



UNIVERSITI PUTRA MALAYSIA

**EFFECTS OF CALCIUM AND COPPER ON LIGNIN BIOSYNTHESIS AND
SUPPRESSION OF *Ganoderma boninense* INFECTION IN OIL PALM
SEEDLINGS**

NUR SABRINA AHMAD AZMI

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SEEDLINGS**

By

NUR SABRINA AHMAD AZMI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Science**

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EFFECTS OF CALCIUM AND COPPER ON LIGNIN BIOSYNTHESIS AND SUPPRESSION OF *Ganoderma boninense* INFECTION IN OIL PALM SEEDLINGS

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September 2011

Chairman : Professor Sariah Meon, PhD

Institute : Tropical Agriculture

Ganoderma boninense the causal pathogen of Basal Stem Rot (BSR) disease is a white rot basidiomycetous fungus. The pathogen infects oil palm (*Elaeis guineensis* Jacq.) mainly through its roots and degrade the lignin component of wood leaving white cellulose exposed. Therefore, by understanding the mode of infection of *G. boninense* on oil palm, the ideal solution to slow down the emergence of basal stem rot disease is to enhance the oil palm physical barriers through lignin biosynthesis by calcium (Ca) and copper (Cu) supplementation. The objectives of our study are to find the best concentration of Ca and Cu that gives optimum production of lignin and to evaluate its effects towards suppressing BSR incidence. Copper have been reported to enhance the production of lignin-related enzymes in plants, and meanwhile, calcium plays a major role in cell wall stiffening. The experiment was carried out to investigate changes in peroxidase activity, laccase activity, hydrogen peroxide concentration and lignin content in roots of Ca-

supplemented and Cu-supplemented oil palm seedlings and their relationships with lignification. 3-month-old oil palm seedlings were supplemented with either Ca or Cu ions (in half- strength Hoagland nutrient solution) in sand culture for 3 months. Ca and Cu-supplemented oil palm seedlings showed significant enhancement in peroxidase activity, laccase activity and lignin content. The amount of hydrogen peroxide was the lowest in 2 mg/L Cu- supplemented tissues among Cu- supplemented tissues and 4000 mg/L Ca- supplemented tissues was the lowest (17.7%, 20.5% respectively) among Ca- supplemented tissues 3 months after supplementation. The peroxidase activity was increased significantly in 2 mg/L Cu (49.0%) among Cu- supplemented tissues and in 4000 mg/L Ca (72.1%) among Ca- supplemented tissues 3 months after supplementation. Laccase activity in 2 mg/L Cu (41.9%) and 4000 mg/L Ca (130.3%) and lignin content in 2 mg/L Cu (61.1%) and 4000 mg/L Ca (86.2%) were also significantly high compared to other treatments. Scanning electron microscopy observations of Cu and Ca-supplemented tissues showed the thickest cell wall (5.55 μm and 6.01 μm respectively) between two adjacent parenchyma cells after 3 months supplementation. The concentrations that give optimum production of lignin content, which are 2 mg/L Cu and 4000 mg/L Ca were chosen to investigate its effect on suppressing the BSR disease. The oil palm seedlings were supplemented with Ca and Cu either singly or as mixture for three months before challenged with *Ganoderma boninense*. The experiment was carried out to determine whether high lignin content in the roots can suppress the infection and spread of *Ganoderma*. Results showed that supplementation with mixture of 2 mg/L Cu with 4000 mg/L Ca gave the best suppression of BSR based on the lowest epidemic rate and highest percentage of disease reduction which suggested that it was effective in suppressing *Ganoderma* infection in oil palm.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains.

KESAN KALSIUM DAN KUPRUM KEATAS BIOSINTESIS LIGNIN DAN PENINDASAN TERHADAP JANGKITAN *Ganoderma boninense* PADA ANAK BENIH KELAPA SAWIT

oleh

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Ganoderma boninense patogen yang menyebabkan penyakit reput pangkal (BSR) ialah kulat basidiomycete reput putih. Patogen ini menjangkiti kelapa sawit (*Elaeis guineensis* Jacq.) melalui akar dengan meleraikan komponen lignin dalam batang dan menyebabkan selulosa putih terdedah. Oleh itu, dengan memahami cara jangkitan *G. boninense* dalam kelapa sawit, penyelesaian yang baik untuk melambatkan proses jangkitan *G. boninense* adalah dengan meningkatkan halangan fizikal dalam kelapa sawit melalui biosintesis lignin menggunakan kalsium (Ca) dan kuprum (Cu). Objektif kajian adalah untuk menentukan kepekatan yang paling baik yang boleh menghasilkan lignin yang banyak serta mengkaji kesan penambahan Ca dan Cu keatas kadar jangkitan penyakit reput pangkal. Cu telah dilaporkan dapat meningkatkan penghasilan enzim berkaitan dengan biosintesis lignin, manakala Ca memainkan peranan penting dalam penebalan dinding sel dalam tumbuhan. Eksperimen dijalankan untuk mengkaji perubahan dalam aktiviti peroksida dan aktiviti laccase, kepekatan hidrogen peroksida dan jumlah kandungan lignin dalam akar kelapa sawit yang dirawat dengan Ca

dan Cu serta mengkaji hubungannya dengan lignifikasi. Anak pokok kelapa sawit yang berusia 3 bulan dirawat dengan ion Ca dan Cu (dalam larutan Hoagland berkepekatan separa) menggunakan medium pasir selama 3 bulan. Anak pokok kelapa sawit yang dirawat dengan Ca dan Cu menunjukkan peningkatan yang signifikan dalam aktiviti peroksida, aktiviti laccase dan jumlah kandungan lignin. Selepas 3 bulan, kepekatan hidrogen peroksida yang paling rendah ditunjukkan oleh anak pokok kelapa sawit yang dirawat dengan 2 mg/L Cu (17.7%) berbanding rawatan Cu yang lain dan 4000 mg/L Ca (20.5%) berbanding rawatan Ca yang lain. Aktiviti peroksida juga meningkat secara signifikan dalam 2 mg/L Cu (49.0%) berbanding rawatan Cu yang lain dan 4000 mg/L Ca (72.1%) berbanding rawatan Ca yang lain 3 bulan selepas rawatan nutrien. Aktiviti laccase dalam 2 mg/L Cu (41.9%) dan 4000 mg/L Ca (130.3%) dan jumlah kandungan lignin dalam tumbuhan 2 mg/L Cu (61.1%) dan 4000 mg/L Ca (86.2%) meningkat secara signifikan berbanding rawatan lain. Pemerhatian yang dibuat melalui mikroskop elektron menunjukkan ketebalan dinding sel parenkima pada tisu akar yang dirawat dengan 2 mg/L Cu (5.55 μm) dan 4000 mg/L Ca (6.01 μm) adalah tinggi. Kepekatan yang menghasilkan kandungan lignin yang optimum iaitu 2 mg/L Cu dan 4000 mg/L Ca dipilih. Anak pokok kelapa sawit dirawat dengan Cu dan Ca secara berasingan atau campuran selama 3 bulan sebelum diinokulasi dengan *G. boninense*. Penilaian tahap jangkitan penyakit dijalankan untuk menentukan samada lignin yang dihasilkan melalui rawatan Ca dan Cu dapat menurunkan kadar jangkitan penyakit BSR pada anak benih kelapa sawit. Keputusan eksperimen menunjukkan campuran Cu dan Ca pada kepekatan 2 mg/L dan 4000 mg/L melambatkan kadar jangkitan berdasarkan kepada kadar epidemik yang paling rendah dan peratusan penurunan jangkitan penyakit yang paling tinggi, mencadangkan ia berkesan untuk melambatkan perebakan jangkitan *Ganoderma* pada kelapa sawit.

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Nur Sabrina Ahmad Azmi

June, 2011

APPROVAL SHEET 1

I certify that a Thesis Examination Committee has met on to conduct the final examination of Nur Sabrina Ahmad Azmi on her thesis entitled "**EFFECTS OF CALCIUM AND COPPER SUPPLEMENTATION ON LIGNIN BIOSYNTHESIS AND SUPPRESSION OF *Ganoderma boninense* Pat. INFECTION IN OIL PALM SEEDLINGS**" in accordance with the Universities and University Colleges 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.

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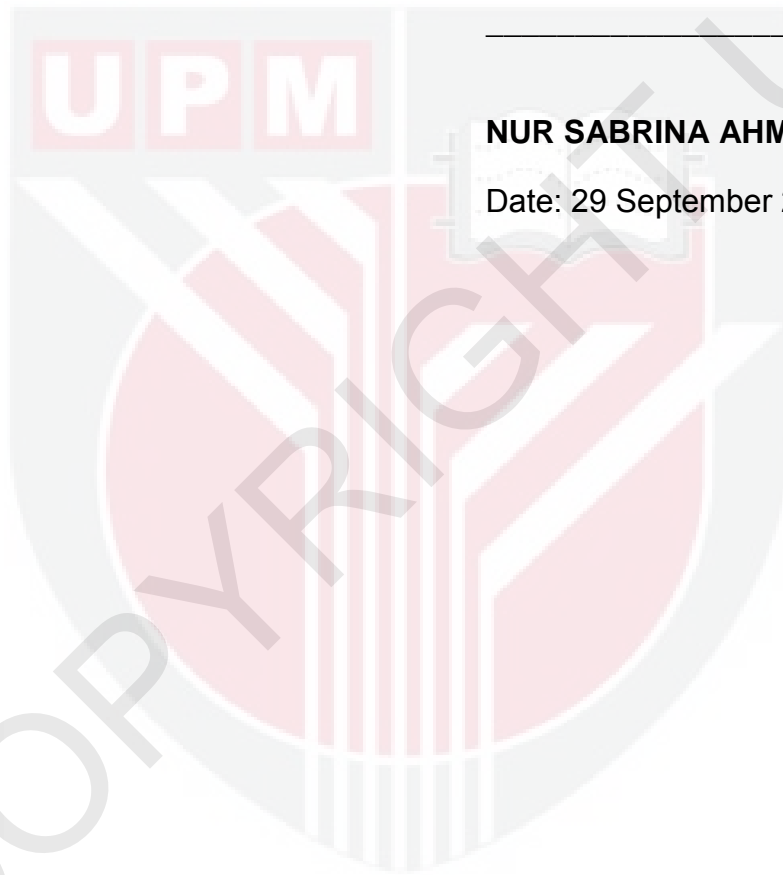
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DECLARATION

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 Universiti Putra Malaysia or at any other institution.

NUR SABRINA AHMAD AZMI

Date: 29 September 2011



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