UNIVERSITI PUTRA MALAYSIA

METABOLITE PROFILING, LIGNIN ACCUMULATION AND GENE EXPRESSION OF OIL PALM GERMINATED SEED TREATED WITH *Hendersonia* GanoEF1

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chair : Rosimah binti Nulit, PhD
Faculty : Science

Oil palm *(Elaeis guineensis* Jacq.*)* is considered as a golden crop and also one of the main contributors to economy of Malaysia. Unfortunately, basal stem rot (BSR) disease remains as a major obstacle in oil palm cultivation and production in Malaysia. The uses of biocontrol agents become the main attention to control and suppress the plant disease. The objective of this study is to compare the metabolite profiling, lignin accumulation and gene expression of root of oil palm germinated seed treated with *Hendersonia toruloidea (GanoEF1)* and *Ganoderma boninense*. Endophytic fungi *Hendersonia* GanoEF1 was obtained from GanoDROP Unit (MPOB) Kajang, Selangor. Four hundred and fifty oil palm germinated seed were used and arranged randomly by using completely randomized design (CRD). The experiment conducted using four-week-old oil palm germinated seed with five following treatments; germinated seed control untreated and uninfected with *G. boninense* (TC), germinated seed treated *Hendersonia* GanoEF1 (TH), germinated seed infected *G. boninense* (TG), germinated seed treated *Hendersonia* GanoEF1 and infected with *G. boninense* (THG), and germinated seed control with uninfected rubber wood block, RWB (TB). Methanol extraction methods were used for metabolite profile analysis. Sixty five compounds from different treatments were selected as main compounds. Three types of sterols detected from these treatments such as *β*-sitosterol, Stigmasterol and Campesterol were used as a potential indicator to detect the present of pathogen (stress) in plant. Using the Venn-digram, few compounds have been identified and used to detect the present of *G. boninense* and *Hendersonia* GanoEF1 in oil palm roots were: Methyl 6-0-[1-methylpropyl]-*β*-D-galactopyranoside; Hexadecanoic acid, methyl ester; Tetradecanoic acid and 9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester. Determination of lignin accumulation and total lignin content in oil palm root is one of the potential methods to indicate the level of plant's stress. Two cm above the root tips were cut to determine the lignin accumulation whereas for total lignin content, 100 mg of root were used to compare the acid-soluble and acid-

insoluble lignin between the treatments. Highest thickness of lignin was found in treatment TG (82.65 ± 2.98) and the lowest in treatment TC (61.11 ± 1.05). For total lignin content, treatment TC shows the highest (263.73 ± 22.52) and the lowest found in treatment TG (188.43 ± 7.42). Study the gene expression of plant defence-related enzymes which are superoxide dismutase (SOD) and catalase (CAT) is also one of the method that possibily used to indicate the stress level in plant. Highest SOD level was found in treatment TC (24.56 ± 0.12) and lowest in treatment THG (23.49 ± 0.19) whereas highest CAT found in treatment THG (17.62 ± 0.06) and lowest in treatment TC (16.98 ± 0.19). This study revealed that *Hendersonia* GanoEF1 is suitable as an effective biological control agent for promoting the growth of oil palm seedlings and controlling BSR disease.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

PROFIL METABOLIT, AKUMULASI LIGNIN DAN EKSPRESI GEN ANAK KELAPA SAWIT YANG DIRAWAT DENGAN *Hendersonia* GanoEF1

Oleh

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Kelapa sawit (*Elaeis guineensis* Jacq.) dianggap sebagai tanaman bernilai dan juga salah satu penyumbang utama kepada ekonomi Malaysia. Malangnya, penyakit reput pangkal batang (BSR) kekal sebagai penghalang utama dalam penanaman dan pengeluaran kelapa sawit di Malaysia. Kegunaan agen biokawalan menjadi perhatian utama untuk mengawal dan menyekat penyakit tumbuhan. Objektif kajian ini adalah untuk membandingkan pemprofilan metabolit, akumulasi lignin, dan ekspresi gen akar anak benih kelapa sawit dengan rawatan *Hendersonia toruloidea* (GanoEF1) dan *Ganoderma boninense*. Kulat endofit *Hendersonia* GanoEF1 diperolehi daripada Unit GanoDROP (MPOB) Kajang, Selangor. Empat ratus lima puluh anak benih kelapa sawit telah digunakan dan disusun secara rawak dengan menggunakan reka bentuk sepenuhnya rawak (CRD). Eksperimen ini dijalankan menggunakan anak benih kelapa sawit yang berusia empat minggu dengan lima rawatan berikut; anak benih kawalan yang tidak dirawat dan tidak dijangkiti dengan *G. boninense* (TC), anak benih yang dirawat dengan *Hendersonia* GanoEF1 (TH), anak benih yang dijangkiti dengan *G. boninense* (TG), anak benih yang dirawat dengan *Hendersonia* GanoEF1 dan dijangkiti *G. boninense* (THG), dan anak benih kawalan dengan blok kayu getah, RWB yang tidak dijangkiti (TB). Kaedah pengekstrakan metanol telah digunakan untuk analisis pemprofilan metabolit. Enam puluh lima sebatian daripada rawatan yang berbeza telah dipilih sebagai sebatian utama. Tiga jenis sterol dikesan dari rawatan ini seperti β-sitosterol, stigmasterol dan campesterol telah digunakan sebagai petunjuk yang berpotensi untuk mengesan kehadiran patogen (stress) dalam tumbuhan. Dengan menggunakan gambar rajah Venn, beberapa sebatian telah dikenal pasti dan digunakan untuk mengesan kehadiran *G. boninense* dan *Hendersonia* GanoEF1 di akar kelapa sawit ialah: Metil 6-0-[1-metilpropil]-*β*-D-galaktopiranosida; Asid heksadekanoik, ester metil; Asid tetradekanoik, dan Asid 9,12-Oktadekanoic (Z, Z) -, 2-hidroksi-1- (hidroksimetil) etil ester. Penentuan akumulasi lignin dan jumlah kandungan lignin dalam akar kelapa sawit adalah salah satu kaedah yang berpotensi untuk menunjukkan tahap tekanan pokok. Dua cm di atas

hujung akar dipotong untuk menentukan akumulasi lignin manakala bagi jumlah kandungan lignin, 100 mg akar digunakan untuk membandingkan lignin asid larut dan asid tidak larut di antara rawatan. Ketebalan lignin yang tertinggi ditemui dalam rawatan TG (82.65 ± 2.98) dan yang paling rendah dalam rawatan TC (61.11 ± 1.05). Untuk jumlah kandungan lignin, rawatan TC menunjukkan paling tinggi (263.73 ± 22.52) dan yang paling rendah dalam rawatan TG (188.43 ± 7.42). Kajian pengekspresan gen terhadap enzim terlibat dalam system pertahanan tumbuhan iaitu superoxide dismutase (SOD) dan catalase (CAT) juga merupakan salah satu kaedah yang mungkin boleh digunakan untuk mengkaji tahap tekanan dalam tumbuhan. Tahap SOD tertinggi ditemui dalam rawatan TC (24.56 ± 0.12) dan terendah dalam rawatan THG (23.49 ± 0.19) manakala tahap CAT tertinggi ditemui dalam rawatan THG (17.62 ± 0.06) dan terendah dalam rawatan TC (16.98 ± 0.19). Kajian ini membuktikan bahawa *Hendersonia* GanoEF1 sesuai sebagai agen biokawalan yang berkesan untuk menggalakkan pertumbuhan anak benih kelapa sawit dan mengawal penyakit BSR.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

GDP	Gross Domestic Product
BSR	Basal Stem Root
FFB	Fresh Fruits Bunch
USR	Upper Stem Rot
RWB	Rubber Wood Block
LCC	Legume Cover Crops
DI	Disease Incidence
SFS	Severity of Foliar Symptoms
DS	Dead Seedlings
DR	Disease Reduction
GC-MS	Gas Chromatography–Mass Spectrometry
SOD	Superoxide Dismutase
CAT	Catalase
ROS	Reactive Oxygen Species
POD	Peroxidase
PPO	Polyphenol Oxidase
GSH	Glutathione
AsA	Ascorbate
O_2	Oxygen
Mn	Manganese
Fe	Iron
Cu	Copper
Zn	Zinc
APX	Ascorbate Peroxidase
PDA	Potatoes Dextrose Agar

MEA	Malt Extract Agar
CRD	Completely Randomized Design
$CHCl_3$	Choloroform
MeOH	Methanol
HCl	Hydrochloric Acid
VPD	Vapor Pressure Deficit
H_2SO_4	Sulphuric Acid
EFBs	Empty Fruit Bunches
VOCs	Volatile Organic Compounds
FAME	Fatty Acid Methyl Esters
CWDE	Cell Wall Degrading Enzyme
RNA	Ribonucleic acid
O_2^-	superoxide ions
OH^-	hydroxyl radicals
H_2O_2	Hydrogen peroxide
GPX	Gluthathione Peroxidise

CHAPTER 1

INTRODUCTION

1.1 Background of study

Oil palm (Elaeis guineensis Jacq.) was originated from West Africa and first introduced to Malaysia as an ornamental plant in the year 1870. Start from 1960, the plantation area in Malaysia had increased rapidly and nowadays the oil palm becomes one of the important commodity crops and was proved to strengthen the economics of Malaysia. Oil palm is considered a golden crop besides being one of the main contributors to the economy of Malaysia. Latest statistics show that the oil palm is the largest contributor in Malaysia by 36.6% whereas other agriculture (20.1%), fisheries and aquaculture at (14.6%) and followed by other sub-sector (Productivity Report 2014/2015). Department of Statistics Malaysia (2014/2015) proved that oil palm sub-sector was the largest contributor to the agriculture sector to the Gross Domestic Product (GDP).

However, the crop faced many pests and disease besides that effects on the yield production and also economic profit (Verheye, 2010). The examples of pest and disease that commonly found on oil palm trees such as rat, caterpillar, bagworm, *Rhinoceros* beetle, fan blight, vascular wilt, upper stem rot, and the most serious disease is Basal Stem Root (BSR) (Turner & Gillbanks, 2003). Breton et al. (2006) stated that the *Ganoderma* is used as a common name for BSR disease. *Ganoderma* is a genus of polypore mushrooms that grow on wood and many come from tropical regions (Kirk et al., 2008). Hepting (1971) reported that the genus *Ganoderma* belongs to the family of Ganodermataceae which is a widespread family of decay wood organism besides causes white rots of hardwoods in many woody plants. This is because, it has the capability to decompose lignin as well as cellulose and other related polysaccharides both angiosperm and gymnosperm host (Singh et al., 2014).

Kirk et al. (2008) state that genus *Ganoderma* has about 80 species in the world besides it had 44 susceptible hosts which from genera and family including oil palm and coconut. In an early report, a few species of *Ganoderma* have been reported by Turner (1981) to be associated with BSR disease and among of them are *G. applanatum*, *G. boninense*, *G. chalceum*, *G. lucidum*, *G. miniatocinctum*, *G. pseudoferreu,* and *G. tornatum*. The latest study found that only three species that related to the BSR disease which is *Ganoderma boninense*, *Ganoderma miniatocinctum,* and *Ganoderma tornatum*. However, *G. boninense* is the main species that pathogenic to the oil palm trees (Turner & Gillbanks, 2003). Idris et al. (2000) reported that *G. tornatum*, *G. applanatum*, *G. lucidum*, *G. pfeifferi,* and *G. philippi* species were not pathogenic. Basal stem rot (BSR) disease was first reported in 1931 infecting oil palms of over 25 years (Thompson, 1931). Now, it becomes the main limitations of long-term oil-palm crop management also major obstacles in oil palm cultivation, especially

in South-East Asia, Malaysia and Indonesia (Lelong et al., 2010; Idris, 2009; Susanto et al., 2005). In additions, Idris et al. (2004) reported that the high incidence of BSR are found in Malaysia and Indonesia while lower recorded in Africa, Papua New Guinea, and Thailand. The external symptoms cause by BSR disease usually can be observed for example decafication of the bole, formation of aerial symptoms and also production of the bracket on the base of the trunk (Flood et al., 2000). Over time, the affected leaves will die, necrosis set in and it starts with oldest leaves and slowly extend upward through the crown. Lastly, the dead fronds will droop at the point of attachment to the trunk or fracture hang down to form a skirt of dead leaves (Mih & Kinge, 2015).

The progression symptoms of BSR are a little bit slow, but usually infected plants definitely die. Formation of basidiocarp also known as the fruiting body is clearly identifiable structure compare to other symptoms however for the public, it may be difficult to identify which species of *Ganoderma* causing that fruiting body. Unfortunately, there are no fixed pattern or progression symptoms of the BSR disease. The progress does not necessarily complete and may stop at any stage due to the quick healing of the wound, natural resistance and also interaction between microorganism (Hushiarian et al., 2013). Nowadays, BSR disease has been labeled as a 'cancer' and also 'silent killer' in major oil palm plantation because of their difficulty to detect at the early stage (Naher et al., 2013).

Controlling the pathogen is a crucial step since the pathogen cause destruction and severe losses of oil palm production especially in Malaysia. BSR disease caused by *Ganoderma* considered the most destructive disease in oil palm. Susanto et al. (2005) stated that controlling the BSR field by using a chemical such as a pesticide and a fungicide have not been very effective besides sometimes can cause pollution. Nowadays, the uses of biocontrol agents become the main attention to control and suppress the plant disease. The main biocontrol agent usually comes from endophytic fungi and endophytic bacteria which will inhabit plant organ at a certain period of the plant life cycle, colonize the host without causing any harm or gain benefit (Azevedo et al., 2000). The potential of biocontrol agent is also capable to reduce the chances of the pathogen to colonize and increase plant defense system.

Biocontrol is used to describe the microbial antagonists to suppress diseases as well as the use of host specific pathogens to control weed populations (Krishna & Gardener, 2006). In addition, the organism that suppresses the pest or pathogen is referred to biological control agent (BCA). The use of *Hendersonia toruloidea* (GanoEF1) as a biological control agent of *Ganoderma* disease was studied (Idris et al., 2012). *Hendersonia* is the genus of the family incertae sedis and classified as an endophytic fungus and also a Nobel strain which successfully isolated and identified by Idris et al. (2012). Characteristics, reproductive, morphology and anatomy of *Hendersonia* especially *Hendersonia* GanoEF1 are still lacked and need further studies, especially in Malaysia. Findings results will be used to improve fertilizer efficiency and to control *Ganoderma* disease.

1.2 Problem statement and significance of the study

As mentioned earlier, BSR disease becomes the main limitations of long-term oil-palm crop management and also major obstacles in oil palm cultivation. The losses caused by BSR disease is not only about reduction of a number of oil palm stand, but also reduction of number as well as the weight of fruit bunches from infected oil palm (Flood et al., 2000). Besides that, it was also categorized as one of the devastating diseases in Malaysia due to the dead percentage which more than 80% of the stand by the time the palm are just halfway through its economic life and losses of 30% quite a frequency occur (Idris et al., 2004).

A survey conducted by Malaysian Palm Oil Board (MPOB) including other agencies show that 632 (59.57%) out of 1061 estates reported the presence of BSR disease (Idris, 2011), the incidence of BSR disease was 3.71% (59,148 hectares). Arif et al. (2011) reported that about RM 1.5 billion estimated losses due to the *Ganoderma* disease in Malaysia. This is because of the capability of the disease to infect in all growth stages of oil palm plants (Naher et al., 2015). Naher et al. (2011) stated that the disease was found in the older plant in the previous study but nowadays, it is also found in the younger palm as young as 1-2 years old. It seems like the disease already started to evolve their ability to adapt to the environmental conditions and food source from plantations to nursery and from old oil palm to younger and lastly to its seedlings. Besides that, the disease also affected the yield of the fresh fruits bunch (FFB) and the average FFB also will decrease (Subagio & Foster, 2003). Nazeeb et al. (2000) reported that the yield of palm with *Ganoderma* infection is between 13 - 21% less than the healthy palm of the same age.

1.3 Objective of the study

The main objectives of the study are:

1. To compare metabolites profile in root of oil palm germinated seed treated with *Hendersonia* GanoEF1 and infected with *G. boninense*.

2. To compare the lignin accumulation in root of oil palm germinated seed treated with *Hendersonia* GanoEF1 and infected with *G. boninense*.

3. To observe and quantify the gene expression of plant defense-related enzyme; superoxide dismutase (SOD) and catalase (CAT) in the root of oil palm germinated seed treated with *Hendersonia* GanoEF1 and infected with *G. boninense*.

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PUBLICATION

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