UNIVERSITI PUTRA MALAYSIA

FUNGAL METABOLITES FOR ASSESSMENT OF *GANODERMA BONINENSE* PAT. INFECTION IN OIL PALM

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By

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

FUNGAL METABOLITES FOR ASSESSMENT OF *GANODERMA BONINENSE* PAT. INFECTION IN OIL PALM

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ROGER LIEU TOH CHOON

September 2011

Chairman : Professor Sariah Meon, PhD

Institute : Tropical Agriculture

Basal Stem Rot (BSR) caused by *Ganoderma boninense* is a serious disease in oil palm. Detection of BSR is based mainly on visible symptoms, and confirmed by plating infected tissues on *Ganoderma* selective media. Unfortunately, these symptoms are only visible when at least half of the basal tissue has been infected. Current research on the plant-pathogen interaction showed that invasion by pathogens can lead to increase of fungal biomass in the healthy plant, making it detectable during the infection. Certain primary fungal metabolites were targeted as they were produced abundantly during the reproduction of mycelium. Therefore, based on the mode of infection and spread of *G. boninense* on oil palm, the probable strategy is to unravel the metabolic pathway of the plant upon infection and possibly use this approach

to differentiate between healthy and infected palms at the early stage of infection. The technique used for analysis and identification of the targeted metabolites were Thin Layer Chromatography (TLC), Ultra Performance Liquid Chromatography (UPLC), Gas Chromatography coupled with Mass Spectrometry (GCMS) and Nuclear Magnetic resonance (NMR). This research focused on the potential of detecting targeted metabolites as indicators for *Ganoderma* infection in field palms. Metabolites from *G. boninense* were extracted and used as indicator for TLC and UPLC analysis. Eighty healthy oil palm seedlings were used as control where no artificial infection applied while eighty seedlings for artificial infection. Metabolites for healthy and infected palms were extracted from root tissues of the experimental seedlings. Meanwhile, two random samplings for the healthy and *Ganoderma*-infected stem tissues were carried out from MPOB Experimental Station, Kluang, Johor and Sime Darby oil palm plantation from Banting, Selangor. By using the same extraction method, these samples were analyzed by TLC with optimum combination of solvent systems to detect and the quantification of the metabolites was done with UPLC with progressive development of BSR symptoms. *G. boninense* extract was used as indicator for TLC and UPLC analysis to distinguish between healthy and infected oil palm samples. Results showed that the best combination solvent system which gave the clearest spot separation were acetone and hexane in the ratio of 30:70. Round and clear separated spots representing different metabolites were detected. Compared with pure *G. boninense* extract as indicator, four clear spots were consistently detected in infected tissues extracts which however were not detected in healthy tissues extracts. These

spots could be used to distinguish between healthy and infected tissues. Two clearest spots were chosen for further analysis named as metabolite A and metabolite B. In UPLC, it showed that metabolites A and B were increased exponentially and showed a positive correlation of the disease severity with time. Metabolites A and B were purified and sent for GCMS for their molecular weight followed by NMR analysis for chemical structure identification. Based on the results from GCMS and NMR, it was concluded that metabolite B was identified to be ergosterol which played an important role in fungal cell wall development. Meanwhile, metabolite A was tentatively suggested to be 2,4-ditert-butylphenol. According to the present work, it could be concluded that *Ganoderma* infection in palms increase the production of metabolites which could be used as indicators for early detection of infection in oil palms. Further studies are needed to produce a better implementation of this finding as disease diagnostic tool in the commercialized oil palm field.

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

METABOLIT KULAT UNTUK PENTAFSIRAN JANGKITAN *GANODERMA BONINENSE* PAT. PADA KELAPA SAWIT

By

ROGER LIEU TOH CHOON

September 2011

Pengerusi : Profesor Sariah Meon, PhD

Institut : Pertanian Tropika

Penyakit reput pangkal (BSR) merupakan sejenis penyakit serius dalam kelapa sawit yang disebabkan oleh *Ganoderma boninense*. Pengesanan BSR biasanya berdasarkan kepada simptom luaran dan disahkan dengan pengasingan *Ganoderma* daripada tisu berpenyakit atas media selektif. Malangnya, simptom-simptom ini hanya menjadi nyata apabila sekurang-kurangnya setengah daripada tisu pangkal telah dijangkiti oleh penyakit ini. Kajian semasa atas interaksi tumbuhan-patogen menunjukkan bahawa jangkitan patogen boleh menyebabkan penambahan biojisim kulat dalam pokok yang sihat, menjadikannya senang dikesan apabila jangkitan berlaku. Sesetengah metabolit kulat primer menjadi sasaran penunjuk apabila ianya banyak dihasilkan semasa reproduksi miselium kulat. Oleh itu, berdasarkan kepada cara jangkitan dan penyebaran oleh *G. boninense* dalam kelapa

sawit, strategi yang sesuai adalah dengan melihat laluan metabolik pokok semasa jangkitan dan menggunakan pendekatan ini untuk membezakan pokok sihat dan pokok terjangkit pada tahap awal jangkitan. Teknik yang digunakan dalam analisis dan pengenalpastian metabolit sasaran adalah kromatografi lapisan nipis (TLC), kromatografi cecair prestasi ultra (UPLC), kromatografi gas digabungkan dengan spektrometri jisim (GCMS) dan resonansi magnetik nuklear (NMR). Kajian ini akan menumpu kepada metabolit sasaran berpotensi sebagai penunjuk dalam mengesan jangkitan *Ganoderma* dalam pokok kelapa sawit. Metabolit *G. boninense* diekstrak dan dijadikan penunjuk untuk analisis TLC dan UPLC. Lapan puluh anak benih kelapa sawit dijadikan sebagai kawalan di mana tiada jangkitan buatan dilakukan sementara lapan puluh anak benih dijangkiti secara buatan. Metabolit untuk pokok sihat dan pokok terjangkit diekstrak daripada tisu akar daripada sampel anak benih yang dikaji. Sementara itu, dua kali penyampelan tisu batang pokok kelapa sawit sihat dan terjangkit dijalankan di stesen ujikaji MPOB, Kluang, Johor dan ladang kelapa sawit Sime Darby, Banting, Selangor. Dengan menggunakan cara ekstrak yang sama, sampel-sampel ini dianalisis dengan TLC dengan kombinasi sistem pelarut optimum untuk mengesan dan mengenalpasti kuantiti metabolit atas kertas TLC. Penentuan kepekatan metabolit dan pembentukan progresif simptom penyakit BSR dilakukan dengan UPLC. Extrak kulat *G. boninense* dijadikan penunjuk dalam analisis TLC dan UPLC untuk membezakan sampel kelapa sawit yang sihat dan berjangkit. Keputusan menunjukkan kombinasi sistem pelarut terbaik yang memberikan bintik pemisahan yang jelas adalah asetone: heksana dengan nisbah 30:70. Bintik- bintik bulat yang jelas

terpisah mewakili metabolit yang berlainan dikesan. Berbanding dengan ekstrak tulen *G. boninense* sebagai penunjuk, empat bintik jelas dikesan dalam ekstrak tisu terjangkit yang tidak dikesan dalam ekstrak tisu yang sihat. Bintik-bintik ini mungkin boleh membezakan tisu-tisu sihat dan terjangkit. Dua bintik yang paling jelas dipilih untuk analisis seterusnya dinamakan sebagai metabolit A dan metabolit B. UPLC menunjukkan bahawa metabolit A dan B meningkat dan memberikan korelasi positif antara keparahan penyakit dan masa. Metabolit A dan B yang tulen dihantar untuk GCMS analisis untuk mendapatkan jisim molekul bagi metabolit. Ini diikuti dengan analisis NMR untuk menentukan struktur kimia metabolit A dan B. Berdasarkan kepada keputusan GCMS dan NMR, metabolit B dikenalpasti sebagai ergosterol yang memainkan peranan penting dalam pembentukan dinding sel-sel kulat. Sementara, metabolit A dinamakan sebagai 2,4-ditert-butylphenol. Menurut kajian semasa, dapatlah disimpulkan bahawa jangkitan *Ganoderma* dalam pokok kelapa sawit boleh menghasilkan metabolit yang boleh digunakan sebagai penunjuk untuk mengesan jangkitan dalam kelapa sawit pada tahap awal. Kajian lanjut perlu dijalankan untuk mendapatkan implimentasi yang lebih baik menggunakan maklumat kajian ini sebagai alat diagnosis penyakit dalam ladang kelapa sawit komersial.

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I certify that a Thesis Examination Committee has met on 27 September 2011 to conduct the final examination of Roger Lieu Toh Choon on his thesis entitled "Fungal Metabolites for Assessment of *Ganoderma boninense* Pat. Infection in Oil Palm" in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P. U. (A) 106] 15 March 1998. The committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

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The thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirements for the degree of Master of Science. The members of the Supervisory committee were as follow:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at UPM or Universiti Putra Malaysia or other Institutes.

ROGER LIEU TOH CHOON

Date: 27th September 2011

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