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PRESENCE OF FOLIAR DISEASES CAUSED BY FUNGI IN MANGROVES ON THE EAST COAST OF PENINSULAR MALAYSIA

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ABSTRACT

Mangrove health is very important for the ecosystem to survive the challenges and threats due to climate change and anthropogenic pressures. However, unhealthy mangroves due to pathogenic fungi causing diseases may demote the survival rate of younger plants to grow and information on the status of foliar disease incidence is limited. This study aimed 1) to observe the foliar disease symptoms that occurred on *Rhizophora apiculata* and *Avicennia marina* and 2) to identify the fungi isolated from the symptomatic leaves. Samples were collected from the mangrove area located in Universiti Malaysia Terengganu (UMT) campus, along the South China Sea. All isolates were identified based on their morphological characteristics. A total of five foliar disease symptoms were observed namely black leaf spot, grey leaf spot, leaf rot, sunken leaf blight, and anthracnose. *Rhizophora apiculata* has a greater number of leaf spots than *A. marina*. Four genera of fungi; *Pestalotiopsis* sp., *Curvularia* sp., *Colletotrichum* sp. and *Rhizopus* sp. were successfully isolated from symptomatic leaves where the most dominant was *Pestalotiopsis* sp. to both mangrove species. This finding highlights the need to obtain the status of foliar diseases and their impact on the resilience of mangroves in Malaysia.

Key words: Fungi, mangrove health, Foliar disease, *Rhizophora apiculata, Avicennia marina,* South China Sea

INTRODUCTION

Mangrove ecosystem plays an important role in balancing the environment quality of marine and coastal environment. It regulates the climate by capturing and storing a high amount of carbon into the mangrove sediment through carbon sequestration (Mcleod *et al*., 2011; Siikamaki *et al*., 2012). An unhealthy mangrove ecosystem could affect the overall ecosystem balance (Purwaka & Siregar, 2012). Mangrove trees are structurally and functionally unique compared to other tropical forests. The unique characteristics of mangrove trees are their adventitious aerial roots, viviparous embryos, complex root tissues, and the presence of salt glands on the leaf surface (Alongi, 2009). Meanwhile, the leaf is considered an important organ in the plant where it enables photosynthesis, transpiration, and other biological processes to occur (Steven, 2012). Moreover, the presence of salt glands on mangrove leaves plays an important function to remove excess salt from their tissue (Kothari *et al*., 2002). Besides, the leaf is the largest component of mangrove litter (Abdullah *et al*., 2010), which becomes an important component in the mangrove food web.

Fungi that inhabit the mangrove ecosystem is known as manglicolous fungi (Kohlmeyer, 1969; Jones & Hyde, 1988, 1990; Jones & Kuthubutheen, 1989). They include mostly marine fungi and a small group of terrestrial fungi that can be categorized into saprophytic, parasitic, and symbiotic fungi. Parasitic fungi may infect the plant's organs such as leaf, stem, and fruits. The spore which is the reproductive body of fungi disperse from one host to another through animals, insects, wind, water, and soil (Agrios, 2005), and attach to the susceptible leaf surface and germinate

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under favorable conditions. After germination, the hyphae developed and penetrated the leaf cell to obtain nutrients and thus, damage the surface of the leaf by creating an abscission layer in the form of spots or lesions. As a result, the photosynthesis and transportation rate of the leaf decreases as the growth of fungus damages the chlorophyll content and reduce the number of stomata on the leaf (Larsen, 2017). This will eventually minimize the light absorption mechanism which is an important process for plant growth.

An infected leaf can be identified and categorized based on the symptom appearance such as shape, color, and size. The symptoms could appear as spots, blights, rusts, and mildew following how the pathogen species invade the leaf tissue (Horst, 2008). The common foliar disease observed is leaf spot and it can be categorized and named after the fungus or the color of the spot, such as black leaf spot (Horst, 2008). Other popular foliar diseases such as anthracnose, scab, and blotch. The leaf spot disease had been observed on the red mangrove in Puerto Rico mangrove forest where *Anthostomella* spp., *Cercospora rhizomorphae,* and *Pestalotia disseminate* were identified (Stevens, 1920). In Florida red mangrove, *Cercospora* spp. was isolated from the leaf spot symptom (McMillan, 1964). Recently, Rossi *et al*. (2017) had discovered *Pestalotiopsis* species infecting the *Rhizophora mangle* in The Bahamas mangrove forest. However, in Malaysia, fungi occurrence in mangroves had been reported from sediment (Alias *et al*., 2010) and little information on foliar diseases. This study aimed to observe the foliar disease symptoms that occurred on *Rhizophora apiculata* and *Avicennia marina* and to identify the fungi isolated from the symptomatic leaves, based on their morphological characteristics.

MATERIALS AND METHODS

Samples collection

Thirty (n = 30) symptomatic mangrove leaves from each six *R. apiculata* and *A. marina* trees (n = 6) were hand-picked randomly and stored in a zip-lock plastic bag with silica gel. The sampling was done during low tide in a mangrove forest located in Universiti Malaysia Terengganu (UMT) campus (Figure 1).

Symptoms observation under Scanning Electron Microscope (SEM)

SEM was used to reconfirm the causal agent of the symptomatic mangrove leaves by detecting the physical presence of fungi such as mycelia, hyphae, and fruiting bodies. The leaves with the disease symptoms were cut into smaller square fragments (0.5 mm × 0.5 mm approx.) by using a sterile sharp razor blade, then were fixed with 2.5% of glutaraldehyde (GA) in 0.1 M sodium cacodylate buffer (pH 7.2) for 4 hr, rinsed three times with ethanol solution and post-fixed the samples with 2% osmium 0.1 M sodium cacodylate buffer (pH 7.2) for 4 hrs. Next, the samples were dehydrated to remove excess water by using a crescent series of ethanol and immersed in hexamethyldisilazane (HMDS) solution for 10 mins before mounted on the Scanning Electron Microscope (SEM) stubs (Purkayastha & Pal, 1998). The samples then sputter-coated with gold and examined under SEM.

Fungal isolation and identification

Potato Dextrose Agar (PDA) media (with antibiotics) was used to isolate fungi. Leaf samples with lesions were cut into small square fragments and immersed in 70% ethanol and 4% sodium hypochlorite (NaOCl) to remove unwanted micro-

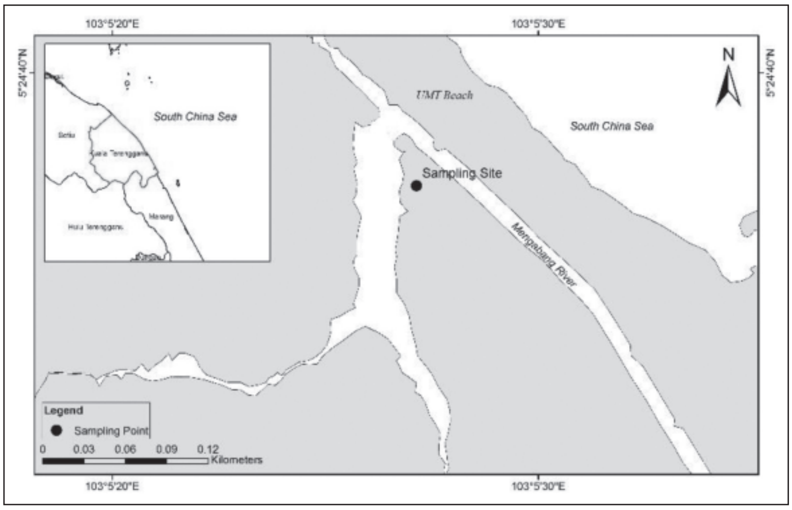

Fig. 1. Mangrove area in Universiti Malaysia Terengganu campus located along the Mengabang river and connected directly to the South China Sea.

organisms such as bacteria from contaminating the culture. The remaining solvents on samples were rinsed using sterile distilled water. The sterile filter paper was used to dry the samples before the samples were placed onto the PDA media (with antibiotics). Full strength media was prepared for direct plating of leaf symptoms and half strength media for the pure colony and pure culture (Table 1). Antibiotic penicillin was added to the media to prevent any contamination from bacteria. All samples were incubated at room temperature (27±2°C). Any fungi that grow out from the leaf samples were isolated onto the new PDA media to obtain a pure colony of fungus (Ravindran *et al.*, 2012). The morphology characteristics of the fungi were identified under an Olympus compound microscope.

RESULTS AND DISCUSSION

Mangrove diseases symptom

A total of five symptoms were observed namely black leaf spot, grey leaf spot, leaf rot, sunken leaf blight, and anthracnose on the *R. apiculata* leaf (Figure 2). However, only two common symptoms appeared on the *A. marina* leaves: grey leaf spot and anthracnose. Although both mangrove species showed the same grey leaf spot and anthracnose symptom, the symptom sizes and appearance on each leaf were slightly different.

Physically, the *A. marina* leaf has thicker water storage tissue than *R. apiculata* and the cuticle layer of *A. marina* is rough compared to other mangrove species due to the presence of uniseriate epidermal hairs (Poompozil & Kumarasamy, 2014). Therefore, those physical barriers on *A. marina* leaf may prevent spore attachment and/or delay germination of spores. The colonization of fungi begins as soon as the spores germinate and produce appressoria to penetrate the leaf tissue where then the fungal invasion will take over (Agrios, 2005). This invasion is depending on the fungi virulence, host susceptibility, and environmental conditions (Agrios 2005; Geeta, 2010). Thus, observation under SEM shown the presence of mycelia and conidia spores at the first stage of infection (attachment) before penetration (Agrios, 2005) (Figure 3). Salt concentration in *A. marina* leaf is higher than *R. apiculata* and this is an advantage to retard the fungal spore germination (Gregory *et al*., 2002).

Morphology characteristics of isolates

Results showed that a total of four fungi genera were successfully identified morphologically from five disease symptoms on both *R. apiculata* and *A. marina*. The leaves of *R. apiculata* were the highest in the number of fungi species that were invaded and isolated. However, the dominant fungus associated with mangrove leaves was identified as *Pestalotiopsis* sp. (leaf spot) and followed by *Colletotrichum* sp. (anthracnose). Each isolate was observed under a compound microscope at a magnification of 100x to identify the genus of fungi. The summary description of the isolates as described in Table 2.

Pestalotiopsis sp. isolated from both black and grey leaf spot symptoms of *R. apiculata* and *A. marina* produced a dense and creamy white color

Table 1. Composition of Potato Dextrose Agar (PDA) media used for fungi isolation

Material	Full strength	Half strength
PDA Powder (20g dextrose, 15g agar, 4g potato starch)	39 g	19.5 g
Distilled water	1 L	1 L
Penicillin powder	0.1 g	0.05 g

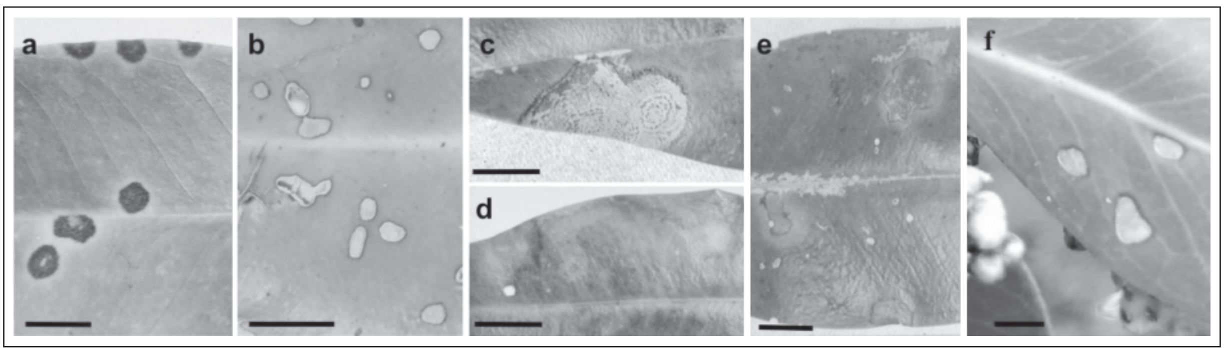

Fig. 2. Symptoms on leaves of *Rhizophora apiculata* (a-d) and *Avicennia marina* (e-f). Several symptoms on leaves observed were (a) black leaf spot, (b) grey leaf spot, (c) anthracnose, (d) sunken leaf blight like the early stage of anthracnose, (e) anthracnose with obvious margin, and (f) grey leaf spot. Black lines are scales at 1 cm.

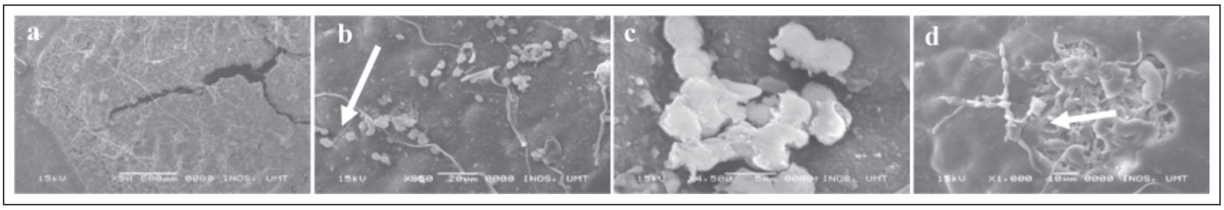

Fig. 3. Observation of fungal morphology characteristics on leaves surface under SEM showing (a) mycelia grows on the leaf lesion, (b) hyphae (arrow), (c) conidia, and (d) chain of microconidia (arrow).

Table 2. Summary of basic morphology of the fungi colony isolated from the symptomatic mangrove leaves of *R. apiculata* and *A. marina* that have been observed a) on a plate and b) under a compound microscope

	Characteristics	*Pestalotiopsis* sp.	*Curvularia* sp.	*Colletotrichum* sp.	*Rhizopus* sp.
a) Colony on plate	Form	Filamentous	Filamentous	Filamentous	Rhizoid
	Elevation	Slightly raised	Convex	Convex	Raised
	Margin	Filiform	Filiform	Entire	Entire
	Color	Creamy white	Light brownish	Creamy white to creamy orange	Light to dark green
	Fruiting body	Black color	Absent	Orange	Absent
	Growth rate	Fast	Medium	Medium	Fast
b) Colony under microscope	Mycelia	Dense	Dense	Dense	Very dense
	Shape of conidia	– Elongated fusiform – Three median cells	– Elongated fusiform – Three median cell	Hyaline cylindrical	Conidia absent
	Hyphae	Septate	Septate	Septate	Septate
	Other characteristics	Presence of two apical appendages on the conidia	Multinucleated segmented conidia	Presence of acervuli covering the fruiting body case	Presence of apophysis

of mycelia with black subepidermal fruiting body structure known as acervuli [Figure 4a(i and ii)]. The growth rate was fast as the mycelia of the culture almost covered the whole petri dish after 10 days of incubation period at room temperature. It has several general characteristics such as the presence of median cell (pigmented), apical and basal appendages, or hairs (Maharachchikumbura *et al*., 2011). The three-median cell with two apical appendages and one or almost absent of basal appendage was pigmented, brownish, elongated fusiform of conidia [Figure 4a(iii)]. Most conidia length ranged from 12 μm to 20 μm but there was conidia size about 35 μm and some still bearing on the branching hyphae [Figure 4a (iv)].

Curvularia sp. isolated from sunken leaf blight symptom was slow growth than other fungi. The margin of the mycelia was filiform with a cottony texture whereas, at an early stage, the pigmentation was white and brownish as it ages [Figure 4b(i and ii)]. The conidia shape almost similar to *Pestalotiopsis* sp. but the conidia length ranging from 25 μm to 35 μm and without appendage at both apical and basal end of the conidia [Figure 4b(iii)]. Some of the conidia were straight to slightly curve and the hyphae are septate and some have branches [Figure 4b(iv)].

The isolates of *Colletotrichum* sp. from anthracnose symptom produced creamy white and robust mycelia [Figure 4c(i and ii)]. The colony appearance was fluffy that raised upward forming like a dome-shape. It produced light orange-colored fruiting bodies of acervuli that contained an abundance of similar in shape and size of conidia. *Colletotrichum* sp. produced hyaline cylindrical shape conidia with a length ranging from 11 μm to 18 μm. It is one of the fungi that could be easily distinguished from other fungi with the presence of acervuli [Figure 4c(iii and iv)].

The rapidly growing colonies were isolated from leaf rot symptoms and were identified as *Rhizopus* species. It only took 5 days for *Rhizopus* sp. to cover the whole agar plate incubated at room temperature. The pigmentation was from light green to dark as they produce spores that are similar to cotton candy in texture [Figure 4d(i and ii)]. Colony produced a round shape of sporangium on top of the apophysis with a diameter length was about 8 μm [Figure 4d(iii)]. The hyphae were wider than other fungi, 3 μm – 3.5 μm and there was an early

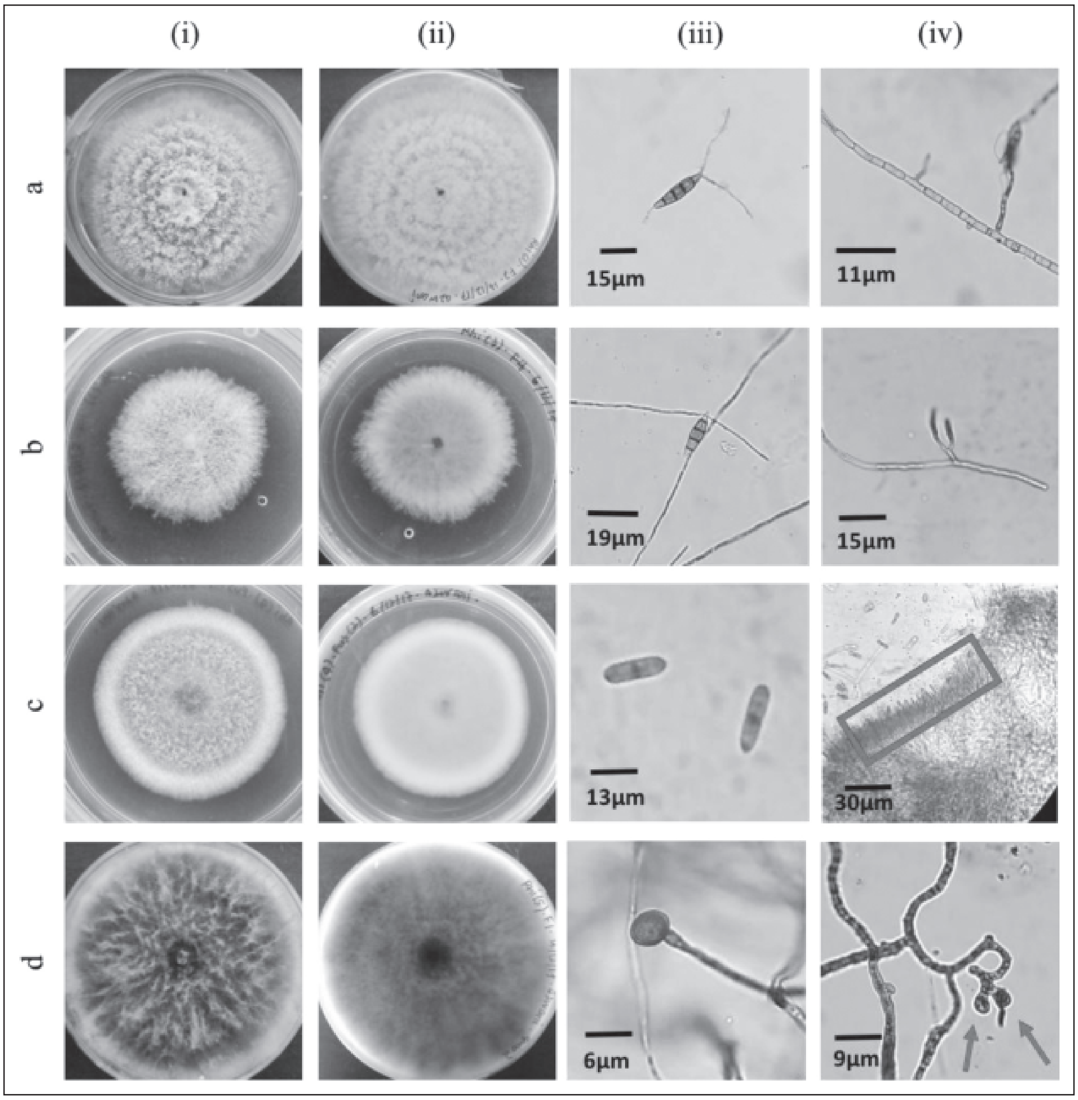

Fig. 4. Morphological characteristics of isolated fungi from foliar diseases of mangroves. On agar plate; (i) the top surface and (ii) bottom surface of (a) *Pestalotiopsis* sp. with a distinguish microscopic of (iii) conidia and appendage and, (iv) septate hyphae; (b) *Curvularia* sp. with (iii) conidia and, (iv) branching hyphae; (c) *Colletotrichum* sp. with (iii) cylindrical conidia and, (iv) acervuli; (d) *Rhizopus* sp. with (iii) sporangium and (iv) apophysis. Cultures were observed under 100X total magnification.

stage of apophysis was observed where it will grow longer forming a sporangiophore to support the sporangium on top of the stolon [Figure 4d (iv)].

Rhizophora apiculata was quite susceptible to foliar disease especially when all four fungi were invading the leaves compared to *A. marina* that had been invaded by only *Pestalotiopsis* sp. and *Colletotrichum* sp. These fungi species are well-known in the crop that cause devastating plant disease (Agrios, 2005). Among the leaf symptoms observed, anthracnose disease caused by *Colletotrichum* sp. is commonly known to affect a wide range of crops such as chili plant (Oo & Oh, 2016) and mango trees (Ismail *et al*., 2015). Rossi *et al*. (2017) had reported on a similar fungus, *Pestalotiopsis* sp. causing leaf spot symptom on *R. mangle*.

This study has confirmed the presence of foliar diseases namely black leaf spot, grey leaf spot, leaf rot, sunken leaf blight, and anthracnose caused by fungi at mangroves area in the UMT campus. However, further work is essential to confirm fungi pathogenicity and to understand its life cycle in the mangrove ecosystem. On top of that, a molecular approach is required to confirm the species.

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