



**UNIVERSITI PUTRA MALAYSIA**

**ISOLATION AND CHARACTERIZATION OF FUNGAL PATHOGEN  
ASSOCIATED WITH OIL PALM BLACK BASE ROT DISEASE**

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**FP 2016 51**

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2015/2016

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BY

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A project report submitted to Faculty of Agriculture, Universiti Putra Malaysia, in fulfillment  
of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor  
of Agricultural Science

Faculty of Agriculture  
Universiti Putra Malaysia

2015/2016

## ABSTRACT

*Elaeis guineensis* Jacq. is a perennial monocotyledonous crop that has been contributing to Malaysia's economy generously. However there are some culprit diseases causing yield reduction and lifespan of the palm. Recently symptoms of a disease caused by a black fungus, which colonized on matured palm bole and trunk tissues were detected in Malaysia. This disease exhibits symptoms such as dry rot of the internal tissues at the base of the stem, upper roots and presence of black sclerotia attached to the vascular bundles in the pith. As the disease progresses, rotting starts on the bole of the palm and presence of hollow hole on the oldest trunk or base of oil palm can be observed. Hence, this project was designed with the objectives i) To isolate the fungal pathogen from BBR disease symptomatic oil palm tissues in the plantation and ii) To characterize and identify the isolated fungal pathogen based on morphological and molecular characteristics. In order to achieve these objectives, sampling of the symptomatic palm tissues from the plantations, followed by isolation of the fungal pathogen from the collected samples were carried out. Successfully isolated pure cultures were then subjected to cultural and morphological identification based on the demonstrated characteristics. In addition, molecular identification was also conducted on extracted fungal genomic DNA using ITS1 and ITS4 primer pairs for amplification purpose. Subsequently, PCR product obtained were subjected to sequencing and BLAST analysis against corresponding sequences in the GenBank database for species level identification. All the five isolates selected from ten isolates after cultural and morphological identification were identified as *Ceratocystis paradoxa*. As a conclusion, pathogenicity test conducted further confirmed *C. paradoxa* isolate as the causal fungal pathogen of black base rot (BBR) disease of oil palm.

## ABSTRAK

*Elaeis guineensis* Jacq. merupakan pokok tanaman jenis monokotiledon yang menyumbang kepada ekonomi Malaysia pada kadar yang tinggi. Walaubagaimanapun, terdapat beberapa jenis penyakit yang menyebabkan penurunan hasil serta jangka hayat sawit. Baru-baru ini, simptom-simptom yang disebabkan oleh kulat hitam yang mengkolonisasi tunggul serta batang sawit yang matang telah diperhatikan di Malaysia. Penyakit ini mempamerkan simptom-simptom seperti kering dan reput pada tisu dalaman pangkal batang serta akar bahagian atas dan diikuti dengan kehadiran sklerotia hitam pada tisu vaskular tengah. Apabila penyakit ini mula menular, proses pereputan akan bermula pada tunggul sawit dan kehadiran lubang berongga pada batang atau pangkal sawit dapat diperhatikan. Oleh itu, objektif-objektif kajian ini adalah, i) Untuk menjalankan pemencilan patogen kulat daripada tisu sawit yang mempamerkan simptom-simptom BBR di ladang, ii) Untuk mengidentifikasi patogen kulat yang dipencilkan berdasarkan ciri-ciri morfologi dan molikul. Bagi mencapai objektif-objektif ini, sampel-sampel di perolehi daripada sawit yang mempamerkan simptom- simptom penyakit BBR di ladang, dan kemudian proses pemencilan patogen kulat dijalankan pada sampel yang diperolehi. Pencirian secara morfologi dijalankan pada isolat-isolat yang berjaya dipencilkan berdasarkan ciri-ciri yang dipamerkan. Disamping itu, pencirian secara molikul dilakukan dengan mengekstrak DNA genomik kulat-kulat yang telah dipencilkan dan diamplifikasi menggunakan set pencetus ITS1 dan ITS4. Produk-produk tindak-balas rantaian polimorfisme yang diperolehi digunakan seterusnya dalam teknik penjujukan nukleotida DNA. Seterusnya, jujukan nukleotida DNA yang diperolehi digunakan bagi menjalankan analisis BLAST pada pangkalan data jujukan di GenBank bagi tujuan identifikasi pada peringkat spesies. Kesemua lima isolat kulat yang telah disaring berdasarkan pencirian morfologi daripada sepuluh isolat yang dipencilkan telah diidentifikasi sebagai *Ceratocystis paradoxa*. Secara kesimpulannya, melalui ujian kepatogenan yang telah dijalankan, *C. paradoxa* telah dikenalpasti sebagai patogen kulat penyebab penyakit reput pangkal hitam (BBR) sawit.

## ACKNOWLEDGEMENTS

I am heartily thankful to my supervisor, DR NUSAIBAH SYD ALI whose encouragement, guidance and support from the initial to the final level enabled me to develop an understanding of the subject. Because of helping and cooperate between me and my supervisor, my project were done successfully. Thankful again to my supervisor which I think she was the important person to ensure all the project were run smoothly and clearly.

I also want to thankful to my supervisor's phd student, Miss MUNIRAH because she willingly to help me to understand more about my project. She also gave encouragement and guidance to me to proceed my project from beginning until final. Futhermore, she willingly share her knowledge about pathology with me and always make sure that I understand all the procedure.

I am grateful because I have my friends that together helping and give support to me to finish my project successful. All my friends willingly their time to help me to conduct the project. I appreciate all their help and support.

I also would like to thank to all lab assistance from Department of Plant Protection because they also give help and guide for me to handle all the lab apparatus with properly. They also willingly to give help for me to finish my project.

Lastly, I offer my regards and blessing to all of those who supported me in any respect during the completion of the project.

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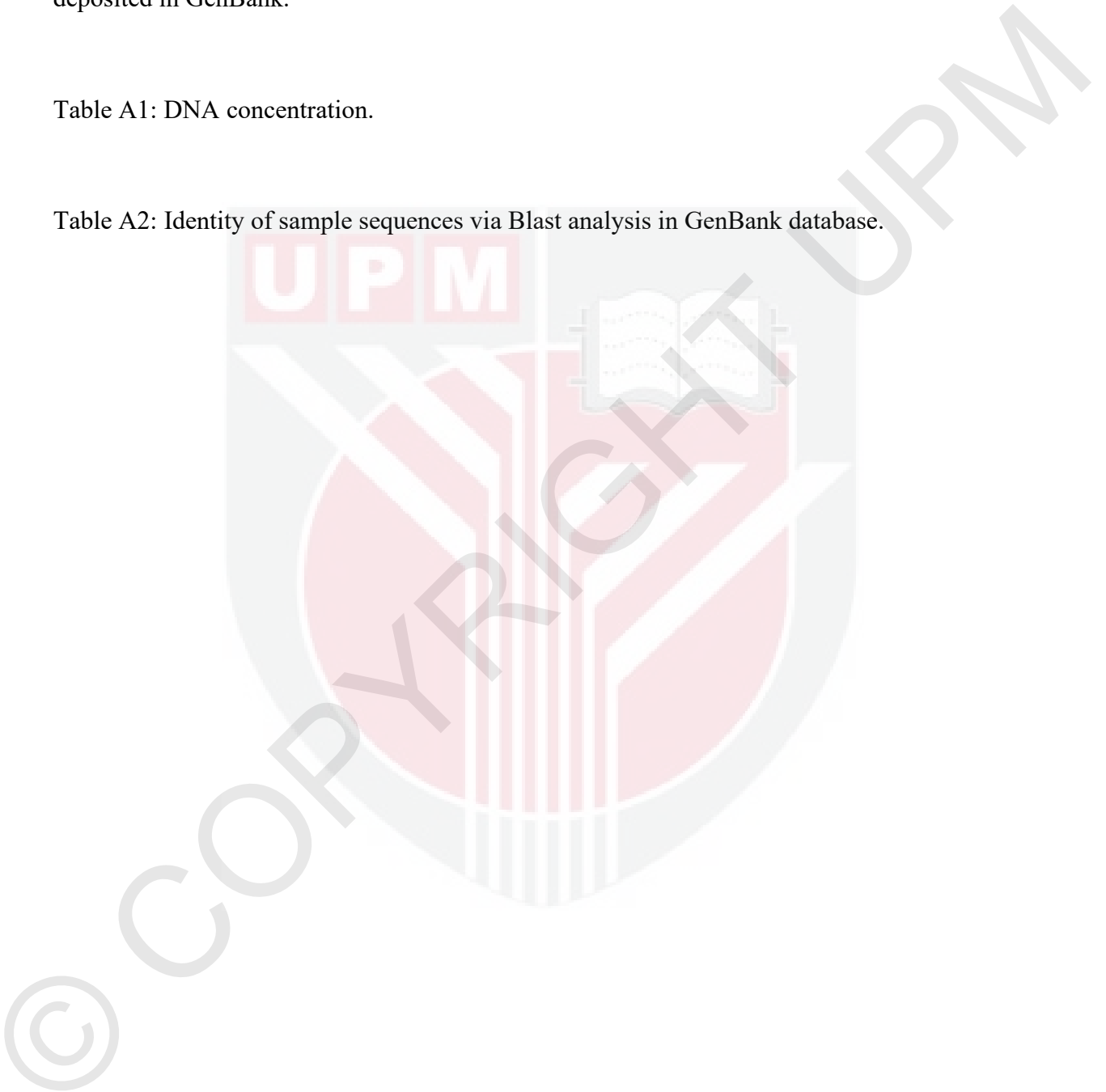
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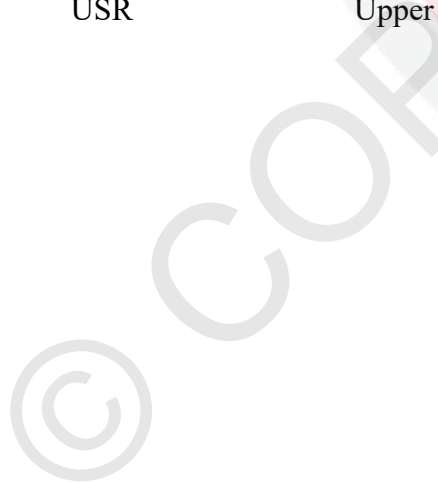
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## LIST OF ABBREVIATIONS

AE	Types of buffer
ANOVA	Analysis of variance
AW	Column wash buffer
AP	Extraction buffer
BBR	Black base rot
BLAST	Basic Local Alignment Search Tool
Bp	Base pair
CP	Ceratocystis paradoxa
DNA	Deoxyribonucleic acid
EB	Buffer purification
ETBR	Ethidium bromide solution
ITS	Internal transcribed spacer
LCB	Lactophenol cotton blue
LCD	Least significant difference
MPOB	Malaysian Palm Oil Board
NCBI	National Center for Biotechnology Information

NKEA	National Key Economic Areas
PCR	Polymerase chain reaction
PDA	Potato Dextrose Agar
QG	Buffer purification
RCBD	Randomized complete block design
RPM	Frequency of rotation
rRNA	Ribosomal RNA
spp.	Species
TAE	Buffer for electrophoresis
TBR	<i>Thielaviopsis</i> bud rot
UPM	Universiti Putra Malaysia
USR	Upper stem rot



# CHAPTER 1

## INTRODUCTION

The oil palm (*Elaeis guineensis* Jacq.) is a perennial monocotyledonous crop from family *Palmaceae*. Genus *Elaeis* was originated from West Africa (Mohd. Basri Wahid *et al.*, 2005). To date this *Elaeis guineensis* Jacq is commonly planted in Malaysia, Indonesia, Sri Lanka, Central America and Madagascar. In addition to that, this multipurpose perennial crop is an imperative commercial crop which has been contributing to Malaysia's economy generously. Furthermore, oil palm industry in Malaysia has been listed as one of the National Key Economic Areas (NKEA) showing the significance of this crop to Malaysia's economy.

However, one of the most common constrain faced by our oil palm industry is disease. To date, the major disease encountered by Malaysian oil palm is *Ganoderma* disease. This disease has been causing tremendous losses in each repeated planting cycles. Thus, many studies and efforts as preventive and curative methods are being focused on *Ganoderma* disease of oil palm. On the other hand, this present study focused on a newly emerging or a disease that has been there unidentified on Malaysian oil palms which can be categorized as deadly to our palms, known as oil palm black base rot (BBR) disease. This disease symptom was commonly found in sorghum, corn, rubber tree, coconut and tea (Tarr, 1962).

Black base rot disease was characterized based on the following symptoms; basal stem lesion, black discoloration of the tissues, dry rot of the internal tissues at the base of the stem and presence of black sclerotia at the vascular bundles in the pith or presence of black mycelium (Dhingra and Sinclair (1977, 1978). *Macrophomina phaseoli* was identified as the common causal agent of BBR disease in most of the crops (Domsch *et al.* 1980).

However from our study, the BBR symptoms of oil palm in Malaysia resembles charcoal base rot disease which displays black hollow hole on the oldest trunk or bole of the oil palm. In addition, rotting starts on bole of the palm and black fungus colonization present on bole and trunk tissues (Elliott *et al.*, 2004). To date, no study in Malaysia has been carried out on oil palm BBR disease and the identity of the causal fungus is still unknown.

Therefore, this study was carried out with the following objectives:

1. To isolate the fungal pathogen from symptomatic oil palm trees in the plantation
2. To characterize and identify the causal fungal pathogen

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