
UNIVERSITI PUTRA MALAYSIA

# MORPHOLOGICAL AND MOLECULAR IDENTIFICATION CHARACTERIZATION OF *Neopestalotiopsis* SPECIES ASSOCIATED WITH LEAF SPOT ON OIL PALM (*Elaeis guineensis Jacq*)

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# MORPHOLOGICAL AND MOLECULAR IDENTIFICATION CHARACTERIZATION OF *Neopestalotiopsis* SPECIES ASSOCIATED WITH LEAF SPOT ON OIL PALM (*Elaeis guineensis* Jacq)

BY

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A project report submitted to Faculty of Agriculture, Universiti Putra Malaysia, in fulfillment of the requirement of PRT4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science

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## CERTIFICATION

This project report entitled MORPHOLOGICAL AND MOLECULAR IDENTIFICATION CHARACTERIZATION OF *Neopestalotiopsis* SPECIES ASSOCIATED WITH LEAF SPOTS ON OIL PALM (*Elaeis guineensis Jacq.*) is prepared by Syahida Binti Norddin and submitted to the Faculty of Agriculture in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science.

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## LIST OF ABBREVIATION

| | |
|---|---|
| **BLAST** | Basic Local Alignment Search Tool |
| **bp** | Base pairs |
| **DNA** | Deoxyribonucleic acid |
| **dntp$_s$** | deoxynucleotide triphosphates |
| **dH$_2$O** | distilled water |
| **EDTA** | Ethylenediaminetetraacetic acid |
| **GelDoc** | Gel Documentation |
| **ITS** | Internal Transcribed Spacer |
| **ITS5-F** | Internal Transcribed Spacer 5- Forward |
| **ITS4-R** | Internal Transcribed Spacer 4 – Reverse |
| **Kb** | Kilo bases |
| **MgCl$_2$** | Magnesium chloride |
| **NCBI** | National Centre for Biotechnology Information |
| **PDA** | Potato Dextrose Agar |
| **PCR** | Polymerase Chain Reaction |
| **RNA** | Ribonucleic acid |
| **rRNA** | Ribosomal ribonucleic acid |
| **UV** | Ultra Violet |
| **UPM** | Universiti Putra Malaysia |
| **TE** | Tris- EDTA |
| **Tris- HCL** | Tris hydrochloride |

## LIST OF UNITS

| | |
|---|---|
| **%** | Percentage |
| **˚C** | Degree celcius |
| **cm** | Centimeter |
| **g** | Gram |
| **h** | Hour |
| **mM** | milimol |
| **ml** | mililitre |
| **µl** | microlitre |

## ABSTRACT

Oil palm (*Elaeis guineensis*) is a tropical perennial plant species in the family Arecacea that grows in Malaysia. It is an economically important crop which the oil palm cultivation area is more than 4.49 million hectares and also producing 17.73 million tonnes of palm oil and 2.13 tonnes of palm kernel oil. Leaf spot is one of the common problem in oil palm plantation, although it does not cause significant impact on oil palm production. The symptoms of these leaf spot are brown, small, oval to irrigular lesions and later, the lesions expand coalesc and form black lesions. In Malaysia, there is limited research on the composition of fungal pathogens causing leaf spot of oil palm. The objectives of this studies are 1) to isolate pure cultures of fungal isolates causing leaf spot of on oil palm; 2) to identify fungal pathogens to species level based on morphological characteristics and polymerase chain reaction (PCR) protocol using ITS 4 and ITS 5 primers; and 3) to construct internal transcribed spacer (ITS) phylogeny of the fungal species using Bayesian analysis. In these studies, symptomatic leaves were collected from five different oil palm trees at Ladang Heavea, University Putra Malaysia (UPM), Selangor. Infected tissues (5× 5mm) from the lesion margin were surface disinfected for 2 min with 10% chlorox and were transferred on potato dextrose agar (PDA). The pure fungal isolate isolated from leaf lesions were identify by *in vitro* morphological and cultural characteristics. The fungal isolates were sub-cultured by single spore isolation and the representative isolate is characterized further. DNA genomic were extracted from fresh fungal mycelium using the Dneasy Plant Mini Kit. The internal transcribed spacer (ITS) region of the ribosomal DNA were amplified using primer ITS4 and ITS5. The PCR

product of the ITS were sequenced and analyzed using BLAST nucleotide search in GenBank. Based on the conidial morphology, the morphotypes of *Pestalotiopsis* were identified. From phylogenetic analysis using maximum likelihood method of combine datasets, the isolates from oil palm leaflets was corresponded to *Neopestalotiopsis*. Therefore, the present study showed that the isolates associated with leafspot of oil palm belong to *Neopestalotiopsis*. Based on the result of morphological characteristics of isolated fungi, it cannot to be distinguished between genus of *Pestalotiopsis* and genus of *Neopestalotiopsis* clearly. Studies on the taxonomy and fungal biodiversity of pathogenic leaf spots are important and a motivation for obtaining this knowledge is that it may set the stage for development of more efficient control management practices in oil palm plantation.

## ABSTRAK

*Kelapa sawit (Elaeisguineensis) merupakan sejenis tumbuhan saka tropikal terdiri dalam keluarga Arecacea yang hidup subur di Malaysia. Kelapa sawit merupakan sejenis tanaman yang dapat memberikan keuntungan dalam ekonomi dimana keluasan tanah tanaman adalah lebih daripada 4.49 juta hektar yang dapat menghasilkan 17.73 juta tan minyak dan 2.13 tan minyak sawit kernal. Penyakit bintik daun merupakan penyakit umum yang membawa permasalahan dalam perladangan kelapa sawit walaupun tidak memberi kesan yang besar dalam penghasilan minyak kelapa sawit.Antara simptom penyakit bintik daun adalah terdapat bintik halus yang berwarna coklat, berbentuk bujur dan bila merebak menghasilkan kesan luka yang berwarna hitam.Di Malaysia, kajian terhadap patogen yang menyebabkan penyakit bintik daun pada kelapa sawit masih berkurang. Objektif utama pembelajaran ini adalah; 1) untuk mengasingkan kultur tulen kulat yang menyebabkan penyakit bintik daun dari daun kelapa sawit yang berpenyakit; 2) mengenalpasti patogen sehingga ke tahap spesis berdasarkan pemerhatian ciri-ciri morfologi kultur dan pengaplikasian protokol 'PCR' menggunakan primer 'ITS 4' dan 'ITS 5'; dan 3) untuk membentuk filogeni 'ITS' spesis kulat menggunakan analisis 'maximum likelihood'. Bagi memenuhi objektif kajian, daun yang mempunyai simptom penyakit bintik daun diambil dari lima pokok kelapa sawit yang berbeza di Ladang Hevea, Universiti Putra Malaysia (UPM), Selangor.Tisu yang dijangkiti penyakit (5x5mm) daripada bahagian permukaan daun yang luka dibasmi kuman selama 2 minit dengan 10% klorok dan dipindahkan kepada 'potato dextrose agar' (PDA).Kultur asli kulat yang diasingkan dari daun yang luka dikenalpasti melalui 'vitro' morfologi dan ciri-ciri kultur.Kulat yang*

*diasingkan di kulturkan semula melalui pengasingan satu spora dan hasilnya dilihat semula. DNA genomik akan diekstrak daripada miselium kulat yang segar menggunakan protokol 'DNAeasy Plant MiniKit.Bahagian 'ITS' ribosom DNA akan dikuatkan menggunakan primer ITS4 dan ITS5.Hasil produk PCR melalui ITS akan disusun dan dianalisis menggunakan carian nukleotida BLAST di GenBank. Berdasarkan morfologi konidial, sifat morfologi tersebut dikenali sebagai Pestalotiopsis. Namun begitu, berdasarkan kajian melalui 'maximum likelihood',kulat yang dikenalpasti bukan dikenali sebagai Pestalotiopsis, tetapi sebagai Neopestalotiopsis. Oleh itu, kajian mendapati Neopestalotiopsis juga boleh menyebabkan peyakit bintik daun kepada daun kelapa sawit. Kajian ke atas taksonomi dan biodiversiti kulat yang patogenik terhadap penyakit bintik daun adalah penting dan motivasi untuk mendapatkan pengetahuan ini boleh menyediakan peringkat kawalan yang lebih cekap dalam pengurusan ladang kelapa sawit.*

# CHAPTER 1

## INTRODUCTION

Oil palm (*Elaeis guineensis*) is a perennial crop belongs to the family Arecacea. Arecacea means that it is comes from palm family. It is the only species of the genus *Elaeis*. Within this genus, two main species can be recognized, *guineensis* called as African oil palm and *oleifera* called as American oil palm. *Elaeis guineensis* may yield about 5-8 tons of oil per hectare, compared to *oleifera* yield much less oil per hectare. Based on the oil production, *guineensis* has been declared as Malaysia's main crop that supplies the raw material; palm oil for production of oil used in foods, cosmetics and biodiesel, while *oleifera* still in research progress.

Oil palm is easy to be cultivated in tropical climate especially Malaysia. According to Sumathi *et al*., 2008, oil palm grown in Malaysia as ornamental plant in 1870, and then in 1917, oil palm was commercialising in the form of estate at Tennamaran Estate, Selangor. Most of the oil palm tree in Malaysia originated from West Africa where initially it was growing as ornamental plant and later developed into an agricultural crop.

Oil palm can grow well in tropical climate country such as Malaysia where the temperature ranges from 25˚ C until 30˚C and rainfall of 2000 mm per year. Although other countries such as Myanmar, Thailand, Philippines, Vietnam, Sudan, Nigeria and Somalia located at 10 degrees latitude of the equator which is suitable for oil palm cultivation, some of them have several month droughts, which

drastically can reduce yields. Only Malaysia and Indonesia are the country that emerged as a major producer of palm oil (Yusof , 2008).

Malaysia and Indonesia have achieved 90% of palm oil world export trade in oil palm industry. This scenario give the sign that both Malaysia and Indonesia will likely remain the key players in the palm oil sector accounting for 28.5 MnT or 85% of the world's palm oil production. According to the Food and Agriculture Organization of the United Nations Statistics Division (FAOSTAT), Malaysia is the largest producer and exporter of palm oil in 2004. Malaysia accounts about 47% of the world's supply of palm oil. Oil palm company now more concern in oil palm renewable energy besides in oil and fat trade (Sumathi, 2008).

The diseases that are common related to oil palm are leaf spots, anthracnose, leaf blight and basal stem rot. Leaf spot is a common disease that attacked oil palm leaves in nursery stage. The symptom of leaf spot begin as dark spots that gradually increase from 1.5 to 20 mm in diameter, changing from circular to elliptical lesions. The symptoms continuous until the black spot are surrounded with a yellow to rust brown zone. Next, anthracnose mainly attack seed in age of two month. The symptomic leaves usually appear yellow colour at the centre and side of leaves, while leaf blight disease symptoms are the leaves become necrotic until the black spot become brittle. Basal stem rot is a major disease causing losses in the oil palm industry. According to Wong *et al*., 2012, basal stem rot was recognized in Malaysia since 1928 when the disease was reported to attack mainly palms aging 30 years and above. Corley and Tinker (2003) reported that young palms that attacked by basal stem rot usually died within one or two years, while mature palms can survive about three years.

*Pestalotiopsis* species is a type of fungus that have related to the leaf spot disease on oil palm. Most of the leaf spot disease in Malaysia is affected by these fungi. The history of *Pestalotiopsis* begin when De Notaris (1839) introduced the genus *Pestalotia De Not* found on the leaves of *Vitis vinifera* in Italy. This fungus is characterized by 6-celled conidia with four deeply olivaceous central cell and simple branching of appendages from the apex. This study is further investigated until *Pestalotiopsis* and *Truncatella*, the new genera proposed by Steyaert (1949), were synonymised with *Pestalotia*. Morphological and molecular characteristics have been used to distinguish *Pestalotiopsis* with other genera, In the view of morphological characteristics, the shape of *Pestalotiopsis*'s conidium are fusiform, curved or straight, and divided into 5 septate cells. Three appendages were present at the apex and one pedicel at the base. However, to identify the fungi to the species level, DNA sequence data for single gene or multiple genes should be used to clarify fungal systematic. Fungi in *Pestalotiopsis* genus can also infect other crops such as coconut tree and other palm tree. The yield of the crop will decrease if there are no disease management to prevent the unnecessary yield loss.

The objectives of this study are; 1) to isolate pure cultures of fungal isolates causing leaf spot of on oil palm; 2) to identify fungal pathogens to species level based on morphological characteristics and polymerase chain reaction (PCR) protocol using ITS 4 and ITS 5 primers; and 3) to construct internal transcribed spacer (ITS) phylogeny of the fungal species using Bayesian analysis.

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