UNIVERSITI PUTRA MALAYSIA

ASSESSMENT ON DEFENSE REGIME CORRESPONDING TO SYSTEMIC RESISTANCE INDUCED UPON OIL PALM–Ganoderma boninense INTERACTION TREATED WITH BIOLOGICAL CONTROL AGENTS

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By

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

ASSESSMENT ON DEFENSE REGIME CORRESPONDING TO SYSTEMIC RESISTANCE INDUCED UPON OIL-PALM–*Ganoderma boninense* INTERACTION TREATED WITH BIOLOGICAL CONTROL AGENTS.

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January 2021

Chairman: Nusaibah Binti Hj. Syd Ali, PhD
Faculty: Agriculture

Oil palm (*Elaeis guineensis*) is a significant contributor to Malaysia's economy, providing both employment and revenue by generating RM 64.9 billion annually in terms of export earnings. However, without positioning efforts in eliminating or controlling major diseases encountered by this commodity crop, sustainability will not be attainable. Thus, it is important to develop early control or preventive measures that will contribute to a sustainable environment that cater the solution to this disastrous disease. Therefore, in this study, effective biological control agents (BCAs) against *Ganoderma boninense* (UPM13) were studied as a mixture for controlling basal stem rot (BSR) disease infestation in oil palm. The objectives employed to achieve this project were i) To evaluate the antagonistic and compatibility activities of *Pseudomonas aeruginosa* and *Trichoderma asperellum* mixture against *G. boninense in vitro*, ii) To assess the *in vivo* effects of *P. aeruginosa* and *T. asperellum* on the suppression of *Ganoderma* infestations and as plant growth promoter in oil palm seedlings, and iii) To determine the metabolites and enzymes induced as defense response in treated oil palm seedlings. In this current study, all the BCAs used were subjected to morphological and molecular identification. In addition, these BCAs were also screened for its antagonistic activity against *G. boninense* via dual culture, culture filtrate test and mycelial growth test. The ability in producing hydrolytic enzymes and plant growth promoting characteristics were also assessed. Both potential BCAs used in this study were identified as *T. asperellum* and *P. aeruginosa*. *Trichoderma asperellum* and *P. aeruginosa* demonstrated strong antagonistic activity against *G. boninense*, with percentages of inhibition of radial growth (PIRG) ranging from 69 to 77 percent in dual culture and culture filtrate tests. View under compound microscope with 400× magnifications demonstrated swelled and discontinued hyphae of *G. boninense* as a result of *T. asperellum* and *P. aeruginosa* treatment respectively in mycelium growth test. Observation of *G. boninense* mycelium in dual culture plate under scanning electron microscope (SEM) exhibited shrink, ruptured and inhibited *G. boninense* mycelium by *P. aeruginosa* and *T. asperellum.* Additionally, the BCAs exhibited growth-promoting characteristics and ability to produce hydrolytic enzymes. Nursery trial was conducted at Ladang 15, Faculty of

Agriculture, UPM for 32 weeks on four months old oil palm seedlings (D×P) using dip, place and drench (DPD) inoculation method. Application of BCA treatments demonstrated a significant increase in the vegetative growth of oil palm seedlings compared to the un-treated ones. In terms of vegetative growth, there was no significant difference in plant height, number of fronds, or chlorophyl content when BCAs were applied singly or in a mixture. However, when BCAs were applied in mixture, the bole diameter and root dry weight were considerably greater with ± 3.98 cm and ±41.46 g respectively than when BCAs were applied as single application. Based on the root symptoms, the BCA mixture treatment successfully reduced disease severity (DS) with the lowest DS observed (50%) compared to the positive control with 83.3 percent DS. The BCA treated oil palm seedlings were also tested for plant secondary metabolites induced via gas chromatography-mass spectrometry (GC-MS) analysis and enzyme produced as defense response against *G. boninense* infection. The treatment with BCAs resulted in the production of plant secondary metabolites which possessed antimicrobial activities and also triggered to the expression of plant defense biochemical regime such as peroxidase (PO), and polyphenol oxidase (PPO) enzymes, total phenolic content (TPC), and lignin content. In conclusion, the BCAs employed in this study demonstrated antagonistic effects against *G. boninense in vitro*. Mixture application of BCAs resulted in efficient suppression of *G. boninense* infection in oil palm seedlings via induction of secondary metabolites and enhanced production of plant biochemical defense regime.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENILAIAN TERHADAP SISTEM PERTAHANAN SISTEMIK YANG TERHASIL DARI INTERAKSI DI ANTARA SAWIT- *Ganoderma boninense* YANG TELAH DIRAWAT DENGAN AGEN KAWALAN BIOLOGI

Oleh

MUNIROH BINTI MD SAAD

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Kelapa sawit (*Elaeis guineensis*) merupakan penyumbang penting terhadap ekonomi Malaysia, di mana ia menyediakan peluang pekerjaan dan menjana pendapatan sebanyak RM 64.9 bilion setiap tahun melalui perolehan eksport. Namun, tanpa meletakkan usaha dalam membasmi atau mengawal penyakit utama yang dihadapi oleh tanaman komoditi ini, kelestarian tidak akan dapat dicapai. Oleh itu, adalah sangat penting untuk membangunkan kawalan awal atau langkah-langkah pencegahan yang dapat menyumbang kepada persekitaran yang lestari bagi mengatasi masalah bencana penyakit ini. Dengan itu, agen kawalan biologi (BCA) yang berkesan terhadap *Ganoderma boninense* (UPM13) telah dikaji sebagai campuran untuk mengawal serangan penyakit busuk pangkal batang (BPB) pada kelapa sawit yang digunakan dalam kajian ini. Beberapa objektif yang digunakan untuk mencapai projek ini ialah i) Untuk menilai aktiviti antagonis dan keserasian campuran *Pseudomonas aeruginosa* dan *Trichoderma asperellum* terhadap *G. boninense* secara *in vitro*, ii) Untuk menilai kesan *P. aeruginosa* dan *T. asperellum* secara *in vivo* terhadap perencatan jangkitan *Ganoderma* dan sebagai penyokong pertumbuhan tanaman bagi anak benih kelapa sawit, dan iii) Untuk menentukan metabolit dan enzim yang terhasil sebagai tindak balas pertahanan anak benih kelapa sawit yang dirawat. Dalam kajian ini, semua BCA yang digunakan telah menjalani pengenalpastian morfologi dan molekul. Selain itu, aktiviti antagonis BCA ini terhadap *G. boninense* juga telah disaring melalui ujian dwi-kultur, ujian kultur turasan dan ujian pertumbuhan miselium. Keupayaan dalam menghasilkan enzim hidrolitik dan ciri-ciri penggalak pertumbuhan tanaman juga dinilai. Kedua-dua BCA berpotensi yang telah digunakan dalam kajian ini dikenal pasti sebagai *T. asperellum* dan *P. aeruginosa*. *Trichoderma asperellum* dan *P. aeruginosa* menunjukkan aktiviti antagonis yang kuat terhadap *G. boninense*, dengan peratusan perencatan pertumbuhan radial (PIRG) dari 69 hingga 77 peratus dalam ujian dwi-kultur dan ujian kultur turasan. Pemerhatian di bawah mikroskop kompaun dengan pembesaran 400× telah menunjukkan hifa *G. boninense* yang bengkak dan terencat melalui hasil rawatan *T. asperellum* dan *P. aeruginosa* dalam ujian pertumbuhan miselium masing-masing. Selain itu, pemerhatian miselium *G. boninense* dalam plat dwi-kultur di bawah mikroskop elektron imbasan (SEM) telah

menunjukkan pengecutan, pemecahan dan perencatan miselium *G. boninense* oleh *P. aeruginosa* dan *T. asperellum*. Di samping itu, BCA telah mempamerkan ciri-ciri yang menggalakkan pertumbuhan dan kemampuan untuk menghasilkan enzim hidrolitik. Percubaan nurseri telah dijalankan di Ladang 15, Fakulti Pertanian, UPM selama 32 minggu dengan anak benih kelapa sawit berusia empat bulan (D×P) menggunakan kaedah inokulasi celup, letak dan siram (DPD). Penggunaan rawatan BCA telah menunjukkan peningkatan yang signifikan dalam pertumbuhan vegetatif anak benih kelapa sawit berbanding dengan yang tidak dirawat. Dari segi pertumbuhan vegetatif, tiada perbezaan signifikan yang didapati pada ketinggian tanaman, jumlah pelepah, atau kandungan klorofil ketika BCA digunakan secara tunggal atau dalam campuran. Walau bagaimanapun, ketika BCA digunakan dalam campuran, diameter batang (bole) dan berat kering akar jauh lebih besar masing-masing dengan ± 3.98 cm dan ± 41.46 g ketika BCA digunakan sebagai aplikasi tunggal. Berdasarkan simptom akar, rawatan campuran BCA berjaya mengurangkan keterukan penyakit (DS) dengan DS terendah yang diperhatikan (50 %) berbanding dengan kawalan positif sebanyak 83.3 peratus DS. Anak benih kelapa sawit yang dirawat dengan BCA juga diuji untuk metabolit sekunder tanaman yang diinduksi melalui analisis kromatografi gas-spektrometri jisim (GC-MS) dan enzim dihasilkan sebagai tindak balas pertahanan terhadap jangkitan *G. boninense*. Rawatan dengan BCA menunjukkan penghasilan metabolit sekunder tumbuhan yang memiliki aktiviti antimikrob dan juga mencetuskan pengekspresan rejim biokimia pertahanan tanaman seperti peroksidase (PO), dan enzim polifenol oksidase (PPO), jumlah kandungan fenolik (TPC), dan kandungan lignin. Kesimpulannya, BCA yang digunakan dalam kajian ini menunjukkan kesan antagonis terhadap *G. boninense* secara in vitro. Penggunaan campuran BCA telah menghasilkan penyekatan yang berkesan terhadap jangkitan *G. boninense* pada anak benih kelapa sawit melalui induksi metabolit sekunder dan meningkatkan pengeluaran rejim pertahanan biokimia tanaman.

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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 Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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µg	microgram
µl	microliter
µm	micrometer
AM	Arbuscular mycorrhizal
AMF	Arbuscular mycorrhizal fungi
AUDPC	Area Under the Disease Progress Curve
BCA	Biological control agent
bp	Base pair
BSR	Basal stem rot
℃	Degree Celsius
CAS	Chrome azurol S
cm	centimeter
CRD	completely randomized design
DAI	Days after inoculation
DI	Disease incidence
DNA	Deoxyribonucleic acid
DPD	Dip, Place and Drench
DS	Disease severity
EB	Endophytic bacteria
ELISA	Enzyme-linked immunosorbent assay
EM	Effective microbes
FW	Fresh weight
g	Gram
GC-MS	Gas chromatography-mass spectrometry
GIS	Geographical information system
GSM	Ganoderma selective media

Ha	Hectares
IAA	Indole acetic acid
ISR	Induced systemic resistance
ITS	The internal transcribed spacer
LCB	Lactophenol cotton blue
LSD	Least significant difference
M	Molar
mA	milliampere
MAI	Month after inoculation
mL	milliliter
mm	millimeter
MVOCs	Microbial volatile organic compounds
NA	Nutrient agar
NB	Nutrient broth
NBRIP	National Botanical Research Institute Phosphate
ng	nanogram
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PDB	Potato dextrose broth
PGPB	Plant growth-promoting bacteria
PIRG	Percentage inhibition radial growth
PLS-DA	Partial least squares-discriminant analysis
PO	Peroxidase
PPO	Polyphenol oxidase
PR	Pathogenesis-related
rDNA	ribosomal Deoxyribonucleic acid
rpm	Revolutions per minute

s	Second
SA	Salicylic acid
SAR	Systemic acquire resistance
SEM	Scanning electron microscope
TPC	Total phenolic content
UV	Ultraviolet
V	Voltage
VOC	Volatile organic compound

CHAPTER 1

INTRODUCTION

1.1 Background of study

Oil palm (*Elaeis guineensis*) is a monocotyledon crop from the family Arecaceae (formerly Palmae) within the subfamily Cocosoideae (Corley and Tinker, 2003). It is a major commodity crop grown in the tropical areas, particularly in Southeast Asia. Oil palm industry contributes to the Malaysian economy enormously and indirectly in the development of country's rural areas (Kui, 2008). Total export revenue increased by 8.4% to RM73.25 billion as compared to the RM67.55 billion in 2019 due to higher prices in world trade (MPOB, 2021). In 2020, palm oil export earnings alone increased by 16.7% to 45.66 billion as against RM39.13 billion in 2019. In Malaysia, cultivation of oil palm has increased year by year with 1.5 million hectares (ha) in the year 1985 to 5.876 million ha in 2020 (MPOB, 2021). However, total Malaysian exports of palm oil and other palm-based products in 2020 amounted to 26.73 million tonnes, lower by 4.1% from 27.88 million tonnes exported in 2019 (MPOB, 2021).

Basal stem rot (BSR) is the major disease encountered by Malaysian palms which is caused by *Ganoderma* species (Idris et al. 2011). The most vigorous species recorded in Peninsular Malaysia plantations as the causal pathogen of BSR has been identified as *Ganoderma boninense* (Utomo et al., 2018; Ahmadi et al. 2017; Bivi et al. 2010). Based on the BSR incident rate, the total area affected in 2020 was estimated to be 443,440 hectares, equivalent to 65.6 million oil palms (Noor Azmi et al. 2020), compared to 59,148 hectares in 2010 (Idris et al. 2011). In Malaysia, the disease was most prevalent in Johor (18,098 ha), Sabah (15,940 ha), Perak (9,869 ha), and other states with less than 3,500 hectares while Perlis was reported as disease-free states. The disease can diminish the yield of infected palms in two ways; (i) by directly killing the infected palms (direct loss) or (ii) by lowering the weight or number of fresh fruit bunches (FFB) produced by infected palms (indirect loss) (Roslan and Idris, 2012). The economic loss owing to the disease was calculated to be 68.73 % of the achievable yield of all affected palms (i.e. 461 palms) after 12 months observation using the yield loss (YL) model developed by Kamu et al. (2021), and if no management measures are applied, this disease will play a significant role in diminishing oil palm output.

Biological control of plant pathogens is an alternative approach in reducing the strong dependence of modern agriculture on synthetic chemical fungicides which causes environmental pollutions (Ab Rahman et al. 2018). Biotechnology advancements have resulted in a major increase in the usage of microorganisms as biological control agents (BCA). The development of BCA as a disease control and management strategy for Ganoderma is an alternate option to supplement existing control techniques (Ramli et al. 2016) and at the same time BC approach supports the effort towards sustainable palm oil production. Most of biocontrol techniques for soilborne plant diseases and plant-parasitic nematodes rely on a single microbial biocontrol agent to suppress infections or

nematodes (Roberts et al. 2005). Unfortunately, individual biocontrol agents are unlikely to act consistently against all crop diseases or under a range of rhizosphere and soil environmental conditions (Roberts et al. 2005). Therefore, to address this inconsistency, a mixture of biocontrol agents in a single preparation could be used. Mixture of antagonists is also more stable and has wider spectrum of activity, enhancing the efficacy and reliability of biological control (Mishra et al. 2011). Moreover, a range of biological control mechanisms may operate in mixed BCA populations (Elad and Stewart, 2007; Guetsky et al. 2002; Guetsky et al. 2001; Whipps 2001).

Endophytic bacteria have been classified as plant growth-promoting bacteria (PGPB) (Coutinho et al. 2015; Ali et al. 2012). *Pseudomonas aeruginosa* has been proven to enhance plant growth by producing growth-stimulating plant hormones (Prathap and Ranjitha, 2015; Ahemed and Kibret, 2014; Bakker et al. 2014). A study conducted by Zaiton et al. (2008) reported that *P. aeruginosa* significantly increased the seedling plant growth and root mass compared to *Bukholderia cepacia* and *P. aeruginosa* more effectively in controlling *Ganoderma* compared to *B. cepacia.* In addition, this study was supported by *in vitro* test conducted by Bivi et al. (2010) which suggested that *P. aeruginosa* can be used as an effective biological control agent against *G. boninense. Trichoderma* spp. have been the most common fungi applied as biological control agents (BCA) as an effort to combat a wide range of plant diseases (Nusaibah and Musa, 2021). According to Nusaibah and Musa (2021), Trichoderma-based biocontrol mechanisms primarily rely on mycoparasitism, the production of antibiotic and/or hydrolytic enzymes, nutrient competition, and induced plant resistance; as well as production of numerous secondary metabolites which could directly inhibit the growth of several plant pathogens. In addition, *Trichoderma* spp were reported to be the most common biological control agents of oil palm rhizosphere are. (Musa et al. 2018; Nusaibah et al. 2017; Susanto et al. 2005). A study conducted by Musa et al. (2018) reported that the mixture of Trichoderma spp was efficient cocktail in controlling BSR disease of oil palm.

1.2 Problem statement and Justification

Oil palm has been recognized as the golden crop of Malaysia and contributed generously to Malaysia's economy. However, the major disease caused by *G. boninense* has been a devastating treat to Malaysia's oil palm industry for many years with the total economic loss was USD 4112.78 per year (Kamu et al. 2021). Therefore, there is an obvious need for understanding and developing early control measures that will contribute to a sustainable environment while catering to this catastrophic disease. Hence, an effort to find substitute preventive and curative methods to control *Ganoderma* disease via biological control agents (BCAs) is on the upsurge. Previous studies have reported on the use of single endophytic bacteria and other beneficial microbes as BCA against *G. boninense* disease and not much studies reported on the use of mixture BCAs. Mixture of BCAs were reported to be more stable and has wider spectrum of activity, enhancing the efficacy and reliability of biological control (Mishra et al. 2011) and a range of biological control mechanisms may operate in mixed BCA populations (Elad and Stewart, 2007). Therefore, in order to fill the research gap, the beneficial microbes that have been isolated and identified in a preliminary work as a potent BCAs against pathogenic fungus were studied as a consortium to control *Ganoderma* infection in oil

palm. The combination of BCAs against *G. boninense* was expected to induced plant growth, reduced disease severity (DS) in the nursery trial via the production of secondary metabolites and defense enzyme. Our aim in this study was to address the following specific questions: (i) Can *P. aeruginosa* and *T. asperellum* work as a mixture against *G. boninense* infection in oil palm?; (ii) Can application of mixture BCAs boost plant growth and the palm immune system against *G. boninense in vivo*? and (iii) Does the mixture BCAs application induces the production of secondary metabolites and defense enzyme.

1.3 Research objectives

The objectives of the study include:

i. To evaluate the antagonistic and compatibility activities of *Pseudomonas aeruginosa* and *Trichoderma asperellum* consortium against *G. boninense in vitro.*
ii. To assess the *in vivo* effects of *P. aeruginosa* and *T. asperellum* on the suppression of *Ganoderma* infestations and as plant growth promoter in oil palm seedlings.
iii. To determine the metabolites and enzymes induced as defense response in treated oil palm seedlings.

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