



UNIVERSITI PUTRA MALAYSIA

**ISOLATION AND IDENTIFICATION OF FUNGAL PATHOGEN CAUSING
LEAF SPOT DISEASE IN OIL PALM AT NURSERY STAGE**

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BY

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LIST OF ABBREVIATIONS

AE	Extraction buffer
AP	Extraction buffer
AW	Column wash buffer
BLAST	Basic Local Alignment Search Tool
Bp	Base pair
CTAB	Cetyl-trimethyl ammonium bromide
DNA	Deoxyribonucleic acid
DxP	Dura X Pisifera
EB	Elution buffer
EPOA	European Palm Oil Alliance
ITS	Internal Transcribed Spacer
LCB	lactophenol cotton blue
LSD	Least significance difference
MPOB	Malaysian Palm Oil Board
MPOC	Malaysian Palm Oil Council
MSPO	Malaysian Sustainable Palm Oil
NCBI	National Centre for Biotechnology Information
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
SFS	Severity foliar symptoms
TBE	Tris borate EDTA

ABSTRACT

Oil palm (*Elaeis guineensis* jacq.) is the most important commodity crop in Malaysia as it contributes 5% of gross national income. Leaf spot disease of oil palm occurs on leaves of all ages particularly on juvenile palms. This disease can cause revenue lost to nursery owners as it affects the growth and quality of the seedlings. Nonetheless, the identity of the causal pathogen is still unknown and often misidentified with other symptoms such leaf blight and anthracnose disease. Therefore the objectives of this study were i. To isolate pathogenic fungi from symptomatic juvenile oil palm leaves ii. To identify the isolated fungus based on morphological and molecular characteristics. Morphological characteristics were studied based on the visible nature and microscopic view of each fungal culture. While for molecular identification, isolated fungal genomic DNA was amplified using ITS1 and ITS4 primer sets and sequenced for identification purpose. The obtained sequences were resulted to BLAST analysis on GenBank sequence database to match the isolates at species level. All the six isolates studied were identified as *Lasidiplodia theobromae*, *Pestalotiopsis microspora*, *Curvularia eragrostidis*, *Phoma herbarum* and *Nigrospora Sphaerica*. Lastly, pathogenicity test was conducted on six months old healthy commercial DxP oil palm seedlings. Two potential fungal isolates which may be related to leaf spot disease were selected, *P. microspora* and *C. eragrostidis*. As a conclusion, in this study both *P. microspora* and *C. eragrostidis* fungi have been identified as the causal pathogen of leaf spot disease on juvenile oil palm seedlings based on the reproducibility of leaf spot symptoms and severity of the foliar symptoms displayed through the pathogenicity test conducted.

ABSTRAK

Kelapa sawit (*Elaeis guineensis* Jacq.) adalah tanaman komoditi yang paling penting di Malaysia kerana ia menyumbang 5% daripada pendapatan kasar negara. Penyakit bintik daun sawit berlaku pada daun dari semua peringkat umur terutamanya di peringkat anak sawit di nurseri. Penyakit ini boleh mendatangkan kerugian kepada pemilik nurseri kerana ia boleh menjejaskan pertumbuhan dan kualiti anak sawit. Namun begitu, identiti patogen penyebab masih tidak diketahui dan sering disalah tafsir dengan gejala penyakit yang lain seperti antraknos daun dan penyakit bintik berpusar. Oleh itu, objektif kajian ini adalah i. Untuk menjalankan pemencilan patogen kulat daripada daun sawit yang mempamerkan simptom-simptom penyakit bintik daun. ii. Untuk mengidentifikasi patogen kulat yang dipencilkan berdasarkan ciri-ciri morfologi dan molikul. Ciri-ciri morfologi setiap kulat yang dipencilkan dikaji berdasarkan diskripsi yang kelihatan pada mata kasar dan secara mikroskopik. Disamping itu, identifikasi secara molikul dilakukan dengan mengekstrak DNA genomik kulat-kulat yang telah dipencilkan dan diamplifikasi menggunakan set pencetus ITS1 dan ITS4 serta diikuti dengan teknik penjujukan nukleotida DNA. Kemudian, jujukan nukleotida DNA yang diperolehi digunakan bagi menjalankan analisis BLAST pada pangkalan data jujukan di GenBank. bagi tujuan identifikasi pada peringkat spesies. Kesemua enam isolat kulat yang dikaji telah berjaya diidentifikasi sebagai *Lasidiplodia theobromae*, *Pestalotiopsis microspora*, *Curvularia eragrostidis*, *Phoma herbarum* dan *Nigrospora Sphaerica*. Akhir sekali, ujian kepatogenan telah dijalankan pada anak sawit DxP komersial yang berumur enam bulan. Dua kulat yang dipencilkan dan dipercayai sebagai patogen yang

menyebabkan penyakit bintik daun; *P. microspora* dan *C. eragrostidis* telah dipilih untuk ujian kepatogenan. Sebagai kesimpulannya, kulat *P. microspora* dan *C. eragrostidis* telah dikenalpasti sebagai penyebab atau patogen yang menyebabkan penyakit bintik daun pada anak sawit berdasarkan penghasilan semula simptom-simptom penyakit bintik daun. serta peratus keterukan simptom pada daun anak sawit yang dipamerkan dalam ujian kepatogenan yang telah dijalankan



CHAPTER 1

INTRODUCTION

Oil palm, (*Elaeis guineensis* jacq.) is a primary commodity crop grown in Malaysia. Oil palm was introduced into Malaya by British in the early 1870's, as an ornamental palm and was first planted as a commercial crop in 1917 and increased rapidly from 1960s when the demand for vegetable oil rise. Even though oil palm is not a native crop, to date Malaysia has become the second largest producer of palm oil after Indonesia. Palm oil contributes 5.1% of Malaysia's gross national income (Ewers *et al.*, 2011). Palm oil is used in a wide range of products in terms of foods, pharmaceutical products, body-care products and most recently as biodiesel. Pessimistically, it has been reported that the economic loss caused by *Ganoderma* disease in Malaysian oil palm was between RM225 million to RM1.5 billion per year (Ommelna *et al.*, 2012). Even though various methods have been introduced to control this disease, unfortunately to date; none of the methods gave promising results in controlling *Ganoderma* disease in plantations. Therefore, quality and resistant seedling materials would be the best solution in countering any field disease and improving the yield of each palm transferred to the field. Thus, this study has undertaken the effort on the identification of symptoms and causal pathogen that is causing leaf spot disease of oil palm seedlings at the nursery

stage that could decrease the immune system of the seedlings and make it prone to other diseases as well when transferred to the field.

Leaf spot can occur at all stages of oil palm life span. Then again the most susceptible stage would be the nursery stage where the metabolism and development of the palm are still in progress (Elliot, 2005). Nursery stage is a critical stage where intense care should be applied to avoid more losses in future, in terms of disease management and yield of production. Generally, leaf spot that occur in palms are caused by fungi. In addition, there are a wide range of fungal pathogens that had been associated with this disease. Manifestation of favorable condition and temperature could worsen and facilitate spread to the adjacent palms (Elliot, 2005). Thus, early detection of this disease is crucial in providing quality and healthy seedlings to the oil palm industry.

Therefore the objectives listed to conduct this study are as follows:

1. To isolate pathogenic fungus from symptomatic oil palm leaves
2. To identify the causal fungus based on morphological characteristics and molecular tools

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