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Isolation and morphological characterization of endophytic fungi isolated from mangrove plants along the Kenyan coastline

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Microorganisms in marine ecosystems are exposed to harsh conditions, thus such systems are of interest in bioprospecting for useful secondary metabolites. The aim of the study was to isolate and identify the fungal endophytes that colonize mangroves. The fungal endophytes were isolated from the leaves, roots, and branches of different mangrove plants (*Bruguiera gymnorrhiza*, *Heritiera littoralis*, *Xylocarpus granatum*, *Rhizophora mucronata,* and *Avicennia marina*) from Mida creek, Tudor creek and Gazi bay using Point-Centered Quarter Sampling method and then, morphologically characterized. A total of 76 fungal isolates were obtained and identified using macro- and micro-morphological features. The isolates were affiliated with eight different genera (*Aspergillus, Cladosporium, Nigrospora, Fusarium, Alternaria, Lasiodiplodia, Chaetomium and Penicillum*). *Aspergillus* spp. were the most prominent with a colonization frequency of 38.9 and 55.6% in root and branch tissues, respectively while *Chaetomium* species were the least frequent appearing only in one branch tissue. Mida creek had the highest total number of endophytic isolates (52.6%) followed by Gazi bay (27.6%). Majority (30.3%) of the endophytic fungal isolates were obtained from *Avicennia marina.* The results indicate that mangrove species are a source of diverse endophytic fungi that may have useful biotechnological applications.

Key words: Mangrove species, endophytic fungi, fungi diversity, colonization frequency.

INTRODUCTION

In Kenya, Mangrove forests cover approximately 61,271 ha and are estimated to make up 3% of the total area occupied by natural forest (Kairo et al., 2017). The forests are spatially fragmented, spanning from Vanga to Lamu along the Kenyan Coastal strip (Kirui et al., 2011). Mangroves are known to play a significant role in

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providing nursery habitat and shelter for juvenile fish, sequester carbon (Chmura et al., 2003), and protect the shoreline from soil erosion and tsunamis (Kathiresan and Rajendran, 2005). Nine species of mangroves (*Rhizophora mucronata, Bruguiera gymnorhiza, Ceriops tagal, Sonneratia alba, Avicennia marina, Lumnitzera racemose, Xylocarpus granatum, Xylocarpus moluccensis,* and *Heritiera littoralis)* have been recorded in Kenya (Abuodha and Kairo, 2001).

Harsh conditions (saline and low oxygen) make mangrove ecosystems ideal in the hunt for novel and unique endophytic fungi (Debbab et al*.,* 2010). Endophytic microorganisms are fungi and bacteria that colonize inter- or intra-cellular spaces of plant tissues during at least one phase of their life cycle as described by Compant and Vacher (2019). Endophytic microorganisms can protect the hosts against several biotic and abiotic factors, such as the attack of insects, pathogens, and herbivores (Bamisile et al., 2018). The interaction between plants and endophytic micro-organisms produce several substances of biotechnological interest. For instance, it has been reported that endophytic fungi are known to produce secondary metabolites with bioabsorption systems application in removing heavy metal ions from water. *Aspergillus niger* has been shown to remove lead, cadmium, copper, and nickel ions from wastewater (Ling et al., 2016). Also, it has pharmaceutical application in the production of antimicrobials that inhibit the development of pathogens (Rao et al., 2020). Fungal endophytes colonize the interior parts of healthy plant tissues without causing symptoms of a disease (Kaul et al., 2014).

Plants and microorganisms provide a leading source of natural products with desirable bioactive properties. Fungi are among the most significant eukaryotic organisms that are being explored for their bioactive secondary metabolites in pharmaceutical applications (Keller, 2019). Previous studies suggest that geographical, rather than plant-linked factors contribute to the composition of endophytes in plants (Cannon and Simmons, 2002). Endophytic fungi have been reported to be a source of anti-inflammatory, antibacterial, antiviral, antitumor, antifungal, and other substances found in terpenoid, alkaloid, flavonoid, and steroid extracts (Selvakumar and Panneerselvam, 2018). Mangrove endophytic fungi constitute the second largest group of marine endophytes (Sridhar et al., 2012) with their leaves harboring more diverse fungal endophytes community compared to other parts of the plant (Hamzah et al., 2018). Endophytic fungi have been reported to be abundant in all tissues such as flowers, fruits, stems, roots, and leaves that are potential sources of natural products (Rana et al*.,* 2019). For instance, nigerasterol A and B compounds obtained from *A. niger,* an endophytic fungus residing inside the inner tissues of *Avicennia marina* collected in Hainan Island, China showed cytotoxic activities against the human A549 cell line with

IC_{50} values of 1.82 µM (Deshmukh and Prakash, 2018). Endophytic fungi isolated from sponges, seaweeds, and plants from the marine environment have also been reported to produce secondary metabolites with therapeutic potential (Kusam et al., 2019). Mangrove endophytes have been identified as potential producers of novel molecules with diverse biological activities (Deshmukh and Prakash, 2018). Therefore this study aimed to characterize the endophytic fungi that colonize some selected mangrove species which will consequently enhance the conservation and value of the mangroves.

MATERIALS AND METHODS

Study sites

This study was conducted on three creeks along the Kenyan Coastline Tudor, Mombasa County (040 00 ' 18.2" S, 0390 38' 17.1" E) located on Mombasa Island and its surround. The Creek extends to about 10 km inland and bounds Mombasa Island on the North West. It is fringed by well-developed mangrove forests composed mainly of *R. mucronata and A. marina* species. The area has human settlements with rural villages that are sparsely populated and lacks formal infrastructures such as sewage and solid waste handling facilities (Mohamed et al*.,* 2008).

Mida creek is situated about 100 km North of Mombasa with its mangrove area estimated to cover 1657.8 ha. The creek has no overland freshwater input and hence, benefit from a high groundwater flow. The area (030 21' 07.5" S, 039 0 56' 30.1" E) is composed of *Ceriops tagal*, *R. mucronata* and *A. marina* as the dominant species which grow on mangrove swamps in soils that are excessively saline, deep, and poorly drained (Alemayehu *et al.,* 2014). Gazi bay is located about 55 km south of Mombasa area (040 25' 09.1" S, 039 0 30' 41.0" E) and is sheltered from storms by a coral reef to the South and Chale Peninsula to the East (Maina et al*.,* 2008). These two natural barriers support the mangroves' growth in the protected bay. The area is surrounded by 6.2 km^2 of mangroves (Hoberg, 2011) (Figure 1).

Sample collection

Sampling was done according to Mitchell (2010) with some modifications during the low tides of the day in the dry season (July) on sunny afternoons. The Kenya Marine and Fisheries Research Institute (KMFRI) High-Low and Hourly Tide Predictions chart was used for planning the sampling time (2019 High-Low and Hourly Tide Predictions for Mombasa and Lamu, 2019). A transect of 150 m was established in each mangrove forest cluster and a prop placed every 50 m to represent the center of four compass directions thus dividing the sampling sites into four quarters. In each quarter, the distance from the prop to the nearest large mangrove tree was measured and recorded. A total of 16 trees were recorded in each transect. With the help of a botanist, the selected mangrove trees were identified and recorded. The mangrove species samples were; *B. gymnorrhiza* (11)*, H. littoralis* (8)*, X. granatum* (8), *R. mucronata* (11), and *Avicennia marina* (10)*.* Five healthy leaves, two aerial branches, and two submerged roots were randomly selected and cut using sterile shears. The samples were further chopped into 5 cm pieces before being packed into labeled sterile Ziploc® bags. All samples were transported to Kenya Marine and Fisheries Research Institute (KMFRI, 2019), Mombasa in cooling boxes and then stored in a refrigerator at 4°C until analyses.
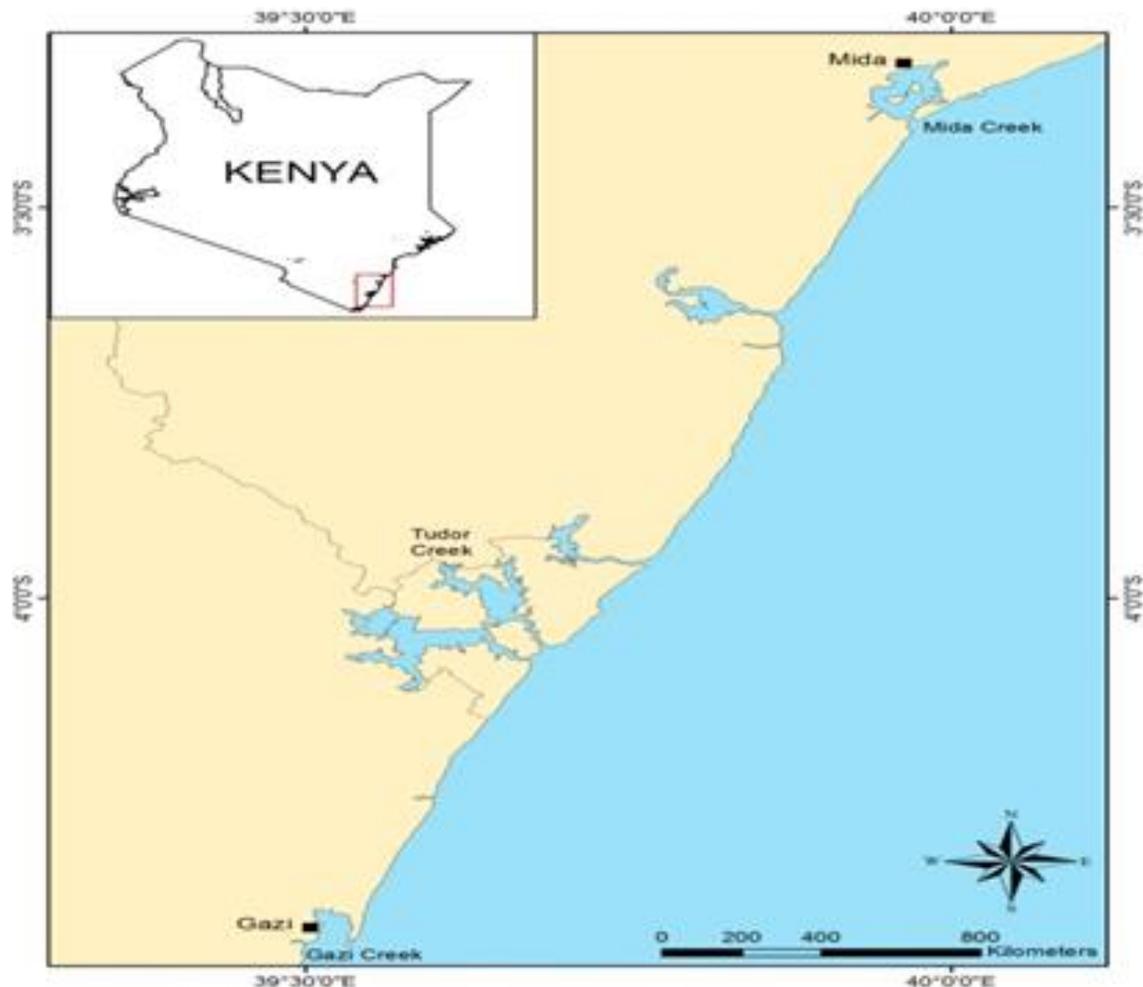
Figure 1. Map of Kenyan coastline showing the locations of selected study sites where mangroves grow.

Sample preparation

The samples were washed with running water for 2 h to remove mud and debris before rinsing thoroughly with sterile distilled water. All the sample surfaces were sterilized by immersing in 75% ethanol for 1 min followed by 5% sodium hypochlorite for 3 min (leaves) and 5 min (branch and root), 75% ethanol for 30 s and finally rinsed three times with sterile distilled water. The leaf samples were aseptically cut into small pieces of approximately 5 × 5 mm while the root and branch samples were cut into 1 cm cross-section and further split longitudinally to expose the interior section under a biological safety cabinet to prevent contamination (Liu et al., 2007).

Isolation of fungi

Sterile Potato Dextrose Agar (PDA) (HiMedia, Mumbai, India) was the medium of choice for this project since it is a general-purpose medium for fungi isolation and was prepared according to the manufacturer's instructions (39 g in 1 L distilled water). The pH of the medium was adjusted to about 4.8, which is low enough to inhibit most bacteria growth by the addition of lactic acid (PDA; with 1:100 lactic acid). Pieces of each sample were placed on labeled PDA Petri plates in triplicates with 3 negative controls and incubated at room temperature for 5 days. Each distinct fungal colony was sub-cultured onto a fresh labeled PDA Petri plate twice to obtain pure isolates which were inoculated into 50 ml sterile Potato Dextrose Broth (TM Media, Rajasthan, India) (24 g in 1 L sterile seawater) and incubated for two weeks at room temperature. For the highest viability when reviving fungal isolates, 80% sterile glycerol was prepared, and an aliquot of 1 ml fungal culture broth and glycerol each were mixed, labeled, and stored at -86°C (Mwamburi et al., 2019).

Morphological characterization of isolates

The pure fungal isolates were grouped based on their morphological features (color, texture) and growth rate. Microscopic analysis was carried out using Lacto phenol cotton blue stain. Observations were made using an Image analyzer microscope (Primo Star ZEISS, JenaGermany) supported by Axiocam camera (ERc5s). The following features were observed, recorded and captured; -type of hyphae, mycelium color, type of spores, characteristics of hyphae and sporangia, features of conidia and arrangement of sporangiophore and conidiophores. Identification of the fungal isolates was aided by an identification guide (Dugan, 2017).

Data analysis

The colonization frequency (CF%) of endophytic fungi was calculated using the formula according to Deepthi et al. (2018). One-way ANOVA was performed to test whether there were significant differences in the colonization rates between the different genera of endophytic fungi obtained from the mangrove species.

RESULTS AND DISCUSSION

Endophytic fungal isolates (76) were obtained from five mangrove species namely; *Bruguiera gymnorrhiza,* *Rhizophora mucronata*, *Avicennia marina, Xylocarpus granatum*, and *Heritiera littoralis* species. All the negative controls did not show the growth of fungi indicating the absence of contamination in the sample preparation and subsequent inoculation steps. Of the 76 fungal isolates recovered, 17.1% were from leaf samples, 59.2% from branch samples, and 23.7% from root samples (Figure 2). Initially, fungal colonies were grouped according to color, shape, and topography followed by microscopic examination of the isolates for characterization of hyphae, mycelium color, type of spores, sporangia,

$$CF\% = \frac{\text{Number of plant tissue colonized by each endophytic fungi (N}_{col})}{\text{Total number of plant tissue studied (N}_{t)}} \times 100$$

conidia, sporangiophore and conidiophores arrangement (Figure 3). This examination led to the classification of the 76 isolates into 8 genera (*Aspergillus, Cladosporium, Nigrospora, Fusarium, Alternaria, Lasiodiplodia, Chaetomium,* and *Penicillum*).

A total of eight isolates were placed in the genus *Alternaria* based on their macro- and micro-morphological features. The colony ranged from white to brown in colour on PDA media at 27°C and was relatively rapid-growing. Some of the white colonies were covered in black or yellow pigmentation in the middle while the brown colonies appeared white at the beginning and later darkened to brown by day 14. These isolates belonging were distinguished by brown hyphae with brown septate conidiophores (Figure 7a). The conidia were branched and large (Meena et al., 2017). These isolates were obtained from leaf and branch tissues of *B. gymnorrhiza, R. mucronata, A. marina,* and *X. granatum* species and were found in the three study sites. Isolates have also been obtained from *R. mucronata* species in the Malaysian mangrove forest (Hamzah et al., 2018) .The genus *Alternaria,* have commercially important species, and is one of the most common fungal genera found ubiquitously (Tibpromma et al., 2018). This genus displayed a high divergence in culture and morphology. In addition, the genus has been reported to produce important bioactive compounds useful in the pharmaceutical industry (Yadav et al., 2019).

In the genus *Penicillium*, five isolates were identified based on the observed macro- and micro-morphological features. The colony ranged from grey to brown in color on PDA media at 27°C. Grey colonies were rapidly growing and were observed by day 3 and appeared to be cottony in texture. The growth rate of the brown colonies was slow and was observed by day 7. The genus *Penicillium* was distinguished by septate hyphae, conidiophores, metulae, phialides, and conidia (Figure 7b). Metulae branches that formed on conidiophores with attached flask-shaped phialides were also observed. These results were consistent with a report by Liu et al. (2007). *Penicillium* was isolated from leaf and root tissues of *B. gymnorrhiza* and *A. marina* species from Mida and Tudor Creeks along the Kenyan coast. The genus has also been isolated from *A. marina* and *R. mucronata* species in mangrove plants of Northeast Brazil (Costa et al*.,* 2012). *Penicillium* is a diverse genus belonging to the Ascomycota phylum which occurs worldwide. Its species play important roles as decomposers of organic materials, destructive rots in the food industry, and source of important drugs (Visagie et al., 2014). *Penicillium,* from earlier studies has been found to produce important bioactive compounds with anticancer, antibacterial, antifungal, and cytotoxic activities (Yadav et al., 2019).

Eight isolates were placed in the genus *Fusarium* based on the observed macro- and micro-morphological features. The colony of the isolates ranged from white, brown to purple, and white in PDA media at 27°C. The growth rate of the colonies was slow and appeared on day 6. They were observed to be cottony in texture. The purple and white colonies showed a white ring formed around the purple colony. Members from this genus *Fusarium* were distinguished by branched conidiophores and sickle-shaped macro conidia (Figure 8a) as observed by Munkvold (2017) and (Deepthi et al., 2018). *Fusarium* was isolated from leaf, root, and branch tissues of *B. gymnorrhiza, R. mucronata, A. marina,* and *H. littoralis* species. It was found in Mida Creek and Gazi Bay along the Kenyan coast. The genus *Fusarium* has also been isolated from *R. mucronata* species in the Malaysian mangrove forest (Hamzah et al., 2018) and in *A. marina* from two different locations of Red Sea mangrove forest (Sea and Shebany, 2012).The genus *Fusarium* includes numerous toxigenic species that are pathogenic to plants or humans and can colonize a wide range of niche. The genus comprises around 70 described species and is also one of the most economically important fungal genera because of yield loss due to plant pathogenic activity (Liu et al., 2007). *Fusarium* has been found to produce bioactive compounds of importance in the medical industry. These include anticancer antimicrobial, immunosuppressive and insecticidal compounds
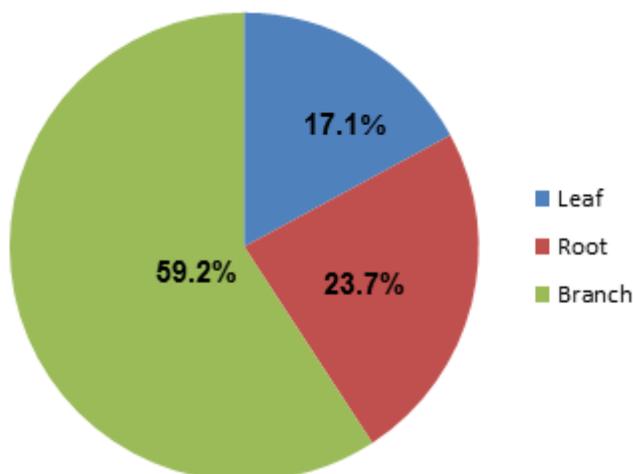
Figure 2. Endophytic fungi (%) isolated from different parts of a mangrove tree.

(Rana et al., 2019).

Thirty-five isolates of the endophytic fungi were placed into the genus *Aspergillus* based on the observed macro- and micro-morphological features. The colony of the isolates ranged from grey, green, brown, white, black to brown, and grey in PDA media at 27°C. The colonies grew rapidly and appeared by day 3 and were powdery in texture. The brown and grey colonies formed a grey ring around the brown colony. The genus *Aspergillus* was distinguished by the unifying feature of the asexual reproductive structure, vesicle formation, and septate hyphae (Figure 8b). The phialides appeared to be flask-shaped and covered the surface of the vesicle. Above the phialides, the conidia formed radial chains (Liu et al., 2007; Makhuvele et al., 2017).

Aspergillus species were isolated from leaf, root, and branch tissues of *B. gymnorrhiza, R. mucronata, A. marina, H. littoralis,* and *X. granatum* species from all the study sites. The genus *Aspergillus* has also been isolated from two different locations of the Red Sea mangrove forest (Sea and Shebany, 2012) and in *A. marina* from the mangrove rich area of Thazhekavu in Madakkara (Gilna and Khaleel, 2011). *Aspergilli* show a large taxonomic divergence in terms of their morphology (Varga and Samson, 2008). They have been reported as the most dominant endophytic fungal inhibiting internal plant tissues and are an enormous source of chemical compounds with promising biological activities (El-Hawary et al., 2020). Some members of this genus are used in the fermentation industry but are also responsible for various plant diseases (Perrone et al., 2007). *Aspergilli* grow in a wide range of habitats, mostly in soil, dead matter and some are capable of colonizing living animal or plant hosts. In total, approximately 350 species have been identified in this genus (Samson et al., 2014). *Aspergillus* has been reported to be useful in the

pharmaceutical industry as a source of anticancer, antitumor, cytotoxic, and antimicrobial compounds (Rana et al., 2019).

In the *Cladosporium* genus, ten isolates were identified based on the observed macro- and micro-morphological features. The colony of the isolates ranged from greyish white to brown in PDA media at 27°C. The growth rate of colonies was slow and appeared by day 7. The texture was observed to be velvety. Septate brown hyphae, erect and pigmented conidiophores and conidia were observed (Figure 9a). The conidia were brown and appeared in branching chains (Wijayawardene et al., 2017). *Cladosporium* was isolated from leaf, root, and branch tissues of *B. gymnorrhiza, R. mucronata,* and *A. marina species.* It was found in Mida and Tudor Creeks along the Kenyan coast. The genus *Cladosporium* has also been isolated from *R. mucronata* species in the Malaysian mangrove forest (Hamzah et al., 2018). It is a large genus comprising species that are saprobes, endophytes, and pathogens (Tibpromma et al., 2018). *Cladosporium* has also been reported to produce antifungal compounds (Selvakumar and Panneerselvam, 2018).

One isolate was identified in the genus *Chaetomium* based on the observed macro- and micro-morphological features. Colony colour ranged from white to chocolate-brown in PDA media at 27°C while the growth rate was slow and appeared by day 7. The colony texture was powdery with non-sporulating hyphae (Figure 9b). Terminal hairs were brown with paler tips, wavy or loosely coiled, and intertwined (Zhai et al., 2018). *Chaetomium* was found in only one of the study sites; Gazi Bay along the Kenyan coast. It was isolated from a branch tissue of *A. marina.* The genus *Chaetomium* has also been isolated from two different locations in the Red Sea mangrove forest (Sea and Shebany, 2012). It has been reported to produce secondary metabolites with
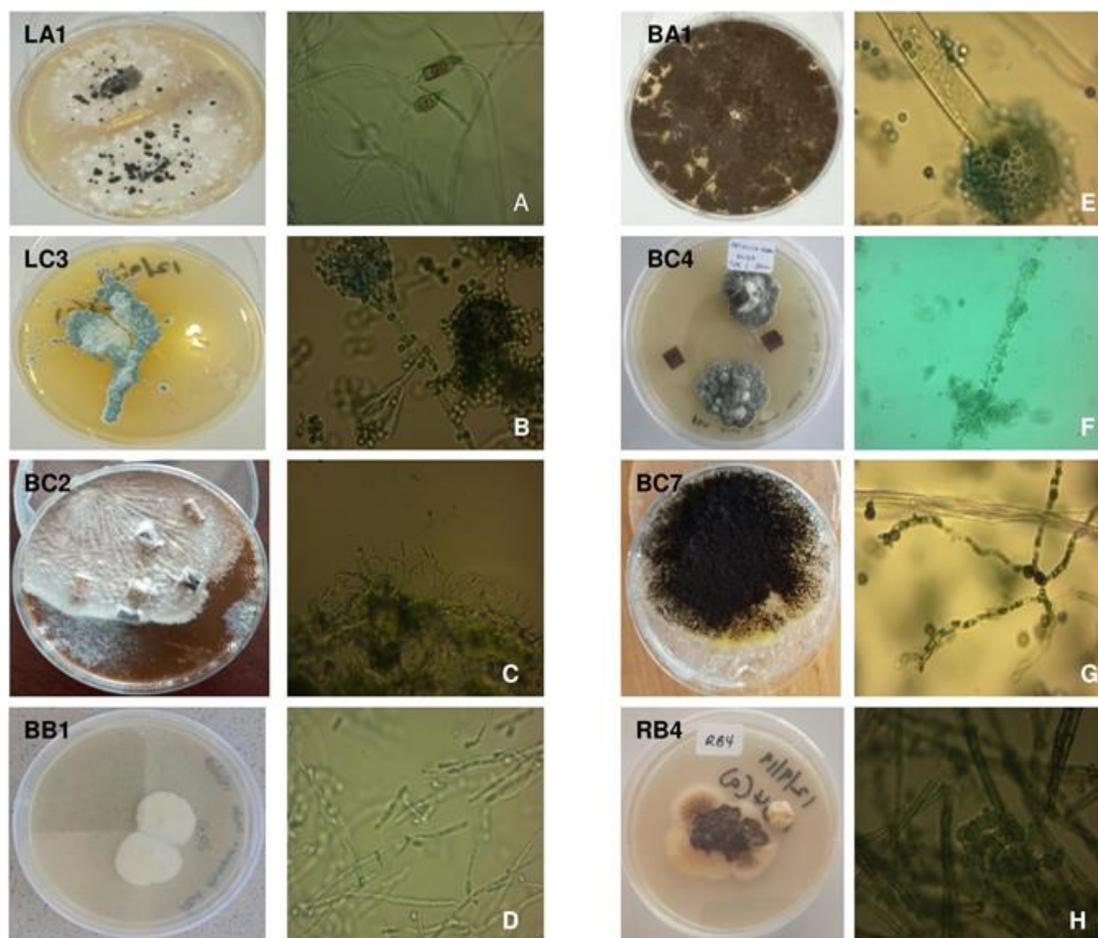
Figure 3. Endophytic fungi isolated from the leaves, roots and branches of mangrove species. Shown here are some representatives of the fungal isolates from the 8 genus identified; **a:** *Alternaria;* b: *Penicillum;* c: *Chaetomium;* d: *Fusarium;* e: *Aspergillus* f: *Cladosporium*; g: *Nigrospora;* h: *Lasiodiplodia.* All fungal isolates were from the inside tissues (leaf, root and branch) of the mangrove species and cultivated on Potato Dextrose Agar media for 7 to 15 days at 27°C.

potential bioactivity (Selvakumar and Panneerselvam, 2018).

Some six isolates were placed in the genus *Nigrospora* based on the observed macro- and micro-morphological features. Colony colour ranged from grey, white to black in PDA media at 27°C. The grey colonies were observed to be velvety, white colonies cottony, and black colonies powdery in texture. Septate mycelia and conidia which were on the swollen conidiophores were observed (Figure 10a) (Gond et al., 2007; Rathod et al., 2014 and Deepthi et al., 2018). *Nigrospora* was isolated from the root and branch tissues of *B. gymnorrhiza* and *A. marina species.* It was found in Mida Creek and Tudor Creek along the Kenyan coast. The genus *Nigrospora* has also been isolated from *A. marina* plant collected from different coastal areas of Pakistan (Tariq et al., 2006). *Nigrospora* is a monophyletic genus belonging to Apiosporaceae. The species in this genus are

phytopathogenic, endophytic, and saprobic on different hosts (Hao et al., 2020). Antifungal bioactive compounds have been found in *Nigrospora* (Rana *et* al.*,* 2019; Deshmukh and Prakash, 2018)*.*

Based on the observed macro- and micro-morphological features, 3 isolates were placed in the genus *Lasiodiplodia*. The colour of the colony ranged from white to brown in PDA media at 27°C and grew rapidly appearing on day 3. Observed to be woolly in texture, hyaline and brown conidia bearing longitudinal striations and conspicuous conidiomatal paraphyses were also observed (Figure 10b) (Abdollahzadeh et al. (2010)). The genus *Lasiodiplodia* was found in Mida and Tudor Creeks and was isolated from the root and branch tissues of *B. gymnorrhiza* and *A. marina* species. This genus has also been isolated from *B. gymnorrhiza* and *A. marina* species in South Africa (Osorio et al., 2017)*.* Deshmukh et al. (2018) reported the presence of bioactive
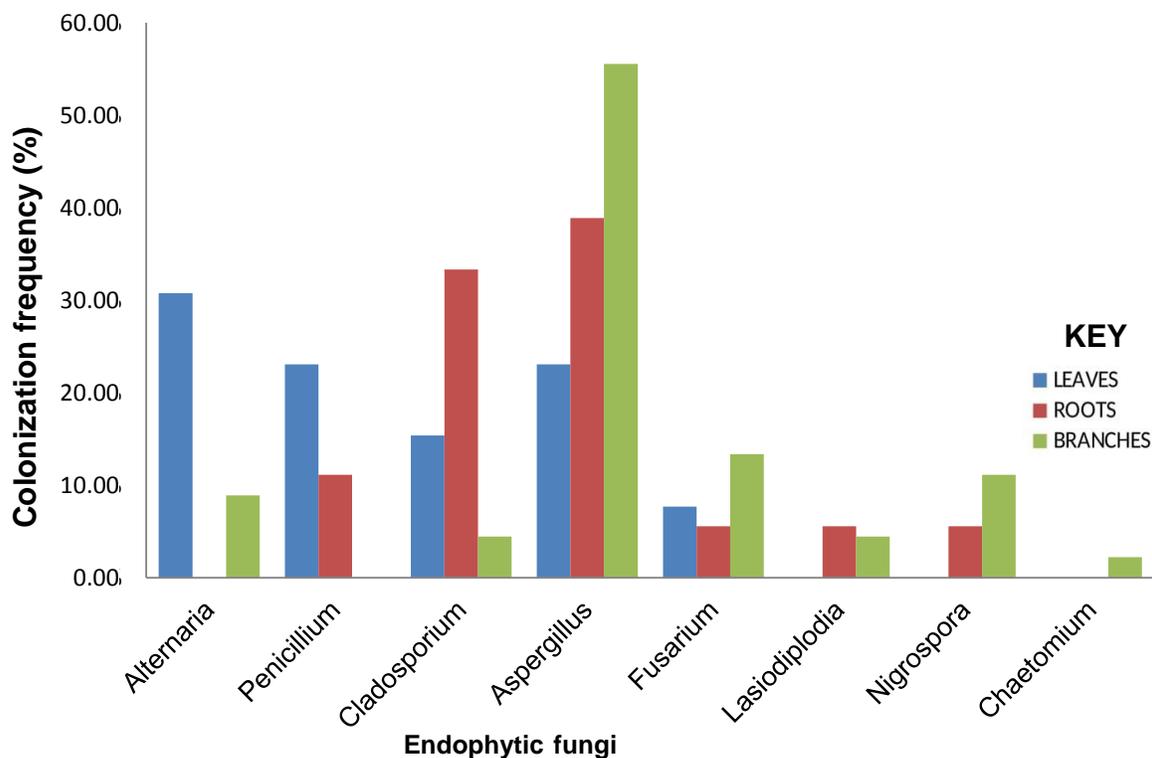
Figure 4. Colonization frequency of endophytic fungi on leaf, root and Branch tissues of mangrove species.

Table 1. Colonizing frequency of endophytic fungi isolated from mangrove trees.

No. of Isolates	Name of Endophytic Fungi (sp.)	Leaves		Roots		Branches	
		Colonization frequency (%)($N_{t=13}$)		Colonization frequency (%)($N_{t=18}$)		Colonization frequency (%)($N_{t=45}$)	
		(N_{col})	% CF	(N_{col})	% CF	(N_{col})	% CF
8	*Alternaria*	4	30.77	-	-	4	8.89
5	*Penicillium*	3	23.08	2	11.11	-	-
10	*Cladosporium*	2	15.38	6	33.33	2	4.44
35	*Aspergillus*	3	23.08	7	38.89	25	55.56
8	*Fusarium*	1	7.69	1	5.56	6	13.33
3	*Lasiodiplodia*	-	-	1	5.56	2	4.44
6	*Nigrospora*	-	-	1	5.56	5	11.11
1	*Chaetomium*	-	-	-	-	1	2.22

N_t = Total number of plant tissue studied; N_{col} = Number of plant tissue colonized by each endophytic fungi%; CF = Colonization frequency__= Absence of isolates. The isolates were coded (A-E)according to the mangrove species *B. gymnorrhiza, R. mucronata, A. marina, X. granatum,* and *H. littoralis,* respectively and part of the tree that was sampled (L, B & R), representing leaf, branch, and root, respectively.

compounds in *Lasiodiplodia* with medical importance. From this study, the overall mean colonization frequency in the genus *Aspergillus* was found to be significantly higher compared to the other genera identified (P<0.05) (Figure 4). *Alternaria* recorded the highest colonization frequency in leaves (30.8%) while *Aspergillus* had the highest colonization frequency in both branches and roots (55.6 and 38.9%, respectively). The genus *Chaetomium* had the least colonization frequency of 2.2% appearing only in a branch tissue (Table 1 and Figure 5).

Of the five mangrove species investigated, *A. marina* and *B. gymnorrhiza* recovered the highest number of endophytic fungal isolates at 30.3 and 26.3%, respectively,
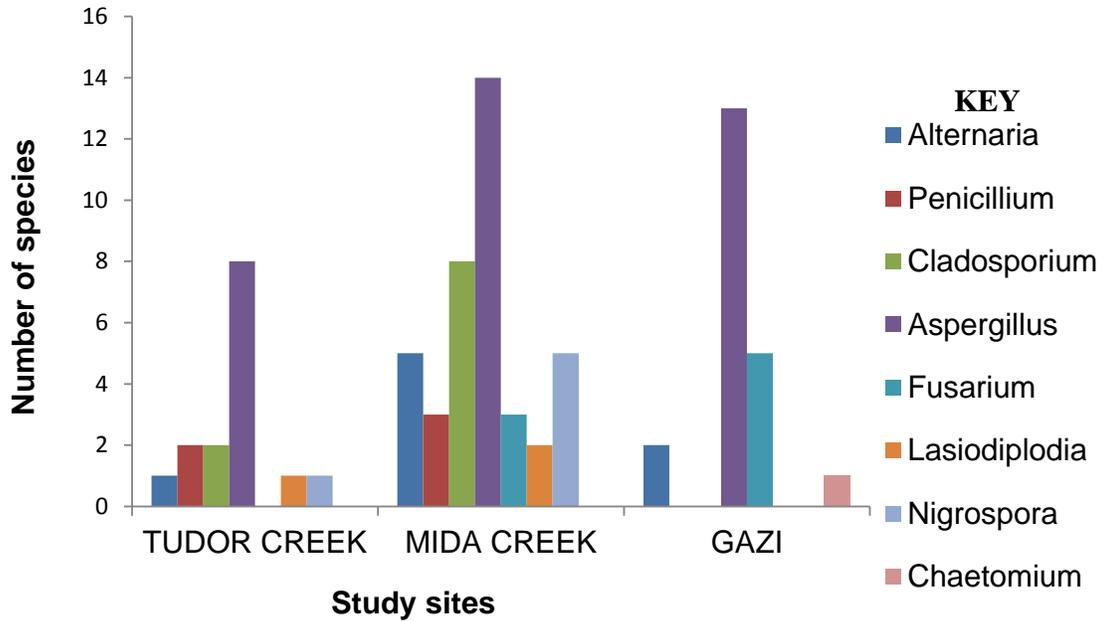
Figure 5. Mangrove endophytic fungi isolated from different sites of the Kenyan coast.
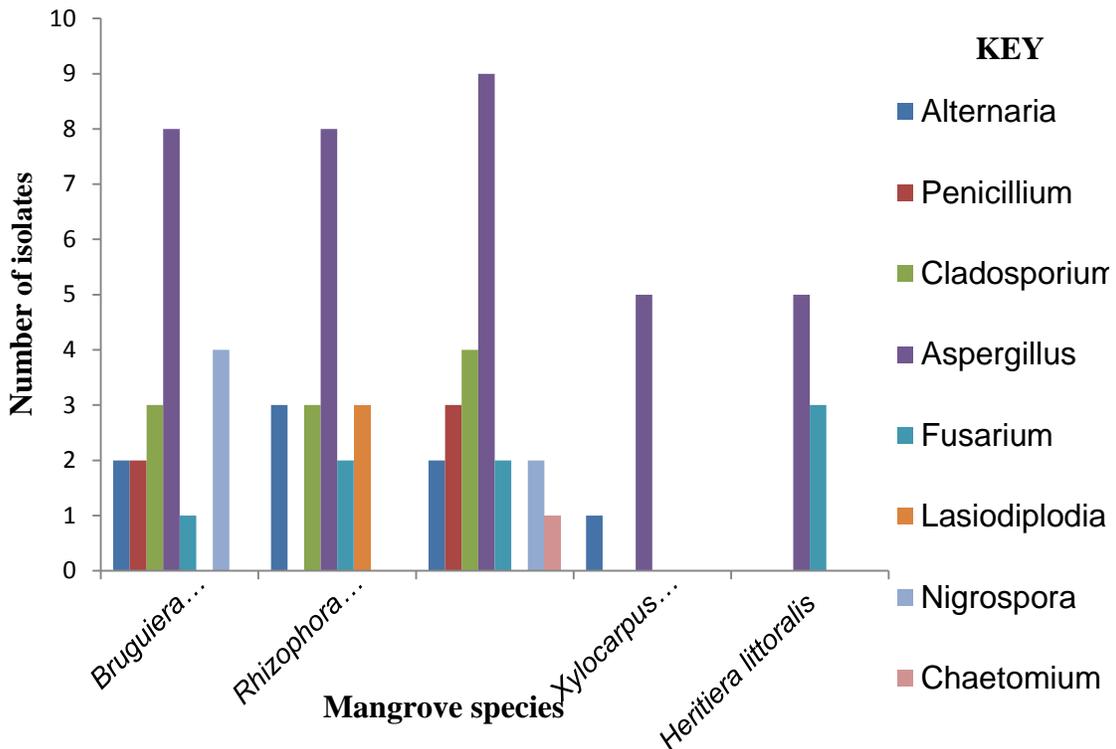

Figure 6. Mangrove endophytic fungi isolated from different mangrove species in the Kenyan coast.

while *X. granatum* had the least recovery rate of 7.9% (Figure 6). *Aspergillus* was dominant in the five mangrove species: *A. marina* (25.7%), *B. gymnorrhiza* (22.9%), *R. mucronata* (22.9%) and *H. littoralis* (14.3%). This was followed by *Nigrospora* from *B. gymnorrhiza* species (66.7%). *Penicillum* was isolated from *A. marina* (60%)
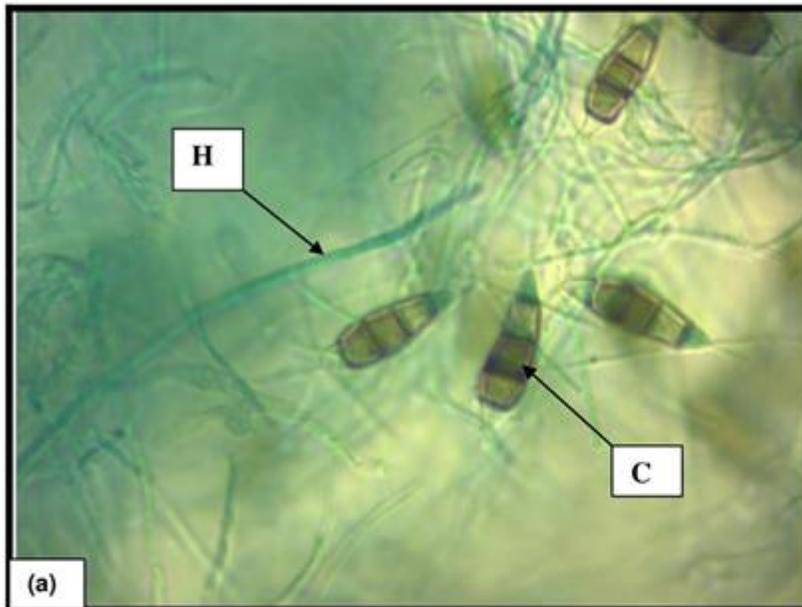
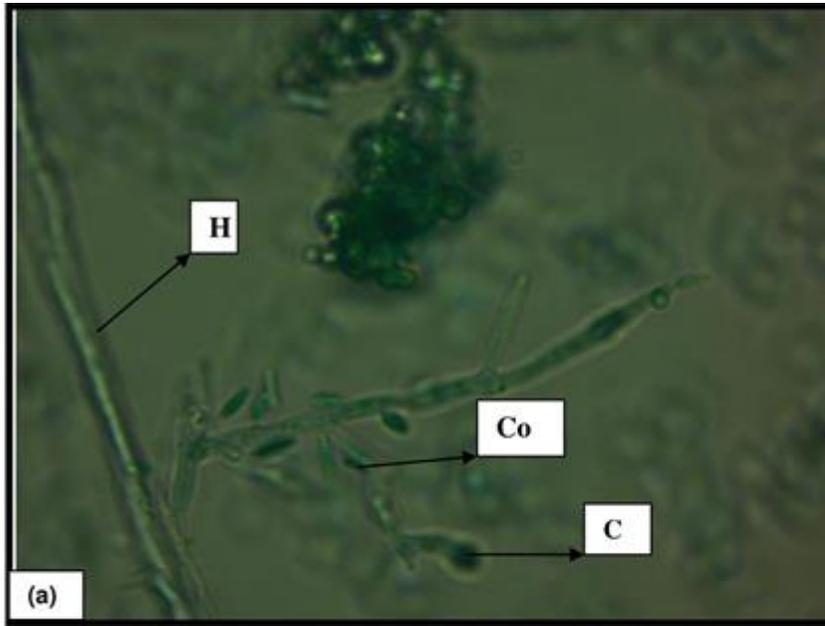**LB1**: Microscopic features of *Alternaria* sp. (10x 40X) H= Hyphae, C=conidia.
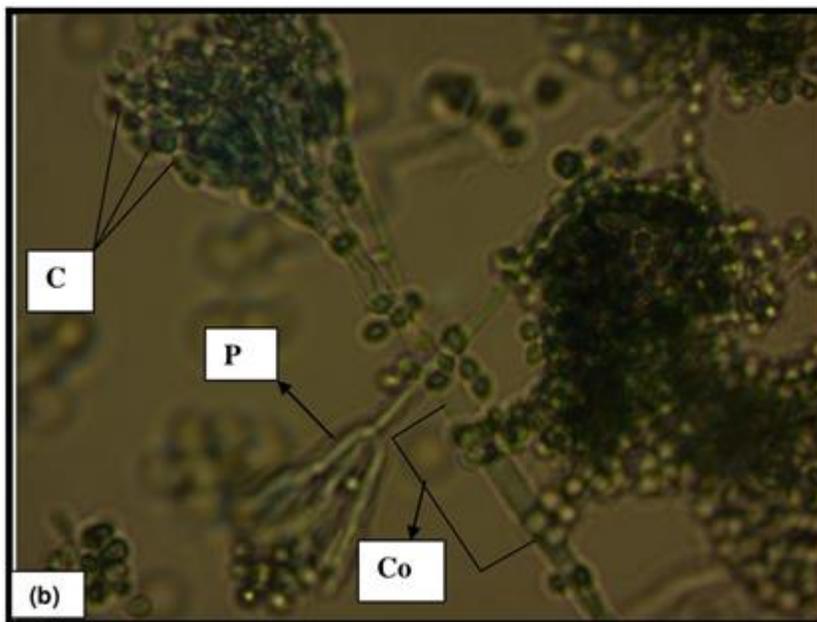
LC3: Microscopic features of *Penincillum* sp. (10x 40X) conidiophores bearing metulae and chains of conidia. C=Conidia, Co=Conidiophore, P=Philades

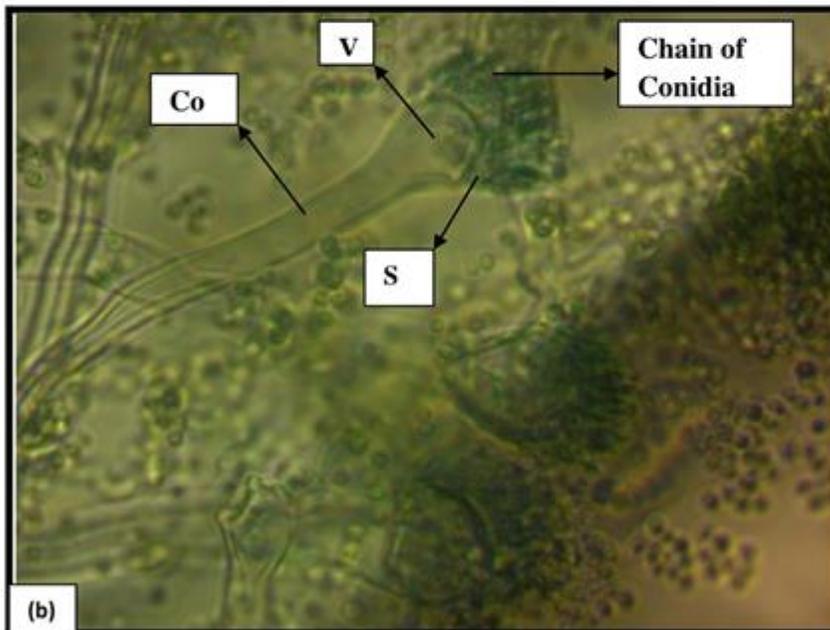**Figure 7.** (a) and (b) Microscopic features of Genus Alternaria and Penicillium respectively.

and *B. gymnorrhiza* (40%).

The genus *Alternaria* was recovered *from R. mucronata* (37.5%) and *X. granatum (*12.5%) while genus *Fusarium (*37.5%) was recovered from *H. littoralis.* The genus *Cladosporium* (30%) was isolated from *R. mucronata.*

The genus *Lasiodiplodia* and *Chaetomium* were recovered from *R. mucronata* and *A. marina*, respectively. Mida Creek had the highest number of fungal isolates (52.6%) as compared to Gazi bay (27.6%) and Tudor Creek (19.7%) (Figure 5). The factors influencing the variations

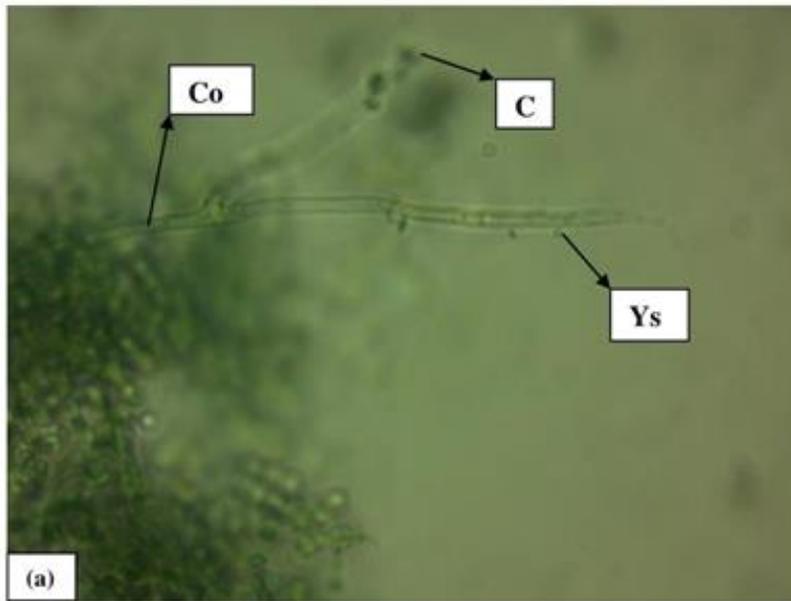RB3: Microscopic features of *Fusarium* sp. (10x40X) H=Hyphae, C=Conidia, Co= Conidiophore.

BE3: Microscopic features of *Aspergillus* sp. (10x 40X) conidiophores bearing sterigmata and chains of conidia C=Conidia, Co=Conidiophore, S=Sterigmata, V=Vescile.

Figure 8. (a) and (b) Microscopic features of genus *Fusarium* and *Aspergillus* respectively.

of these fungal species on different plant parts, mangrove trees and locations are still obscure.

Biodiversity analysis of endophytic fungi showed that all the recovered endophytes belong to the division Ascomycota and three classes (Figure 11). *Alternaria, Cladosporium* and *Lasiodiplodia* species belong to class

RC8: Microscopic features of *Cladosporium* sp. (10x 100X) Ys=Young spore, C=Conidia, Co=Conidiophore.
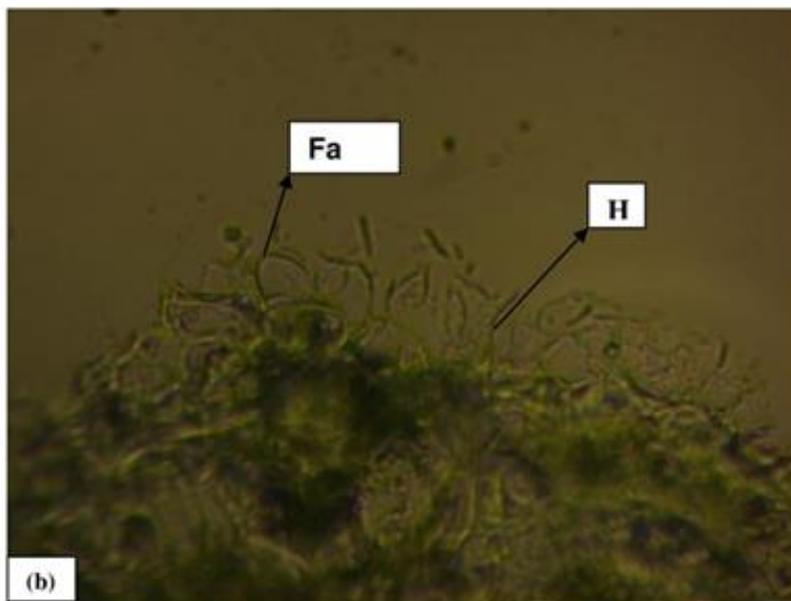
BC2: Microscopic features of *Chaetomium* sp. (10x 100X) Fa= Floccose aerial, H=Hyphae,

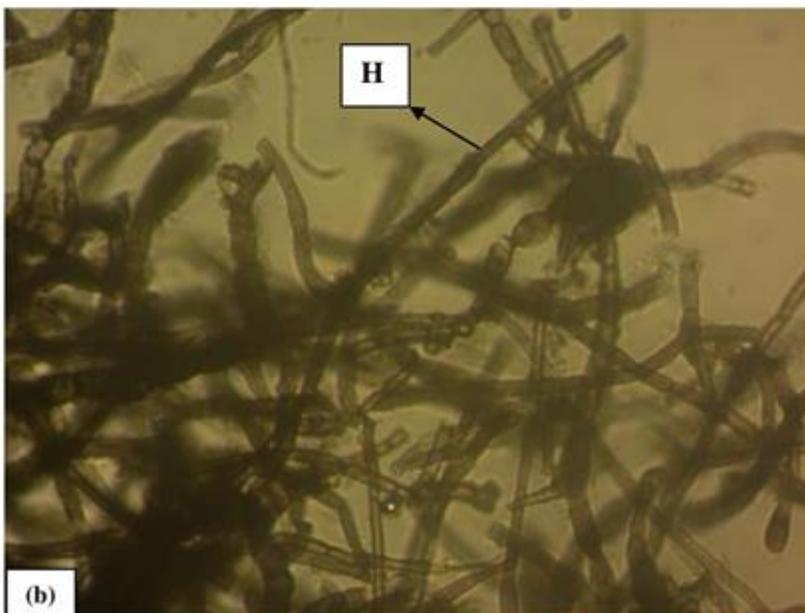**Figure 9.** (a) and (b) Microscopic features of Genus Cladosporium and Chaetomium respectively.

Dothiodeomycetes, *Fusarium, Nigrospora* and *Chaetomium* species belong to class Sordariomycetes while *Penicillium* and *Aspergillus* species belong to class Eurotiomycetes. Of the seventy-six recovered isolates, 40 belong to Eurotiomycetes, 21 to Dothideomycetes and 15 to Sordariomycetes.

Conclusion

In this study, the 76 endophytic fungi recovered were associated with eight genera (*Aspergillus, Cladosporium, Nigrospora, Fusarium, Alternaria, Lasiodiplodia, Chaetomium,* and *Penicillum), and were obtained* from

**BC7:** Microscopic features of *Nigrospora* sp. (10x 100X) C=Conidia, Co=Conidiophore

BB7: Microscopic features of *Lasiodiplodia* sp. (10x 100X) H=Hyphae.

Figure 10. (a) and (b) Microscopic features of Genus Nigrospora and Lasiodiplodia respectively.

leaves, roots, and branches of *B. gymnorrhiza, R. mucronata*, *A. marina, X. granatum*, and *H.* colonization frequency in both branches and roots (55.6% and 38.9%, respectively), while the genus *Alternaria* recorded the highest colonization frequency in leaves (30.8%). The results show the distribution of mangrove fungal endophytes within the studied sites and contribute

littoralis species. Eurotiomycetes was the most dominant fungal class. The genus *Aspergillus* had the highest to the growing inventory of mangrove endophytic fungi. Further characterization of mangrove fungal endophytes might offer valuable information about their biotechnological potential, a baseline for subsequent functional and bioprospecting studies.

Endophytic Isolates from Different Fungal Classes
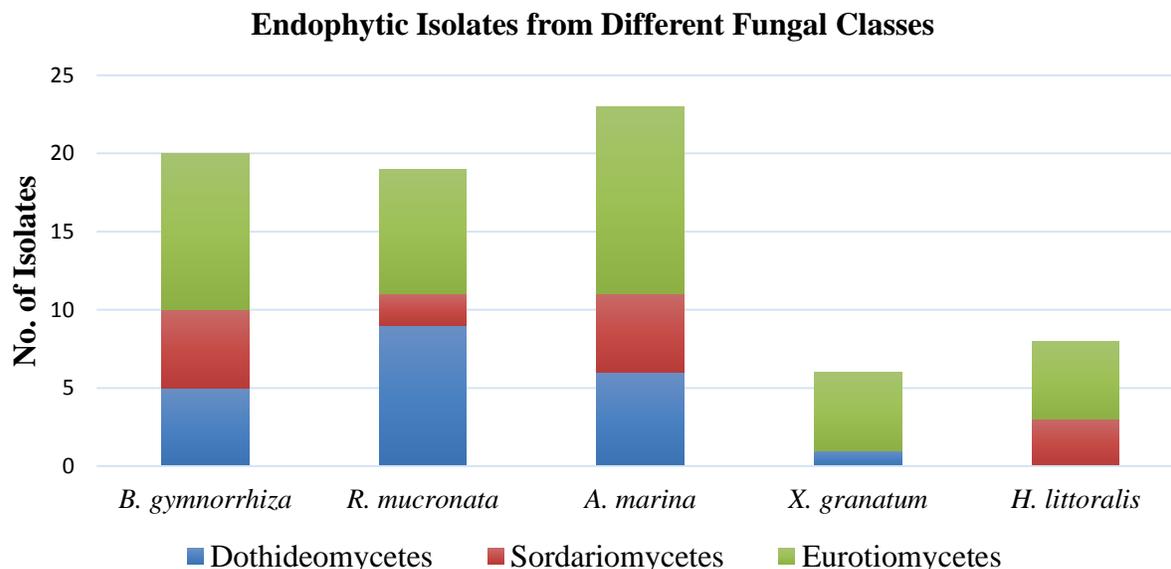
Figure 11. Mangrove endophytic fungi biodiversity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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