UNIVERSITI PUTRA MALAYSIA

INFLUENCE OF SOIL pH ON MICROBIAL COMMUNITY AND INCIDENCE OF BASAL STEM ROT (BSR) DISEASE DEVELOPMENT IN OIL PALM SEEDLINGS PLANTED ON BSR AFFECTED AND FOREST SOILS

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By

KHUNAW ABDULLA RAHMAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillments of the Requirements for the Degree of Doctor of Philosophy

September 2017

DEDICATION

I would like to dedicate this work to those who taught, motivated and helped me throughout my study.
To my memory of late father Abdulla Rahman

This work is also dedicated to my dearest husband, Shihab Ahmed
and to my sweet kids Janna, Dana, & Dema.
This work is also dedicated to all my family members

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

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September 2017

Chairman : Associate Professor Radziah Othman, PhD
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Basal stem rot (BSR) disease caused by *Ganoderma boninense*, remains as the most challenging disease in oil palm industry. In search for a practical approach to halt *Ganoderma* incidence and development in oil palm plantations, experiments were conducted with the following objectives; (i) to evaluate the diversity of microbial communities in BSR affected oil palm and forest soils, (ii) to determine the role of indigenous microbial community in suppressing the development of *G. boninense* in oil palm seedlings, (iii) to determine the effect of soil pH on growth of oil palm seedlings and BSR disease development in *Ganoderma* inoculated seedlings, and (vi) to evaluate the influence of pH and calcium ion in soil amended with liming material on *Ganoderma* development in the roots of oil palm seedlings. Four seperate experiments were conducted under laboratory and glasshouse conditions. Soil samples from three different oil palm locations (BSR affected, unaffected and forest) were collected and analyzed for physicochemical properties, the microbial biomass carbon and total microbial enzyme at different depths. Metagenomic determination of soil bacterial diversity in *Ganoderma* affected and unaffected soils was also dertemined (Experiment 1). Soil sterilization technique was used to eliminate the presence of indigenous microorganisms in the three different soils. Rubber wood blocks fully colonized with *G. boninense* UPM13 mycelium were used to inoculate 3-month old oil palm seedlings. Seedlings with (G+) and without (G-) *Ganoderma* inoculation were then grown in the respective soils for four months. Plant growth (shoot and root dry weights), nutrient uptake, soil microbial properties, disease incidence (DI) and disease severity index (DSI) were determined at harvest (Experiment 2). Soil from BSR affected soil of pH 4.5 was adjusted with liming material, calcium carbonate ($CaCO_3$) to pH 5, 6, and 7. Oil palm seedlings were then grown with (G+) and without without (G-) *Ganoderma* inoculation. Plant growth and disease incidence were

determined as in Experiment 2 (Experiment 3). Acid washed sand culture was adjusted with $CaCO_3$ to pH 5, pH 6 and pH 7 were each applied with four levels of calcium (0, 0.27, 0.40 and 0.60 g Ca/pot) using $CaCl_2$ as the calcium source. Oil palm seedlings were grown with and without *Ganoderma* inoculation. Plant growth and disease incidence were determined as in Experiment 2 (Experiment 4). Results of Experiment 1 showed that the populations of bacteria, fungi, and actinomycetes in BSR affected, unaffected and forest soils significantly ($P \leq 0.05$) decreased with increased in soil depth. The observed pattern corresponded with the microbial biomass carbon with the highest value in forest soil. The total microbial enzymes showed decreasing trend with increasing depth, with higher values in forest and BSR unaffected soils. The BSR unaffected soil had the highest relative abundance of Acidobacteria and Actinobacteria compared to other bacterial groups. Both of the bacteria phyla relative abundance exhibited a strong link with soil pH and total carbon. Results of Experiment 2 showed that sterilized soil without presence of indigenous microbial community gave lower plant growth and higher disease incidence. Seedlings grown in non-sterilized and non-*Ganoderma* inoculated forest soil had the highest ($P \leq 0.05$) shoot and root biomass as well as root length, root surface area and root tips number. Mean while, seedlings grown in non-sterilized and non-*Ganoderma* inoculated BSR affected soil had the lowest plant biomass. Higher bacterial populations were observed in the forest soil compared to BSR affected and unaffected soils. Seedlings grown on forest soil showed better growth with lower DI and DSI and having the highest uptake of N (724 mg/plant), P (60 mg/plant) and K (605 mg/plant) compared to others treaments. Experiment 3 showed that the non-*Ganoderma* inoculated seedlings of pH 6 + G- displayed higher top biomass (8.46 %) and highest root growth. Seedlings grown at higher pH of 7 had lower shoot growth compared to that with *Ganoderma* inoculation. *Ganoderma* infected seedlings at pH 5 had the lowest growth. The DI and DSI of seedlings with *Ganoderma* inoculation pH 5 were higher that at other pH. The *Ganoderma* (G+) treated plants displayed lower populations of bacteria, fungi, and actinomycetes than without *Ganoderma* treated groups at all pH levels, though pH 6 + G- soil had the highest bacterial population. Higher nutrient contents, chlorophyll and photosynthesis were observed in plants without *Ganoderma* inoculation as compared to that with *Ganoderma*. The highest shoot N and Ca were observed at pH 6 while the P, K and Mg at pH 5. Experiment 4 showed that addition of Ca to non-*Ganoderma* inoculated seedlings resulted in higher shoot and root biomass with highest yields observed at pH 6 G- (Ca 0.27). Addition of higher Ca (0.6 Ca/pot) increased root length, surface area, and volume (2.99, 3.24 and 2.34 %, respectively) and the bole size compared to at other pH. Higher lignin content of *Ganoderma* infected plants were oberved at pH 6 G+ Ca (0.60) compared to at pH 4.5. The pH 6 together with 0.6 Ca g/pot application significantly reduced the disease infection in Ganoderma affected seedlings and improved biomass yield and activity of indigenous microorganisms. Under controlled condition, pH 6 with addition of Ca appeared to be effective in controlling BSR disease in oil palm seedlings.

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

PENGARUH pH TANAH TERHADAP KOMUNITI MIKROORGANISMA DAN INSIDEN PEMBENTUKAN PENYAKIT REPUT PANGKAL BATANG (BSR) DALAM ANAK BENIH KELAPA SAWIT DI ATAS TANAH TERJEJAS BSR DAN HUTAN

Oleh

KHUNAW ABDULLA RAHMAN

September 2017

Pengerusi : Profesor Madya Radziah Othman, PhD
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Penyakit reput pangkal batang (BSR) yang disebabkan oleh *Ganoderma boninense*, kekal sebagai penyakit yang paling mencabar dalam industri kelapa sawit. Dalam mencari pendekatan praktikal untuk menghentikan kejadian *Ganoderma* dan pembangunan di ladang kelapa sawit, kajian berikut telah dijalankan dengan objektif (i) untuk menilai kepelbagaian komuniti mikrob dalam tanah kelapa sawit yang dijangkiti BSR dan tanah hutan, (ii) untuk menentukan peranan komuniti mikrob asli dalam menghalang pembentukan *G. boninense* dalam anak benih kelapa sawit, (iii) untuk menentukan kesan pH tanah pada pertumbuhan anak benih kelapa sawit dan perkembangan penyakit reput pangkal batang di dalam benih yang diinokulasi *Ganoderma*, dan (vi) untuk menilai pengaruh pH dan kalsium dalam tanah yang dipinda dengan bahan kapur pada pembentukan *Ganoderma* dalam akar anak benih kelapa sawit. Empat eksperimen yang berasingan telah dijalankan di makmal dan rumah kaca. Sampel tanah dari tiga lokasi kelapa sawit berbeza (terjejas BSR, tidak terjejas BSR dan hutan) dikumpulkan dan dianalisa sifat fizikokimia, biojisim karbon, jumlah enzim mikrob di kedalaman yang berbeza. Penentuan metagenomik kepelbagaian bakteria tanah dalam tanah terjejas dan tak terjejas *Ganoderma* ditentukan (Eksperimen 1). Teknik pensterilan tanah telah dilakukan untuk menghapus mikroorganisma asli dalam tiga sampel tanah berbeza. Blok kayu getah yang penuh koloni mesilium *G. boninense* UPM13 digunakan untuk inokulasi pada anak benih kelapa sawit yang berusia 3 bulan. Anak benih kemudiannya dengan inokulasi (G+) dan tanpa (G-) *Ganoderma* ditanam selama 4 bulan. Pertumbuhan pokok (berat kering pucuk dan akar), pengambilan nutrien, sifat mikrob tanah, insiden penyakit (DI) dan indeks keterukan penyakit (DSI) ditentukan pada masa tuaian (Eksperimen 2). Tanah daripada kawasan terjejas BSR pada pH 4.5 diubahsuaikan dengan bahan pengapuran, kalsium karbonat ($CaCO_3$) kepada pH 5, 6 dan 7. Anak

benih kelapa sawit kemudiannya ditanam dengan inokulasi (G+) dan tanpa (G-) *Ganoderma*. Pertumbuhan pokok dan insiden penyakit ditentukan seperti dalam Eksperimen 2 (Eksperimen 3). Kultur pasir dicuci asid diubahsuai dengan $CaCO_3$ kepada pH 5, pH 6 dan pH 7, setiap satu ditambah empat tahap kalsium (0, 0.27, 0.40 dan 0.60 g Ca/pasu) menggunakan $CaCl_2$ sebagai sumber kalsium. Anak benih kelapa sawit ditanam dengan dan tanpa inokulasi *Ganoderma.* Pertumbuham pokok dan insiden penyakit ditentukan seperti dalam Eksperimen 2 (Eksperimen 4). Keputusan Eksperimen 1 menunjukkan bahawa populasi bakteria, kulat, dan aktinomicetes dalam tanah terjejas BSR, tanah tidak terjejas dan tanah hutan berkurangan dengan ketara ($P \leq 0.05$) dengan peningkatan kedalaman tanah. Pola ini diperhatikan bersesuaian dengan hasil biojisim karbon mikrob dimana tanah hutan menunjukkan nilai tertinggi. Jumlah enzim mikrob menunjukkan trend menurun dengan pengikatan kedalaman, dengan nilai tertinggi dalam tanah hutan dan tanah tidak terjejas BSR. Tanah tidak terjejas BSR mempunyai kelimpahan relatif tertinggi Acidobakteria dan Aktinobakteria berbanding kumpulan bakteria lain. Kedua-dua phyla bakteria ini menunjukkan hubungan yang kuat dengan pH tanah dan jumlah karbon. Keputusan Eksperimen 2 menunjukkan tanah yang disteril tanpa kehadiran komuniti mikrob asli menyebabkan pertumbuhan pokok yang rendah dan tinggi insiden penyakit. Anak benih yang ditanam dalam tanah hutan tidak steril dan tanpa diinokulasi *Ganoderma* mempunyai dengan ketara ($P \leq 0.05$) biojisim pucuk dan akar yang tertinggi serta panjang akar, luas permukaan akar dan bilangan hujung akar. Sebaliknya anak benih ditanam dalam tanah terjejas BSR tidak steril dan tanpa inokulasi *Ganoderma* mempunyai biojisim pokok terendah. Populasi bakteria lebih tinggi diperhatikan di dalam tanah hutan berbanding dengan tanah terjejas dan tidak terjejas BSR. Anak benih ditanam atas tanah hutan menunjukkan pertumbuhan lebih baik dengan DI dan DSI rendah dan mempunyai pengambilan N yang tertinggi (724 mg/pokok), P (60 mg/pokok) dan K (605 mg/pokok) berbanding rawatan lain. Eksperimen 3 menunjukkan bahawa anak benih tanpa inokulasi *Ganoderma* pada pH 6 + G- memaparkan biojisim bahagian atas lebih tinggi (8.46%) dan pertumbuhan akar tertinggi. Anak benih ditanam pada pH lebih tinggi pH 7 menunjukkan pertumbuhan pucuk lebih rendah berbanding yang diinokulasi *Ganoderma*. Anak benih terjangkit Ganoderma pada pH 5 mempunyai pertumbuhan terendah. Nilai DI dan DSI anak benih diinokulasi *Ganoderma* pada pH 5 adalah lebih tinggi daripada pH lain. Tanaman dirawat Ganoderma (G +) menunjukkan populasi bakteria, kulat, dan aktinomicetes yang lebih rendah daripada kumpulan tanpa *Ganoderma* (G-) yang dirawat di semua tahap pH, walaupun tumbuhan pH 6+ G- mempunyai populasi bakteria tertinggi. Kandungan nutrien, klorofil dan fotosintesis tertinggi diperolehi dalam pokok tanpa inokulasi *Ganoderma* berbanding ada *Ganoderma*. Peratusan N dan Ca pucuk yang paling tinggi dicatat dalam pH 6 dan P, K dan Mg pada pH 5. Eksperimen 4 mendedahkan penambahan Ca pada anak benih tanpa inokulasi Ganoderma menghasilkan pertumbuhan pucuk dan akar lebih tinggi dengan hasil tertinggi diperolehi pada pH 6 G- (Ca 0.27). Penambahan Ca (0.6 Ca/pasu) meningkatkn panjang akar, luas permukaan, dan isipadu (2.99, 3.24 dan 2.34%, masing-masing) dan saiz bol berbanding pada pH lain. Kandungan lignin lebih tinggi dalam pokok terjangkit Ganoderma didapati pada pH 6 G+ Ca (0.60) berbanding pada pH 4.5. Pada pH 6 dengan 0.6 Ca g/pasu ketara merencat jangkitan *Ganoderma* dalam tanah terjejas BSR dan memperbaiki hasil biojisim dan aktiviti mikroorganisma asli.

Dalam keadaan terkawal, pH 6 dengan penambahan Ca menunjukkan efektif dalam kawalan penyakit BSR pada anak benih kelapa sawit.

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This thesis was submitted to the Senate of the 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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ANOVA	Analysis of variance
AAS	Atomic Adsorption Spectrometer
BSR	Basal stem rot
CEC	Cation exchange capacity
CFU	colony forming unit
DI	Disease incidence
DNA	Deoxyribonucleic acid
DS	Disease severity
EC	Electrical conductivity
G-	non-treated with Ganoderma
G+	treated with Ganoderma
H_2O_2	Hydrogen peroxide
MEB	Malt extra agar
NA	Nutrient agar
NH_4OAC	Ammonium acetate
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PDB	Potato dextrose broth
RCBD	Randomized Complete Block Design
SAS	Statistical Analysis System
SE	Standard error
TC	Total carbon

CHAPTER 1

GENERAL INTRODUCTION

One of the major and devastating diseases in oil palm is basal stem rot (BSR) caused by *Ganoderma boninense* (Chong et al., 2011). *Ganoderma boninense* has been identified as the main causative species in Malaysia and Indonesia (Hushiarian et al., 2013; Mercière et al., 2015; Lee and Chang, 2016). Basal stem rot is a disease that appears as root rot with lower stem rot. The disease commonly spreads by direct root contact and basidiospore, via yet unidentified means (Rees et al., 2009; 2012). Likely, the primary route of infection occurs after the oil palm roots come into contact with inoculant from colonized debris left previously in the soil (Idris et al., 2002; Flood et al., 2005) or roots of adjacent infected palms (De Oliveira et al., 2005). *Ganoderma* species are characterized as white rot fungi with the ability to break down plant cell wall (Paterson, 2007; Naidu et al., 2016a). These fungi interrupt the nutrient and water transportation across the cell wall of oil palm, causing the leaf to wilt, yellowness, canopy mottling, unopened leaves, and lower stem appearance of basidiocarps (Turner, 1981; Chung, 2011). This disease is of a major concern in Malaysian oil palm industry. An average of 50 % yield losses of 13 years old oil palms in Malaysian coastal areas was previously reported to be infected by BSR (Parthiban et al., 2016). The infected oil palms are associated with poor yield and finally collapse of their stands (Mazliham et al., 2007). The incidence of BSR disease appears to be influenced by soil pH level (Parthiban et al., 2016). Soil pH is critical for plant nutrient uptake, nutrition, and disease resistance (Sariah and Zakaria, 2000; Huber, 2007; Tajudin et al., 2016). It has been previously demonstrated that low pH may has some influence in suppressing the BSR disease incidence in oil palm (Parthiban et al., 2016).

Several approaches that include cultural (Naher et al., 2013; Assis et al., 2016a), biological (Bivi et al., 2016; Goh et al., 2016; Ramli et al., 2016), chemical (Nadhrah et al., 2015), and mechanical (Abdul Razak et al., 2004) methods have been previously employed to control the BSR incidences in oil palms. However, no current control approaches have been proven effective and satisfactory in inhibiting the *Ganoderma* incidences in oil palm seedlings (Susanto et al., 2005; Assis et al., 2016b). As a result of increasing incidences and economic implication of this disease in Malaysia, there is a need for an effective and feasible approach to controlling the disease incidences and development in oil palm seedlings. It is factual to hypothesize that increasing the soil pH level can prevent the oil palm seedlings from BSR pathogens attack.

The main objectives were:

1. To evaluate the diversity of microbial communities in basal stem rot (BSR) infected oil palm and forest soils.
2. To determine the role of indigenous microbial community in suppressing the development of *Ganoderma boninense* in oil palm seedlings.

3. To determine the effect of soil pH on growth of oil palm seedlings and BSR disease development in *Ganoderma* inoculated seedlings.
4. To evaluate the influence of pH and calcium ion in soil amended with liming material on *Ganoderma* development in the roots of oil palm seedlings

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LIST OF PUBLICATIONS

Khunaw, A.R., Radziah, O., Nusaibah .S.A., Mohamed Hanafi. M., Rabara.F.S. (2017). Role of Microbial Community in Suppressing Development of Ganoderma in Oil Palm Seedlings. International Journal of Plant & Soil Science.Applied.http://www.sciencedomain.org/review-19(1):1-14,2017; Article no. IJPSS.36192. ISSN: 2320-7035.

Khunaw, A.R., Radziah, O., Nusaibah .S.A., Mohamed Hanafi. M. (under review). Effect of pH on growth of oil palm seedlings and basal stem rot disease incidence in Ganoderma inoculated plants. The Indian Journal of Agricultural Sciences. (Manuscript ID: 71037).

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