UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF BIOORGANIC FERTILIZER CONTAINING Pseudomonas GanoEB3 IN PROMOTING OIL PALM GROWTH AND INHIBITING Ganoderma boninense

NURUL ISMA BINTI ABDUL WAHAB

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By

NURUL ISMA BINTI ABDUL WAHAB

Thesis Submitted to the School of Graduate Studies Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

June 2014

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DEDICATION

Dedicated to the Glory of God and to my dearest mom and dad, Rokiah Ismail and Abdul Wahab Mamat. Special thanks to my siblings for their unconditional love and support.

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

DEVELOPMENT OF BIOORGANIC FERTILIZER CONTAINING *Pseudomonas* GanoEB3 IN PROMOTING OIL PALM GROWTH AND INHIBITING *Ganoderma boninense*

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June 2014

Chairman : Rosimah binti Nulit, PhD
Faculty : Science

Oil palm is one of the important crops in Malaysia. Basal Stem Rot (BSR) disease is the biggest threat for oil palm production in Malaysia caused by *Ganoderma boninense* has been causing a huge damage for the oil palm industry in Malaysia. The objective of this study was to develop the bioorganic fertilizer containing *Pseudomonas* GanoEB3 as oil palm growth enhancer and for inhibiting *Ganoderma boninense*. Endophytic bacteria *Pseudomonas* GanoEB3 was isolated from healthy oil palm roots and cultured on nutrient agar media. A suspension containing 10^8 CFU/mL of the bacteria cells being mixed with vermiculite powder and stored at room temperature. The vermiculite powder (V) containing *Pseudomonas* GanoEB3 has been formulated with Bioorganic EFB and Bioorganic BF with following ratios; 0 g (EFB/BF): 60 g (V), 10 g (EFB/BF): 50 g (V), 20 g (EFB/BF): 40 g (V), 30 g (EFB/BF): 30 g (V), 40 g (EFB/BF): 20 g (V), 50 g (EFB/BF): 10 g (V) and 60 g (EFB/BF): 0 g (V). This study found that 30 g Bioorganic EFB mixed with 30 g vermiculite containing *Pseudomonas* GanoEB3 showed the best viability test with the percentage inhibition of radial growth (PIRG) value of 57.75% against *G. boninense* after one day product storage. Whereas, the PIRG value was 14.38% after twelve months product storage. Meanwhile, 10 g Bioorganic BF mixed with 50 g vermiculite containing *Pseudomonas* GanoEB3 showed 53.75% and 18% PIRG value after one day and twelve months product storage, respectively. Plant growth result showed that seedlings treated with Bioorganic BF gave the highest result in plant height (91.61 ± 0.80 cm), girth (46.86 ± 0.49 mm), frond length (64.61 ± 0.58 cm), number of leaflets (17.67 ± 0.30), number of frond (14.33 ± 0.18), chlorophyll content (54.95 ± 0.47 µg/L), leaf area index (2175.30 ± 88.08 cm^2) and biomass (53.97 ± 0.76 g) respectively, followed by seedlings treated with Bioorganic EFB and control treatment. Pathological analysis showed that Bioorganic EFB containing *Pseudomonas* GanoEB3 (T3) and Bioorganic BF containing *Pseudomonas* GanoEB3 (T4) also have a good potential in inhibiting the growth of *G. boninense*. The reduced percentage of disease

incidence (DI) for T3 (40%) and T4 (50%), area under the disease progress curve (AUDPC) for T3 (42 units^2) and T4 (76 units^2), disease severity of foliar index (DSFI) for T3 (37.5%) and T4 (45%), disease severity of bole index (DSBI) for T3 (40%) and T4 (47.5%), disease severity of root index (DSRI) for T3 (55%) and T4 (52.5%) and dead seedlings for T3 (30%) and T4 (40%) compared with control treatment (T2). It shows that both treatments have a good potential in inhibiting BSR disease in oil palm seedlings. This study revealed that Bioorganic EFB and Bioorganic BF containing *Pseudomonas* GanoEB3 are suitable as an effective biological control agent for promoting the growth of oil palm seedlings and suppressing *G. boninense*.

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

PENGHASILAN BAJA BIOORGANIK YANG MENGANDUNGI *Pseudomonas* GanoEB3 UNTUK MENGGALAKKAN PERTUMBUHAN POKOK KELAPA SAWIT DAN MERENCATKAN *Ganoderma boninense*

Oleh

NURUL ISMA BINTI ABDUL WAHAB

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Pengerusi : Rosimah binti Nulit, PhD
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Kelapa sawit merupakan salah satu tanaman penting di Malaysia. Penyakit reput pangkal batang (RPB) adalah ancaman terbesar terhadap pengeluaran kelapa sawit di Malaysia yang disebabkan oleh kulat *Ganoderma boninense* dan telah menyebabkan kerosakan yang besar kepada ladang kelapa sawit di Malaysia. Objektif kajian ini adalah untuk menghasilkan baja bioorganik yang mengandungi *Pseudomonas* GanoEB3 sebagai penggalak pertumbuhan kelapa sawit dan sebagai agen kawalan biologi untuk *Ganoderma boninense*. Endofitik bakteria *Pseudomonas* GanoEB3 telah diasingkan daripada akar kelapa sawit yang sihat dan dihidupkan di dalam medium agar nutrien. Bakteria yang mengandungi 10^8 CFU/mL telah dimasukkan ke dalam serbuk vermikulit dan disimpan pada suhu bilik. Serbuk vermikulit (V) mengandungi *Pseudomonas* GanoEB3 telah dirumuskan dengan Bioorganik EFB dan Bioorganik BF dengan nisbah berikut; 0 g (EFB/BF): 60 g (V), 10 g (EFB/BF): 50 g (V), 20 g (EFB/BF): 40 g (V), 30 g (EFB/BF): 30 g (V), 40 g (EFB/BF): 20 g (V), 50 g (EFB/BF): 10 g (V) dan 60 g (EFB/BF): 0 g (V). Kajian ini mendapati bahawa 30 g Bioorganik EFB dicampur dengan 30 vermikulit yang mengandungi *Pseudomonas* GanoEB3 menunjukkan ujian daya hidup yang terbaik dengan perencatan peratusan pertumbuhan jejari (PIRG) ialah 57.75% terhadap *G. boninense* selepas satu hari penyimpanan produk. Nilai PIRG ialah 14.38% selepas dua belas bulan penyimpanan produk. Sementara itu, 10 g Bioorganik BF dicampur dengan 50 g vermikulit masing-masing menunjukkan nilai PIRG 53.75% dan 18% menunjukkan bahawa pokok yang dirawat dengan Bioorganik BF memberikan hasil yang tertinggi dalam keputusan ketinggian pokok (91.61 ± 0.80 cm), ukur lilit batang (46.86 ± 0.49 mm), panjang pelepah (64.61 ± 0.58 cm), bilangan daun (17.67 ± 0.30), bilangan pelepah (14.33 ± 0.18), kandungan klorofil (54.95 ± 0.47 µg/L), indeks luas daun (2175.30 ± 88.08 cm^2) dan biojisim (53.97 ± 0.76 g) diikuti dengan pokok yang dirawat dengan Bioorganik EFB dan pokok kawalan. Analisis patologi

mendapati bahawa Bioorganik EFB yang mengandungi *Pseudomonas* GanoEB3 (T3) dan Bioorganik BF yang mengandungi *Pseudomonas* GanoEB3 (T4) juga berkesan menghalang pertumbuhan *G. boninense*. Pengurangan peratusan terhadap serangan penyakit (DI) untuk T3 (40%) dan T4 (50%), perkembangan penyakit bawah lengkung (AUDPC) untuk T3 (42 units^2) dan T4 (76 units^2), serangan penyakit pada dedaun (DSFI) untuk T3 (37.5%) dan T4 (45%), serangan penyakit pada batang (DSBI) untuk T3 (40%) dan T4 (47.5%), serangan penyakit pada akar (DSRI) untuk T3 (55%) dan T4 (52.5%) dan peratusan anak pokok mati untuk T3 (30%) dan T4 (40%) berbanding dengan pokok rawatan kawalan (T2). Ini menunjukkan bahawa kedua-dua baja bioorganik EFB and BF mempunyai potensi yang bagus untuk menghalang penyakit RPB terhadap pokok sawit. Kajian ini membuktikan bahawa bioorganik yang mengandungi *Pseudomonas* GanoEB3 sesuai dijadikan agen kawalan biologi yang berkesan untuk menggalakkan pertumbuhan anak benih kelapa sawit dan merencat *Ganoderma boninense*.

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I certify that a Thesis Examination Committee has met on 24 June 2014 to conduct the final examination of Nurul Isma binti Abdul Wahab on her thesis entitled "Development of Bioorganic Fertilizer Containing *Pseudomonas* GanoEB3 in Promoting Oil Palm Growth and Inhibiting *Ganoderma boninense*" 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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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
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Signature : _____	Signature : _____
Name of Member of Supervisory Committee : **Mohd. Puad Abdullah**	Name of Member of Supervisory Committee : **Idris Abu Seman**

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LIST OF ABBREVIATIONS

BSR	Basal Stem Rot
PDA	Potato Dextrose Agar
MA	Malt Agar
MEA	Malt Extract Agar
CMA	Corn Meal Agar
CDA	Czapekôs Dox Agar
AMF	Arbuscular Mycorrhiza Fungi
MPOB	Malaysian Palm Oil Board
PGPR	Plant Growth Promoting Rhizobacteria
ISR	Induced Systemic Resistance
PO	Peroxidase
PPO	Polyphenol oxidase
EFB	Empty Fruit Bunch
FFB	Fresh Fruit Bunch
C	Carbon
H	Hydrogen
N	Nitrogen
O	Oxygen
K	Potassium
P	Phosphorus
Mg	Magnesium
Ca	Calsium
S	Sulphur
Cl	Chlorine

B	Boron
Cu	Copper
Zn	Zinc
Mn	Manganese
Co	Cobalt
Si	Silicon
Na	Sodium
GSM	Ganoderma selective medium
NA	Nutrient agar
NB	Nutrient broth
CMC	Carboxy methyl cellulose
PIRG	Percentage inhibition of radial growth
CFU/g	Colony forming unit per gram
mL	Mililiter
NaCl	Sodium chloride
ºC	Degree celcius
mm	Milimeter
g	Gram
cm	Centimeter
%	Percentage
CRD	Completely Randomized Design
LAI	Leaf area index
RGR	Relative growth rate
DI	Disease Incidence
AUDPC	Area Under the Disease Progress Curve
DS	Dead Seedlings

DSFI	Disease Severity of Foliar Index
DSBI	Disease Severity of Bole Index
DSRI	Disease Severity of Root Index
RWB	Rubber Wood Blocks
ANOVA	One way Analysis of Variance

CHAPTER 1

INTRODUCTION

Oil palm (*Elaeis guineensis*) is one of the important crops in Malaysia and it proved strengthening the economics of Malaysia. Current production of crude palm oil (CPO) of Malaysia has reached 19 million metric tons (Mmtons) which contributed [illegible] income (GNI). The oil palm estates produce an estimated 80 Mmtons of dry weight biomass which includes fronds, trunks, empty fruit bunches (EFB) and other biomass fractions.

Since 2007, Indonesia overtook Malaysia to become the world's largest oil palm producer in the world and these two countries have accounted for nearly 90% of the annual growing demand for palm oil in the global market. As one of the biggest exporters and producers of oil palm products, Malaysia has to fulfill the global needs especially for fats and oils sustainability. Palm oil is now a major source of sustainable raw material for the food, oleo chemical and also bio fuels industries.

Unfortunately, the growth of oil palm is influenced by pests and diseases. The crop is exposed to pest and disease problems start from seed germination up to field planting. Brown germ, upper stem rot and bagworm are the examples of pest and disease problems, and the most serious disease is basal stem rot (BSR). The BSR disease has caused a huge damage especially to the oil palm plantation in Malaysia for the past 50 years.

Basal stem rot (BSR) disease is considered the biggest threat to sustainable palm oil production especially in South East Asia. Previous study reported that the most severe losses from BSR occurred in Malaysia and Indonesia with lower incidences was being recorded in Thailand, Africa and Papua New Guinea. Meanwhile, Lim *et al*. (1992) recorded an average of 50% yield losses from 80% of 13-year-old plantings in coastal area of Malaysia. Estates in Indonesia also encountered similar problems which by the time of replanting, 40% of oil palms were lost in the fields with standing palms showing BSR disease symptoms. Once the disease affects more than 10% of the stand, losses begin to have a big financial effect and the yield of fresh fruit bunch (FFB) on average will decline as well.

Controlling the pathogen is an important factor since the pathogen caused severe losses of oil palm production. BSR field control by using chemicals, such as pesticide and fungicide have not been very successful even with *in vitro* efficacy of fungicides against *G*. *boninense* have not been reported. This is probably attributed to the fact that the palms might already have the disease by the time the treatment was applied.

To overcome this problem, a new strategy of using biological control agents may be developed in order to find an effective and environmental friendly treatment against BSR disease. Biological control is ecologically safe and it will not pollute the environment. The approach may not necessarily be a cure for the disease but it could arrest the spread of the disease.

The manipulation of microbes such as fungi, bacteria, mychorrhiza and actinomycetes as biocontrol agents is being studied to control BSR disease. According to Petrini (1990), beneficial endophytic microorganisms consist of fungi and bacteria that colonize internal plant tissues without causing visible damage to their hosts. Previous studies showed that some of endophytic microorganisms had isolated from a wide range of plants including cultivated crops like woody plants and grass such as *Serratia* spp., *Burkohlderia* spp., *Pseudomonas* spp., *Bacillus* spp., and *Fusarium* spp. could induce systemic resistance in plants and possessed antimicrobial properties like lysis and antibiotic activity.

The suppression of plant diseases due to the action of endophytic microorganisms has been demonstrated in several pathosystems. Several mechanisms may control this suppression, either indirectly by induction of plant defense response or directly inside the plant by antibiosis and competition for nutrients. Nasyaruddin and Idris (2011) reported that *Pseudomonas* GanoEB3 pure culture has the capability in inhibiting the growth of *G*. *boninense in vitro*, and effective in controlling *G. boninense* infection in oil palm seedlings.

Thus, the main objectives of this study were:

1. to develop the powder formulation of bioorganic fertilizer containing *Pseudomonas* GanoEB3 for suppressing *Ganoderma boninense in vitro*.

2. to evaluate the effectiveness of bioorganic fertilizer containing *Pseudomonas* GanoEB3 in promoting oil palm growth.

3. to determine the potential of bioorganic fertilizer containing *Pseudomonas* GanoEB3 in inhibiting basal stem rot disease in oil palm seedlings.

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