

# FIRST REPORT OF *Diaporthe phaseolorum* AND *Neopestalotiopsis clavispora* CAUSING LEAF SPOT DISEASE ON OIL PALM (*Elaeis guineensis*)

NUR AIN IZZATI MOHD ZAINUDIN<sup>1,2\*</sup>; NURÁIN AZHAR<sup>1</sup>; NOOR AISYAH MD NOORDIN<sup>1</sup> and HARUNA USMAN BAITA<sup>3</sup>

## ABSTRACT

Oil palm is a significant agricultural crop, highly valued for its role in oil production. This study identified two fungal pathogens, *Diaporthe phaseolorum* and *Neopestalotiopsis clavispora*, as causal agents of leaf spot disease in oil palm (*Elaeis guineensis*), marking their first reported occurrence. Over 85% of surveyed trees in Pahang, Malaysia, were affected, posing a significant threat to oil palm productivity. Morphological, pathogenicity and molecular analyses confirmed their roles, with *Lasiodiplodia theobromae* causing the most severe lesions. These findings underscore the importance of early detection and targeted management to mitigate economic losses.

**Keywords:** crop protection, fungal pathogen, fungi, necrotic, plant disease.

**Received:** 23 November 2024; **Accepted:** 13 October 2025; **Published online:** 3 February 2026.

## INTRODUCTION

Malaysia plays a significant role in the global oil palm industry, making substantial contributions to the nation's economic development. As the world's second-largest producer of oil palm (*Elaeis guineensis* Jacq.), the country accounted for approximately 26% of global production in the 2023/2024 marketing year, producing 19.71 million tonnes as reported by the Foreign Agricultural Service, U. S. Department of Agriculture (USDA).

Basal stem rot (BSR) is one of the common diseases of oil palm, which is caused by *Ganoderma boninense*. Economic losses due to BSR in Malaysia are estimated to be up to USD500 million annually (Zakaria, 2023). Apart from BSR, leaf spot and anthracnose, other foliar diseases affecting oil palm seedlings have also been reported (Kittimorakul et al., 2019; Muhamad et al., 2018; Nasehi et al., 2020; Wibowo et al., 2024). Compared to

anthracnose, leaf spot infections are more common. Two of the prevalent fungal pathogens associated with these diseases are *Cercospora elaeidis* and *Curvularia oryzae* (Pornsuriya et al., 2013).

Previous study has explored various fungal pathogens, including *Ganoderma boninense*, *Fusarium oxysporum*, *Marasmius palmivorus* and *Phytophthora palmivora*, which affect oil palm (Daval et al., 2022; Flood et al., 2022; Maizatul-Suriza et al., 2019; Pham et al., 2020). However, limited attention has been given to leaf spot diseases, as most current studies primarily focus on seed, bark and root diseases rather than those affecting the leaves. Leaf spot diseases are often neglected, yet understanding their impact is crucial, as they significantly reduce the leaf surface area available for photosynthesis, consequently diminishing growth and yield production.

This study aimed to contribute to and enhance the existing study on leaf spot disease pathogens, especially in oil palm. It seeks to identify the pathogens, provide insights into disease symptoms and propose preventative measures to mitigate widespread infections and significant losses. Hence, this study was carried out to isolate and identify fungi associated with diseased oil palm leaves using morphological approaches, assess the pathogenicity of the fungi isolated from the leaves, and identify the pathogenic isolates through Internal Transcribed Spacer (ITS) sequence analysis.

<sup>1</sup> Department of Biology,  
Faculty of Science, Universiti Putra Malaysia,  
43400 Serdang, Selangor, Malaysia.

<sup>2</sup> Laboratory of Sustainable Agronomy and Crop Protection,  
Institute of Plantation Studies,  
Universiti Putra Malaysia,  
43400 Serdang, Selangor, Malaysia.

<sup>3</sup> Department of Biological Sciences,  
Sule Lamido University,  
Kafin Hausa, P.M.B. 048, Jigawa State, Nigeria.

\* Corresponding author e-mail: [ainizzati@upm.edu.my](mailto:ainizzati@upm.edu.my)

## MATERIALS AND METHODS

### Sampling Locations, Duration and Sample Collection

Leaf spot-infected oil palm leaf samples were collected during a series of samplings in a FELDA plantation in Jengka, Pahang, with coordinates 3° 36' 49.39" N, 102° 30' 37.01" E, and 3° 37' 12.71" N, 102° 29' 24.10" E from March to April 2022. Using a visual check, the percentage of infected plants was determined. The infected leaf samples were collected using an opportunistic sampling method, in which specimens are gathered whenever and wherever they are encountered in the field, allowing for the capture of a broad range of naturally occurring variations (Stratton, 2021). A total of 20 infected leaf samples were collected from 40 ha across three distinct sites at each location based on visible symptoms of infection, such as discolouration, necrotic spots, and fungal growth (Nazarov et al., 2020).

### Isolation and Purification of Fungal Isolates

Infected leaf samples, including both symptomatic and asymptomatic leaf areas, were excised into 3 mm<sup>2</sup> sections and surface-sterilised by soaking in 70% ethanol for 3 min, followed by 1% NaOCl for 3 min and rinsed three times with sterile distilled water, each rinse lasting 1 min. The samples were air-dried on sterile filter paper before being transferred to potato dextrose agar (PDA) plates. The inoculated plates were incubated at 28 ± 2°C for 3 days. Fungal purification was completed using the hyphal tip technique (Brown, 1924).

### Morphological Identification of Fungal Cultures

The purified fungal isolates were grouped into genus levels based on macro- and micro-morphological characteristics, including colony features and pigmentation of cultures (Watanabe, 2002). Observations were made and recorded for both top and bottom views of the PDA plates. Different media were employed to promote fungal sporulation and growth. The media utilised included PDA, carnation leaf agar (CLA) and Spezieller Nahrstoffarmer agar (SNA) (Fisher et al., 1982; Zainudin et al., 2011).

The microscopic characteristics (shape and size of conidia, hyphae, fruiting bodies, phialides, chlamydospores and conidiophores) were examined using an Olympus CX31 compound microscope (Salvamani & Nawawi, 2014). The length and width of the conidia were measured with DinoCapture 2.0 software.

### Pathogenicity Test

The healthy, 9-month-old (variety FELDA Deli × Yangambi) oil palm seedlings used in this test were obtained from Jempol Green Plantations, Bahau, Negeri Sembilan, Malaysia. The leaves were surface sterilised using 70% alcohol for 30 s and then air-dried. Six wound points were created on the leaf using a sterile needle: Two at the apical, two in the middle and two at the basal part of the leaf. A 5 mm mycelial plug of the fungal isolate was inoculated onto the wounded areas for the treatment set, while a PDA plug was used for the control set. All treatments and controls were incubated for 24 hr by wrapping a plastic sheet around the plant, allowing fungal penetration into the leaves (Zainudin et al., 2011). The plants were maintained under greenhouse conditions for 21 days. The inoculated area and symptom development were examined daily. For the infected leaf, the lesion sizes were measured and documented. To analyse the produced lesion sizes, one-way ANOVA and Tukey's HSD were employed using IBM SPSS v.26.0 for Windows (Di Marco, 2004).

### Species Identification of the Pathogenic Isolates Using ITS Sequence Analysis

The species identification of pathogenic isolates that produced leaf spot lesions was further confirmed by ITS sequence analysis. The pathogenic isolates were cultured in PDA and incubated at 28 ± 2°C for 5 days. Genomic DNA was extracted using the Ultra Clean® microbial DNA isolation kit (M0-BIO, Carlsbad, CA, USA) according to the manufacturer's instructions.

ITS sequences were amplified using the primer pair ITS1/ITS4 (White et al., 1990). The PCR reaction mixture was produced in a total volume of 20 µL and contained 4.0 µL of 5 × Go *Taq* buffer, 1.0 µL of 10 mM primers ITS1 and ITS4, 2.0 µL of 2 mM dNTPs, 2.0 µL of 2 mM MgCl<sub>2</sub>, 0.1 µL of 5u *Taq* Polymerase, 8.9 µL of sterile distilled water (ddH<sub>2</sub>O) and 1.0 µL of 20 ng gDNA template. The PCR reactions were conducted using a Professional Standard Thermocycler (Biometra Company, USA). The amplification protocol began with an initial denaturation at 95°C for 30 seconds, followed by 35 cycles of denaturation at 95°C for 10 s, annealing at 59°C for 15 s and extension at 72°C for 30 s ending with a final extension step at 72°C for 5 min (Tsui et al., 2011).

The PCR products were then sent to Apical Scientific Sdn. Bhd. (Selangor, Malaysia) for sequencing. The purified ITS fragments were sequenced in both directions using an Applied Biosystems 3730xl DNA Analyser (Thermo

Scientific). DNA sequenced results were aligned using the Molecular Evolutionary Genetics Analysis (MEGA) software version 11.0 (Tamura et al., 2021). The resulting sequences were aligned and compared with GenBank database entries via Basic Local Alignment Search Tool (BLAST) to confirm species identity. The sequences generated were deposited into the GenBank database.

## RESULTS AND DISCUSSION

### Observation of Leaf Spot Disease in the Field

From March to April 2022, irregularly shaped spots with a dotted necrotic appearance were observed on oil palm leaves. The affected leaves displayed chlorosis to extensive necrotic lesions, potentially contributing to yield losses. The leaf samples showed dark brown or yellowish spots, some of which had irregular holes, while others featured light and dark circular halos. Several spots grew and eventually merged into large, angular or irregular necrotic lesions (Figure 1). A detailed survey conducted during this period revealed that over 85% of the oil palm trees in the area were infected. Observations indicated that the disease was widespread across the plantation, raising concerns about its potential impact on oil palm productivity.

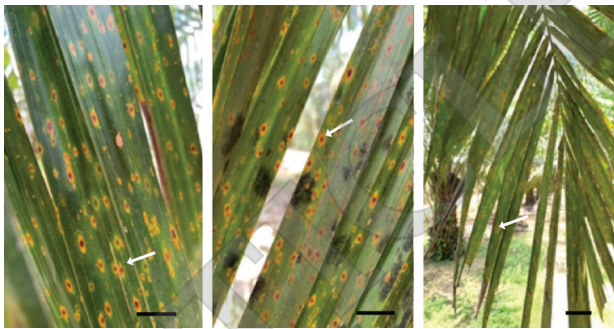


Figure 1. Infected leaf spots were observed in the field. Bar: 1 cm.

Leaf spot diseases in oil palm plantations are influenced by various environmental factors, including climate conditions and plantation management practices. High humidity, frequent rainfall, and warm temperatures prevalent in tropical regions like Malaysia create favourable conditions for fungal pathogens (Ismail et al., 2017). Plantation practices also play a significant role in disease emergence. Monoculture systems, improper pruning, and inadequate removal of infected plant debris can facilitate the spread of pathogens. A study assessing leaf spot and anthracnose diseases in oil palm nurseries across Peninsular Malaysia found that disease incidence was higher in

younger seedlings, indicating that age and possibly related management practices influence disease susceptibility (Nor Azlan et al., 2018).

### Morphological Characteristics of Fungal Genera Isolated from Leaf Spot Samples of Oil Palm

Forty-one isolates of fungi obtained from oil palm leaves were grouped into their genus based on their macroscopic and microscopic characteristics (Table 1). Colony features of *Annulohyphoxylon* species were formed with white cottony with buff to dark brown pigmentation (Figure 2-A1 and A2). Conidia of *Annulohyphoxylon* species are ellipsoidal, ranging from 4.34 to 5.71  $\mu\text{m}$   $\times$  2.65 to 3.21  $\mu\text{m}$  in size. Conidiophores of *Annulohyphoxylon* species displayed details of periconiella-like branching patterns that are hyaline and septate.

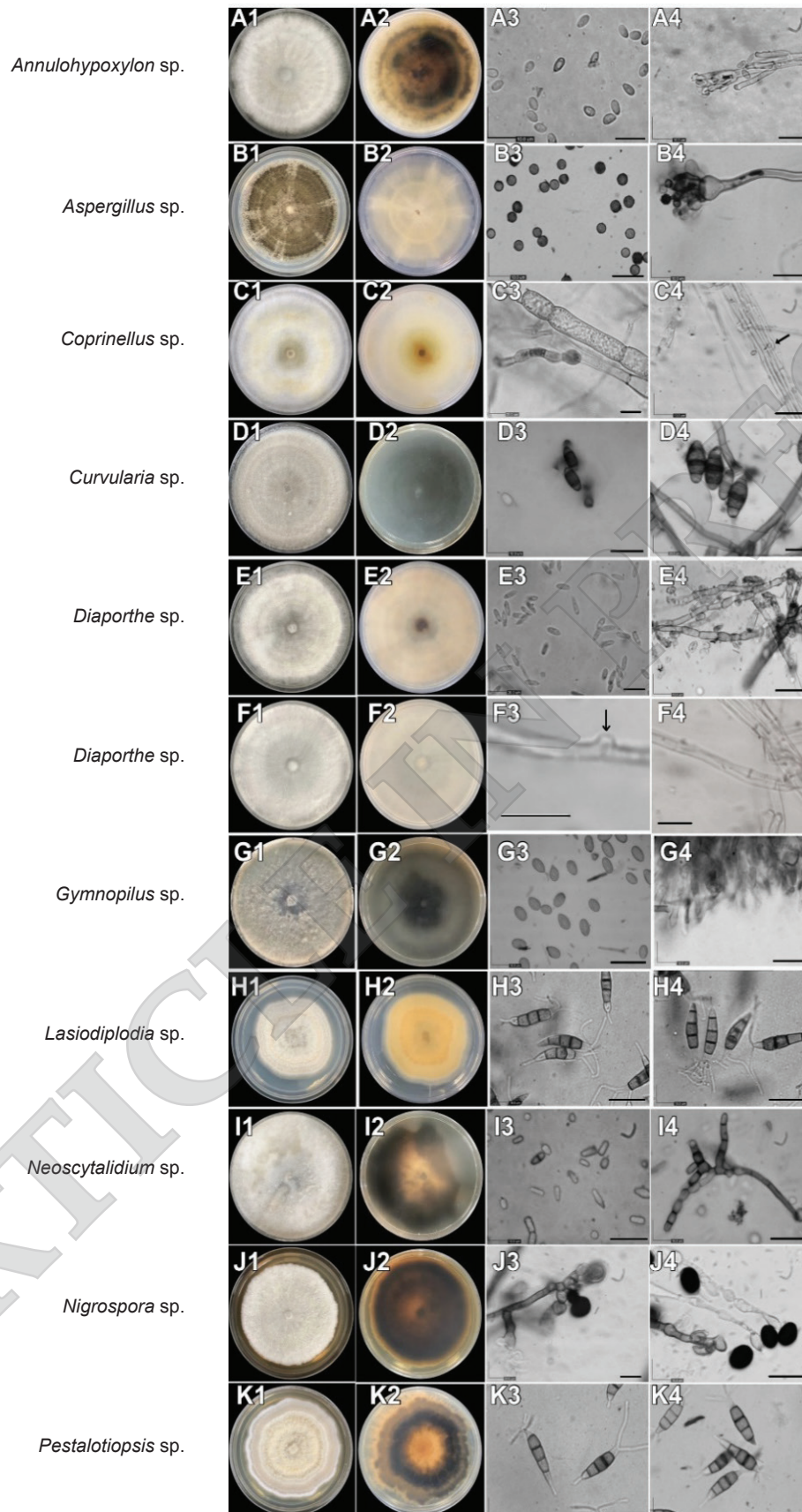
Species of *Aspergillus* had a black powdery colony surface with creamy pigmentation (Figure 2-B1 and B2). The tip of the *Aspergillus* species conidiophore is enlarged, creating a vesicle-like swelling. Flask-shaped phialides entirely or partially cover the vesicles. Globose, aseptate conidia are generated by the phialides. The conidia had a size range of around 1.82–4.24  $\mu\text{m}$   $\times$  1.89–3.82  $\mu\text{m}$ .

The colonies of *Coprinellus* species exhibited white, cottony aerial mycelia with buff to creamy pigmentation on the reverse side of the culture plate (Figure 2-C1 and C2). For *Coprinellus* sp. and *Gymnopilus* sp., no conidia were produced under standard culture conditions; however, conidia could be observed when fruiting body formation was induced. Clamp connections (Figure 2-C4) were present on their hyaline, septate hyphae. Clamp connections are specialised hyphal structures found in many Basidiomycota fungi. Their primary function is to maintain the dikaryotic state (two genetically distinct nuclei per cell) during cell division. They ensure the even distribution of nuclei by forming a bridge that allows one of the nuclei to migrate into the newly formed cell, preserving genetic balance.

From oil palm leaves, only one isolate of *Curvularia* sp. was isolated. The colony features of this species are that it has a grey cottony surface, which is flat and filiform. The reverse plate also showed greyish-black pigmentation (Figure 2-D1). This fungus had single conidiophores with single conidia that were straight, brown and solitary. *Curvularia* sp. produced conidia that have 3-distoseptate (Figure 2-D4), barrel-shaped, with the largest cell being the second from the base. The intermediate cells were brown. The conidia measured roughly around 14.32–21.51  $\mu\text{m}$   $\times$  7.39–11.89  $\mu\text{m}$ . The hyphae were dark hyaline and septate.

TABLE 1. MORPHOLOGICAL CHARACTERISTICS OF FUNGAL ISOLATES FROM OIL PALM LEAVES IN JENGGKA, PAHANG

Isolate No.	Colony features					Microscopic features			Species ID (total isolates)
	Form	Elevation	Margin	Surface	Pigmentation	Shape of conidia	Conidia size (length x width, µm)	Hyphae	
C3337P, C3352P, C3354P, C3371P	Filamentous	Raised	Undulate	White cottony	Dark brown	Dark globose, no septate	8.38–12.34 x 10.06–13.89	Dark hyaline, septate	<i>Nigrospora</i> sp. (4 isolates)
C3338P, C3348P, C3491P, C3345P, C3349P, C3368P, C3357P, C3370P	Filamentous	Flat	Filiform	Grey, brown cottony/feathery	Light brown/ creamy	Fusoid-cylindrical, no septate	5.11–7.43 x 2.15–2.61	Hyaline, septate	<i>Diaporthe</i> sp. (8 isolates)
C3339P, C3490P, C3367P, C3359P	Filamentous	Flat	Entire	Brown/grey/ black prower	Creamy	Globose, no septate	1.82–4.24 x 1.89–3.82	Hyaline, septate,	<i>Aspergillus</i> sp. (4 isolates)
C3340P, C3343P, C3344P, C3346P, C3350P, C3361P, C3492P, C3493P, C3494P	Filamentous	Flat	Filiform	Grey-cottony	Grey to black	Ellipsoid, 0-1 septate	17.99–21.95 x 10.34–14.23	Hyaline, septate	<i>Lasiodiplodia</i> sp. (9 isolates)
C3341P, C3365P, C3372P	Filamentous	Flat	Filiform	Hairy grey to white	Creamy	-	-	Hyaline, septate	<i>Gynnopitius</i> sp. (3 isolates)
C3342P, C3347P, C3360P, C3373P	Filamentous	Flat	Filiform	White cottony	Buff with irregular dark brown rings	Ellipsoid, no septate	4.34–5.71 x 2.65–3.21	Hyaline, septate	<i>Annulohyphogylon</i> sp. (4 isolates)
C3351P, C3366P	Filamentous	Raised	Filiform	White-yellow cottony	Creamy	-	-	Hyaline, septate	<i>Coprinellus</i> sp. (2 isolates)
C3353P, C3362P, C3369P	Filamentous	Raised	Undulate	Pale brown feathery	Buff with irregular concentric rings	Fusoid with 2-3 appendage, 3-4 septa	20.33–28.49 x 4.88–6.09	Hyaline, septate	<i>Pestalotiopsis</i> sp. (3 isolates)
C3355P	Filamentous	Flat	Filiform	Grey cottony	Greyish-black	Barrel-shaped, 3 septa	14.32–21.51 x 7.39–11.89	Dark hyaline, septate	<i>Curvularia</i> sp. (1 isolate)
C3356P	Filamentous	Flat	Filiform	Hairy grey to white	Brown to black	Globose, ellipsoid, cylindrical, no septum	5.20–7.94 x 2.36–2.96	Dark hyaline, septate, Arthroconidia	<i>Neoscytalidium</i> sp. (1 isolate)
C3363P, C3364P	Filamentous	Raised	Undulate	White to pale brown feathery	Buff with irregular concentric rings	Fusoid with 2-3 appendages, 3-4 septa	21.26–24.50 x 5.07–5.81	Hyaline, septate	<i>Neopestalotiopsis</i> sp. (2 isolates)



Note: Column 1 = top view, column 2 = bottom view, column 3-4 = microscopic photos. Arrows indicated the clamp connection.  
Bars: 10  $\mu$ m

Figure 2. Morphological characteristics of the different fungal species grown on PDA. The isolated fungi show varying colony features and conidial shape and size.

Colony features of *Diaporthe* species range from a white, grey to brown feathery surface. After two weeks' incubation, dark-coloured stromata were observed on the media; these matured stromata produce pycnidial conidiomata. The cultures produced buff to creamy pigment (Figure 2-E2) produced swollen hyaline, and septate hyphae. *Diaporthe* species formed three types of conidia: Alpha, beta and gamma conidia. The alpha conidia were biguttulate, hyaline and aseptate. The conidia shapes were fusoid-cylindrical, with sizes ranging from 5.11 to 7.43  $\mu\text{m} \times$  2.15 to 2.61  $\mu\text{m}$ . The beta conidia were filiform, one-celled, hyaline, aseptate and had straight or curved ends.

Colony of *Lasiodiplodia* species produced abundant grey-cottony aerial mycelia that have a range of colours of colonies from dark grey to black (Figure 2-F1 and F2). Initially, the colony was white to pale grey, but changed to a dark grey colour when matured. The colony produced dark grey to black pigment. *Lasiodiplodia* sp. paraphyses were hyaline in colour, cylindrical and septate. The conidia of *Lasiodiplodia* species are cylindrical with sub-ovoid shapes, 17.99–21.95  $\mu\text{m} \times$  10.34–14.23  $\mu\text{m}$  with pointed bases and rounded apices. The mature conidia were thick-walled, single-septate, dark brown, septate and had longitudinal striations on the inner surface.

*Neopestalotiopsis* species have white to pale brown feathery colony morphology, which was also raised and undulated (Figure 2-H1 and H2). The reverse plate was buff with irregular concentric rings, and the surface of the colony had conidiomata that exuded dark conidial masses. Fusoid conidia with one to two tubular apical appendages and one tubular basal appendage are seen. It had three to four brown and versicolor septa. Sizes of *Neopestalotiopsis* sp. conidia ranged from 21.26 to 24.50  $\mu\text{m} \times$  5.07 to 5.81  $\mu\text{m}$ .

The colony of *Neoscytalidium* species was hairy, grey to white, with flat elevation and filiform margin (Figure 2-I1 and I2). The species produced black pigment. This species produced hyaline to brown arthroconidia occurring singly or in arthric chains. They have varied shapes and sizes, such as globose, ellipsoid or cylindrical, with sizes in a range of 5.20–7.94  $\mu\text{m} \times$  2.36–2.96  $\mu\text{m}$ .

*Nigrospora* species initially appear white and cottony, expand rapidly and gradually change colour from white to grey and eventually to black over time (Figure 2-J1 and J2). On SNA, colonies are flat and have a lot of aerial mycelia surface, black without any spots and sporulate quickly. A lot of single, globose, black and smooth aseptate conidia were produced. They ranged from sizes of 8.38–12.34  $\mu\text{m} \times$  10.06–13.89  $\mu\text{m}$  for the *Nigrospora* species. Closer to the conidiogenous area, hyphae became brown and were branching, septate, smooth and hyaline. Conidiophores gathered

in black sporodochia, and were multiseptate, greatly branching, and occasionally condensed to conidiogenous cells. The cells that are monoblastic and determinate congregate in clusters on hyphae.

*Pestalotiopsis* sp. had white to pale brown to brown feathery aerial mycelium that was raised. Over time, the presence of conidiomata can be seen on PDA, which exudes globose with dark brown to black pigment. Colony pigmentation on the reverse plates of the isolates was buffed with irregular concentric rings (Figure 2-K1 and K2). The conidia are seen to be fusoid with two to three tubular apical and one tubular basal appendages (Figure 2-K3 and K4). It had three to four septa that were brown and concolourous. The conidia of *Pestalotiopsis* had sizes ranging from 20.33 to 28.49  $\mu\text{m}$  in length and 4.88 to 6.09  $\mu\text{m}$  in width.

### Several Fungi Cause Leaf Spots

About 10 pathogenic isolates formed chlorosis and necrotic spots on oil palm leaves. The leaves showed varying symptoms after 21 days post-inoculation. Some include chlorosis to severe necrotic lesions at the point of inoculation (Figure 3). The lesion sizes were analysed, and 10 isolates were significantly bigger ( $p < 0.05$ ) compared with control leaves (Table 2). All pathogenic isolates include *Lasiodiplodia theobromae* (isolates C3340P, C3344P, C3346P and C3361P), *Diaporthe phaseolorum* C3349P, *Nigrospora oryzae* C3352P, *Pestalotiopsis microspora* C3353P, *Curvularia eragrostidis* C3355P, *Aspergillus niger* C3359P and *Neopestalotiopsis clavispora* C3363P. *Lasiodiplodia theobromae* had the highest number of pathogenic isolates. The ITS sequences of the fungal isolates that produced disease lesions were successfully amplified. The bands were consistently reproducible.

Accession numbers of the representative fungal isolates associated with leaf spot disease are presented in Table 3, along with their closest sequence matches obtained from BLAST analysis. These matches provide a reference for the molecular identification of each isolate based on ITS region sequences. The tabulated data offer insights into the species-level identity of the pathogens and support the morphological and pathogenicity findings of the study. This molecular characterisation is crucial for accurate diagnosis and understanding of the causal agents involved in leaf spot disease.

Phylogenetic analysis based on the ITS sequences was carried out to determine the genetic relationships of the pathogenic fungal isolates. The sequences were aligned and compared with reference sequences retrieved from GenBank using BLASTn. A phylogenetic tree was then constructed

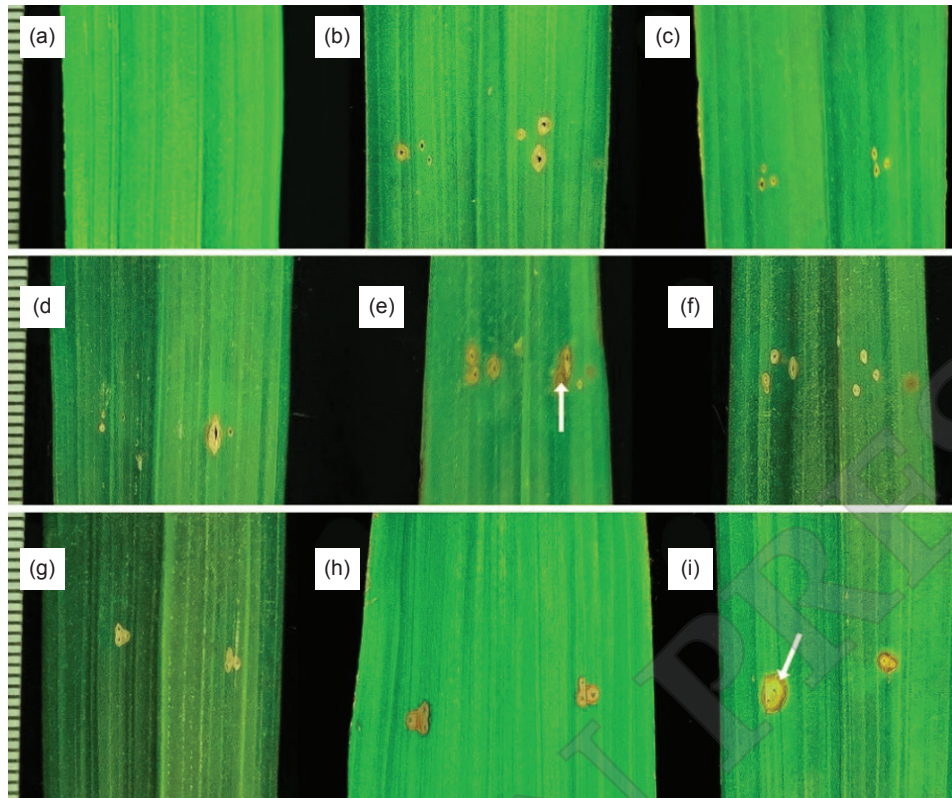


Figure 3. The lesions on oil palm leaves produced by representative isolates of pathogenic species after 21 days of fungal inoculation. (a) Non-wounded control, (b) *Lasiodiplodia theobromae* C3340P, (c) *Lasiodiplodia theobromae* C3344P, (d) *Diaporthe phaseolorum* C3349P, (e) *Nigrospora oryzae* C3352P, (f) *Pestalotiopsis microspora* C3353P, (g) *Curvularia eragrostidis* C3355P, (h) *Aspergillus niger* C3359P, (i) *Neopestalotiopsis clavispora* C3363P. The arrow indicates the necrotic area. Saturation and color differences are due to lighting effects during photography. One division of the ruler on the left side of the leaf equals 1 mm.

TABLE 2. LEAF SPOT LESION SIZES 21 DAYS AFTER FUNGAL INOCULATION

Isolate No.	Species	*Mean lesion size $\pm$ SD (mm)	Description	*Pathogenicity
Control	-	0 <sup>a</sup>	-	-
C3337P	<i>Nigrospora</i> sp.	0 <sup>a</sup>	No visible lesion observed	N
C3338P	<i>Diaporthe</i> sp.	0 <sup>a</sup>	No visible lesion observed	N
C3339P	<i>Aspergillus</i> sp.	0 <sup>a</sup>	No visible lesion observed	N
<b>C3340P</b>	<b><i>Lasiodiplodia theobromae</i></b>	<b>0.93 <math>\pm</math> 0.54<sup>de</sup></b>	<b>Yellow to brownish necrotic lesion</b>	<b>P</b>
C3341P	<i>Gymnopilus</i> sp.	0 <sup>a</sup>	No visible lesion observed	N
C3342P	<i>Annulohyphoxylon</i> sp.	0 <sup>a</sup>	No visible lesion observed	N
C3343P	<i>Lasiodiplodia theobromae</i>	0 <sup>a</sup>	No visible lesion observed	N
<b>C3344P</b>	<b><i>Lasiodiplodia theobromae</i></b>	<b>0.62 <math>\pm</math> 0.76<sup>bcd</sup></b>	<b>Small, light necrotic lesion</b>	<b>P</b>
C3345P	<i>Diaporthe</i> sp.	0 <sup>a</sup>	No visible lesion observed	N
<b>C3346P</b>	<b><i>Lasiodiplodia theobromae</i></b>	<b>1.76 <math>\pm</math> 0.87<sup>g</sup></b>	<b>Yellow to brownish necrotic lesion</b>	<b>P</b>
C3347P	<i>Annulohyphoxylon</i> sp.	0 <sup>a</sup>	No visible lesion observed	N
C3348P	<i>Diaporthe</i> sp.	0 <sup>a</sup>	No visible lesion observed	N
<b>C3349P</b>	<b><i>Diaporthe phaseolorum</i></b>	<b>0.69 <math>\pm</math> 0.99<sup>cde</sup></b>	<b>Small, light necrotic lesion</b>	<b>P</b>
C3350P	<i>Lasiodiplodia theobromae</i>	0.18 $\pm$ 0.19 <sup>abc</sup>	Small, light necrotic lesion	N
C3351P	<i>Coprinellus aureoconulatus</i>	0 <sup>a</sup>	No visible lesion observed	N
<b>C3352P</b>	<b><i>Nigrospora oryzae</i></b>	<b>1.79 <math>\pm</math> 0.72<sup>g</sup></b>	<b>Yellow to brownish necrotic lesion</b>	<b>P</b>

TABLE 2. LEAF SPOT LESION SIZES 21 DAYS AFTER FUNGAL INOCULATION (continued)

Isolate No.	Species	*Mean lesion size ± SD (mm)	Description	*Pathogenicity
C3353P	<i>Pestalotiopsis microspora</i>	0.80 ± 0.29 <sup>de</sup>	Small, light necrotic lesion	P
C3354P	<i>Nigrospora</i> sp.	0 <sup>a</sup>	No visible lesion observed	N
C3355P	<i>Curvularia eragrostidis</i>	0.74 ± 0.29 <sup>de</sup>	Small, light necrotic lesion	P
C3356P	<i>Nigrospora dimidiatum</i>	0.13 ± 0.19 <sup>ab</sup>	Small, light necrotic lesion	N
C3357P	<i>Diaporthe</i> sp.	0 <sup>a</sup>	No visible lesion observed	N
C3359P	<i>Aspergillus niger</i>	1.65 ± 0.95 <sup>fg</sup>	Yellow to brownish necrotic lesion	P
C3360P	<i>Annulohyphoxylon</i> sp.	0 <sup>a</sup>	No visible lesion observed	N
C3361P	<i>Lasiodiplodia theobromae</i>	1.20 ± 0.84 <sup>ef</sup>	Yellowish brown necrotic lesion with chlorosis	P
C3362P	<i>Pestalotiopsis microspora</i>	0.04 ± 0.07 <sup>a</sup>	Small, light necrotic lesion	N
C3363P	<i>Neopestalotiopsis clavispورا</i>	2.56 ± 0.32 <sup>h</sup>	Large necrotic lesion with yellow chlorosis	P
C3364P	<i>Neopestalotiopsis</i> sp.	0 <sup>a</sup>	No visible lesion observed	N
C3365P	<i>Gymnopilus</i> sp.	0 <sup>a</sup>	No visible lesion observed	N
C3366P	<i>Coprinellus</i> sp.	0 <sup>a</sup>	No visible lesion observed	N
C3367P	<i>Aspergillus niger</i>	0.40 ± 0.19 <sup>abcd</sup>	Yellow to brownish necrotic lesion	N
C3368P	<i>Diaporthe</i> sp.	0 <sup>a</sup>	No visible lesion observed	N
C3369P	<i>Pestalotiopsis</i> sp.	0 <sup>a</sup>	No visible lesion observed	N
C3370P	<i>Diaporthe</i> sp.	0 <sup>a</sup>	No visible lesion observed	N
C3371P	<i>Nigrospora sphaerica</i>	0.50 ± 0.33 <sup>abcd</sup>	Yellow to brownish necrotic lesion	N
C3372P	<i>Gymnopilus</i> sp.	0 <sup>a</sup>	No visible lesion observed	N
C3373P	<i>Annulohyphoxylon</i> sp.	0 <sup>a</sup>	No visible lesion observed	N
C3490P	<i>Aspergillus</i> sp.	0 <sup>a</sup>	No visible lesion observed	N
C3491P	<i>Diaporthe</i> sp.	0 <sup>a</sup>	No visible lesion observed	N
C3492P	<i>Lasiodiplodia theobromae</i>	0 <sup>a</sup>	No visible lesion observed	N
C3493P	<i>Lasiodiplodia theobromae</i>	0 <sup>a</sup>	No visible lesion observed	N
C3494P	<i>Lasiodiplodia theobromae</i>	0 <sup>a</sup>	No visible lesion observed	N

Note: \*Different superscript letters in each column indicate that there were significant differences ( $p < 0.05$ ). #N= Non-pathogenic, P= Pathogenic.

TABLE 3. REPRESENTATIVE PATHOGENIC ISOLATES WITH GENBANK ACCESSION NUMBERS AND THEIR CLOSEST SEQUENCE MATCHES

Isolate No.	Species	GenBank accession number	GenBank accession number (Closest Match)	Query coverage (%)	E-value	Similarity/Identity (%)
C3340P	<i>Lasiodiplodia theobromae</i>	OQ132547	OP882285	100	0.0	99.82
C3344P	<i>Lasiodiplodia theobromae</i>	OQ132549	MK530029	100	0.0	99.82
C3346P	<i>Lasiodiplodia theobromae</i>	OQ132550	OP882285	100	0.0	99.82
C3349P	<i>Diaporthe phaseolorum</i>	OQ132539	MT043783	100	0.0	99.47
C3352P	<i>Nigrospora oryzae</i>	OQ132553	PV770025.1	100	0.0	99.64
C3353P	<i>Pestalotiopsis microspora</i>	OQ132554	MN635619.1	100	0.0	99.82
C3355P	<i>Curvularia eragrostidis</i>	OQ132555	OR135782.1	100	0.0	100.00
C3359P	<i>Aspergillus niger</i>	OQ132541	MH091025.1	100	0.0	99.84
C3361P	<i>Lasiodiplodia theobromae</i>	OQ132542	KR260791.1	100	0.0	99.81
C3363P	<i>Neopestalotiopsis clavispورا</i>	PV888892	MH397088.1	100	0.0	100.00

using the Maximum Likelihood method with bootstrap support to assess the reliability of the groupings. The resulting tree grouped the isolates with their respective closely related taxa, confirming their identity at the species or genus level (Figure 4). This molecular approach provided robust support for the morphological identification and clarified the evolutionary relationships among the disease-causing fungi.

*Lasiodiplodia theobromae* is the most common pathogen responsible for leaf spot disease in plants. However, there have been limited studies documenting this species on oil palm leaves. These fungi can act as endophytes or secondary pathogens and can acquire the ability to cause disease when subjected to stress (Huda-Shakirah et al., 2022). However, in *Diaporthe* species, only one pathogenic isolate (C3349P) was found to cause lesions on oil palm leaves. There have been multiple reports of *Diaporthe* species functioning as saprobes, non-pathogenic endophytes or plant pathogens across various hosts (Gomes et al., 2013). However, no report of this species infecting oil palm.

Another pathogenic fungal species is *Nigrospora* C3352P, which causes visible lesions on the leaves of oil palms. In a previous study, *Nigrospora* species were known to be pathogenic on several host plants (Hao et al., 2020). It exhibits clear pathogenic behaviour, but it is typically classified as a weak

pathogen (Zhang et al., 2012). The wind, rain splash and insect vectors help *Nigrospora* disperse its spores, causing the disease to spread quickly. Hao et al. (2020), also explained that on released spores, a sticky, mucilaginous material was seen to be present.

Several cases of leaf spot diseases caused by *Pestalotiopsis*, include oil palm leaves which have been reported in several countries such as China and Malaysia (Mohamed-Azni et al., 2022; Shen et al., 2014). The infection can occur initially by the conidia or fragmented spores of fungal pathogens contacting the host (Espinoza et al., 2008). These inocula could endure weather conditions and result in initial infections. The severity of the disease may worsen if secondary inocula develop on leaf spot-infected tissue which can lead to secondary infections (Borrero et al., 2018).

*Neopestalotiopsis* species is a common leaf spot pathogen worldwide, mostly causing disease in blueberry and strawberry plants (Borrero et al., 2018; Chamorro et al., 2016). To date, no report of this pathogen in oil palm leaves. In Malaysia, there have been many reported cases of *N. clavispora* on the tea plant, *Camellia sinensis* (Chen et al., 2017; Shahriar et al., 2022). The capacity of *Neopestalotiopsis* species to create phytotoxins, pestalopyrones, hydroxypestalopyrones and pestalosides determines how pathogenic they are (Prasannath et al., 2020).

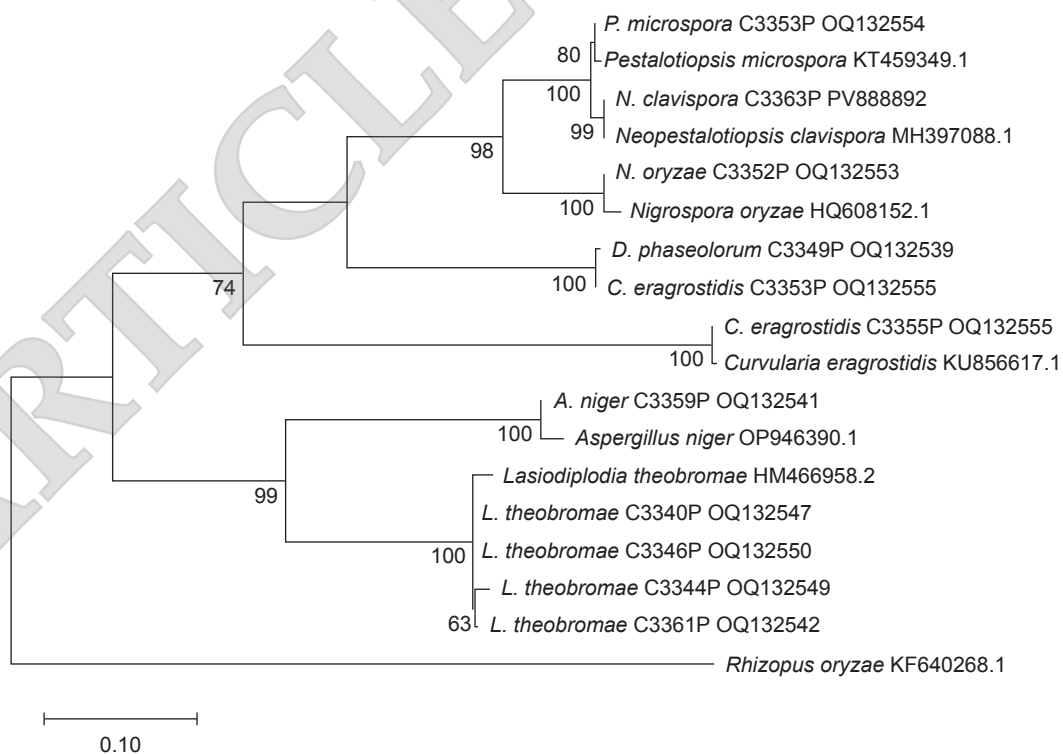


Figure 4. Neighbor-joining (NJ) tree based on internal transcribed spacer (ITS) sequences of fungal species associated with leaf spot disease of oil palm. Bootstrap values ( $\geq 70\%$ ) are presented at nodes of the tree. The number on the side denotes the GenBank accession number of the sequence.

*Aspergillus* species is a common postharvest pathogen and has been reported as a leaf spot causing pathogen in various plants (Zhang et al., 2016). This species can survive at 15°C–53°C and is tolerant to variations in pH, humidity and heat, making it a prevalent pathogen (Dania et al., 2021).

The findings indicate that *Curvularia* species cause small, light necrotic lesions on oil palm leaves. In Thailand, Kittimorakul et al. (2013) reported, *C. eragrostidis* has an incidence rate of 61.01% on oil palm leaves and is a major contributor to *Curvularia* leaf spot disease of the plant. In Malaysia, there has not been any reported cases of this specific fungal species infecting oil palm leaves. Reports of this species in Malaysia were only found on the plant, *Sansevieria trifasciata* (Kee et al., 2020).

*Neoscytalidium* sp. is primarily recognised as an opportunistic plant pathogen responsible for internal black rot in fruits, as well as pit canker and spots on plant stems and fruits (Nouri et al., 2018; Yi et al., 2015). In this study, two out of four isolates produced infection symptoms on the leaves of oil palm. Pakdaman (2022) reported that this species caused infection in an extensive host range. Although no vector has been identified for this pathogen, it is classified as a soilborne and airborne pathogen capable of surviving in infected debris and damaged trees (Pakdaman, 2022). However, there are no prior reports of this species infecting oil palm (Farr & Rossman, 2023). In addition to pathogenic species, some fungal genera such as *Annulohyphoxylon*, *Coprinellus* and *Gymnopilus* species are non-pathogenic. These genera did not cause any lesions on inoculated oil palm leaves and are classified as saprophytic fungi. Saprophytes are microscopic organisms that feed on detritus or non-living organic material (Wilson, 2018).

The presence of these pathogens raises concerns regarding their potential impact on oil palm productivity. The high infection rate observed suggests that environmental factors, such as temperature and humidity, may favour disease development. Given that *L. theobromae* is known for its aggressive necrotrophic behaviour, it is likely to exacerbate disease severity, leading to premature leaf senescence and reduced photosynthetic capacity. *Diaporthe* species are commonly reported as endophytes or latent pathogens, which may indicate that *D. phaseolorum* could persist asymptomatically before causing disease under stress conditions. Similarly, *Neopestalotiopsis* species have been associated with a broad host range, including economically significant crops, suggesting that *N. clavispora* could also pose a risk beyond oil palm if favourable conditions arise.

The findings of this study emphasise the need for targeted disease management strategies.

Since these pathogens have different infection mechanisms compared to previously reported leaf spot fungi, integrated control approaches, including early detection, cultural practices and fungicidal applications, should be explored. Future study should focus on understanding the environmental triggers that influence disease outbreaks and the potential interactions among these fungal species in mixed infections. Additionally, molecular studies on the genetic diversity of these pathogens could provide insights into their evolutionary adaptations and potential resistance to management strategies.

By identifying additional species, including *L. theobromae*, *D. phaseolorum* and *N. clavispora*, as potential causal agents of leaf spot disease, further disease management strategies should be considered.

## CONCLUSION

Leaf spot disease was observed in oil palm plantations in Jengka, Pahang, Malaysia with a high incidence of infection among trees. Pathogenicity tests identified *L. theobromae*, *D. phaseolorum* and *N. clavispora* as the primary causal agents, marking the first report of the latter two species affecting oil palm. Early identification of these pathogens is crucial for implementing effective disease management strategies, which may include an integrated approach combining cultural practices, chemical control and biological methods to mitigate their impact.

## ACKNOWLEDGEMENT

The authors would like to express their sincere appreciation to the Universiti Driven Research Programme (UDRP)-Biodiversity and Conservation (ConBioD) for its continuous support and encouragement, which greatly contributed to the success of this research.

## REFERENCES

- Borrero, C., Castaño, R., & Avilés, M. (2018). First report of *Pestalotiopsis clavispora* (*Neopestalotiopsis clavispora*) causing canker and twig dieback on blueberry bushes in Spain. *Plant Disease*, 102(6), 1178. <https://doi.org/10.1094/pdis-10-17-1529-pdn>
- Brown, W. (1924). A method of isolating single strains of fungi by cutting out a hyphal tip. *Annals of Botany*, 38(150), 402–404.

- Chamorro, M., Aguado, A., & De Los Santos, B. (2016). First report of root and crown rot caused by *Pestalotiopsis clavispora* (*Neopestalotiopsis clavispora*) on strawberry in Spain. *Plant Disease*, 100(7), 1495. <https://doi.org/10.1094/pdis-11-15-1308-pdn>
- Chen, Y., Zeng, L., Shu, N., Jiang, M., Wang, H., Huang, Y., & Tong, H. (2017). *Pestalotiopsis*-like species causing gray blight disease on *Camellia sinensis* in China. *Plant Disease*, 102(1), 98–106. <https://doi.org/10.1094/pdis-05-17-0642-re>
- Dania, V. O., Fajemisin, A. O., & Azuh, V. O. (2021). Morphological and molecular characterization of *Aspergillus niger* causing postharvest rot of white yam (*Dioscorea rotundata* Poir). *Archives of Phytopathology and Plant Protection*, 54(19–20), 2356–2374. <https://doi.org/10.1080/03235408.2021.1983365>
- Daval, A., Pomiès, V., Dossa, J. S. B., Riou, V., Lopez, D., Poncet, C., Pernaci, M., Cochard, B., Jacob, F., Billotte, N., & Tisné, S. (2022). Identification of *Fusarium* wilt resistance loci in two major genetic backgrounds for oil palm breeding. *Industrial Crops and Products*, 187, 115291. <https://doi.org/10.1016/j.indcrop.2022.115291>
- Di Marco, S., Calzarano, F., Osti, F., & Mazzullo, A. (2004). Pathogenicity of fungi associated with a decay of kiwifruit. *Australasian Plant Pathology*, 33(3), 337–342. <https://doi.org/10.1071/ap04024>
- Espinoza, J. G., Briceño, E. X., Keith, L. M., & Latorre, B. A. (2008). Canker and twig dieback of blueberry caused by *Pestalotiopsis* spp. and a *Truncatella* sp. in Chile. *Plant Disease*, 92(10), 1407–1414. <https://doi.org/10.1094/PDIS-92-10-1407>
- Farr, D. F., & Rossman, A. Y. (2023). *Fungal databases*. U.S. National Fungus Collections, ARS, USDA. Retrieved January 28, 2023, from <http://nt.ars-grin.gov/fungaldatabases/>
- Fisher, N. L., Burgess, L. W., Toussoun, T. A., & Nelson, P. E. (1982). Carnation leaves as a substrate for preserving cultures of *Fusarium* species. *Phytopathology*, 72(1), 151–153.
- Flood, J., Bridge, P. D., & Pilotti, C. A. (2022). Basal stem rot of oil palm revisited. *Annals of Applied Biology*, 181(2), 160–181. <https://doi.org/10.1111/aab.12772>
- Gomes, R. R., Glienke, C., Videira, S. I., Lombard, L., Groenewald, J. Z., & Crous, P. W. (2013). *Diaporthe*: A genus of endophytic, saprobic and plant pathogenic fungi. *Persoonia*, 31, 1–41. <https://doi.org/10.3767/003158513x666844>
- Hao, Y., Aluthmuhandiram, J. V. S., Chethana, K. W. T., Manawasinghe, I. S., Li, X., Liu, M., Hyde, K. D., Phillips, A. J. L., & Zhang, W. (2020). *Nigrospora* species associated with various hosts from Shandong Peninsula, China. *Mycobiology*, 48(3), 169–183. <https://doi.org/10.1080/12298093.2020.1761747>
- Huda-Shakirah, A. R., Mohamed, N. N. M. I., Zakaria, L., Leong, Y. H., & Mohd, M. H. (2022). *Lasiodiplodia theobromae* as a causal pathogen of leaf blight, stem canker, and pod rot of *Theobroma cacao* in Malaysia. *Scientific Reports*, 12(1), 1–14. 8966. <https://doi.org/10.1038/s41598-022-13057-9>
- Ismail, S. I., Zulperi, D., Norddin, S., & Ahmad-Hamdani, S. (2017). First report of *Neopestalotiopsis saprophytica* causing leaf spot of oil palm (*Elaeis guineensis*) in Malaysia. *Plant Disease*, 101(10). <https://doi.org/10.1094/PDIS-02-17-0271-PDN>
- Kee, Y. J., Zakaria, L., & Mohd, M. H. (2020). *Curvularia asianensis* and *Curvularia eragrostidis* associated with leaf spot of *Sansevieria trifasciata* in Malaysia. *Journal of Phytopathology*, 168(5), 290–296. <https://doi.org/10.1111/jph.12890>
- Kittimorakul, J., Pornsuriya, C., Sunpapao, A., & Petcharat, V. (2013). Survey and incidence of leaf blight and leaf spot disease of oil palm seedlings in Southern Thailand. *Plant Pathology Journal*, 12(3), 149–153. <https://doi.org/10.3923/ppj.2013.149.153>
- Kittimorakul, J., Sunpapao, A., Duangpan, S., Anothai, J., & Eksomtramage, T. (2019). Screening of oil palm (*Elaeis guineensis* Jacq.) varieties for resistance to *Curvularia* leaf spot disease. *Australian Journal of Crop Science*, 13(4), 507–512. <https://doi.org/10.21475/ajcs.19.13.04.p1276>
- Maizatun-Suriza, M., Dickinson, M., & Idris, A. S. (2019). Molecular characterization of *Phytophthora palmivora* responsible for bud rot disease of oil palm in Colombia. *World Journal of Microbiology and Biotechnology*, 35(3), 44. <https://doi.org/10.1007/s11274-019-2618-9>
- Mohamed-Azni, I. N. A., Sritharan, K., Ho, S., Roslan, N. D., Arulandoo, X., & Sundram, S. (2022). Isolation, identification and pathogenicity

- of fungi associated with leaf blotches in *Tenera x Tenera* (TxT) variety of oil palm in Malaysia. *Journal of Plant Pathology*, 104(1), 167–177. <https://doi.org/10.1007/s42161-021-00953-8>
- Muhamad, A., Azlan, N., Abidin, Z., Ahmad, M., Seman, I. A., & Vadamalai, G. (2018). Assessment of leaf spot and anthracnose diseases in nurseries and its relationship with oil palm seedling ages. *International Journal of Advanced Multidisciplinary Research*, 5(1), 19–26.
- Nasehi, A., Sathyapriya, H., & Wong, M. Y. (2020). First report of leaf spot on oil palm caused by *Phyllosticta capitalensis* in Malaysia. *Plant Disease*, 104(1). <https://doi.org/10.1094/PDIS-06-19-1232-PDN>
- Nazarov, P. A., Baleev, D. N., Ivanova, M. I., Sokolova, L. M., & Karakozova, M. V. (2020). Infectious plant diseases: Etiology, current status, problems and prospects in plant protection. *Acta Naturae*, 12(3), 46–59. <https://doi.org/10.32607/actanaturae.11026>
- Nor Azlan, A. M., Mior Ahmad, Z. A., Idris, A. S., & Vadamalai, G. (2018). Assessment of leaf spot and anthracnose diseases in nurseries and their relationship with oil palm seedling ages. *International Journal of Advanced Multidisciplinary Research*, 5(1), 19–26. <https://doi.org/10.22192/ijamr.2018.05.01.005>
- Nouri, M. T., Lawrence, D. P., Yaghmour, M. A., Michailides, T. J., & Trouillas, F. P. (2018). *Neoscytalidium dimidiatum* causing canker, shoot blight and fruit rot of almond in California. *Plant Disease*, 102(8), 1638–1647. <https://doi.org/10.1094/pdis-12-17-1967-re>
- Pakdaman, B. S. (2022). *Neoscytalidium dimidiatum*: An emerging threat in global warmth era. *Journal of Biotechnology and Bioresource*, 3(4), 1–4.
- Pham, M. T., Huang, C. M., & Kirschner, R. (2020). First report of the oil palm disease fungus *Marasmius palmivorus* in Taiwan causing stem rot on *Arenga engleri*. *Letters in Applied Microbiology*, 70(3), 143–150. <https://doi.org/10.1111/lam.13257>
- Pornsuriya, C., Sunpapao, A., Srihanant, N., Worapattam, K., Kittimorakul, J., Phithakkit, S., & Petcharat, V. (2013). A survey of diseases and disorders in oil palms of Southern Thailand. *Plant Pathology Journal*, 12(4), 169–175. <https://doi.org/10.3923/ppj.2013.169.175>
- Prasannath, K., Galea, V. J., & Akinsanmi, O. A. (2020). Characterisation of leaf spots caused by *Neopestalotiopsis clavispora* and *Colletotrichum siamense* in macadamia in Australia. *European Journal of Plant Pathology*, 156(4), 1219–1225. <https://doi.org/10.1007/s10658-020-01962-6>
- Salvamani, S., & Nawawi, N. M. (2014). Macroscopic and microscopic approaches for identification of fungi from plant soil of Cameron Highlands. *Bioremediation Science and Technology Research*, 2(1), 14–18.
- Shahriar, S. A., Nur-Shakirah, A. O., & Mohd, M. H. (2022). *Neopestalotiopsis clavispora* and *Pseudopestalotiopsis camelliae-sinensis* causing grey blight disease of tea (*Camellia sinensis*) in Malaysia. *European Journal of Plant Pathology*, 162(3), 709–724. <https://doi.org/10.1007/s10658-021-02433-2>
- Shen, H. F., Zhang, J. X., Lin, B. R., Pu, X. M., Zheng, L., Qin, X. D., Li, J., & Xie, C. P. (2014). First report of *Pestalotiopsis microspora* causing leaf spot of oil palm in China. *Plant Disease*, 98(10), 1429. <https://doi.org/10.1094/pdis-02-14-0163-pdn>
- Stratton, S. J. (2021). Population research: Convenience sampling strategies. *Prehospital and Disaster Medicine*, 36(4), 373–374. <https://doi.org/10.1017/s1049023x21000649>
- Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, 38(7), 3022–3027. <https://doi.org/10.1093/molbev/msab120>
- Tsui, C. K. M., Woodhall, J., Chen, W., Lévesque, C. A., Lau, A., Schoen, C. D., Baschien, C., Najafzadeh, M. J., & de Hoog, G. S. (2011). Molecular techniques for pathogen identification and fungus detection in the environment. *IMA Fungus*, 2, 177–189. <https://doi.org/10.5598/imafungus.2011.02.02.09>
- Watanabe, T. (2002). Morphology of soil fungi. In T. Watanabe (Ed.), *Pictorial atlas of soil and seed fungi* (2nd ed., pp. 39–183). CRC Press.
- White, T. J., Bruns, T., Lee, S. J. W. T., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR protocols* (pp. 315–322). Academic Press.
- Wibowo, C. S., Susilo, R., Ernawan, R., Apriyanto, A., Alshaharni, M. O., Smith, G. R., Gatehouse, A. M. R., & Edwards, M. G. (2024). Molecular

basis of resistance to leaf spot disease in oil palm. *Frontiers in Plant Science*, 15, 1458346. <https://doi.org/10.3389/fpls.2024.1458346>

Wilson, A. W. (2018). *Saprotroph*. Encyclopedia Britannica. Retrieved December 30, 2021, from <https://www.britannica.com/science/saprotroph>

Yi, R. H., Mo, J. J., Wu, F. F., & Chen, J. (2015). Fruit internal brown rot caused by *Neoscytalidium dimidiatum* on pitahaya in Guangdong Province, China. *Australasian Plant Disease Notes*, 10, 13. <https://doi.org/10.1007/s13314-015-0166-1>

Zainudin, N. A. I. M., Siti Nordahliawate, M. S., Johari, N. A., Azmi, A. R., & Salleh, B. (2011). Isolation and identification of *Fusarium* species associated with *Fusarium* ear rot disease of corn.

*Pertanika Journal of Tropical Agricultural Science*, 34(2), 325–330.

Zakaria, L. (2023). Basal stem rot of oil palm: The pathogen, disease incidence, and control methods. *Plant Disease*, 107(3), 603–615. <https://doi.org/10.1094/pdis-02-22-0358-fe>

Zhang, L. X., Li, S. S., Tan, G. J., Shen, J. T., & He, T. (2012). First report of *Nigrospora oryzae* causing leaf spot of cotton in China. *Plant Disease*, 96(9), 1379. <https://doi.org/10.1094/pdis-04-12-0349-pdn>

Zhang, X., Xi, H., Lin, K., Liu, Z., Yu, Y., Sun, Y., & Zhao, J. (2016). *Aspergillus* leaf spot of field bindweed (*Convolvulus arvensis* L.) caused by *Aspergillus niger* in China. *SpringerPlus*, 5(1), 605. <https://doi.org/10.1186/s40064-016-2292-4>

ARTICLE IN PRESS