THE EFFECTIVENESS OF SELECTED PHENOLIC ACIDS AGAINST *Ganoderma boninense* AND ITS EFFECTS TO SOIL MICROBIAL COMMUNITY

JEE WEI REN

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2017

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JEE WEI REN

THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

FACULTY OF SCIENCE AND NATURAL RESOURCES
UNIVERSITI MALAYSIA SABAH
2017

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MS1211001T

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DEGREE	:	**MASTER OF SCIENCE (BIOTECHNOLOGY)**
VIVA DATE	:	**14 JUNE 2017**

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1. SUPERVISOR
Assoc. Prof. Dr. Chong Khim Phin _____

ACKNOWLEDGEMENT

I would like to take this opportunity to express my utmost appreciation to my supervisor, Assoc. Prof. Dr. Chong Khim Phin. Thanks for giving me opportunity to gain the invaluable experience in conducting my master research. I am truly grateful for his patience, encouragement, advice, support and effort in guiding me to accomplish my master thesis.

I also wish to express my appreciations to my friends and course mates who have assisted me during the laboratory work and through all the challenges in this study and during the progression of my thesis writing. I am very thankful for their patience and tolerance. In addition, my gratitude also extends to the laboratory assistants who provided their technical support and cooperation in making my study a smooth one.

Furthermore, I acknowledged the grant (PRGS) awarded by Ministry of Higher Education Malaysia and the contribution of oil palm sites from Sawit Kinabalu Sdn Bhd. I would also like to express my sincere thanks to Mr. Richard Gudang, representatives of Sawit Kinabalu Sdn Bhd for his kind assistance during the field research at Langkon Estate, Kota Marudu, Sabah.

Last but not least, I would like to take this opportunity to dedicate my heartfelt thanks to my beloved parents, brother, sisters and relatives who gave me moral support and endless love in encouraging me to keep going.

Jee Wei Ren

14 June 2017

ABSTRACT

Basal Stem Rot (BSR) disease caused by *Ganoderma boninense* is thriving rapidly within oil palm plantations, especially in Indonesia and Malaysia. This study illustrates the potential of combination of three phenolic acids at the ratio of 1:1:1 (w/w); caffeic acid (CA), syringic acid (SA) and 4-hydroxybenzoic acid (4-HBA) in suppressing *G. boninense* colonization and its effect to soil microbial community. There were four objectives being carried out in this master's thesis. The first objective is to evaluate the *in vitro* antifungal activity of phenolic acids against *Ganoderma boninense* and possible development of resistance. Cell assay plates with different concentrations of phenolic acids combination; 0.0, 0.1 mg, 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg and 2.5 mg mL^{-1} were prepared by incorporating the respective phenolic acids into Potato Dextrose Agar (PDA). For antifungal assays, the growth of *G. boninense* was inhibited as the concentration of phenolic acids increased. *G. boninense* in 0.1 to 0.3 mg mL^{-1} concentrations of phenolic acids was able to reach the maximum growth as in the control plates at the end of the experiment. Phenolic acids with concentrations of 0.4 and 0.5 mg mL^{-1} gave a significant slower growth rate of the pathogen compared to control, thus *G. boninense* was unable to reach the maximum growth as controls after 14 days of incubation. The highest concentration of phenolic acids (2.5 mg mL^{-1}) had inhibited the growth of *G. boninense* completely. While for the study on possible resistance of *G. boninense* to phenolic acids, *G. boninense* was transferred to new cell assay plates containing higher concentration of phenolic acids than the previous cell assay plates. The results showed that *G. boninense* was incapable to develop resistance to higher concentrations of phenolic acids (0.5 and 2.5 mg mL^{-1}). Throughout the incubation period where *G. boninense* was cultured in cell assay plates containing phenolic acids, there were colour changes occurred to the culture and media itself which indicated the process of phenolic acids degradation. The colour intensity of culture was changed from light brown to dark brown when the concentration of phenolic acids increased. The second objective is to observe the *in planta* antifungal activity of phenolic acids against *Ganoderma boninense* through Scanning Electron Microscope (SEM) examination. The Scanning Electron Microscope (SEM) examination and *in vitro* bioassay showed that the phenolic acids reduced the branching of mycelium and affected the growth of *G. boninense* mycelium. When the infected oil palm seedling was treated with the phenolic acids, the roots of oil palm seedling showed a reduction in disease symptoms, such as the obstruction on xylem bundles and vessels. The third objective is to quantify the ergosterol content (fungal biomass) of *Ganoderma*-infected oil palm trees after treated with phenolic acids. There were four concentrations of phenolic acids combination being tested in this study, which were 1.0, 2.0, 3.0 and 4.0 g. All concentrations were capable to suppress the colonization of *Ganoderma* in infected oil palm trees. However, the concentration 4.0 g was the most effective in suppressing *Ganoderma* ergosterol, the fungal sterol in infected tissues. *Ganoderma* Selective Media (GSM) was used to further confirm the presence of *Ganoderma* besides extraction of ergosterol from the infected tissues. *Ganoderma* was successfully isolated on GSM after one week of incubation from all infected tissues. The last objective is to investigate the effect of phenolic acids to soil microbial community. Positive changes were observed in the composition of the soil

microbes after the application of phenolic acids although it was a slightly increase. The application of phenolic acids into the soil had introduced the presence of some beneficial microorganisms. Besides, the application of phenolic acids did not cause an adverse effect on the microorganism communities in soil. This study showed that the combination of three phenolic acids; caffeic acid, syringic acid and 4-hydroxybenzoic acid at the ratio 1:1:1 (w/w) provided a promising result in suppressing the colonization of *G. boninense* under different environmental circumstances. Therefore, the combination of phenolic acids could be a potential fungicide to suppress the colonization of *G. boninense* in oil palm plantation in near future.

Keywords: Basal Stem Rot, *Ganoderma boninense*, phenolic acids, syringic acid, caffeic acid, 4-hydroxybenzoic acid

ABSTRAK

KEBERKESANAN ASID FENOLIK TERPILIH DALAM MENGAWAL Ganoderma boninense DAN KESANNYA TERHADAP KOMUNITI MIKROB TANAH

Penyakit Reput Pangkal Batang (RPB) yang disebabkan Ganoderma boninense tersebar luas dalam ladang kelapa sawit, terutamanya di Indonesia dan Malaysia. Kajian ini menunjukkan potensi gabungan tiga asid fenolik pada nisbah 1:1:1 (w/w); asid caffeic (CA), asid syringic (SA) dan asid 4-hydroxybenzoic (4-HBA) dalam membendung penularan G. boninense dan kesannya terhadap komuniti mikrob tanah. Terdapat empat objektif yang dijalankan dalam tesis sarjana ini. Objektif yang pertama adalah untuk menilai aktiviti anti-kulat in vitro asid fenolik terhadap Ganoderma boninense dan kemungkinan kewujudan resistan. Plat cerakin sel dengan kepekatan gabungan asid fenolik yang berbeza; 0.0, 0.1 mg, 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg dan 2.5 mg mL^{-1} telah disediakan dengan menggabungkan asid fenolik masing-masing ke dalam Potato Dextrose Agar (PDA). Untuk ujian anti-kulat, pertumbuhan G. boninense telah dihalang apabila kepekatan asid fenolik meningkat. G. boninense dalam kepekatan asid fenolik 0.1 sehingga 0.3 mg mL^{-1} dapat mencapai pertumbuhan maksimum seperti dalam plat kawalan. Asid fenolik dengan kepekatan 0.4 dan 0.5 mg mL^{-1} memberikan kadar pertumbuhan patogen yang ketara lebih perlahan berbanding dengan kawalan, jadi G. boninense tidak dapat mencapai pertumbuhan maksimum seperti dalam plat kawalan selepas 14 hari pengeraman. Kepekatan tertinggi asid fenolik (2.5 mg mL^{-1}) telah menghalang pertumbuhan G. boninense sepenuhnya. Manakala bagi kajian ke atas kemungkinan resistan G. boninense terhadap asid fenolik, G. boninense telah dipindahkan ke plat cerakin sel baru yang mengandungi kepekatan asid fenolik yang lebih tinggi daripada plat cerakin sel yang sebelumnya. Hasil kajian menunjukkan bahawa G. boninense tidak mampu untuk membentuk resistan terhadap kepekatan asid fenolik yang lebih tinggi (0.5 dan 2.5 mg mL^{-1}). Sepanjang tempoh pengeraman di mana G. boninense dikulturkan dalam plat cerakin sel yang mengandungi asid fenolik, terdapat perubahan warna berlaku kepada kultur dan media itu sendiri yang menunjukkan proses degradasi asid fenolik. Keamatan warna kultur telah bertukar daripada coklat terang kepada coklat gelap apabila kepekatan asid fenolik meningkat. Objektif yang kedua adalah untuk memerhatikan aktiviti anti-kulat in planta asid fenolik terhadap Ganoderma boninense melalui pemeriksaan Scanning Electron Microscope (SEM). Pemeriksaan Scanning Electron Microscope (SEM) dan bioesei in vitro menunjukkan bahawa asid fenolik mengurangkan cabangan miselium dan menjejaskan pertumbuhan miselium G. boninense. Apabila anak pokok kelapa sawit yang dijangkiti telah dirawat dengan asid fenolik, akar anak pokok tersebut menunjukkan pengurangan dalam gejala penyakit, seperti halangan pada bahagian xilem. Objektif yang ketiga adalah untuk mengukur kandungan ergosterol (biomass kulat) pokok kelapa sawit yang dijangkiti oleh Ganoderma selepas dirawat dengan asid fenolik. Terdapat empat kepekatan gabungan asid fenolik yang diuji dalam kajian ini, iaitu 1.0, 2.0, 3.0 dan 4.0 g. Kesemua kepekatan ini mampu untuk menghalang penularan Ganoderma dalam

pokok-pokok kelapa sawit yang dijangkiti. Walau bagaimanapun, kepekatan 4.0 g adalah yang paling berkesan dalam mengurangkan ergosterol Ganoderma, sterol fungi dalam tisu yang dijangkiti. Ganoderma Selective Media (GSM) telah digunakan untuk mengesahkan lagi kehadiran Ganoderma selain pengekstrakan ergosterol dari tisu yang dijangkiti. Ganoderma telah berjaya diasingkan di GSM dari semua tisu yang dijangkiti selepas pengeraman selama seminggu. Objektif yang terakhir adalah untuk menyiasat kesan asid fenolik terhadap komuniti mikrob tanah. Perubahan positif telah diperhatikan dalam komposisi mikrob tanah selepas aplikasi asid fenolik walaupun ia menunjukkan sedikit peningkatan. Aplikasi asid fenolik ke dalam tanah telah menarik kehadiran beberapa mikroorganisma berfaedah. Selain itu, aplikasi asid fenolik tidak menyebabkan kesan yang buruk terhadap komuniti mikroorganisma dalam tanah. Kajian ini menunjukkan bahawa gabungan tiga asid fenolik; asid caffeic, asid syringic dan asid 4-hydroxybenzoic pada nisbah 1:1:1 (w/w) telah memberikan hasil yang memberangsangkan dalam mengurangkan penularan G. boninense di bawah keadaan alam sekitar yang berbeza. Oleh itu, kombinasi asid fenolik ini boleh dijadikan racun kulat yang berpotensi untuk mengawal G. boninense di ladang kelapa sawit pada masa hadapan.

Kata kunci: *Reput pangkal batang, Ganoderma boninense, asid fenolik, asid syringic, asic caffeic, asid 4-hydroxybenzoic*

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%	Percentage
°C	Degree celcius
±	plus-minus
µg mL^{-1}	Microgram per milliliter
µg g^{-1}	Microgram per gram
µg	Microgram
µL	Microliter
µm	Micrometer
4-HBA	4-hydroxybenzoic acid
a.i	Active Ingredients
ANOVA	Analysis of Variance
BSR	Basal Stem Rot
CA	Caffeic acid
CFU	Colony Forming Unit
cm	Centimeter
CPO	Crude palm oil
CRD	Completely Randomized Design
D x P	Dura x Pisifera
et al.	et alia (and others)
FF	Filamentous Fungi
FFB	Fresh fruit bunches
g	Gram
G.B	*Ganoderma boninense*
GSM	*Ganoderma* Selective Media
h	hour
ha	Hectare
HPLC	High Performance Liquid Chromatography
IF	Inoculation Fluid
kg	Kilogram
kPa	Kilopascal

L	Liter
m	Meter
MEA	Malt extract agar
mg	Milligram
min	Minute
mL min^{-1}	Milliliter per minute
mL	Milliliter
mm	Millimeter
MPOB	Malaysian Palm Oil Board
NA	Nutrient agar
nm	Nanometer
PCR	Polymerase Chain Reaction
PDA	Potato dextrose agar
PVC	Polyvinyl chloride
RWB	Rubber wood block
SA	Syringic acid
SEM	Scanning Electron Microscope
spp.	Species
SPSS	Statistical Package for Social Science
USR	Upper Stem Rot