EVALUATION ON THE EFFECTIVENESS OF POTENTIAL ENDOPHYTIC *Trichoderma* spp. FROM NORTH SUMATRA, INDONESIA IN PREVENTING AND SUPPRESSING *Ganoderma boninense* INFECTION IN OIL PALM SEEDLINGS

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DECLARATION

I hereby declare that the material in this thesis is my own except for quotations excepts, equations, summaries and references, which have been duly acknowledged.

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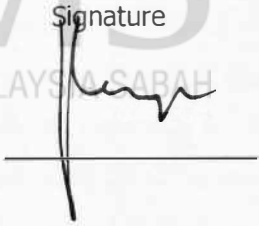

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ABSTRACT

Basal stem rot (BSR) caused by the fungus *Ganoderma boninense* is regarded as the most destructive disease of oil palm (*Elaeis guineensis* Jaqc.) and causes significant economic losses in the oil palm industry of South East Asia. High incidence of BSR affects to the tremendously reduced weight and number of fruit bunches in infected but living palms. Various approaches including chemical, mechanical, cultural and biological measures have been reported to control BSR disease. Unfortunately, no single strategy has yet been able to halt the spreading of the disease. Therefore, a more reliable and integrated strategy that could manage this BSR disease is urgently needed to be further investigated. Application of endophytic biological control agents (BCAs) especially *Trichoderma* spp.have been used for decades and shows promising results in supressing *Ganoderma* infection, beside environmentally safe and sustainable. In this respect, evaluation on the effectiveness of potential endophytic *Trichoderma* spp. in preventing and suppressing *G. boninense* infection in oil palm seedlings were investigated in this study. This study aimed to investigate the identity of BSR causal pathogen from North Sumatra, Indonesia and to identify the endophytic *Trichoderma* strain from healthy oil palm roots of this area. Thus, to evaluate the antibiosis activity and resistance induced by endophytic *Trichoderma* in oil palm seedlings.

Identification of *Ganoderma* isolate from Negeri Lama Estate in North Sumatra, Indonesia was confirmed using Ganoderma selective medium (GSM) and DNA sequence analysis. That latter method showed the isolate from Negeri Lama was closely related to virulent *G. boninense* isolate GB001 (NCBI accession number: KX092000.1), with a maximum similarity of 99%. Prior to *in vitro* assessment, all the endophytic BCA isolates from oil palm roots in Negeri Lama Estate studied were first confirmed using Trichoderma selective medium (TSM). Subsequently, DNA sequence analysis identified the BCAs as *Trichoderma* species with 99% of similarity for all isolates, where endophytic ET501 identified as *Trichoderma reesei* strain RHa (NCBI accession number: KM246746.1), while endophytic ET523 identified as *Trichoderma asperellum* isolate F1 (NCBI accession number: KP281701.1) and endophytic ET537 identified as *Trichoderma asperellum* strain Q1 (NCBI accession number: HQ293149.1).

Antagonistic effects of potential endophytic *Trichoderma* spp. against *G. boninense* growth were evaluated via dual culture test and culture filtrate test. The results demonstrated that the percentage inhibition of radial growth (PIRG) of *G. boninense* were 95.1% in endophytic *T. reesei* ET501, 87.1% in endophytic *T. asperellum* ET523, and 88.9% in endophytic *T. asperellum* ET537. In addition, the ability of *Trichoderma* spp. in suppressing *G. boninense* growth was also assessed through percentage inhibition of mycelia growth (PIMG). Endophytic *T. reesei* ET501 showed the strongest PIMG activity (100%), followed by endophytic *T. asperellum* ET537 and *T. asperellum* ET537, 90.5% and 12.3%, respectively. Scanning electron microscopy (SEM) observation was done to reveal the mycoparasitic effect of endophytic *Trichoderma* spp. to the *G. boninense* mycelia. Exposure of endophytic *T. reesei* ET501 caused the most severe disruption and lysed effect to the *G. boninense* mycelium, followed by *T. asperellum* ET537 and *T. asperellum* ET523, respectively.

Potential bioactive compounds produced by single endophytic *Trichoderma* spp. and/or during this interaction which inhibiting the *G. boninense* growth was further investigated using gas chromatography mass spectrometry (GCMS). Several potential antimicrobial compounds such as 3-furaldehyde; pyrazole,1,4-dimethyl-;

propanoic acid; 5-methyloxazolidine; pyrrolizin-1,7-dione-6-carboxylic acid, methyl(ester); butanedioic acid; 9-hexadecenoic acid; thiophene, 2,5-dihydro-; 2-bromotetradecanoic acid; 3,4-furandiol, tetrahydro-trans-; 3-methyloxirane-2-carboxylic acid; hydrazine; octadecenoic acid; 2,5-methano-2H-furo[3,2-b]pyran-8-one, hexahydro-; α-bisabolol, etc were also detected. These compounds shall contribute to the antagonistic effect of endophytic *Trichoderma* spp. against *G. boninense.*

Moreover, to measure the potential of these BCAs in *G. boninense* suppression, an *in-vivo* trial with ten months duration was conducted on oil palm seedlings via *G. boninense* artificial inoculation (rubber wood blocks method) treated with endophytic *Trichoderma* spp. in the nursery condition. The application of endophytic *T. reesei* ET501 found to be the most effective in suppressing BSR with only 13.4% of disease incidence (DI), compared to endophytic *T. asperellum* ET23 and *T. asperellum* ET537 with 71.8% and 48.4 % of DI, respectively. The ability of endophytic *Trichoderma* spp. in inducing resistance of oil palm seedlings was also measured through total phenolic content (TPC) analysis of treated oil palm roots. The results showed all the of endophytic *Trichoderma* spp. treated seedlings were significantly induced of TPC compared to the control seedlings. Application of endophytic *Trichoderma* spp. in the nursery exhibited the potential of triggering oil palm seedlings' TPC amount and help in developing resistance in oil palm seedlings, thus protect them from *G. boninense* infection.

Therefore, the application of endophytic *Trichoderma* spp. to the oil palm seedling roots before transplant (at nursery stage) could provide a promising sustainable strategy to manage BSR disease of oil palm. Despite this, there is also a need for further study in evaluating the potential endophytic *Trichoderma* spp. as a single or consortium approach under field conditions.

Keywords : Basal stem rot, *Ganoderma boninense*, biocontrol agents, resistance, endophytic *Trichoderma* spp.

ABSTRAK

PENILAIAN KEATAS KEBERKESANAN DAN POTENSI TRICHODERMA *SPP. ENDOFITIK DARI SUMATRA UTARA, INDONESIA DALAM MENCEGAH DAN MERENCAT JANGKITAN* GANODERMA BONINENSE *DALAM ANAK POKOK SAWIT*

Reput pangkal batang (RPB) yang disebabkan oleh kulat Ganoderma boninense *merupakan penyakit kelapa sawit (Elaeis guineensis Jaqc.) yang paling membinasakan dan menyebabkan kerugian ekonomi dalam industri kelapa sawit di Asia Tenggara. Kejadian RPB yang tinggi memberi kesan yang besar terhadap penurunan berat dan jumlah tandan pada kelapa sawit yang dijangkiti. Pelbagai pendekatan termasuk kaedah kimia, mekanikal, kultura dan biologi telah dilaporkan untuk mengawal penyakit RPB. Malangnya, tiada strategi tunggal yang dapat menangani penyebaran penyakit ini. Oleh itu, strategi yang lebih boleh dipercayai dan bersepadu untuk mengurus penyakit RPB perlu dikaji selanjutnya. Penggunaan agen kawalan biologi (AKB) endofitik terutamanya* Trichoderma *spp. telah digunakan selama beberapa dekad dan menunjukkan keputusan yang baik dalam mengawal jangkitan* Ganoderma, *di samping ianya selamat terhadap alam sekitar dan mampan. Oleh itu, penggunaan* Trichoderma *dalam mencegah dan membasmi jangkitan* G. boninense *pada anak kelapa sawit telah diselidik dalam kajian ini. Matlamat utama kajian ini adalah mengenalpasti patogen penyebab RPB dari Sumatera Utara, Indonesia dan mengenalpasti strain* Trichoderma *endofitik dari akar kelapa sawit yang sihat dari kawasan ini. Selain itu, kajian ini juga menilai aktiviti antibiosis dan rintangan yang disebabkan oleh* Trichoderma *endofitik dalam anak kelapa sawit.*

Identiti isolat Ganoderma *dari Ladang Negeri Lama di Sumatera Utara, Indonesia, telah dikenalpasti dengan menggunakan medium selektif* Ganoderma *dan analisis urutan DNA. Kaedah ini menunjukkan bahawa isolat dari Negeri Lama berkait rapat dengan* G. boninense *virulen isolat GB001 (nombor aksesi NCBI : KX092000.1), dengan kesamaan maksimum 99%. Sebelum penilaian in vitro, AKB endofitik yang diasingkan daripada akar kelapa sawit dalam kajian Ladang Negeri Lama telah disahkan menggunakan medium selektif* Trichoderma. *Selepas itu, analisis DNA urutan mengenalpasti semua AKB sebagai spesies* Trichoderma *dengan kesamaan 99% untuk semua isolat, di mana endofitik ET501 dikenalpasti sebagai* Trichoderma reesei *strain RHa (nombor aksesi NCBI: KM246746.1), manakala endofitik ET523 dikenalpasti sebagai* Trichoderma asperellum *isolat F1 (nombor aksesi NCBI : KP281701.1) dan endofitik ET537 yang dikenalpasti sebagai* Trichoderma asperellum *strain Q1 (nombor aksesi NCBI : HQ293149.1).*

Kesan potensi antagonistik endofitik Trichoderma *spp. terhadap pertumbuhan* G. boninense *telah dinilai melalui ujian dwikultur dan ujian tapisan kultur. Hasil menunjukkan bahawa peratusan perencatan pertumbuhan radial* G. boninense *adalah 95.1% dengan endofitik T. reesei ET501, 87.1% dengan endofitik* T. asperellum *ET523, dan 88.9% dengan endofitikT. asperellum ET537. Di samping itu, keupayaan* Trichoderma *spp. dalam menindas kadar pertumbuhan* G. boninense *juga dinilai melalui perencatan pertumbuhan mycelia. Endofitik* T. reesei *ET501 menunjukkan aktiviti PIMG terkuat (100%), diikuti oleh endofitik* T. asperellum *ET537 dan* T. asperellum *ET537, masing-masing 90.5% dan 12.3%. Pemerhatian menggunakan elektron mikroskop telah menunjukkan kesan mikoparasitik endofitik* Trichoderma *spp. ke atas miselia* G. boninense. *Endofitik T. reesei ET501 menyebabkan kerosakan dan*

kesan lisis paling teruk ke atas miselia G. boninense*, diikuti oleh* T. asperellum *ET537 dan* T. asperellum *ET523.*

Beberapa sebatian bioaktif perencat pertumbuhan G. boninense *yang dihasilkan oleh endofitik* Trichoderma *spp. tunggal dan/ atau semasa interaksi telah dianalisa dengan menggunakan kromatografi gas dan spektrometri jisim. Beberapa sebatian antimikrob yang berpotensi dikenalpasti antaranya adalah 3-furaldehid; pyrazole,1,4-dimetil-; propanoik asid; 5-metiloxazolidine; pyrrolizin-1,7-dione-6-carboxylic asid, metil(ester); butanedioik asid; 9-hexadecenoik asid; thiophene, 2,5-dihydro-; 2-bromotetradecanoic asid; 3,4-furandiol, tetrahidro-trans-; 3-methyloxirane-2-carboxylic asid; hidrazine; oktadecenoik asid; 2,5-metano-2H-furo[3,2-b]pyran-8-one, hexahydro-; α-bisabolol, dan lain-lain. Sebatian-sebatian ini sepatutnya menyumbang terhadap kesan antagonistik endofitik* Trichoderma *spp. ke atas* G. boninense.

Selanjutnya, untuk menilai potensi endofitik ini dalam menyekat jangkitan G. boninense*, percubaan in vivo selama sepuluh bulan dilakukan pada anak kelapa sawit melalui inokulasi tiruan* G. boninense *(kaedah blok kayu getah) yang kemudiannya dirawat dengan endofitik* Trichoderma *spp. di nurseri. Aplikasi endofitik* T. reesei *ET501 didapati paling berkesan dalam merencat RPB dengan hanya 13.4% insiden penyakit (IP) berbanding dengan endofitik* T. asperellum *ET23 dan* T. asperellum *ET537 masing-masing dengan 71.8% dan 48.4% IP. Keupayaan endofitik* Trichoderma *spp. dalam mengaruh ketahanan anak kelapa sawit juga diukur melalui analisis kandungan jumlah kandungan fenolik (JKF) akar kelapa sawit. Hasil yang diperolehi menunjukkan kesemua anak pokok yang dirawat dengan endofitik* Trichoderma *spp. menaikkan jumlah KTF yang ketara berbanding kawalan. Penggunaan endofitik* Trichoderma *spp. di nurseri mempamerkan potensi untuk mencetuskan jumlah KTF anak kelapa sawit dan membantu dalam meningkatkan ketahanan dalam anak kelapa sawit, seterusnya melindungi dari jangkitan* G. boninense.

Oleh itu, aplikasi endofitik Trichoderma *spp. kepada akar anak kelapa sawit sebelum pemindahan (pada peringkat nurseri) dapat menyediakan strategi yang mampan dalam menguruskan penyakit kelapa sawit RPB. Walaupun begitu, kajian yang lebih lanjut juga diperlukan dalam menilai potensi endofitik* Trichoderma *spp. sebagai pendekatan tunggal atau konsortium di bawah keadaan lapangan.*

Keywords : *Reput pangkal batang*, Ganoderma boninense, *agen kawalan biologi*, *ketahanan*, *endofitik* Trichoderma *spp.*

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LIST OF ABBREVIATIONS

ANOVA	:	Analyzed using analysis of variance
ARISA	:	Amplified rRNA intergenic spacer analysis
BCAs	:	Biological control agents
BLAST	:	Basic local alignment search tool
BSR	:	Basal stem rot
CRBD	:	Completely randomized block design
CRD	:	Completely randomized design
CWDEs	:	Cell wall degrading enzymes
DGGE	:	Denaturing gradient gel electrophoresis
DI	:	Disease incidence
DNA	:	Deoxyribonucleic acid
DMRT	:	Duncan's multiple range test
ELISA	:	Enzyme-linked immunosorbent assay
ET	:	Endophytic *Trichoderma*
FFB	:	Fresh fruit bunch
FOL	:	*Fusarium oxysporum* f. sp. *Lycopersici*
GC-MS	:	Gas chromatography – mass spectrometry
GSM	:	Ganoderma selective medium
IDM	:	Integrated disease management
IPM	:	Integrated pest management
ISR	:	Induced systemic resistance
ITS	:	Internal transcribed spacer
MEGA	:	Molecular evolutionary genetics analysis

MM	:	Master mix
NCBI	:	National center for biotechnology information
NEB	:	New england biolabs
NIST	:	National institute standard and technology
PCR	:	Polymerase chain reaction
PCNB	:	Pentachloronitrobenzene
PDA	:	Potato dextrose agar
PDB	:	Potato dextrose broth
PIMG	:	Percentage inhibition of mycelia growth
PIRG	:	Percentage inhibition of radial growth
RAFLP	:	Random amplification polymorphic
RAPD	:	Random amplified polymorphic DNA
RFLPs	:	Restriction fragment length polymorphisms
ROS	:	Reactive oxygen species
RWB	:	Rubber wood block
SAR	:	Systemic acquired resistance
SEM	:	Scanning electron microscopy
SMs	:	Secondary metabolites
SPSS	:	Statistical package for social science
SSCP	:	Single-strand conformation polymorphism
TGGE	:	Thermal gradient gel electrophoresis
TPC	:	Total phenolic content
TSM	:	Trichoderma selective medium
USR	:	Upper stem rot
VOCs	:	Volatile organic compounds

CHAPTER 1

INTRODUCTION

1.1 Research Background

Basal stem rot (BSR) caused by the fungus *Ganoderma boninense* is regarded as the most economically debilitating disease of oil palms in Malaysia and Indonesia (Idris, 2009; Susanto, 2013; Turner, 1981). Cooper *et al.* (2011) reported that 50% of palms are lost with the majority of standing palms showing disease symptoms at the same time of replanting in North Sumatra, Indonesia. Losses begin to have a financial effect once the disease affects more than 10% of the stand (Hasan & Turner, 1998). Yield decline on average was 0.16 tonnes per hectare fresh fruit bunch (FFB) for every palm lost, and when the stand had declined by 50% mean losses were 35% (Subagio & Foster, 2003).

BSR also the most serious disease of oil palm in Asian Agri Group estates in North Sumatra. The disease has been particularly severe in first generation oil palm plantings established on volcanic and peat soils. Up to 2017, a total of 30 palms per hectare (22% per hectare) have been killed by the BSR disease in Asian Agri Group (Asian Agri R&D internal report, 2018). Apart from a significant decline in yield, the high loss of palm stand has significantly shortened the economic life-span of the oil palm plantings necessitate premature replanting. This becomes a serious threat to oil palm plantations sustainability.

However, the research about *Ganoderma* pathogenicity and controlling the pathogen are still in sufficient, especially in North Sumatra, Indonesia. Through this research, *Ganoderma* pathogenicity and *Ganoderma* species proper identification as one of the early steps in controlling strategy were done followed by evaluating the efficacy of some antagonistic bioagents in controlling the disease. Basic identification to confirm the identity of BSR causal pathogen in North Sumatra, through molecular

approach also has never been performed before, especially in Negeri Lama Estate, North Sumatra.

In order to control the spread of BSR in the second generation area, Asian Agri Group has adopted an integrated disease management (IDM) strategy based on four principles i.e. proper land preparation before replanting, the use of more tolerant oil palm varieties in the new planting area, application of antagonistic bioagents to protect oil palms from the disease and early detection of infected palms (Asian Agri R&D internal report, 2010). One of main strategies to control BSR is by utilization of potential antagonistic bioagents to protect and strengthen the newly planted oil palm seedlings and controlling the BSR infection in mature oil palms.

Zeilinger *et al.* (2016) reported that species of the *Trichoderma* genus inhabit diverse environments and undergo a variety of interactions with different organisms. Mycoparasitic *Trichoderma* species have been successfully applied as bio-fungicides due to their plant-protecting abilities and they are prolific producers of secondary metabolites (Zeilinger *et al.*, 2016). The wide range application of selected metabolites to induce host resistance and/or to promote crop yield may become a reality in the near future and represents a powerful tool for the implementation of integrated pest management (IPM) strategies (Vinale *et al.*, 2014).

Trichoderma species have been used as biocontrol agents against plant pathogens and could be a possible source of biofungicides as part of IDM strategies especially against fungal pathogens due to its secondary metabolites. The comprehensive information of secondary metabolites, mechanism of action and applications would be useful and important for integrated and sustainable pest and disease management. It is therefore important to identify and perform bioassay on the secondary metabolites from the interaction of *Trichoderma* isolates and *G. boninense* to provide basic information for *Trichoderma* utilization against *Ganoderma* of BSR.

Preliminary study on potential antagonistic bioagents has been done by the Asian in Agri Research and Development Centre and numerous other research organizations including Universiti Malaysia Sabah has shown very promising results in controlling *Ganoderma* (Asian Agri R&D internal report, 2014; Alexander & Chong, 2013). From the numerous bioagents that have been evaluated, endophytic *Trichoderma* showed great potential against the activity of *G. boninense* through various mechanisms of action including mycoparasitism, antibiosis, induced systemic

resistance, and others (Sundram, 2013). This potential need to be further explored to optimize the utilities of the bioagents in controlling BSR. Therefore, this project is designed with the following research objectives.

1.2 Hypothesis

1. *Ganoderma boninense* allegedly to be the causal pathogen of BSR in North Sumatra, Indonesia.
2. Endophytic *Trichoderma* could suppress *G. boninense* growth.
3. Endophytic *Trichoderma* plays a role in preventing oil palm from BSR attacks.
4. Endophytic *Trichodema* could induced oil palm resistance to BSR.

1.3 Research Objectives

1. To isolate and confirm the identity of the causal pathogen of Basal Stem Rot (BSR) from North Sumatra, Indonesia.
2. To isolate and identify the endophytic *Trichoderma* strain from healthy oil palm roots in North Sumatra, Indonesia.
3. To evaluate the effectiveness of endophytic *Trichoderma* in preventing and suppressing the infection of *G. boninense*.
4. To evaluate the antibiosis activity and resistance induced by endophytic *Trichoderma* in oil palm seedlings.

CHAPTER 2

LITERATURE REVIEW

2.1 Oil Palm

2.1.1 History of oil palm

African oil palm originated from Africa, along the coastal strip (200–300 km wide) between Liberia and Angola, from whence it spread north, south and east to Senegal, the Indian Ocean, Zanzibar (Tanzania) and Madagascar (NewCROP, 1996). The African oil palm was named by Jacquin in 1763 (Williams & Hsu, 1970). The genus name *Elaeis* originated from the Greek word "elaion", which means oil, and the specific name *Guineensis* indicates its origin at the Guinea Coast (Hartley, 1988). Since its domestication, oil palm has been introduced and cultivated throughout the humid tropics (16°N to 16°S) (NewCROP, 1996). Since the early 1980s, the total area of land allocated to mature oil palm has more than tripled globally, reaching approximately 20.3 million hectares across the world in 2018 (Oil World, 2018). Few developments of oil palm plantation generate as much controversy as the rapid expansion of oil palm area into forest-rich developing countries such as Indonesia (Koh & Wilcove, 2007).

In Indonesia, oil palms have been cultivated commercially since 1911, when they were first developed in the east coast area of Sumatra under Dutch administration (Corley & Tinker, 2003). While the oil palm was successfully cultivated in this area in large plantations, the native population did not replace their coconut palms with this new palm species. They planted it only for decorative purposes.

Nowadays, expansion of oil palm plantation in Indonesia could not dammed. Indonesia became the biggest oil palm producer sifted Malaysia in 2009 and supplied more than 40% of global oil palm demand (Rofiq, 2013). Ministry of Agriculture of Republic of Indonesia reported that oil palm areal in Indonesia rapidly growing with