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EVALUATION OF FOLIAR DISEASE INCIDENCE AND SEVERITY OF MANGROVES IN UNIVERSITI MALAYSIA TERENGGANU

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Abstract: Mangroves are an important ecosystem because of their ecological roles and services, particularly in nutrient cycling and carbon sequestration. However, habitats have declined over the year, mostly due to anthropogenic and natural threats. Mangrove plant diseases caused by pathogenic fungal invasion are poorly described. The status of diseased mangroves remained unknown, primarily in Malaysia. The current study evaluates the disease incidence and severity of mangrove trees in the Universiti Malaysia Terengganu (UMT) campus. This study aimed to determine the dominant type of foliar disease in the UMT campus and identify the most affected mangrove species using Disease Incidence and Disease Severity approach. 30 leaves from each of five mangrove species; *Lumnitzera racemosa, Rhizophora apiculata, Hibiscus tiliaceus, Avicennia alba* and *Sonneratia caseolaris* were collected, observed and analysed for the Disease Incidence and Disease Severity. A total of six types of foliar diseases were observed, with leaf blight as the dominant, followed by brown leaf spot, insect graze, grey leaf spot, black leaf spot and anthracnose. Mangrove trees at Jalan Biawak have the highest percentage value of Disease Incidence and Disease Severity compared to mangroves at Pusat Islam UMT. Fungal invasion rate variation could be caused by factors such as temperature, humidity and mangrove species' resilience toward fungal invasion. Therefore, further study needs to be done to understand this issue better.

Keywords: Undergraduate research, mangrove, disease assessment, foliar disease, Universiti Malaysia Terengganu.

Introduction

The mangrove ecosystem supports trees and shrubs that grow along sheltered coastlines such as rivers, estuaries or tidal marsh in the subtropics and tropics. Mangroves tree species are unique in terms of morphological and ecophysiological such as having aerial roots, viviparous seed and surviving saline conditions while sustaining their water and carbon balance (Alongi, 2002). These unique characteristics made them tolerate extreme conditions such as tides, waves, and storm surges (NOAA, 2009). Despite their ability to adapt to harsh environmental condition, mangrove forest is decreasing by 1% per year globally, at the estimated rate of about 1282 hectares (ha) per year since 1990 (Kanniah *et al*., 2015).

Invasion of microorganisms such as bacteria, viruses and fungi is one of the factors that could cause foliar disease in a mangrove. The disease is a condition of the plant body that ruins normal functioning and is typically expressed by distinguishing signs and symptoms (Tampakaki *et al.,* 2009; Manikandan *et al*., 2019). These microorganisms can attack different parts of the host plant: the leaf, root, flower and fruit. When the invasion starts through the cell wall penetration, they will extract nutrients from the plant host and leave the wound on the leaf surface, which appears as a lesion (Agrios, 2005). This invasion process also affects the biological processes of a normal plant. The photosynthesis and transportation rate will decrease and eventually could cause the plant host to die.

According to Garcia-Guzman *et al*. (2016), foliar disease in tropical rainforest communities is mostly caused by fungi and is the most important agent causing plant damage. The living nature of fungi are mostly parasites

that depend on the live host to obtain food and nutrients and negatively affect the host. These fungi are also pathogens (Hrudayanath *et al*., 2013). Most of the 44 terrestrial fungi isolated from Avicennia, Hibiscus, Languncularia, and Rhizophora are identified as parasites on living mangrove leaves, while other fungi that do not affect the health of mangroves are known as saprophytes (Kohlmeyer *et al*., 1969).

Doehlemann *et al*. (2017) reported that fungi use different strategies to invade and cause disease in plants. Pathogenic fungi are often divided into two, which are necrotrophic and biotrophic. Necrotrophic fungi kill the host cells and feed on dead materials, while biotrophic fungi depend on the host cell to extract nutrients. There are many different types of fungi associated with mangroves because the dispersal of the fungi can be achieved by spores or fruiting bodies (Kohlmeyer *et al*., 1969). Different symptoms appearing on the plant leaf could also be caused by similar or different species of pathogenic fungi.

Numerous studies have focused on the foliar diseases caused by fungi in commercial plants that describe fungi species and their invasion process. However, foliar diseases in mangrove trees are still poorly understood. Information on pathogenic fungi, specifically in mangrove environments, will contribute to more understanding of the fungi behaviour and could be useful for better managing mangrove forests. Therefore, this study aimed to determine the dominant type of foliar disease in the UMT campus and identify the most affected mangrove species using Disease Incidence and Disease Severity indices.

Materials and Method

Study area

The sampling sites were located at Jalan Biawak and Pusat Islam UMT (Figure 1) where three transects were laid out at each site.

Figure 1: Map of sampling sites at (A) Jalan Biawak and (B) Pusat Islam Universiti Malaysia Terengganu (UMT). Colours indicate Transect 1: Red, Transect 2: Yellow and Transect 3: Blue

A 15-meter transect was laid out from the reference tree and divided into three sampling points, with the distance between each point being five meters. A reference tree was chosen as a mark to lead the transect laid from the riverbank (Figure 2). Then, four individual trees closest to each sampling point were chosen, identified and recorded for Diameter at Breast Height (DBH) and height.

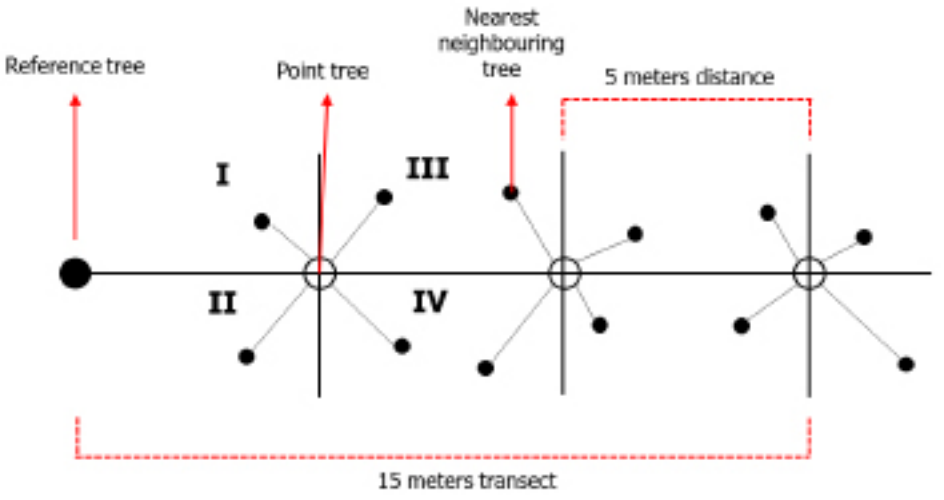

Figure 2: Sampling design of transect that show reference tree, point tree and nearest neighbouring trees

Symptoms Observation and Occurrences

A total of 30 leaves from each chosen mangrove tree were randomly collected and brought to the laboratory for further analysis. Different symptoms were observed on each leaf under a dissecting microscope.

Disease Incidence and Disease Severity

The number of infected leaves was counted and the formula shown below was used to calculate the value of Disease Incidence in percentage units. Then, Image J software was then used to analyse the severity of each mangrove leaf (Figure 3). All mangrove leaves were photographed and uploaded into the software. The scale of the leaf was set up in centimetres for length unit. The area of the leaf surface and the lesion area was calculated after setting up the threshold. Then, Disease Severity was calculated using the formula below:

$$Disease\ Incidence\ (DI) = \frac{Number\ of\ infected\ leaves}{Total\ number\ of\ leaves\ assessed} X100$$

$$Disease\ Severity\ (DS) = \frac{Area\ of\ infected\ tissue}{Total\ area\ of\ leaf\ surface} X100$$

(Zones *et al*., 2017)

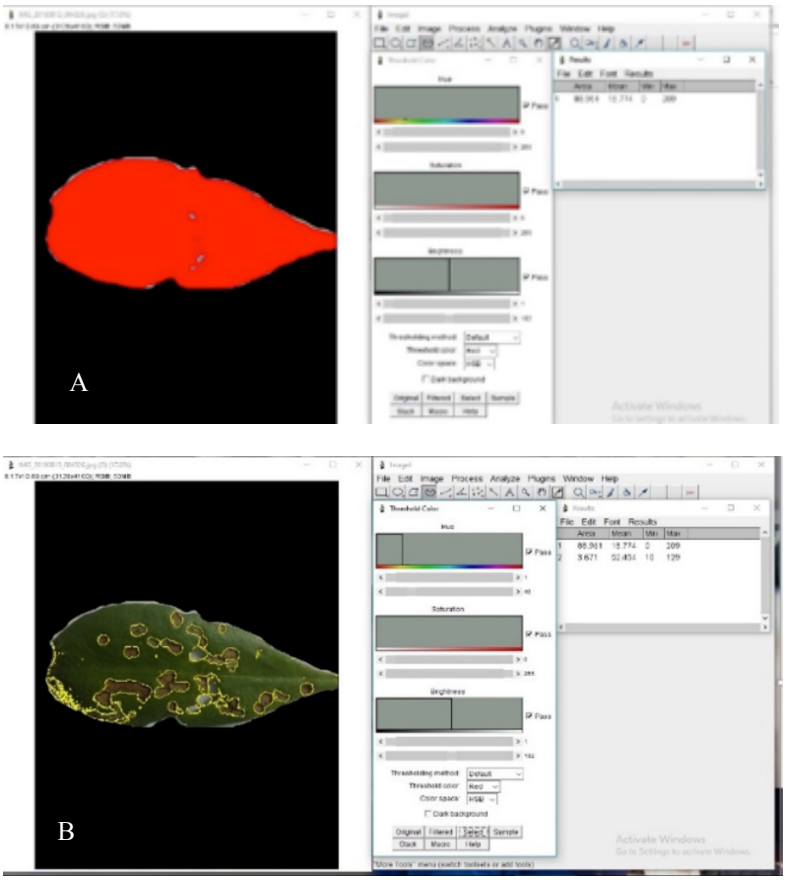

Figure 3: Image J software used to analyse (A) total surface area and (B) infected lesion area on the leaf of a mangrove

Results and Discussion

Symptoms of Mangrove Foliar Disease

Both sampling areas are dominated by different mangrove species recorded within the transects, *Rhizophora apiculata* at Jalan Biawak and *Sonneratia caseolaris* at Pusat Islam UMT, respectively. This is likely contributed by the zonation between both areas as transect lines at Jalan Biawak were laid near the riverbank with muddy sediment mostly influenced by the tide, whereas transect lines at Pusat Islam UMT are situated towards the land where the area will be flooded only during the highest tide.

Table 1: Range of Diameter at Breast Height (DBH) and height of mangrove trees at both sampling areas in UMT

Sampling area	Mangrove species	Range of DBH (cm)	Range of height (m)
Jalan Biawak	*Lumnitzera racemosa*	2.5 – 7.9	1.3 – 7.5
	Hibiscus tiliaceus	2.3 – 9.5	1.0 – 7.5
	Rhizophora apiculata	1.3 – 8.8	1.4 – 12.0
	Avicennia alba	3.4 – 3.5	1.4 – 1.5
Pusat Islam UMT	*Lumnitzera racemosa*	0.9 – 2.7	2.2 – 4.8
	Hibiscus tiliaceus	4.0 – 4.1	3.6 – 3.7
	Rhizophora apiculata	1.0 – 3.0	1.6 – 3.7
	Avicennia alba	1.8 – 1.9	2.1 – 2.2
	Sonneratia caseolaris	0.8 – 9.6	1.7 – 10.0

Rhizophora is the dominant mangrove species forming pure stands at exposed mangrove forest areas were frequently flooded with normal high tides and this species usually grow on deep, soft, and muddy soils, while *Sonneratia* usually settled near the banks of tidal rivers in brackish water (Shin *et al*., 2015). Mangrove trees in Jalan Biawak had higher DBH values and height compared to the mangrove trees in Pusat Islam UMT (Table 1). The range of DBH and height for mangroves at Jalan Biawak were 1.3 cm – 9.5 cm and 1.0 m – 12.0 m, while mangroves at Pusat Islam only recorded 0.8 cm – 9.6 cm and 1.6 m – 10.0 m, respectively.

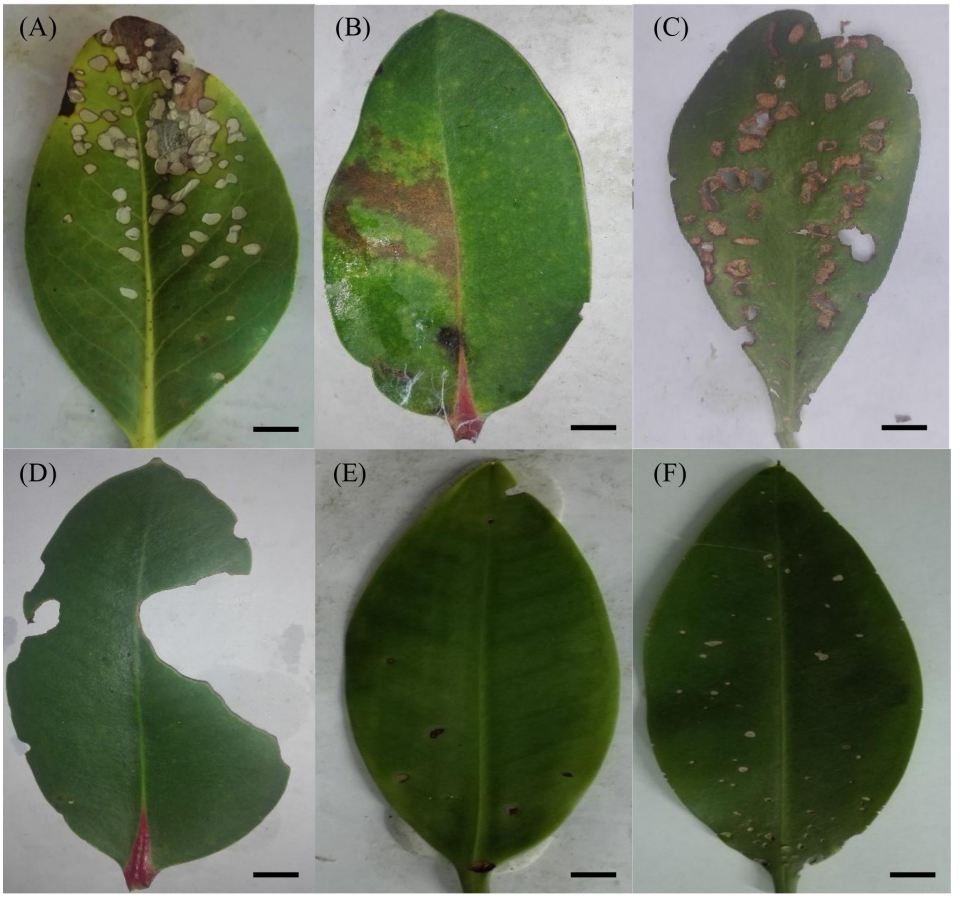

Figure 4: Symptoms of (A) anthracnose, (B) leaf blight, (C) brown leaf spot, (D) insect graze, (E) black leaf spot and (F) grey leaf spot observed on mangrove species

Six different types of symptoms were observed on the leaf surface of mangroves which are anthracnose, leaf blight, insect graze, black leaf spot, brown leaf spot and grey leaf spot (Figure 5). The symptoms were identified based on the morphological characteristics of the lesion on the leaf surface such as colour, shape, size and texture or elevation, concerning Plant Pathology Book (Agrios, 2005). A similar result was also reported in a study by Sahibu *et al*. (2020) where black leaf spot, grey leaf spot and leaf blight were observed affecting *Rhizophora apiculata* while grey leaf spot and anthracnose affecting *Avicennia marina* at mangrove forests in UMT campus.

Leaf spot is considered the most common symptom caused by pathogenic fungi affecting a wide range of plants (Grant, 2020) and the colour of the leaf spot can be varied, which can be observed in the present study; black, grey and brown leaf spot. Symptoms are typically observed as a small circular to oval, but the shape can be irregular depending on the host and pathogen species. Black leaf spots are usually surrounded by a yellow chlorotic border known as "halo" and the texture of the centre lesion is rough with the presence of pustule easily observed under dissecting microscope. Grey leaf spots have a smooth texture and are brittle at the centre lesion as degradation of dead tissue happens, while brown leaf spots usually have

sunken elevation at the centre and the spots can group, forming larger lesions (Ward *et al*., 1999; Lin *et al*., 2016; Piątek & Yorou, 2019). Leaf spot symptom observed on the mangroves has been reported in previous studies where *Pestalotiopsis* spp. was consistently isolated from symptomatic leaves of *Rhizophora mangle* in the Bahamas mangrove (Rossi, 2018), and *Alternaria alternata* was confirmed as the pathogen causing leaf spot on the leaves of *Bruguiera gymnorhizza* in Zhanjiang, China (Lin *et al*., 2016).

Melissa (2017) defined blight as any plant disease with symptoms such as sudden and severe yellowing, browning or dying of leaves or the entire part of the plant. Leaf blight symptoms usually appear in a small, irregular shape with a brownish colour of the lesion at an early stage and grow larger in size as the symptoms progressively invade other parts of leaf tissue (Agrios, 2005; Larsen, 2007; Small, 2011). The later stage of invasion can cause a hole in the leaf as the centre of the lesion starts to degrade. Meanwhile, insects and pests are also the factors that could promote fungal invasion as they graze on the leaves and cause direct opening of leaf tissue (Geeta, 2010). A wounded leaf increases the chances of the attachment of fungi spores that are always present in the surrounding air compared to the unwounded leaf, as the spores need to penetrate the thick cuticle layer and the mangrove leaf cell wall (Agrios, 2005).

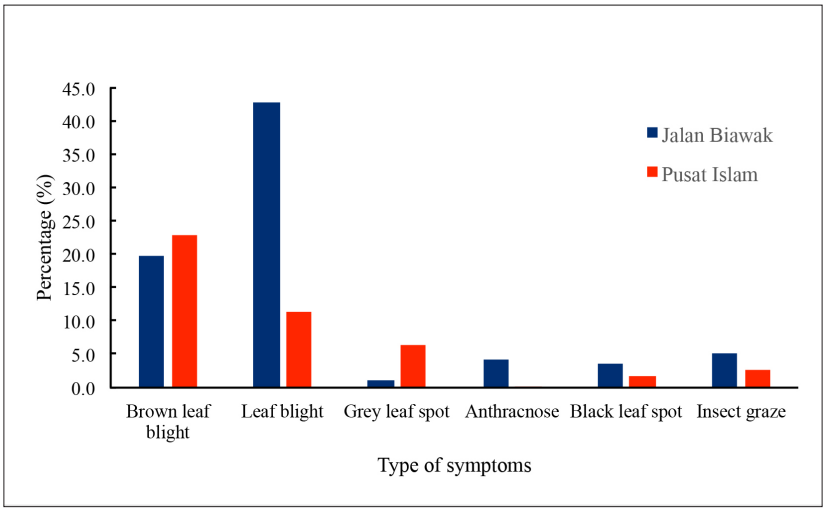

Figure 5: Graph percentage of different symptom types in both sampling areas

The graph above showed that leaf blight and brown leaf spot were the dominant symptoms observed on the mangroves, followed by insect graze, grey leaf spot, black leaf spot and anthracnose (Figure 5). Leaf blight was first reported in India, caused by several species of pathogenic fungi. It is a common disease in agricultural plants and causes substantial economic losses in Mexico, Brazil, India and some part of countries (Butler, 1918; Das & Rajendrakumar, 2016). Meanwhile, anthracnose disease which is only found affecting *Avicennia alba* at Jalan Biawak mainly caused by *Colletotrichum* usually invading plants in warm and humid areas. The symptom appeared almost similar to the normal leaf spot symptom but with the presence of tiny spots at the centre of the lesion (Than *et al*., 2008).

Disease Incidence and Disease Severity

Mangrove trees at Jalan Biawak showed a higher Disease Incidence value, with more than 70% of leaves showing symptoms as compared to mangrove trees at Pusat Islam UMT, with DI

value recorded below 50% leaves were infected (Figure 6). The higher the DI percentage value indicates that the foliar disease infected more mangrove trees. Thus, mangroves in both sampling areas are considered as susceptible to foliar diseases caused by fungi as the DI value is above 31% based on the DI scale by Nepal Agricultural Research Council (Manandhar *et al.,* 2016).

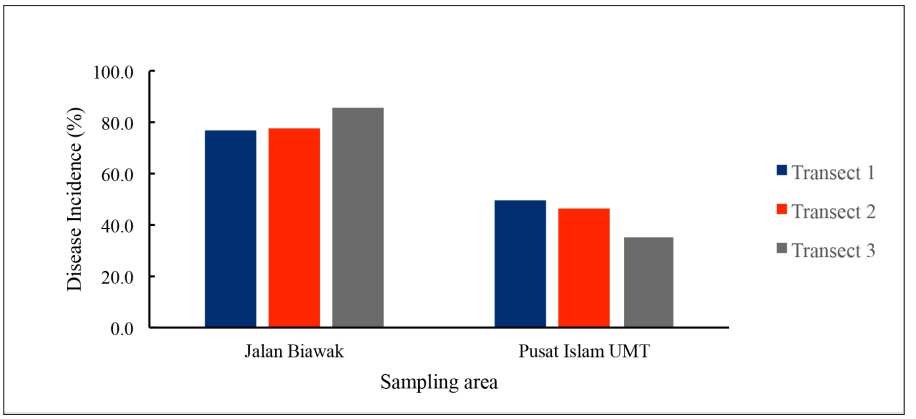

Figure 6: Graph of Disease Incidence of mangroves in both sampling areas

Based on the incidence data, the severity of the disease can be estimated. Similar results were recorded for Disease Severity where the mangroves at Jalan Biawak showed higher severity than trees at Pusat Islam UMT (Figure 7). This is because as incidence values increase, the severity values associated with the incidence also increase (Seem, 2003). *Rhizophora apiculata* at Jalan Biawak had the most severe infection with the highest percentage value at 12.87%, followed by *L. racemosa* (12.28), *A. alba* (9.5%) and *H. tiliaceus* (4.3%). Meanwhile, *S. caseolaris* at Pusat Islam UMT recorded the highest DS value (9.0%), followed by *R. apiculata* (2.8%), *L. racemosa* (0.5%), *A. alba* (0.4%) and *H. tiliaceus* (0.3%).

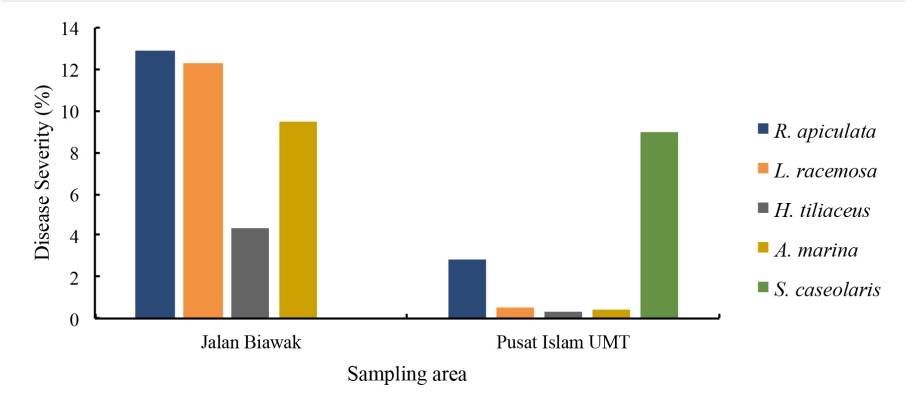

Figure 7: Graph of Disease Severity of different mangrove species in both sampling areas

Once the fungi successfully invade the leaf host, it will leave marks that appear as a lesion. As the fungal invasion progresses, the lesions will grow larger, extracting nutrients from other parts of leaf tissue. This will reduce the green leaf surface area, minimise the light absorption for the photosynthesis process, and eventually decrease the photosynthesis rate, which is an important process for plants (Jennings & Lysek, 1999). The percentage of the infected trees in the study area varied the species of mangroves, likely due to the difference in the resilience of each mangrove tree species and the presence of pathogens at different areas. The higher severity of disease on the leaf shows weaker tree resilience against fungal invasion.

Besides, mangrove trees at Jalan Biawak are considered older than mangrove trees at Pusat Islam UMT based on the recorded DBH and height (Table 1). The different developmental stage of plant is considered one of the factors that affect the response of pathogens toward host plants. Older trees tend to have more symptoms on the leaf surface as the fungi have already invaded most of the leaf tissue, while younger trees show most of the early stage of fungal invasion (Kus *et al*., 2002; Whalen, 2005). The density of fungal inoculum (ID) in different areas also affects the severity of the disease as high ID might disrupt the plant barriers in resisting the fungal invasion (Raftoyannis & Dick, 2002). The DI and DS were high at Jalan Biawak may be due to the location near the sea that allowed the wind to disseminate the spore while Pusat Islam UMT is near buildings that block wind from some directions.

Conclusion

The present study identified a total of six different types of symptoms from different species of mangrove leaves. The dominant symptom was leaf blight and brown leaf spots at both Jalan Biawak and Pusat Islam UMT. Disease Incidence and Disease Severity were analysed and found that Jalan Biawak has the highest percentage value of DI and DS, indicating that mangrove trees in Jalan Biawak are severely affected compared to Pusat Islam UMT. The present result from this study is important to provide an early insight into the health status of mangroves in both sampling areas. However, further understanding of pathogenic fungi causing foliar disease in mangroves should be done in the future, which could contribute to better managing the mangrove ecosystem.

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