranging from 70 to 100% was recorded in the different species.

Isolation of endophytic fungi in different plant parts revealed that during monsoon, a higher number of fungal isolates were recovered from *R. apiculata,* whereas a higher number of fungal isolates during winter and summer were recovered from *Av. marina*. The diversity index indicated a significant difference in the number of colonies in different plant parts. Shannon diversity index recorded a diversity index between 2.42 to 2.43. Moderate diversity of the endophytic community was recorded in all the seasons at the study site. Similarly, Simpson's diversity index revealed moderate diversity of 0.8 to 0.9 at the study site.

The seasonal distribution of endophytes revealed a maximum number of fungal endophytes in the leaves. During summer, the maximum number of isolates was recorded in leaves, followed by stem and root. While during winter, maximum number of endophytes were recorded in leaves, followed by root and stem. Among the season's highest relative abundance and isolation, frequency was noticed during monsoon. *Aspergillus* sp. was the most frequently recovered isolate. Besides this, *Fusarium* sp., *Dreshslera* sp., and *Cladosporium* sp. were the few other species recovered during the study period. During monsoon, *Ae. corniculatum* and *Av. marina* represented the highest similarity coefficient.

Distribution of the endophytic fungi in mangrove leaves revealed the presence of 35 fungal colony-forming units (CFU) belonging to seven genera isolated from different leaf growth stages. *Ceriops tagal* and *Av. marina* hosted the highest number of colony-forming units. There was a progressive change in the number of fungal isolates at each leaf stage. Species belonging to *Phoma* sp. and *Pestalotiopsis* sp. appeared at stage I, which were also the most representative species, recovered during all the leaf growth stages. Among 35 isolates, *Fusarium* was the only

species associated with two host plants. The study also revealed that species such as *Alternaria alternata*, and *Aspergillus* sp. appear soon after leaf fall (Stage 4) and therefore appear to be late plant colonizers/degraders. Isolation frequencies increased with the age of the leaf sample. *Rhizopus* sp. and sterile mycelia were rarely found during the early stages of growth (stage I and II), indicating these species participate in leaf degradation. The results showed that older leaves (stage 3 and 4) recorded the highest colonization. Besides, the persistence of two endophytic isolates *viz.*, *Phoma* sp., and *Pestalotiopsis* sp., in all the leaf growth stages indicated their active role in leaf degradation.

In the present study variation in leaf degradation patterns by the inoculated endophytes was recorded. It was observed that a few of the endophytes exhibited sporulation. Bleaching of leaf material was recorded in the treated plates. In contrast, there was no degradation or bleaching in the control plates. Seven of the 14 endophytic isolates tested, *viz*., MEn10, MEn06, MEn37, MEn27, MEn36, MEn42, and MEn10 showed significant litter degradation activity. Two sterile isolates *viz.*, MEn37 (*A. officinalis*) and MEn38 (*B. cylindrica*) exhibited sporulation in the medium amended with host leaf litter. Based on the sporulation, the two fungal isolates were identified as species of *Cladosporium* (MEn37) and *Curvularia* (MEn38). The isolate MEn10 (*Aspergillus* sp.) recorded maximum degradation activity in the leaves of *A. marina* and *E. agallocha*. Complete degradation of *E. agallocha* leaf was achieved using MEn42 (*Pestalotiopsis* sp.). Isolates MEn27 (*Aspergillus niger*) and MEn36 (*Nigrospora* sp.), recorded maximum leaf degradation activity in *A. illicifolius*.

The results of the *in vivo* leaf degradation activity indicated that endophytic isolates decompose the litter at a relatively higher rate compared to the control. Litter samples in inoculated bags showed more pigmentation and tissue softening with high moisture content compared to the control, where leaves were the least pigmented and had low moisture content. Five of the isolates, *viz*., MEn30, MEn04, MEn34, MEn36, and MEn09 recorded a higher amount of litter degradation compared to other isolates. The degraded litter upon CHNS analysis, confirmed the degradation of C, H, N, and S.

Out of 14 endophytic isolates, nine recorded positive activity for amylase production. The maximum amylase activity was observed in *Drechslera* sp., followed by *Aspergillus* sp. Isolates MEn06 and MEn37 showed positive activity for amylase and protease, indicating their involvement in the degradation of organic matter from the litter. MEn09 and MEn42 recorded positive activity for cellulase production, indicating their role in the degradation of

cellulose/glucose matrix from the plant material. Isolates MEn04, MEn10, MEn27, MEn38, and MEn42 recorded the production of amylase and cellulase, thus contributing to nutrient flow. MEn16 showed the production of amylase, cellulase, and lipase, while the isolate MEn38 was involved in the production of all four enzymes.

Fungal endophytes showed clear halo zones in the culture plates indicating their role in P solubilization. The results indicated that the P solubilizing index (PSI) ranged from 2 to 3.8 cm. The highest solubilizing index (3.8 cm) was recorded in MEn19 (*Drechslera* sp.). The total P solubilized by endophytic isolates ranged from 9.70 to 0.1 μg mL^{-1}. The highest P solubilization was recorded using *Drechslera* sp. (9.70 μg mL^{-1}). In liquid medium, a drastic decrease in pH was observed from 5.7 to 3.

Biodegradation of residual petroleum hydrocarbon using fungal endophytes revealed higher hydrocarbon degradation activity in two- and four-wheeler oil samples by seven isolates. However, these isolates recorded partial degradation of boat oil. This study confirms the FT-IR reports. Among the various isolates studied, *Nigrospora* sp. was the most promising isolate degrading maximum functional groups from two- and four-wheeler oil, followed by *Aspergillus* sp. However, a few isolates did not show much activity to degrade hydrocarbon. *Fusarium* sp. recorded a higher amount of two-wheeler hydrocarbon degradation than other isolates.

The study indicated that eight endophytic isolates recorded varied growth-promoting activity in *Av. marina*. Maximum shoot length was recorded in *A. marina* plants inoculated with MEn24, followed by MEn27, MEn30, MEn10, MEn37, and MEn36, while the least growth was recorded in plants inoculated with MEn06. Plants of *Av. marina* inoculated with various fungal endophytes recorded higher amounts of chlorophyll b than chlorophyll a. The inoculated plants recorded higher biomass compared to the control. Maximum biomass was recorded in plants inoculated with T_6 (MEn30) followed by T_2 (MEn04), and T_3 (MEn06). However, the least biomass was recorded in T_5 (MEn27). The seedling inoculated with fungal endophytes produced dense coiling of lateral as well as adventitious root hairs with dense septate colonization. The control plants, however, showed the least colonization. This may be attributed to the vertical transmission of the endophytes.

Molecular identification of the pathogenic culture revealed 99% similarity with *Elsinoe embeliae*. Eight endophytic fungal cultures, *viz*., MEn24 (*Curvularia* sp.), MEn27 (*Aspergillus niger)* MEn10 *(Aspergillus* sp.), MEn30 (*Junctospora pulchra*), MEn37 *(Cladosporium* sp.),

MEn36 (*Nigrospora* sp.), MEn04 (*Drechslera* sp.), MEn06 *(Pestalotiopsis* sp.), MEn30 (*Junctospora pulchra*), and MEn36 (*Nigrospora* sp.) were isolated and tested against the plant pathogen using dual culture plates. Three fungal isolates, *viz*., MEn06, MEn30, and MEn36 recorded 70% inhibition followed by MEn27 (61.66%), and MEn10 (53.33%). While the two isolates *viz*., MEn04 and MEn37 showed the least inhibition (45%).

Endophytic fungal extracts indicated a clear difference in yield. Fermented potato dextrose broth yielded approximately 1 to 3 mg of the dried compound after 6 - 8 weeks of culturing. The results of qualitative phytochemical analysis inferred the presence of alkaloids, flavonoids, phenols, and steroids. In the present study, two solvent systems *viz*., acetone: chloroform and chloroform: methanol was used, of which the latter system with chloroform: methanol in the ratio 95:5 resulted in a clear separation of compounds. Some of the endophytic extracts showed well-separated spots on TLC, while others *viz*., MEn01, MEn87, MEn102, MEn89, MEn67, and MEn85 did not show clear separation.

The GC-MS revealed the presence of Sulfurous acid, 2-ethylhexyl isohexyl ester (at 24.44), 1-lodo-2-methylundecane (at RT 26.57), Heptacosane (at RT 27.63, 28.70 and 35.5), 1-lodo-2-methylnonane (at RT 29.96, 31.46, 33.30, 35.34, 35.49 and 35.48) in MEn87 isolate (*A. alternata*). Metabolites isolated from endophytic isolate MEn102 (*Pestalotiopsis*) showed the presence of Hexadecane (RT 8.73), n-Hexadecanoic acid (14.74), Octadecanoic acid (18.73), Myristic anhydride (23.15), 9-Octadecanoic acid (Z), 2-hydroxy-3-[(1-oxohexadecyl) oxy] propyl ester. Chromatogram of endophytic extract belonging to MEn89 (*Cladosporium* sp.) revealed the presence of Octane,3-ethyl-2,7, dimethyl- ($Rt_{(min)}$7.88), Octatriacontyl trifluoroacetate (8.74), Hexadecane (10.75, 13.71), Dodecane,2,6,11-trimethyl (10.98), Tetradecanoic acid (11.56), 7,9-Di-tert-butyl-1-Ocaspiro (4,5) deca-6,9-diene-2,8-dione (14.53), n-hexadecanoic acid (14.74), Octadecanoic acid (18.43), 2-Oxepanol,5-(1,1-dimethylethyl)- (24.24), 12-Hydroxydodecanoic acid (27.26), and Oleic acid (27.79). Isolate MEn67 (*Curvularia* species) indicated the presence of Tetradecane (6.05), Dodecane, 2,6,11-trimethyl (7.86), Hexadecane (8.73), Tetradecanoic acid (10.98), Hexadecane (11.55), n-Hexadecanoic acid (14.73), Octadecanoic acid (18.42), 10-undecanoic acid, and octyl ester (at 26.75, 26.76 and 18.41). Endophytic fungi belonging to MEn85 (*Nigrospora* sp.) revealed the presence of B-D-Glucopyranose,1,6-anhydro- (11.34), Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (26.54, 26.94), Hexadecanoic acid,1-(hydroxymethyl)-1,2-ethanediyl ester (27.48), and Oleic acid (32.25).

Of the 14 fungal crude extracts prepared in ethyl acetate, the extract of *S. monoceras* exhibited antibacterial activity. It inhibited all the bacterial pathogens tested in the study showing relatively large zones of inhibition. It exhibited efficacy even at low concentrations (10 µL) against the target pathogens. However, it did not exhibit inhibitory activity against mold (*Aspergillus brasiliensis*) and yeast (*Candida albicans*), indicating the absence of antifungal activity. DMSO (Dimethyl sulphoxide) used as a negative control, did not show any zone of inhibition. The MIC results revealed a decrease in the growth of pathogenic bacterial strains, ranging from 3.1- 6.25 µg mL^{-1} as observed by the decreasing absorbance values at 6.25 µg mL^{-1}. Further MBC study, the aliquots from the MIC tubes were sub-cultured on the Muller Hinton agar medium, the plates with aliquots from 3.12 to 6.25 µg mL^{-1} showed no bacterial colony, indicating its strong bactericidal property.

The IC_{50} values of the extracts ranged from 2.4 to 3.4 µg mL^{-1}. Results of the anticancer study revealed maximum inhibitory activity in MEn89 (*Cladosporium* sp.) at 1000 µg mL^{-1} concentration with an IC_{50} value of 2.672 µg mL^{-1}, while MEn87 (*Alternaria alternata*) had an IC_{50} value of 3.012 µg mL^{-1}. However, the results of the biocompatibility assay using Mouse Fibroblast cell line L929 revealed nontoxic effects as evident from their cells with normal proliferation and growth.

Although MEn67 showed promising anticancer activity against the A549 cell line, it recorded least number of viable cells when tested for bio-compatibility assay against the L929 cell line. Among the other isolates, MEn102 showed moderate inhibition against the A549 cell line and showed the maximum viability percentage in the L929 cell line. Two isolates *viz*., MEn87 and MEn85 inhibited the growth of the A549 cell line and had no lethal effect on L929.

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Presentation at Conferences:

1. **Sawant, A.S.** and Rodrigues, B. F. 2015. Diversity of mangrove plant species from Chorao Island Goa. In: National conference on mangroves of Konkan coast. Organized by Mangrove society of India in collaboration with R.P. Gogate College of Arts and Sciences and R.V. Joglekar College of commerce, Ratnagiri from 2nd - 3rd February, 2015 (Poster).

2. **Sawant, A. S.** and Rodrigues, B. F. 2015. Diversity of endophytic fungi from Chorao Island Goa. In: Asian Mycological Congress. Co-organized by Department of Botany, Goa University, Goa, Asian Mycological Association (AMA) and Mycological Society of India (MSI) from 7th - 10th October 2015 (Poster).

3. **Sawant, A. S.** and Rodrigues, B. F. 2017. Isolation, characterization and identification of secondary metabolite using GC-MS from fungal endophytes associated with *Avicennia marina*. In: National conference on Reaching the unreached through science and technology: recent advances in physical, chemical, mathematical and biological sciences for energy, health and environment organized by Mangalore University from 8th - 9th September 2017 (Poster).

4. **Sawant, A. S.** and Rodrigues, B. F. 2020. Distribution and phytosociological study of mangrove plant species at Chorao Island Goa. In: National conference on New Vistas in Botany, organized by UGC-SAP and Department of Botany, Goa University, Goa from 13th -14th February 2020 (Poster).

5. **Sawant, A. S. and** Rodrigues, B. F. 2021. Characterization of bioactive compounds isolated from mangrove endophytic fungi and its potential activity against A549 lung cancer cell line. In: National Conference on Biodiversity and Biotechnology of Fungi. Co-organized by Department of Botany, Punjabi University, Patiala from 22nd - 24th February 2021 (Oral).

Research Publications:

1. **Sawant, A.S**., Rodrigues, B. F. and Sardessai, Y. 2018. Antimicrobial and anticancer activity of *Seatosphaeria monoceras*, an endophytic fungus associated with tropical mangrove plant. *World Journal of Pharmacy and Pharmaceutical Sciences*, 7(6): 1315-1329.

2. **Sawant, A.S.** and Rodrigues, B.F. 2020. Isolation and identification of phosphate (P) solubilizing halophytic fungal endophytes. *Kavaka*, 55:71-76.

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4. Kripa, M. K., Lele, N., Panda, M., Das, S. K., Hari Nivas, A, Divakaran, N. **Sawant, A.S**., Naik-Gaonkar, S., Pattnaik, A. K., Samal, R. N., Thangaradjou, T., Saravanakumar, A., Rodrigues, B. F., Roy, S. D., Sankar, R. K., Kumar, L., Angel, R. J., Raghavan, P., Mankad, A. U. and Murthy, T. V. R. 2020. Biodiversity assessment of Indian mangroves using *in situ* observations and remotely sensed data. *Biodiversity*, 21:4, 198-216.

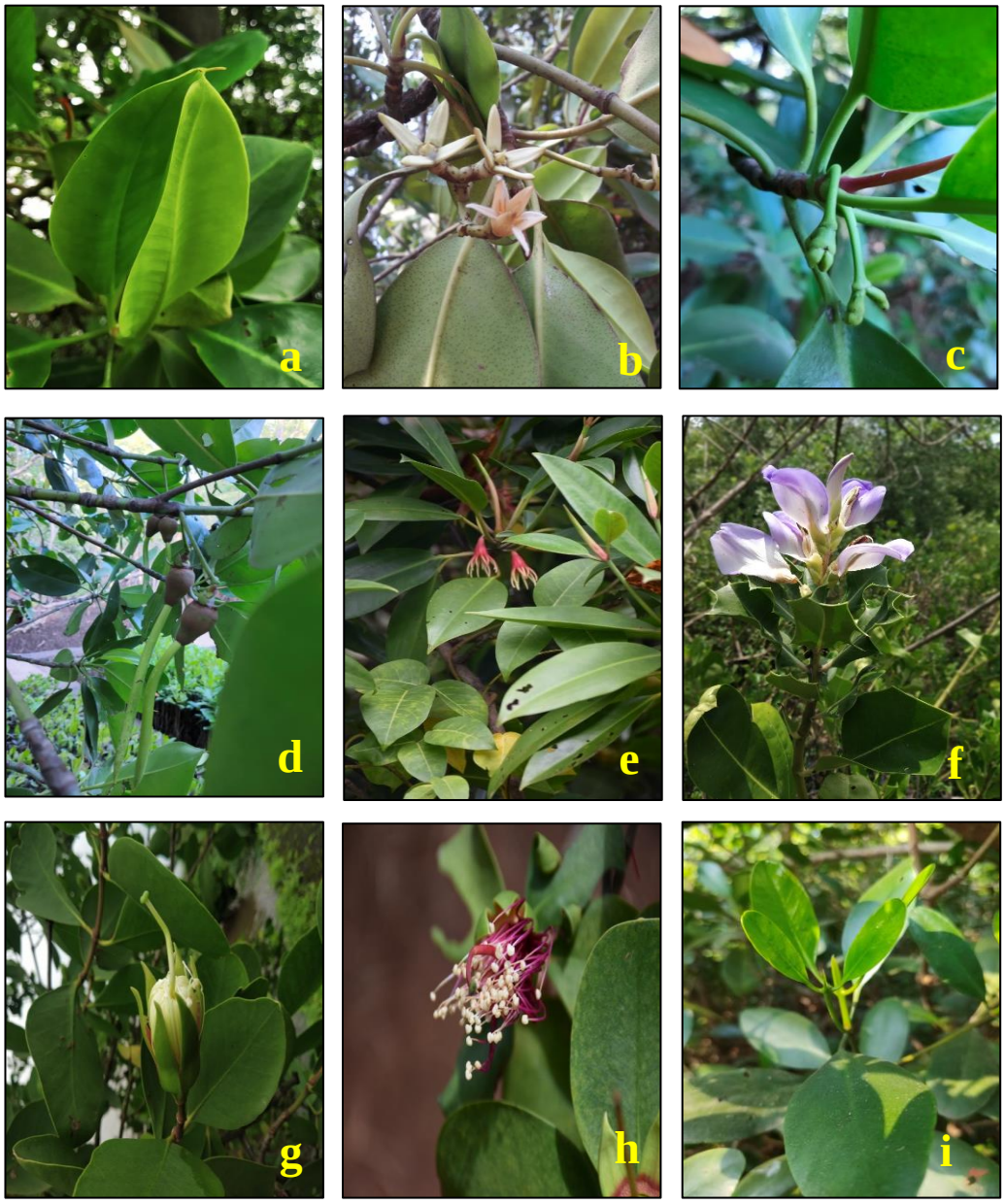


Plate 3.1: Diversity of mangrove plant species. a-d: *Rhizophora mucronata* – phenotypic characters; **e:** *Rhizophora apiculata* - flower; **f:** *Acanthus ilicifolius* - flower; **g:** *Sonneratia alba* - inflorescence; **h:** *Sonneratia ceaseolaris* - inflorescence; i**:** *Ceriops tagal* - habit.

Plate 3.2: Diversity of mangrove plant species. a: *Kandelia candel* - inflorescence; **b:** *Rhizophora apiculata* - habit; **c:** *Clerodendron inermis* - habit; **d-e:** *Excoecaria agallocha* - inflorescence and latex; **f-g:** *Avicennia marina* - leaf and fruits; **h-i:** *Aegiceras corniculatum* - inflorescence.

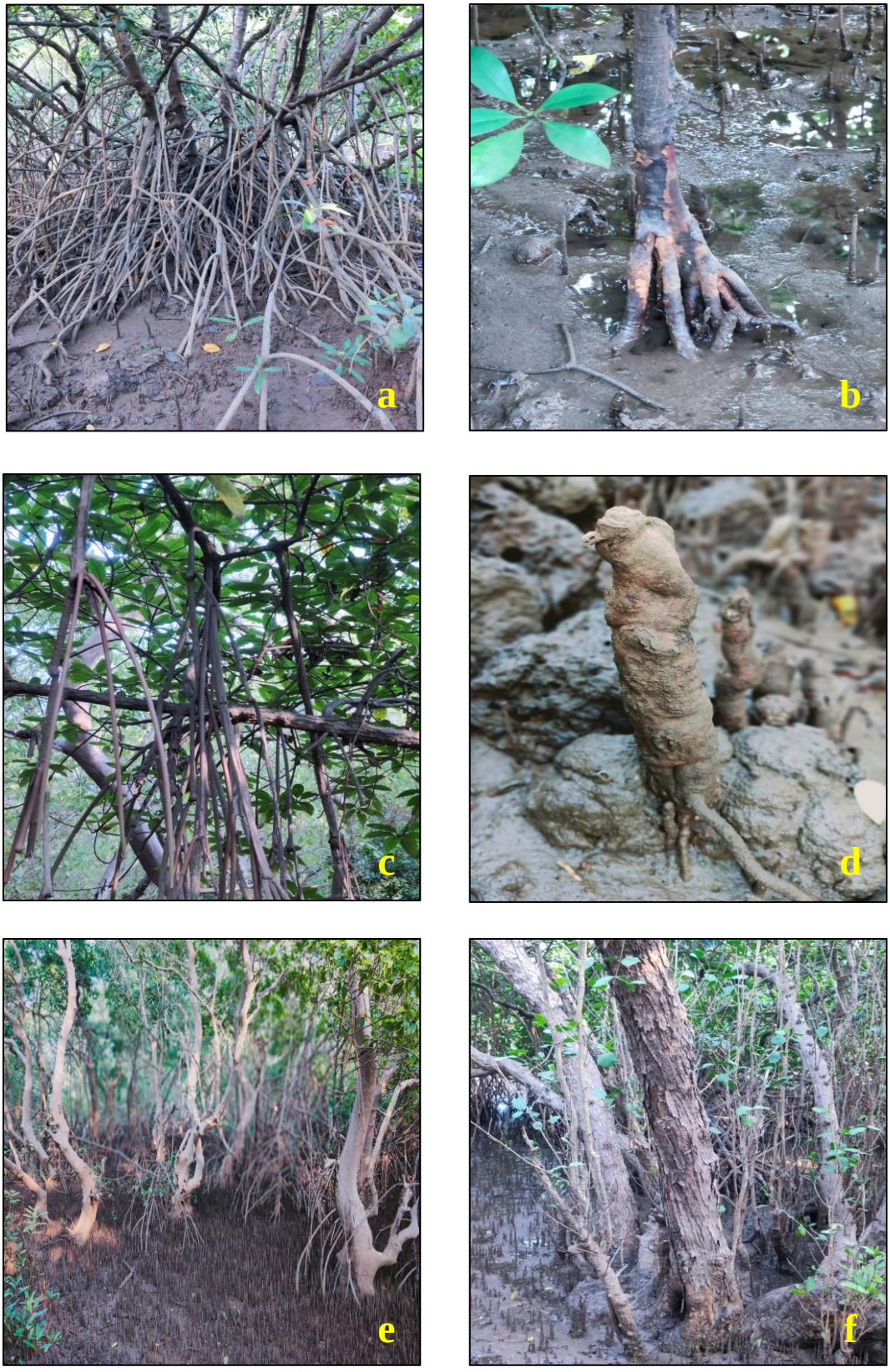


Plate 3.3: Modifications in root morphology in mangrove species. a: *Rhizophora mucronata* - Hanging prop roots; **b:** *Brugueira cylindrica* - Knee roots; **c:** *Rhizophora apiculata* - Hanging prop roots; **d-e:** *Avicennia marina* - root and pneumatophores; **f:** *Sonneratia alba* – root.

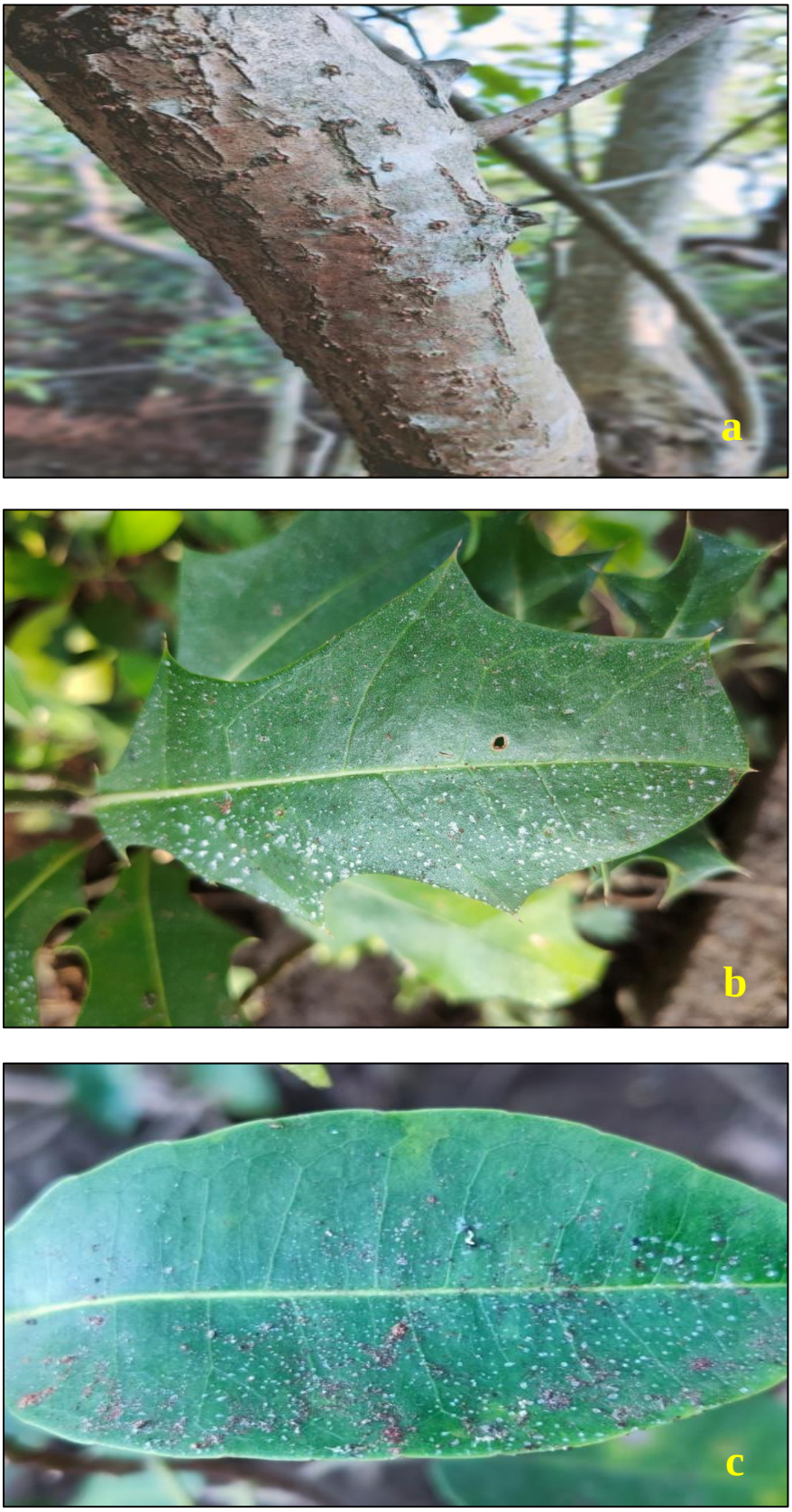


Plate 3.4: Salt excretion in mangrove plant species. a: *Sonneratia alba* - lenticels; **b:** *Acanthus illicifolius* - salt crystals on leaf surface; **c:** *Excoecaria agallocha* - salt crystals on leaf surface.

Plate 3.5: Seed propagation in mangrove plant species. a: *Rhizophora mucronata* - viviparous seed germination; **b-c:** *Kandelia candel* - viviparous seed germination; **d:** *Ceriops tagal* - viviparous seed germination; **e:** *Aegiceras corniculatum* - incipient vivipary; **f:** *Avicennia marina* - incipient vivipary; **g:** *Sonneratia alba* - mature fruit; **h:** *Excoecaria agallocha* - mature fruit; **i:** *Derris heterophylla* - fruit.

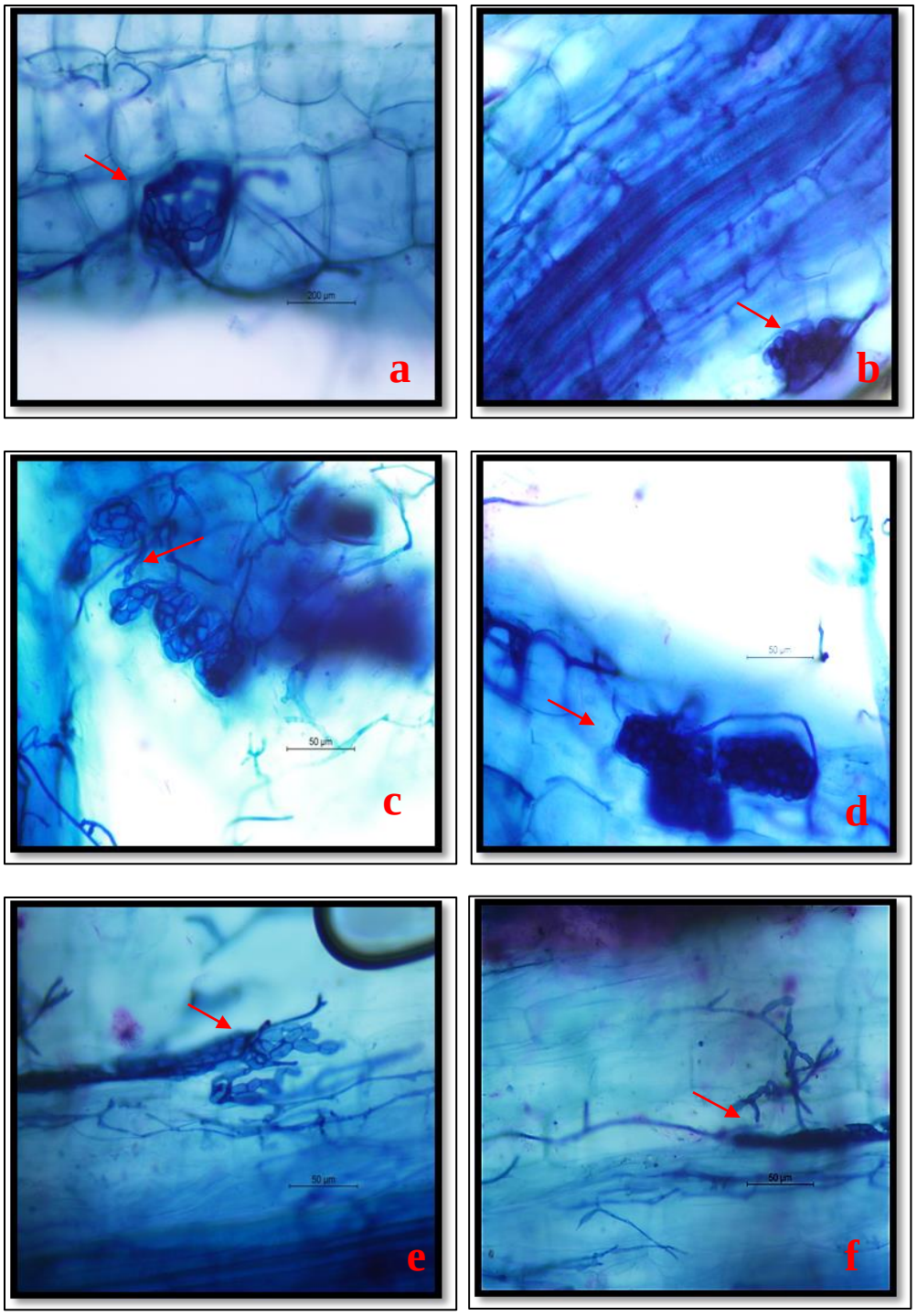


Plate 4.1: Localization of endophytes in mangrove roots. a: *Avicennia marina* - hyphal coils; **b:** *A. officinalis* - septate endophyte*;* **c.** *Brugueira cylindrica* - coiled septate hyphae; **d.** *Acanthus illicifolius* - endophytic structure; **e.** *Excoecaria agalocha* - endophytic structure; **f.** *Ceriops tagal* - coiled hyphae.

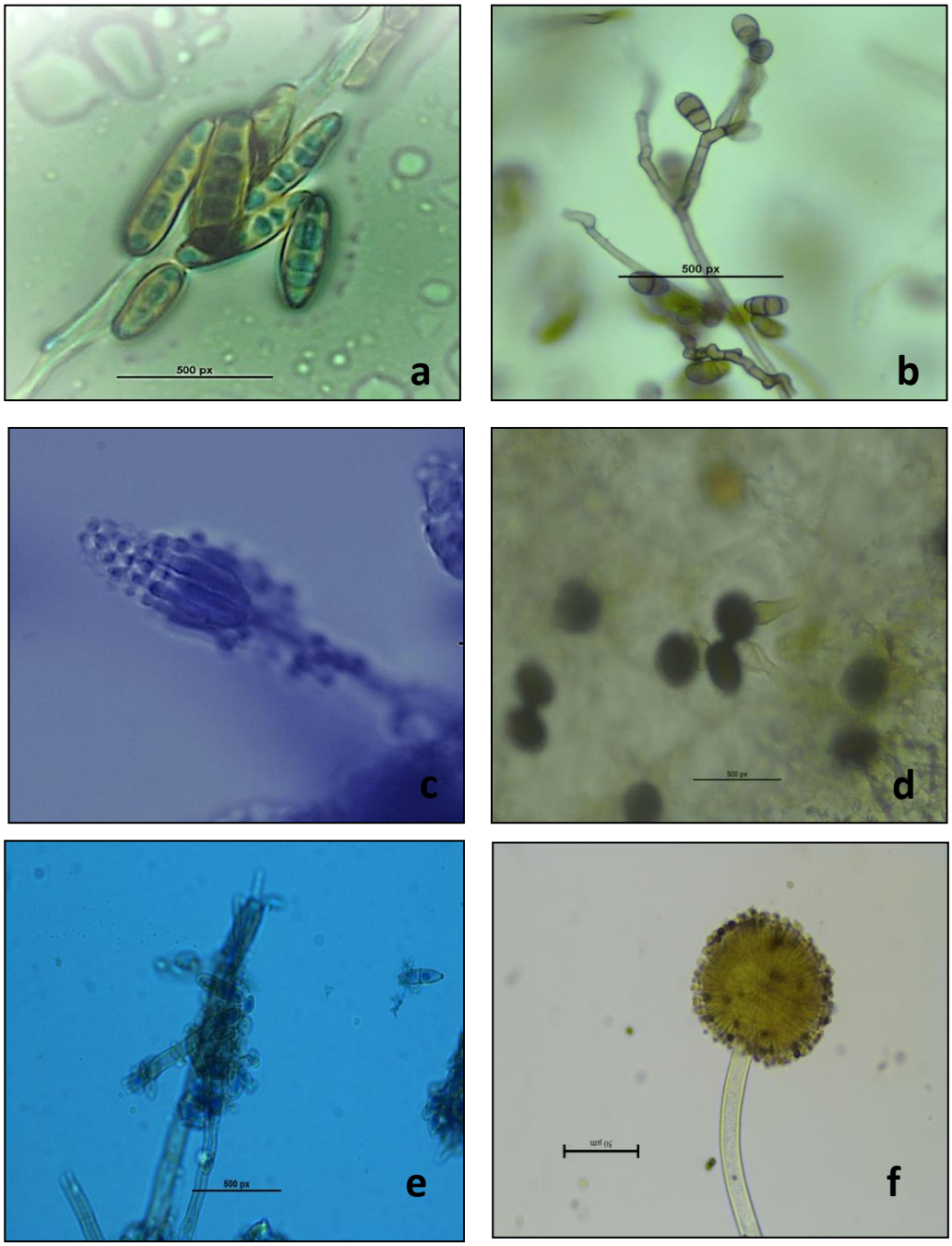


Plate 4.2: Diversity of endophytic fungi in mangrove plants. a: *Drechslera* sp. 2; **b:** *Scolecobasidium* sp.; **c:** *Penicillium* sp. 1; **d:** *Nigrospora* sp.1; **e:** *Cladosporium* sp.1; **f:** *Aspergillus* sp. 1.

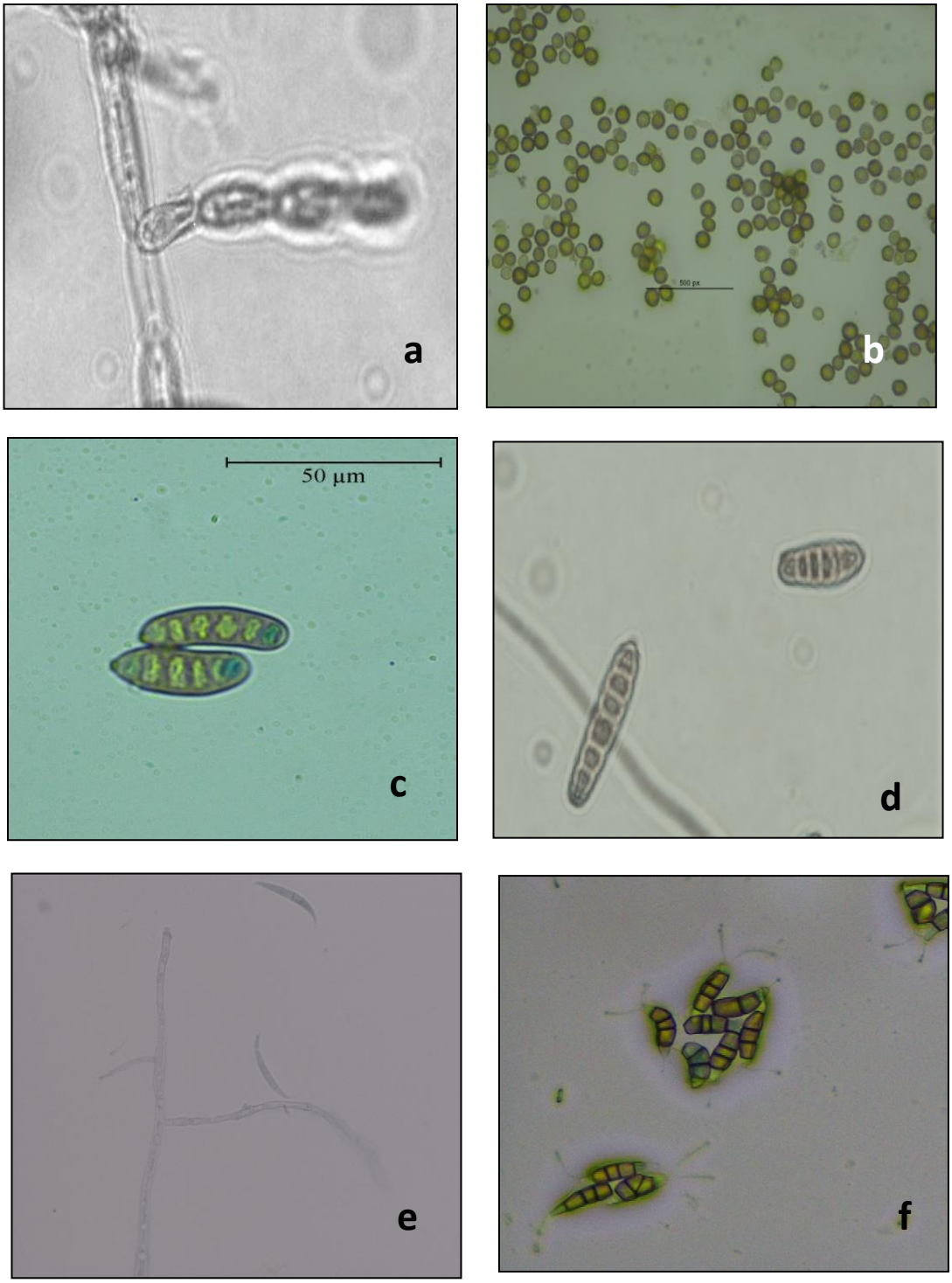


Plate 4.3: Diversity of endophytic fungi from mangrove plants. a: *Scytalidium lignicola*; **b:** *Aspergillus niger*; **c:** *Drechslera* sp.1; **d:** *Drechslera* sp. 2; **e:** *Fusarium* sp.1; **f:** *Pestalotiopsis* sp.1.

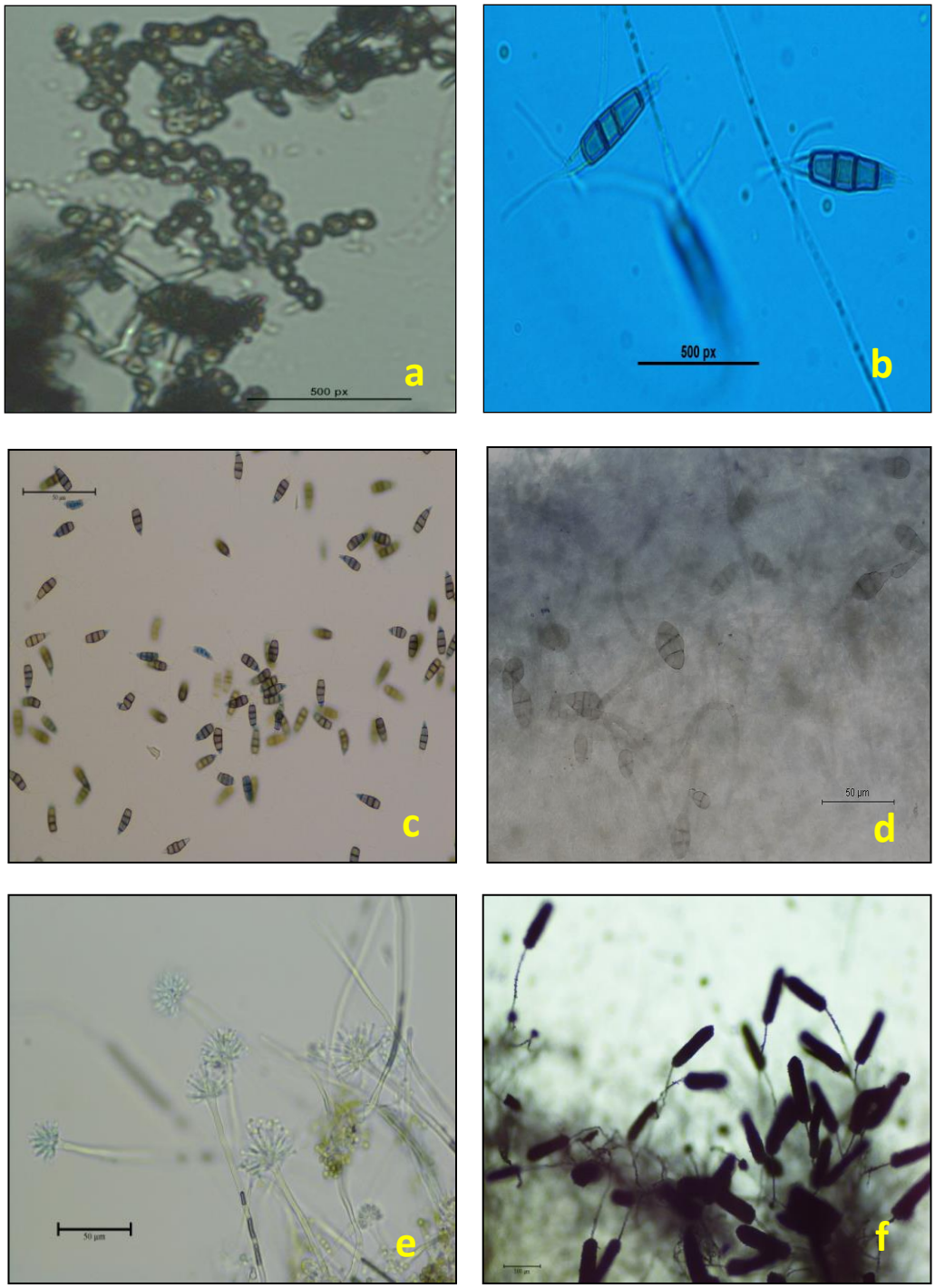


Plate 4.4: Diversity of endophytic fungi from mangrove plants. a: *Aureobasidium pullulans*; **b:** *Pestalotiopsis* sp. 1; **c:** *Pestalotiopsis* sp. 2; **d:** *Alternaria* sp. 1; **e:** *Penicillium* sp. 2; **f:** *Aspergillus* sp. 3.

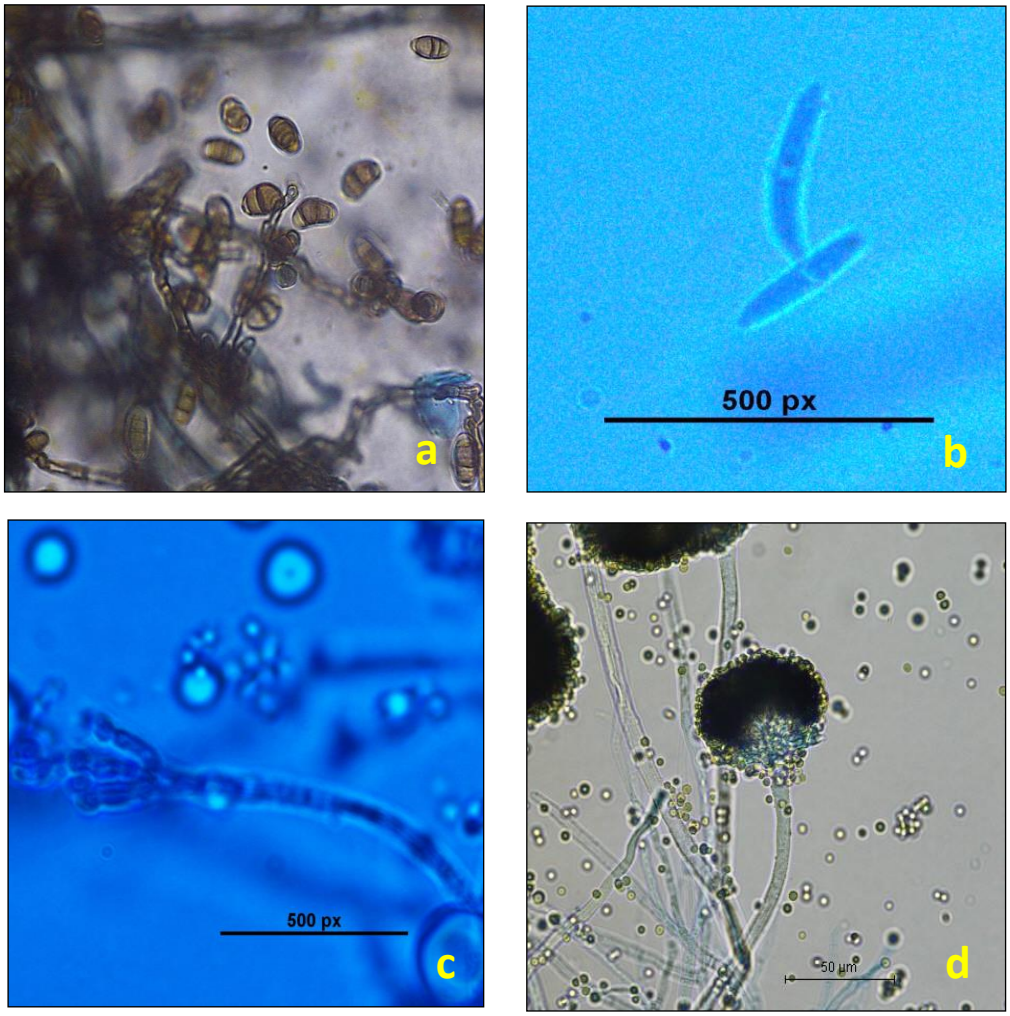


Plate 4.5: Diversity of endophytic fungi from mangrove plant. a: *Curvularia* sp. 6; **b:** *Fusarium* sp. 8; **c:** *Penicillium* sp. 9; **d:** *Aspergillus* sp. 10.

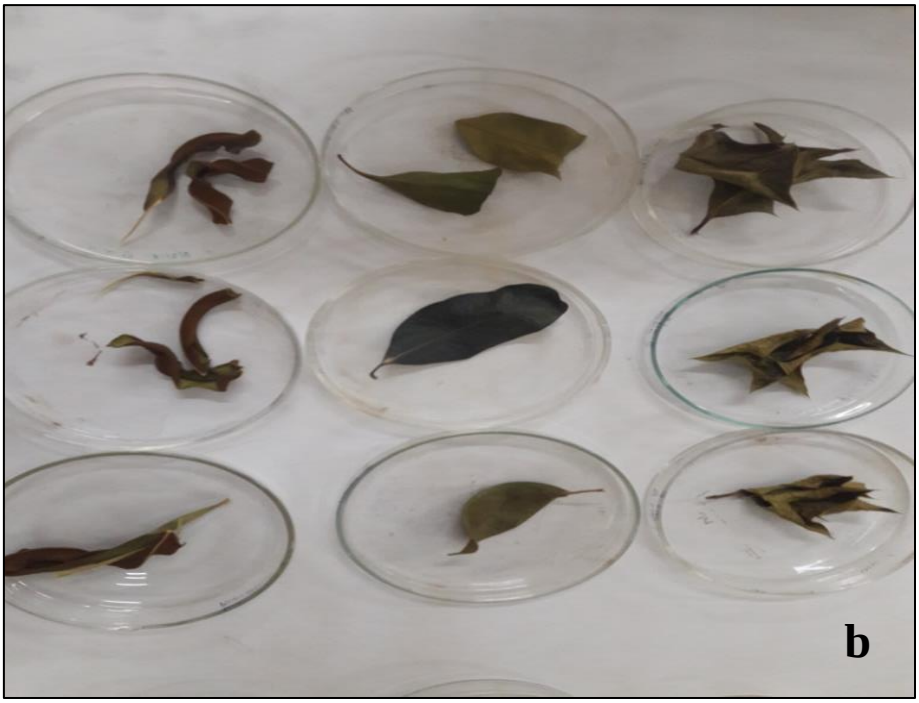


Plate 5.1: Sterilization of mangrove leaves. a: Sterilized healthy leaf samples; **b:** Oven-dried leaf samples.

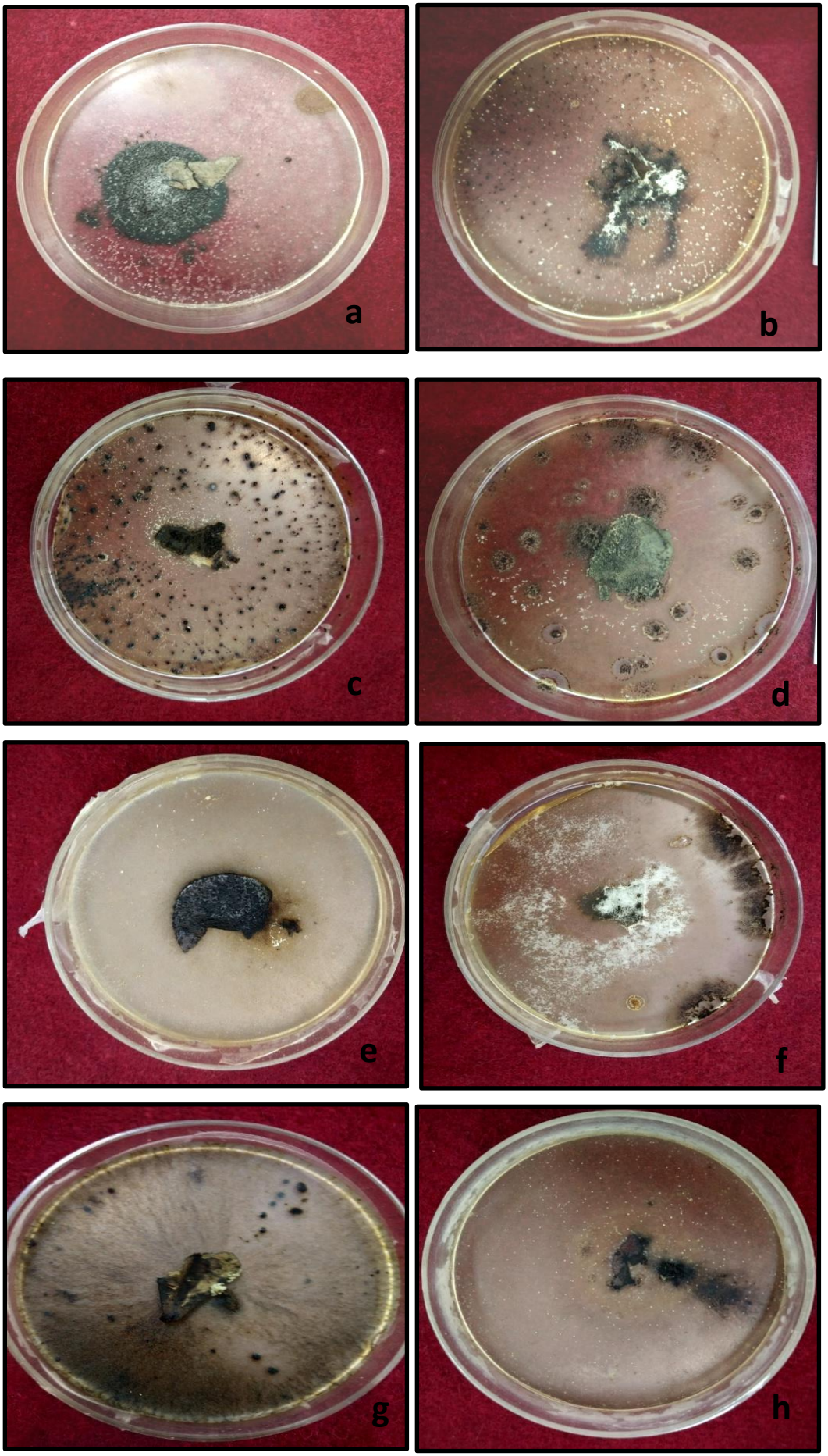


Plate 5.2: ***In vitro*** **degradation of mangrove leaf litter by fungal endophytes**. **a:** MEn10; **b:** MEn06; **c:** MEn37; **d:** MEn27; **e:** MEn36; **f:** MEn42; **g:** MEn38; **h:** MEn30.

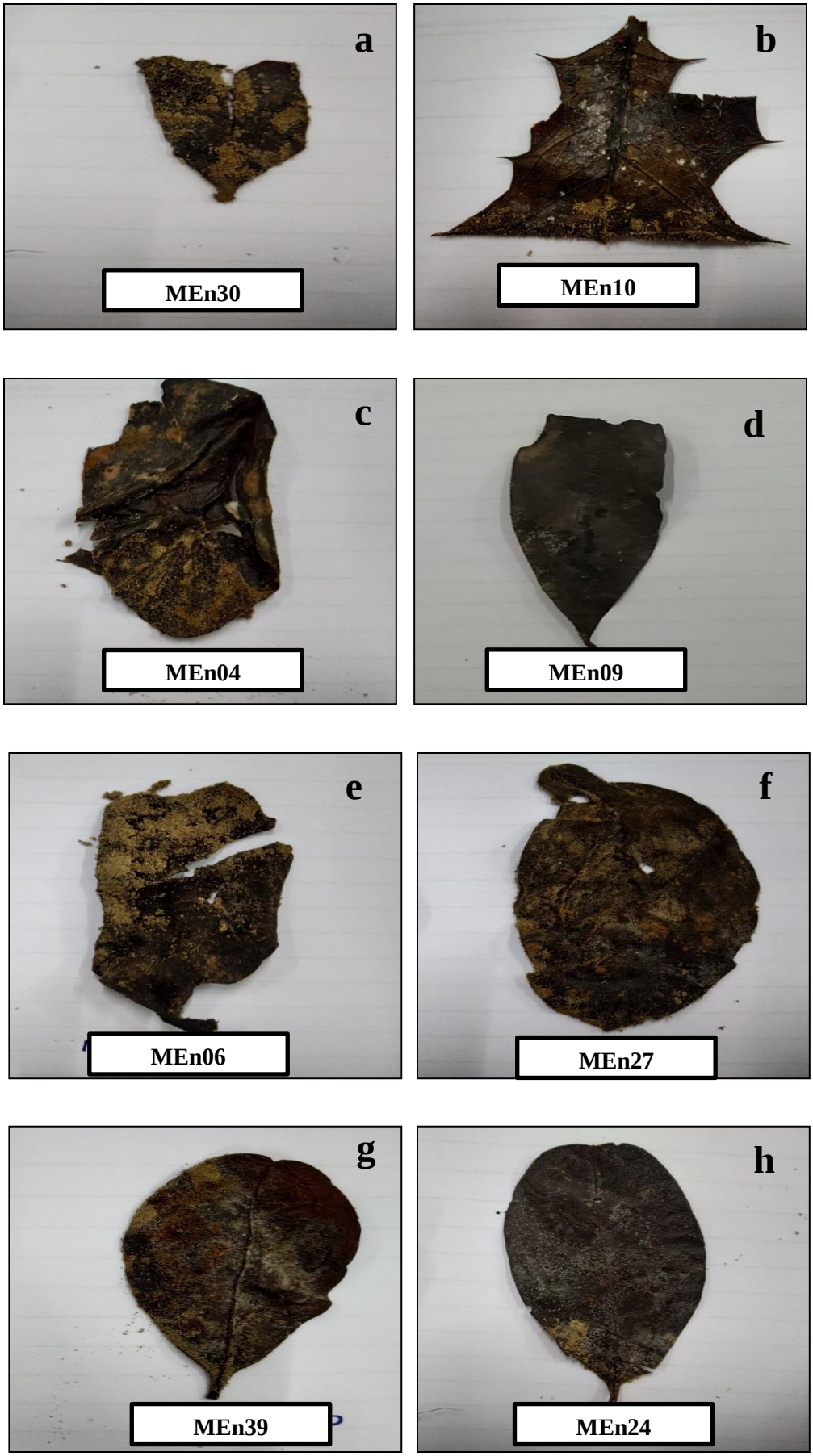
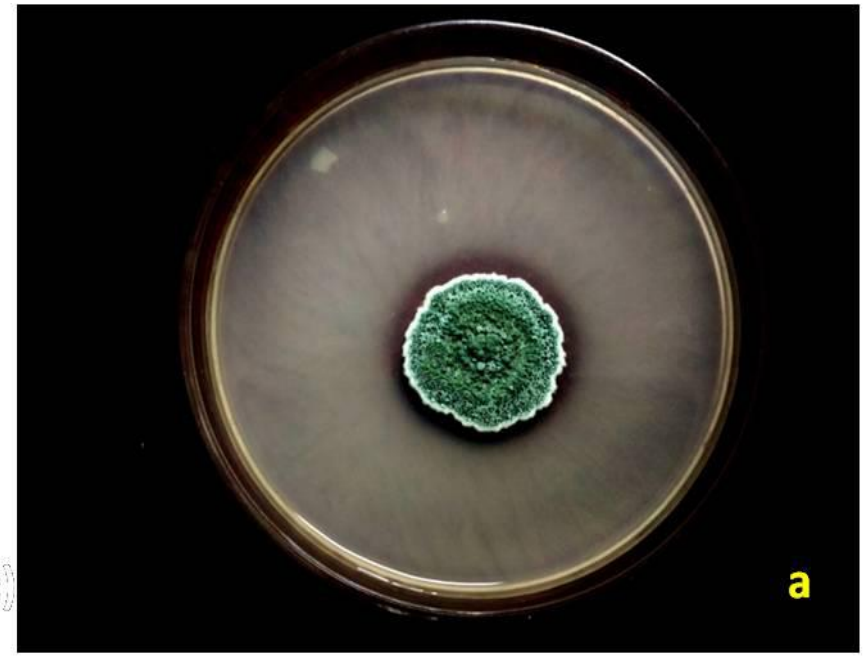


Plate 5.3: ***In vivo*** **leaf degradation by endophytic fungi. a:** MEn30; **b:** MEn10; **c:** MEn04; **d:** MEn09; **e:** MEn06; **f:** MEn27; **g:** MEn39; **h:** MEn24.



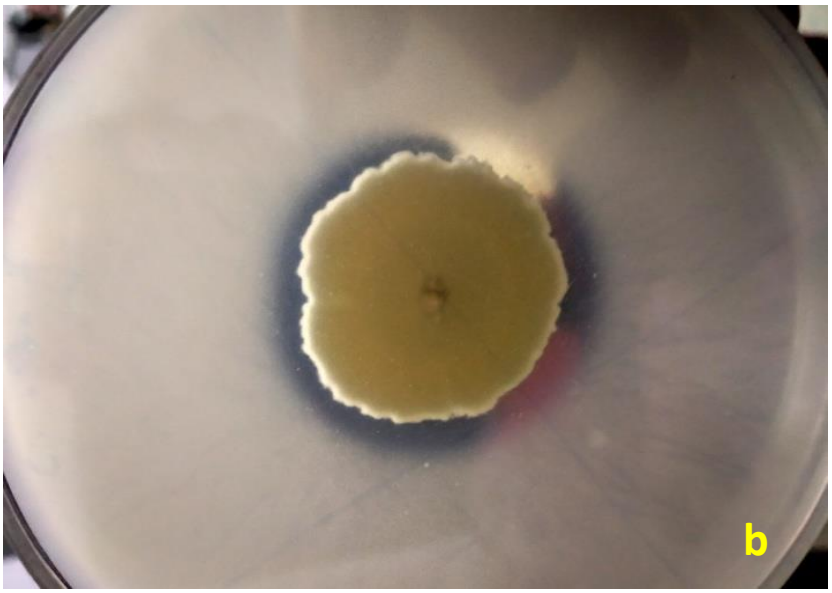


Plate 5.4: P solubilization activity of endophytic fungi. a: P solubilization by *Penicillium* sp**. b:** P solubilization by *Drechslera* sp.

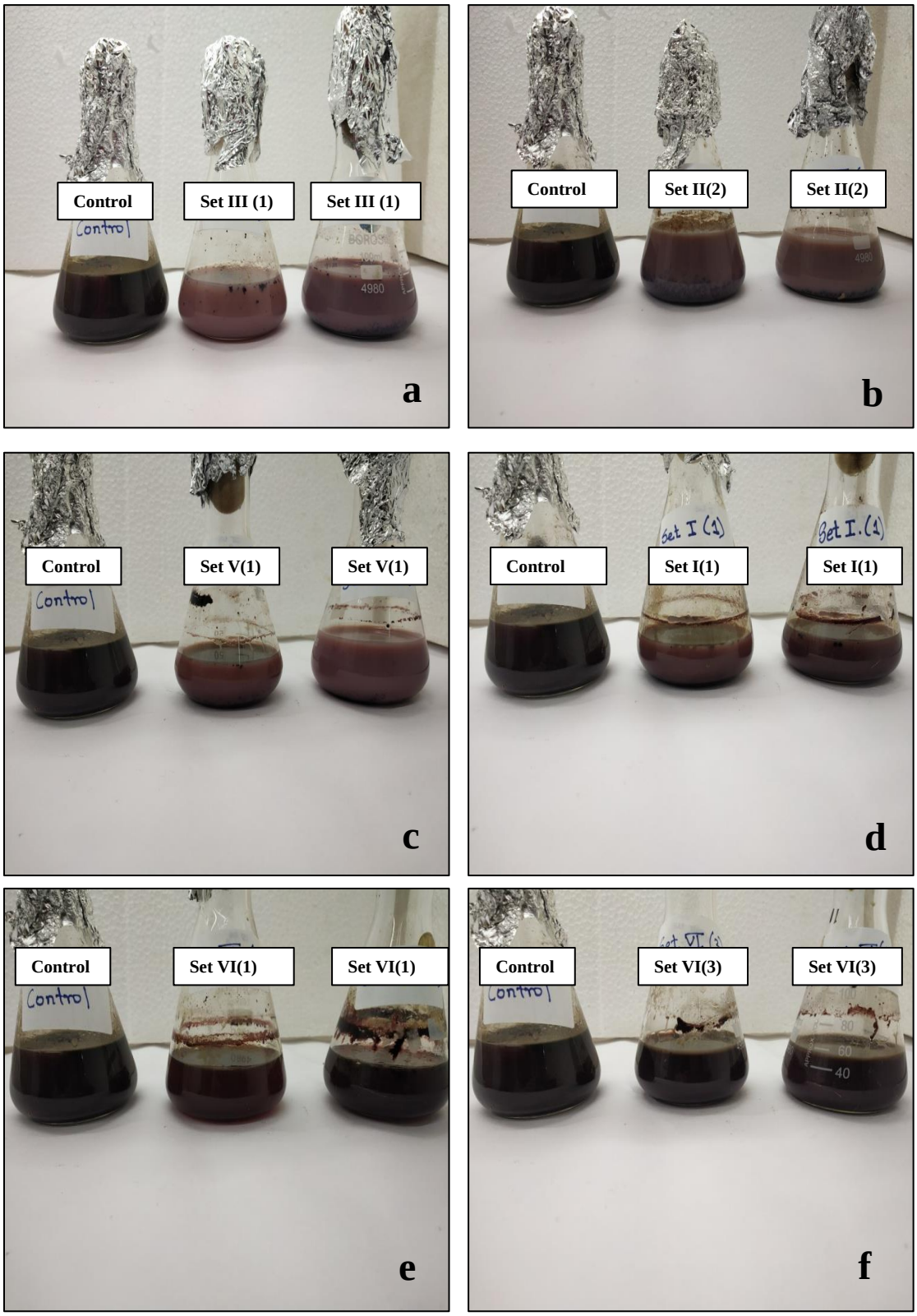


Plate 5.5: **Qualitative analysis *in vitro* biodegradation of four-wheeler residual oil by. a:** *Fusarium* sp.; **b:** *Pestalotiopsis* sp.; **c:** *Curvularia* sp. **Two-wheeler residual oil by d:** *Aspergillus niger*; **e:** *Aspergillus* sp.; **f**: *Curvularia* sp.

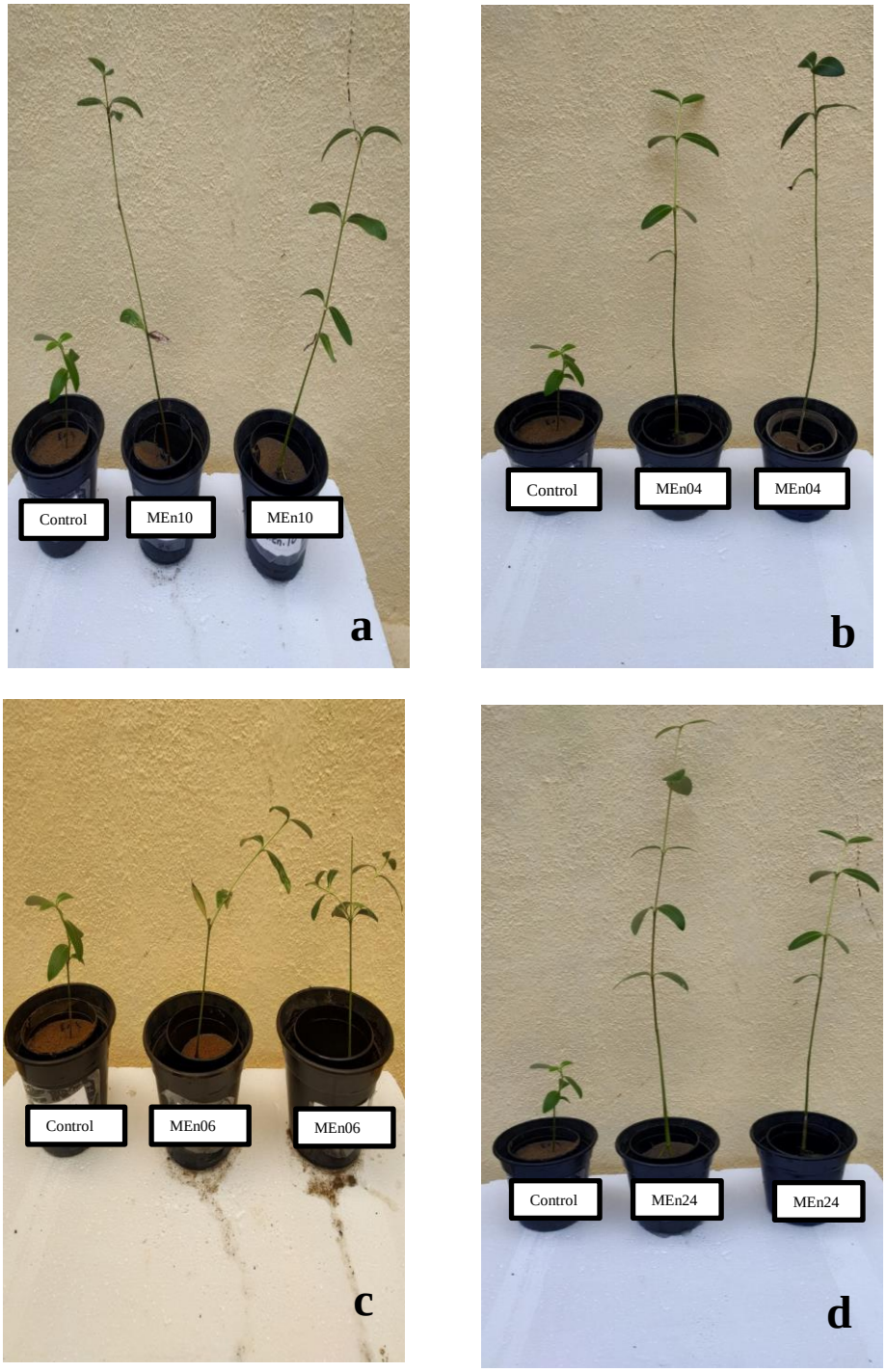
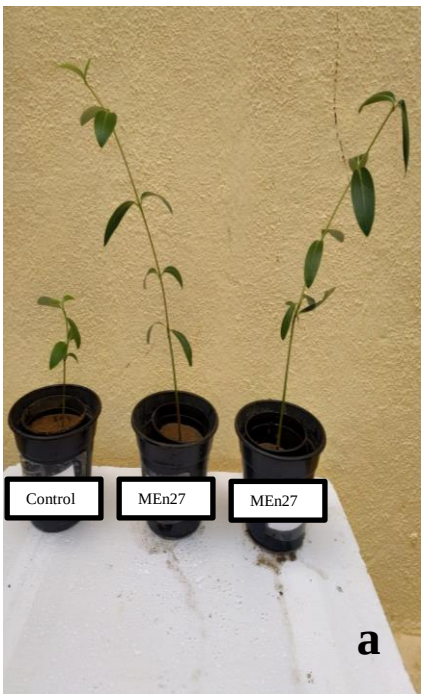
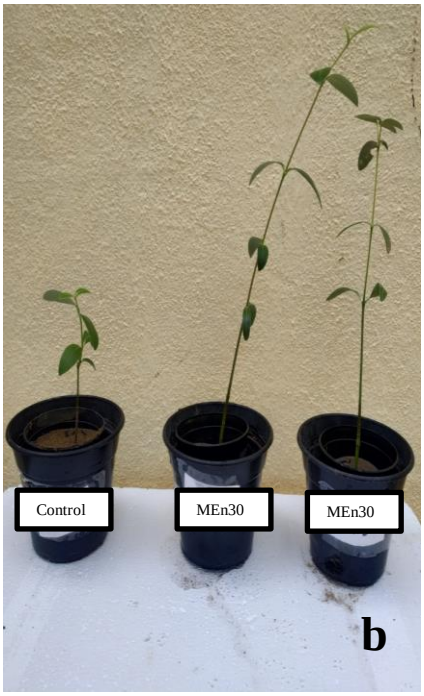


Plate 5.6. Effect of fungal endophytes on the growth of *Avicennia marina* seedlings inoculated with a: MEn10 (T_1), **b:** MEn04 (T_2), **c:** MEn06 (T_3), **d:** MEn24 (T_4).





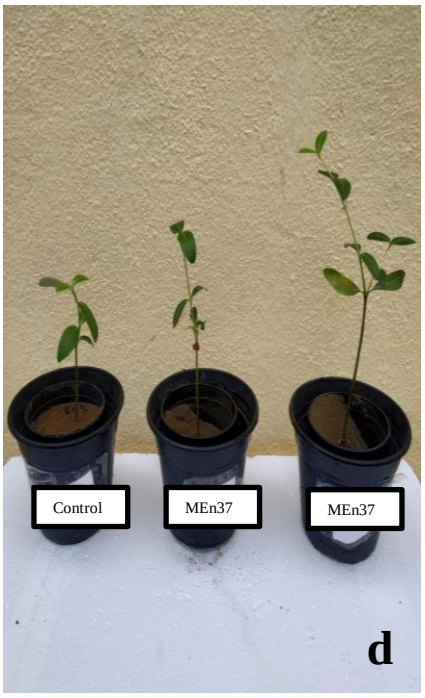


Plate 5.7. Effect of fungal endophytes on the growth of *Avicennia marina* seedlings inoculated with a: MEn27 (T_5); **b:** MEn30 (T_6); **c:** MEn36 (T_7); **d:** MEn37 (T_8).

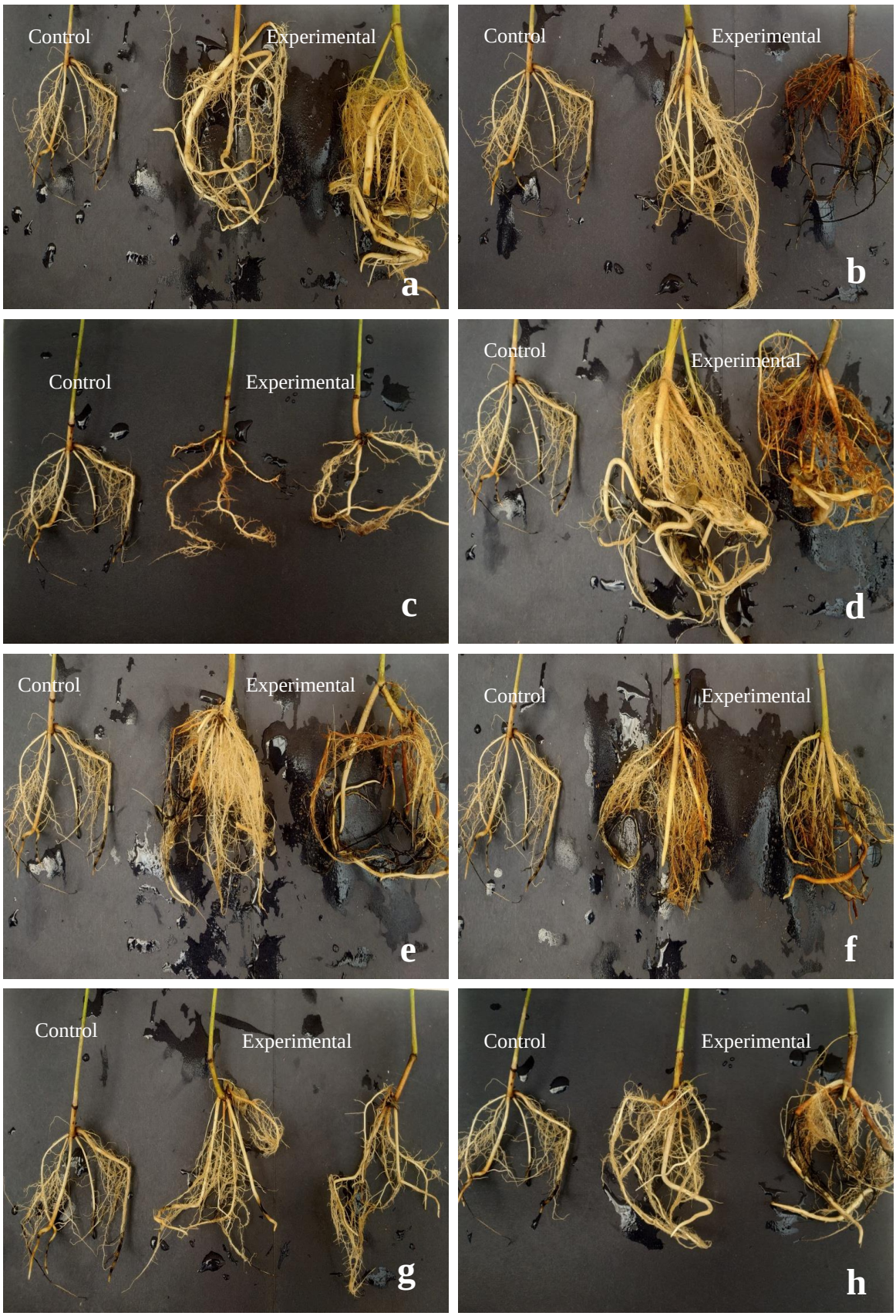


Plate 5.8: Effect on endophytic inoculation on *A. marina* roots inoculated with a: MEn10 (T_1); **b:** MEn04 (T_2); **c:** MEn06 (T_3); **d:** MEn24 (T_4); **e:** MEn27 (T_5); **f:** MEn30 (T_6); **g:** MEn36 (T_7); **h:** MEn37 (T_8).

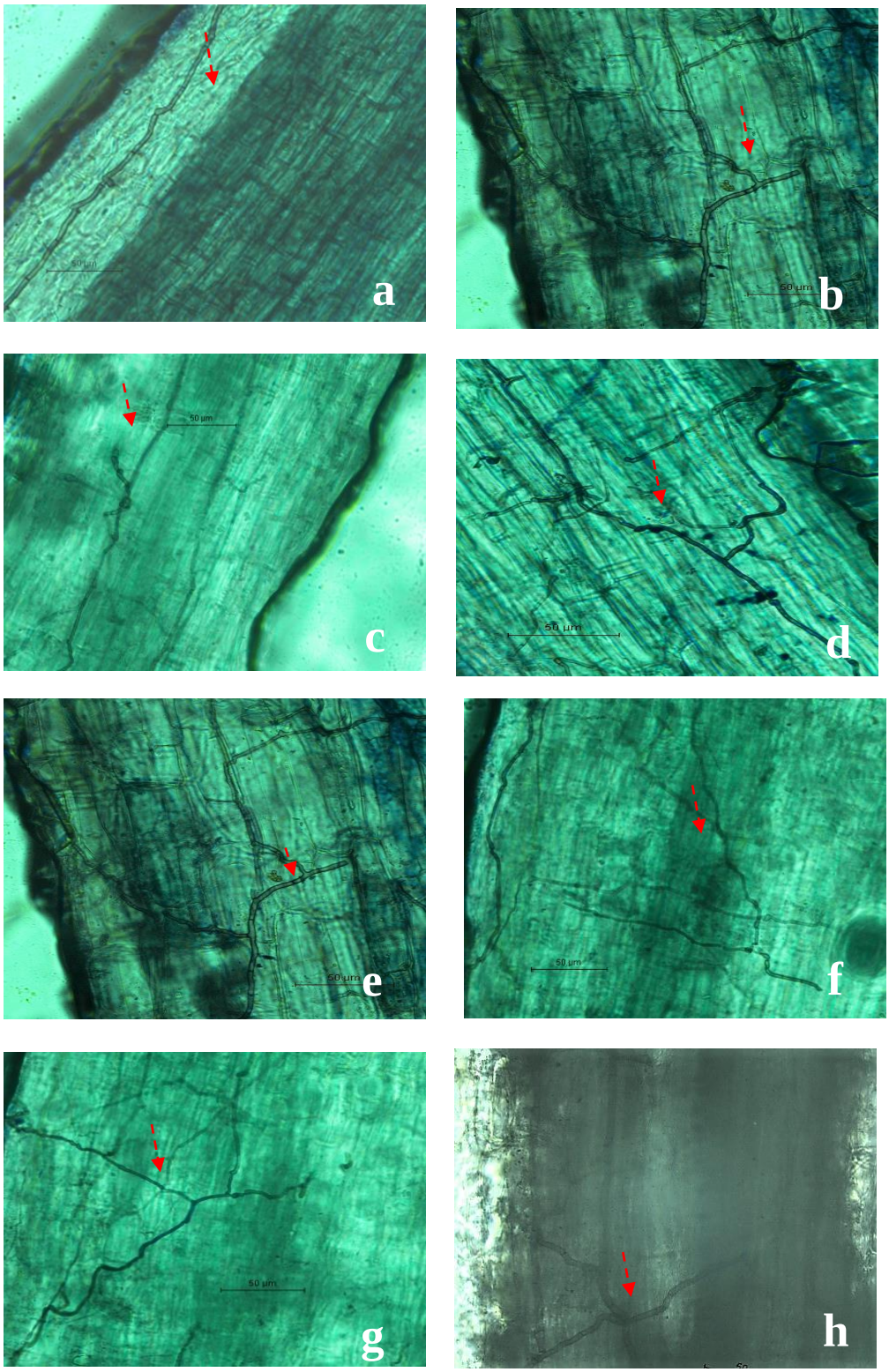


Plate 5.9: Localization of endophytic fungal colonization in roots of *Avicennia marina*. a: MEn10 (T_1); **b:** MEn04 (T_2); **c:** MEn06 (T_3); **d:** MEn24 (T_4); **e:** MEn27 (T_5); **f:** MEn30 (T_6); **g:** MEn36 (T_7); **h:** MEn37 (T_8).

Plate 5.10: Isolation of plant pathogen from infected leaf. **a**. *Avicennia marina* - leaf; **b:** *Elsinoe embeliae* - culture tube.

Plate 5.11: Extraction of bioactive metabolites. a-b: *Setosphaeria monoceras* - liquid culture (day 1) and fermented culture (after 15 days of incubation).

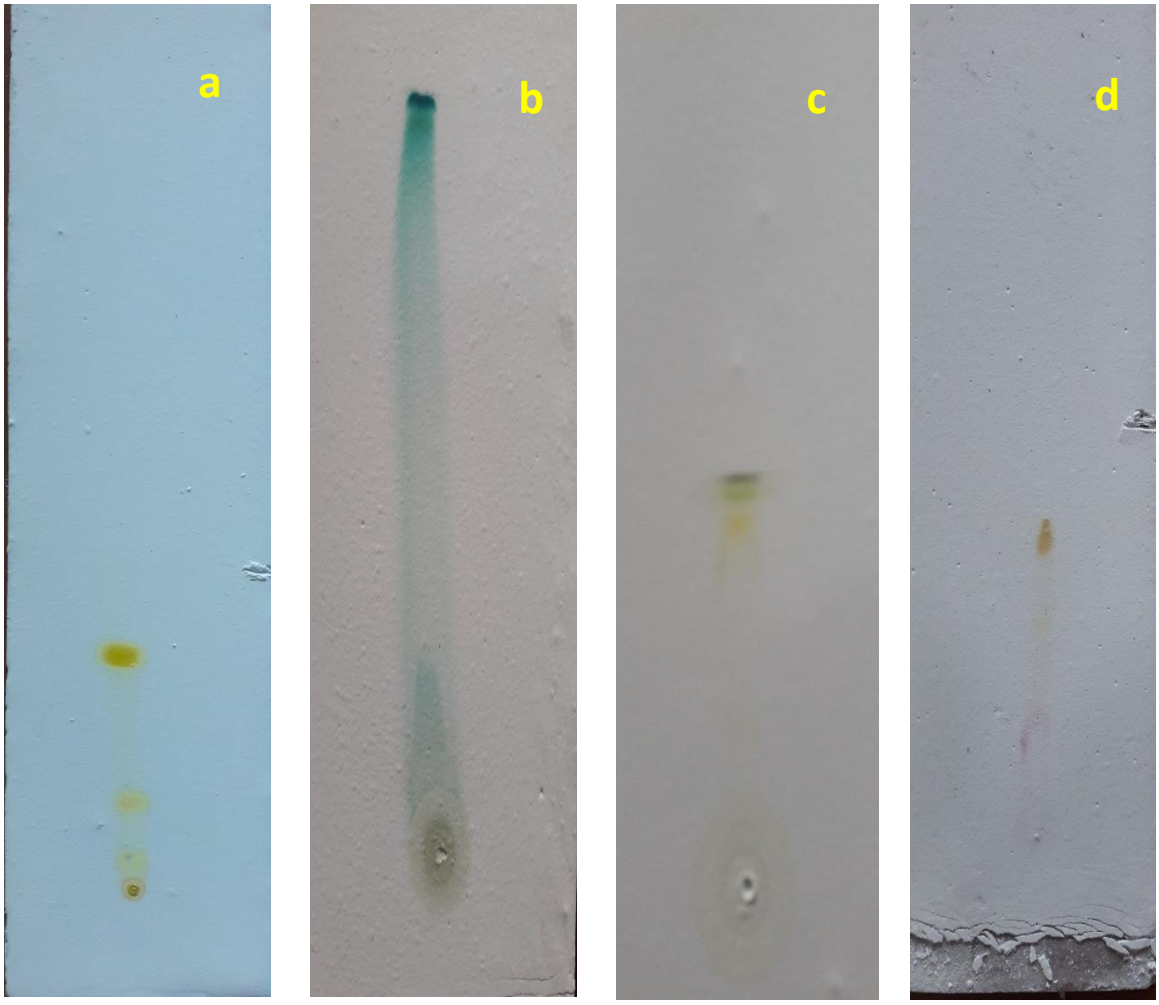


Plate 5.12: Separation of bioactive compounds using TLC. a: *Dreshslera* sp. **(**MEn04**); b:** *Aspergillus* sp. (MEn10); **c:** *Curvularia* sp. (MEn24); **d:** *Cladosporium* sp. (MEn37).

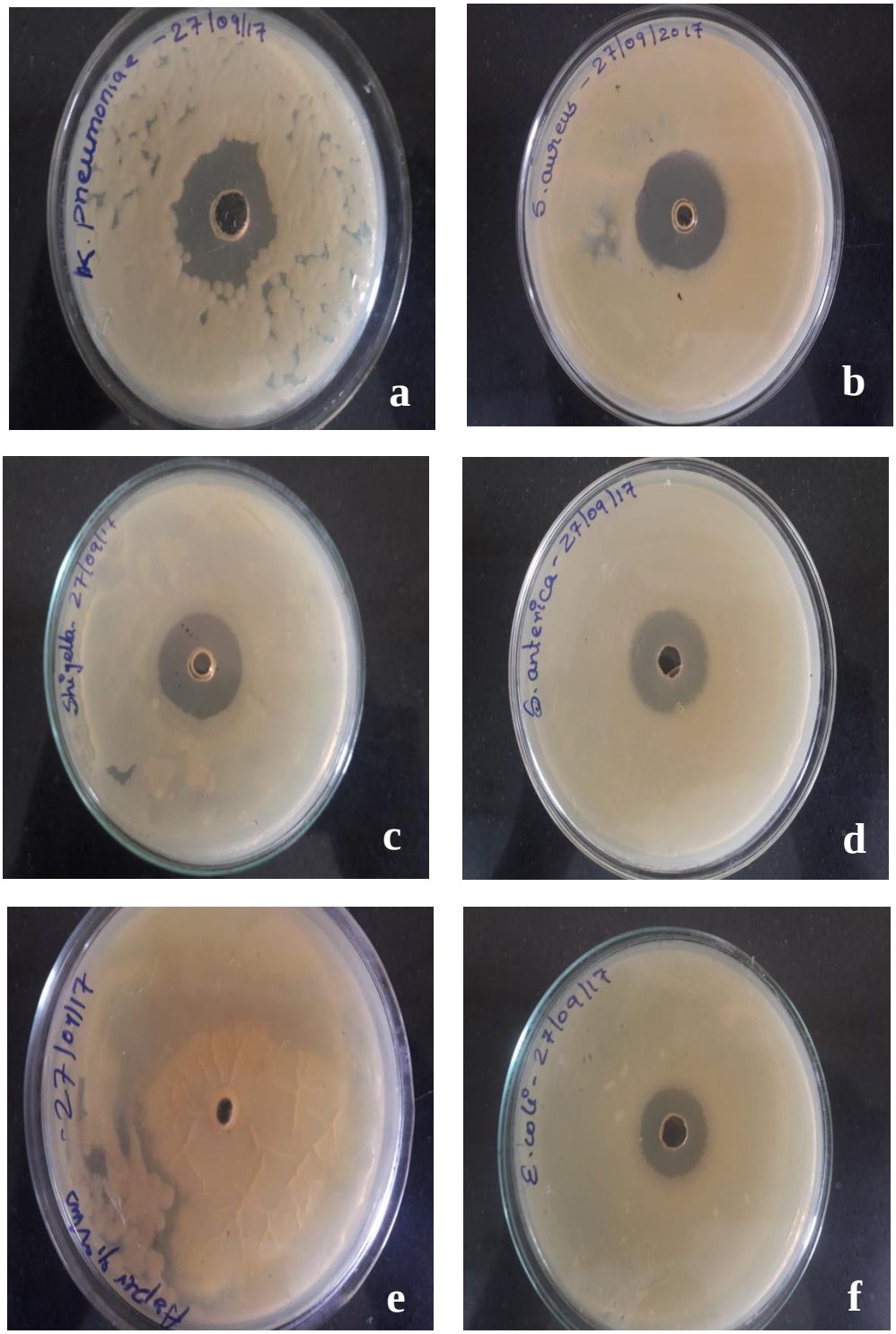


Plate 5.13: Antimicrobial activity of *Setosphaeria monoceras* against a: *Klebsiella pneumonia*; **b:** *Steaphylococcus aureus;* **c:** *Shigella boydii;* **d:** *Salmonella enterica*; **e:** *Aspergillus brasiliensis*; **f:** *Escherichia coli.*

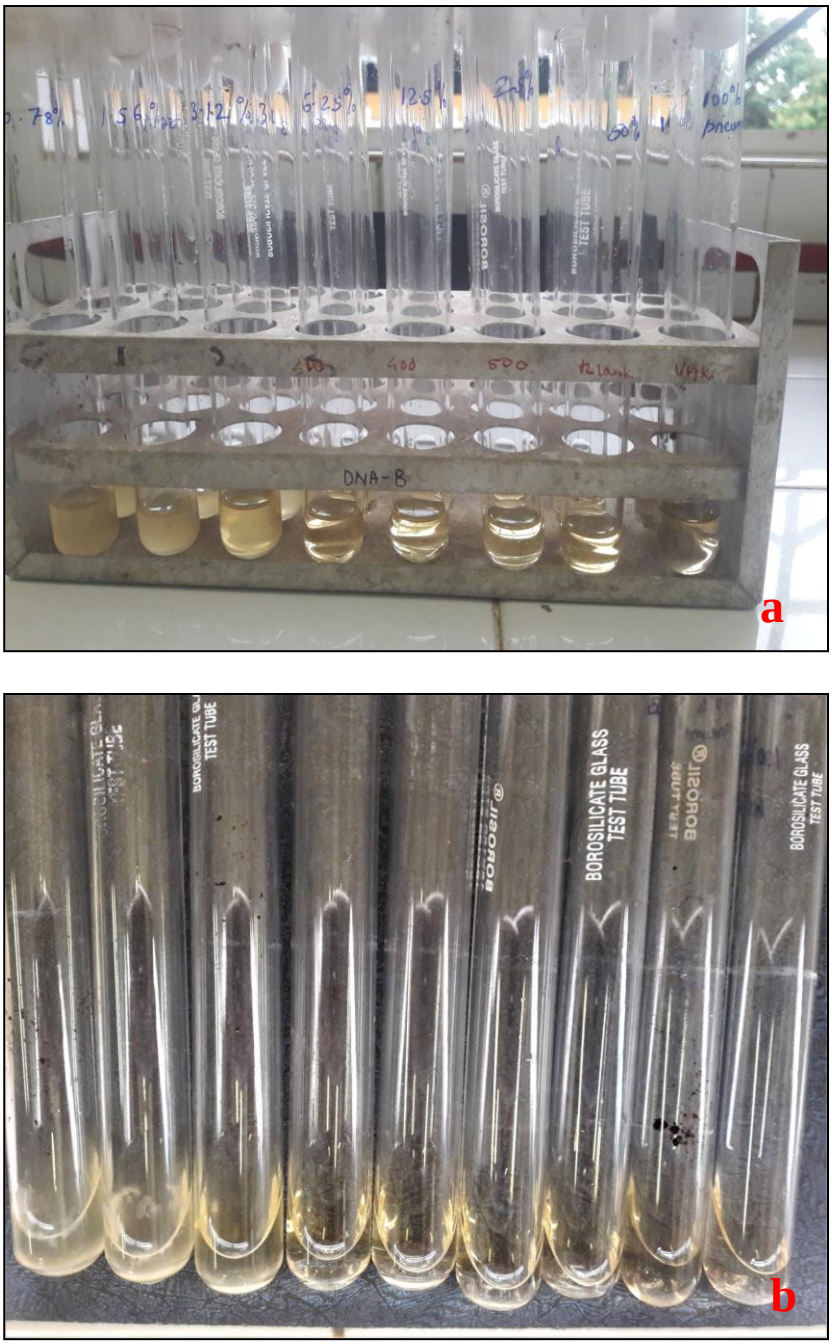


Plate 5.14: Minimum inhibition concentration (MIC) of *Setosphaeria monoceras.* a-b: *S. monoceras* - MIC tubes extract after 24h and 48h of incubation

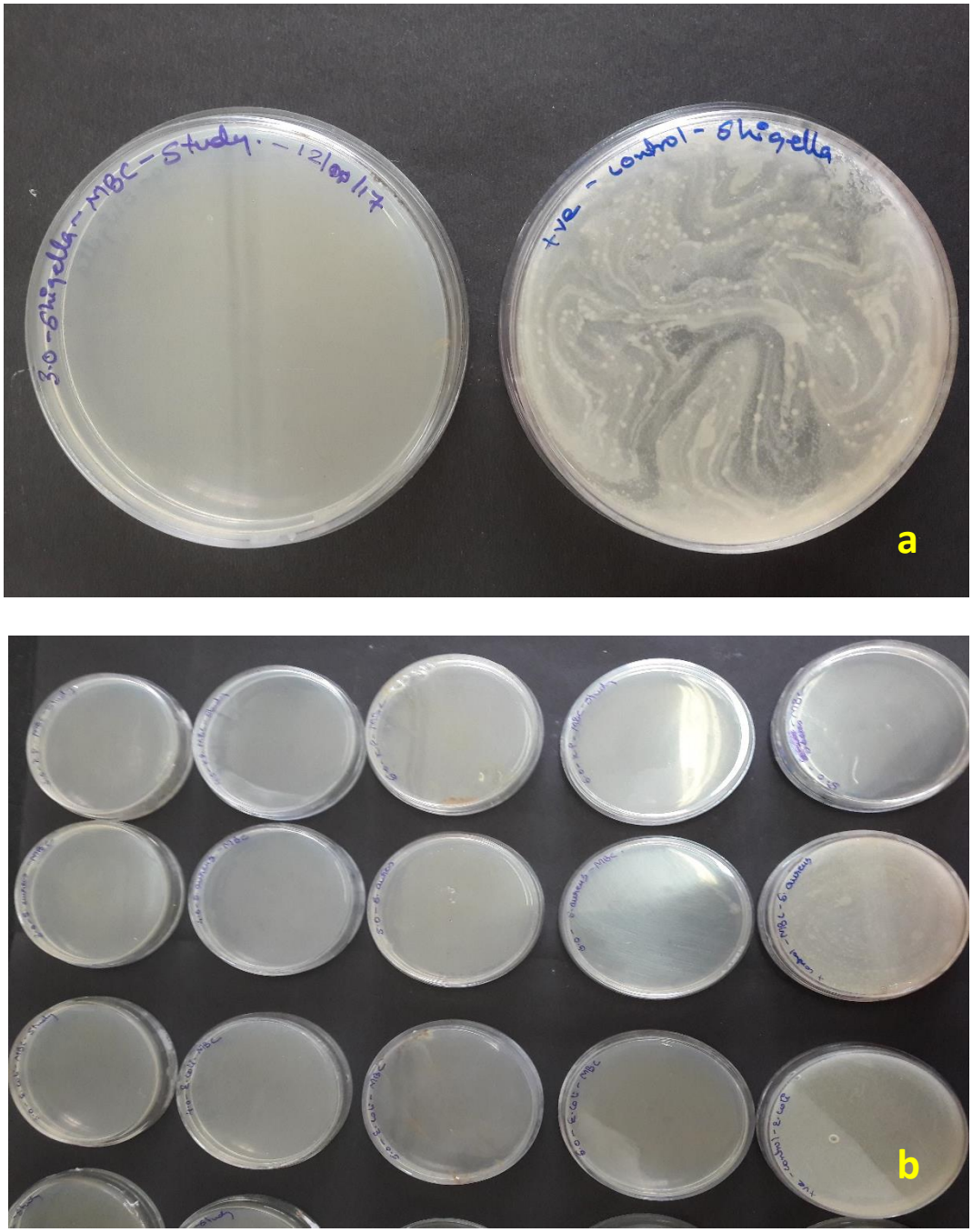
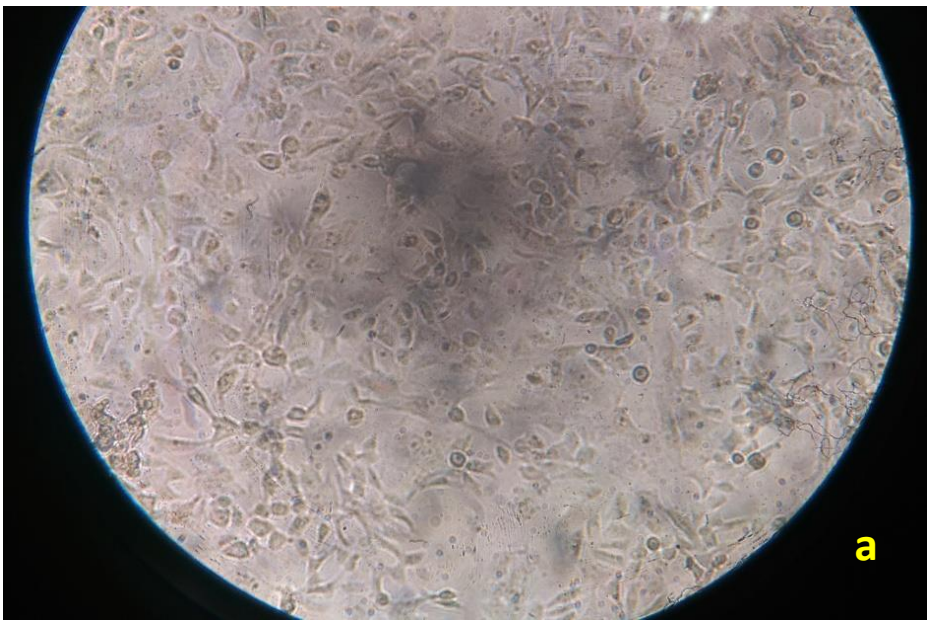


Plate 5.15: Bactericidal property (MBC) of *Setosphaeria monoceras* against a: *Shigella boydii***; b:** Test pathogens.



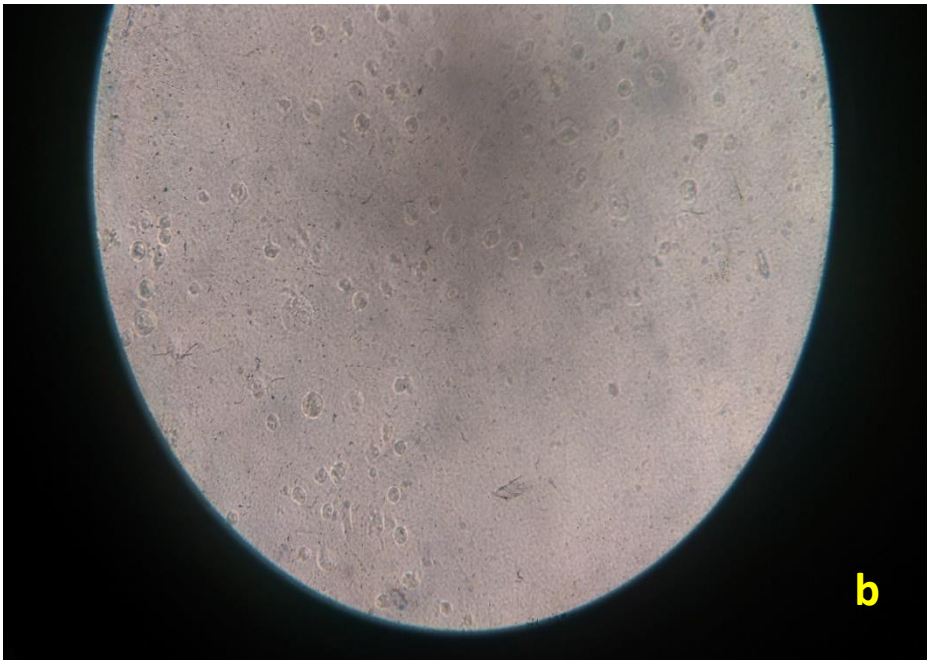


Plate 5.16: Anti-cancer activity of *Setosphaeria monoceras.* a: A549 lung cancer cell line; **b:** A549 lung cancer cell line treated with crude endophytic extract of *S. monoceras*.