UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF BIOORGANIC CONTAINING ENDOPHYTIC FUNGUS Phlebia GanoEF3 FOR OIL PALM GROWTH AND CONTROL OF BASAL STEM ROT DISEASE

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By

ERMA NADIA BINTI ANUAR

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

April 2014

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DEDICATION

Special dedication to:

My Husband and My Twin Babies

My Mother

My Family

My Three Beautiful Friends, Inside and Out

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

DEVELOPMENT OF BIOORGANIC CONTAINING ENDOPHYTIC FUNGUS *Phlebia* GanoEF3 FOR OIL PALM GROWTH AND CONTROL OF BASAL STEM ROT DISEASE

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ERMA NADIA BINTI ANUAR

April 2014

Chairman : Rosimah Nulit, PhD

Faculty : Science

The oil palm, *Elaeis guineensis*, is the highest oil-yielding among the oil-producing crops. Like any other crops, oil palm is prone to infection by a number of diseases, and one of the most serious diseases is basal stem rot (BSR). The disease is caused by species of *Ganoderma.* Recent control measure to control *Ganoderma* associated with BSR is now focused on the use of biological control agents such as endophytic fungi. Therefore, the objectives of this study were; 1) to develop powder formulations of endophytic fungus *Phlebia* GanoEF3 by incorporating organic and inorganic fertilizer as a carrier, 2) to study the potential of bioorganic containing endophytic fungus *Phlebia* GanoEF3 as a biological fertilizer for enhancing the growth of oil palm seedlings and 3) to study the capability of bioorganic containing endophytic fungus *Phlebia* GanoEF3 to suppress BSR disease in oil palm seedling. An initiative to isolate endophytic microorganisms from trunk and root tissues of oil palms by the Malaysian Palm Oil Board (MPOB) has found promising fungi, including *Hendersonia* GanoEF, *Amphinema* GanoEF2 and *Phlebia* GanoEF3. Two types of organic fertilizers; Empty Fruit Bunches (EFB) powder and Real Strong Bioorganic Fertilizer (RSBF) were incorporated into the formulation to develop biofertilizers containing endophytic *Phlebia* GanoEF3. Five ratios of fertilizer (EFB/RSBF) to vermiculite containing endophytic *Phlebia* GanoEF3 (10:50, 20:40, 30:30, 40:20 and 50:10) for each formulation were prepared and *in vitro* study and the shelf life of viable cell of *Phlebia* GanoEF3 in the powder formulations during storage were determined. It was found that the ratio of 30 g of EFB powder to 30 g of vermiculite containing endophytic *Phlebia* GanoEF3 (30:30 g) and 10 g of RSBF to 50 g of vermiculite containing endophytic *Phlebia* GanoEF3 (10:50 g) recorded the highest number of fungal colony population among other ratios, where product with EFB was with 51% percentage inhibition of radial growth (PIRG) against *G. boninense*, a month

after storage of the product and 15% at the end. Meanwhile, 10 g of RSBF to 50 g of vermiculite containing endophytic *Phlebia* GanoEF3 (10:50 g) recorded 56% PIRG value after a month of product storage, and 17% after eight months of storage. Plant growth result showed that Treatment 3 seedlings *(*10 g of RSBF to 50 g of vermiculite containing endophytic *Phlebia* GanoEF3) gave the highest result in number of frond (18.23), frond length (73.56 cm), plant height (107.5 cm), number of leaflets (38.33), stem diameter (60.06 mm) and leaf area index (4355.92 cm^2), followed by Treatment 2 seedlings (30 g of EFB powder to 30 g of *Phlebia* GanoEF3) which gave the highest result for total chlorophyll content (70.43 μg/L*)* and total biomass (105.95 cm), and control treatment (Treatment 1). Pathological parameter analysis showed that ratio of 30 g of EFB powder to 30 g of vermiculite containing endophytic *Phlebia* GanoEF3 (Treatment 3) *and* 10 g of RSBF to 50 g of vermiculite containing endophytic Phlebia GanoEF3 (Treatment 4) also have good potential in inhibiting the growth of G. boninense in order to suppress basal stem rot disease (BSR) on oil palm seedlings. It was found that the percentage of disease incidence (DI) for T3 and T4 seedlings were only at 50% and 40%, respectively, compared to the control seedlings (T2) which suffered a total of 100% diseased seedlings. The area under the disease progress curve (AUDPC) for T3 (130 $units^2$) and T4 (140 $units^2$), disease reduction (DR) for T3 (63.89%) and T4 (61.11%), disease severity of foliar index (DSFI) for T3 (35%) and T4 (20%), disease severity of bole index (DSBI) for T3 (40%) and T4 (32.5%), disease severity of root index (DSRI) for T3 (50%) and T4 (41%) and dead seedlings for T3 (30%) and T4 (20%), all showed reduction in values compared to control seedlings (T2). Based on all the results, it was found that Bioorganic containing *Phlebia* GanoEF3; Bioorganic EFB *Phlebia* GanoEF3 and Bioorganic RSBF *Phlebia* GanoEF3 were effective in promoting oil palm seedlings growth and suitable as an effective biological control agent for controlling BSR disease.

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

PEMBANGUNAN BIOORGANIK MENGANDUNGI KULAT ENDOFITIK *Phlebia* GanoEF3 UNTUK PERTUMBUHAN DAN KAWALAN PENYAKIT REPUT PANGKAL BATANG

Oleh

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Kelapa sawit, *Elaeis guineensis*, merupakan tanaman yang menghasilkan minyak paling banyak berbanding tanaman-tanaman penghasil minyak yang lain. Seperti tanaman yang lain, kelapa sawit juga cenderung terhadap serangan beberapa jenis penyakit; satu daripada penyakit-penyakit yang penting itu ialah reput pangkal batang (RPB). Penyakit RPB, disebabkan oleh spesis *Ganoderma*, merupakan penyakit kelapa sawit yang paling serius di Malaysia. Kaedah kawalan yang terbaru untuk mengatasi masalah *Ganoderma* kini adalah dengan memfokuskan pada penggunaan kaedah kawalan biologikal, iaitu mikroorganisma endofitik, terutamanya kulat endofitik. Objektif kajian ini ialah; 1) untuk membangunkan formulasi produk serbuk yang mengandungi kulat endofitik *Phlebia* GanoEF3 dengan mencampurkan baja organik dan tidak organik sebagai pembawa, 2) untuk mengkaji potensi bioorganik mengandungi kulat endofitik *Phlebia* GanoEF3 sebagai baja biologikal yang membantu pertumbuhan anak sawit, dan 3) untuk mengkaji kemampuan bioorganik mengandungi kulat endofitik *Phlebia* GanoEF3 merencatkan penyakit RPB pada anak benih. Pemencilan tisu akar dan batang pokok kelapa sawit yang dilakukan oleh Lembaga Minyak Sawit Malaysia (MPOB) telah menemui kulat yang berpotensi, *Hendersonia* GanoEF, *Amphinema* GanoEF2 dan *Phlebia* GanoEF3. Dua jenis baja organik; serbuk Tandan Buah Kosong (EFB) dan Baja Bioorganik Real Strong (RSBF) telah dicampurkan ke dalam formula untuk menghasilkan baja biologikal yang mengandungi kulat endofitik *Phlebia* GanoEF3. Lima nisbah baja (EFB/RSBF) kepada vermikulat mengandungi *Phlebia* GanoEF3 (10:50, 20:40, 30:30, 40:20 and 50:10) untuk setiap formula telah disediakan dan kajian *in vitro* and jangka hayat sel *Phlebia* GanoEF3 yang berdaya hidup di dalam serbuk formulasi ketika di dalam storan telah ditentukan. Nisbah 10 g bioorganik RSBF dicampur dengan 50 g vermikulat mengandungi *Phlebia* GanoEF3 dan 30 g bioorganik EFB dicampur dengan 30 g vermikulat mengandungi *Phlebia* GanoEF3 mencatatkan bilangan populasi koloni kulat yang paling tinggi, dimana

produk mengandungi EFB dengan 51% perencatan peratusan pertumbuhan jejari (PIRG) terhadap *G. boninense* selepas sebulan penyimpanan produk dan 15% diakhir eksprimen. Sementara itu, 10 g RSBF dicampur dengan 50 g vermikulat mengandungi *Phlebia* GanoEF3 mencatatkan nilai PIRG sebanyak 56% selepas sebulan penyimpanan produk, dan 17% selepas lapan bulan. Kajian pertumbuhan menunjukkan bahawa Rawatan 3 (30 g bioorganik EFB dicampur dengan 30 g vermikulat mengandungi *Phlebia* GanoEF3) memberikan hasil yang tertinggi dalam bilangan pelepah *(*18.23), panjang pelepah (73.56 sm*)*, tinggi pokok (107.5 sm), bilangan daun (38.33*)*, ukur lilit batang *(*60.06 mm*)*, dan indeks luas daun (4355.92 sm^2), diikuti dengan Rawatan 2 (10 g RSBF dicampur dengan 50 g vermikulat mengandungi kulat endofitik *Phlebia* GanoEF3) yang memberikan hasil yang tertinggi dalam kandungan klorofil (70.43 μg/L) dan biomass total (105.95 g), dan kawalan (Rawatan 1). Analisis parameter patologi menunjukkan nisbah 30 g bioorganik EFB dicampur dengan 30 g vermikulat mengandungi *Phlebia* GanoEF3 (Rawatan 3) dan 10 g RSBF dicampur dengan 50 g vermikulat mengandungi *Phlebia* GanoEF3 (Rawatan 4) juga berkesan merencat pertumbuhan *G. boninense* seterusnya menyekat penyakit RPB. Didapati peratusan serangan penyakit (DI) untuk T3 dan T4 adalah pada 50% dan 40%, setiap satu. Perkembangan penyakit (AUDPC) untuk T3 (130 $units^2$) dan T4 (140 $units^2$), pengurangan penyakit (DR) untuk T3 (63.89%) dan T4 (61.11%), serangan penyakit pada dedaun (DSFI) untuk T3 (35%) dan T4 (20%), serangan penyakit pada batang (DSBI) untuk T3 (40%) dan T4 (32.5%), serangan penyakit pada akar (DSRI) untuk T3 (50%) dan T4 (41%) dan peratusan anak pokok mati untuk T3 (30%) dan T4 (20%), kesemuanya menunjukkan pengurangan nilai berbanding dengan kawalan (Rawatan 1). Berdasarkan keputusan diperolehi, didapati bahawa Bioorganik mengandungi kulat *Phlebia* GanoEF3; Bioorganik EFB *Phlebia* GanoEF3 dan Bioorganik RSBF *Phlebia* GanoEF3 berkesan dalam menggalakkan pertumbuhan anak benih kelapa sawit dan sesuai dijadikan agen kawalan biologi untuk mengawal penyakit RPB.

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I certify that a Thesis Examination Committee has met on (28th of April 2014) to conduct the final examination of Erma Nadia binti Anuar on her thesis entitled Development of Bioorganic containing Endophytic Fungus *Phlebia* GanoEF3 for Oil Palm Growth and Control of Basal Stem Rot Disease 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 degree.

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LIST OF ABBREVIATIONS

AMF	Arbuscular Mychorrhiza Fungi
ANOVA	Analysis of Variance
AUDPC	Area Under Disease Progress Curve
BF	Bioorganic fertilizer
BSR	Basal stem rot
CFU	Colony forming unit
cm	Centimeter
CRD	Completely randomized design
°C	Degree Celsius
DI	Disease incidence
DR	Disease reduction
DS	Disease severity
DSBI	Disease severity of bole index
DSFI	Disease severity of foliar index
DSRI	Disease severity of root index
EFB	Empty Fruit Bunch
g	Gram
GSM	Ganoderma selective media
m	Meter
mg	Miligram
ml	Mililiter
mm	Milimeter

%	Percent
PIRG	Percentage inhibition of radial growth
PDA	Potato Dextrose Agar
RBA	Rose Bengal Agar
RSBF	Real Strong Bioorganic Fertilizer

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CHAPTER 1

INTRODUCTION

The oil palm (*Elaeis guineensis)* is a major plantation crop in the tropics because of its contribution in the production of palm oil and palm kernel oil. Malaysia has helped shape the status of palm oil in the global market through significant contributions and commitment to the industry (Goh *et al*., 2010). The growing global demand for edible oils and fats has further fuelled the Malaysian palm oil industry, which has grown over the last few decades, and is undeniably an important component of the Malaysian economy. Malaysia currently accounts for 39 % of world palm oil production and 44% of world exports, including 12% and 27% of the world's total production and exports of oils and fats (MPOC, 2013).

Oil palm is prone to diseases and one of them being the basal stem rot (BSR) disease. Malaysia and Indonesia are the two countries that were suffering the most severe losses from BSR caused by several species of *Ganoderma* (Idris *et al*., 2004). Idris *et al*. (2000a; 2000b) had identified four species of *Ganoderma* associated with oil palm in Malaysia, which are *G. boninense, G. zonatum, G. tornatum* and *G. miniatocinctum.* Study has shown that the presence of *G. boninense* in the two areas that were considered as moderate (15% - 30%) and high (>30%) of BSR incidence suggests it to be the primary pathogen causing outbreaks of BSR in oil palm plantation, thus considered to be the most aggressive, while *G. miniatocinctum* is considered as the least aggressive of all (Idris, 1999).

As agricultural production intensified over the past few decades, the industry became more and more dependent on agrochemicals as a relatively reliable method of crop protection. However, increasing use of chemical inputs causes several negative effects, for example, development of pathogen resistance to the applied agents and their non target environmental impacts. Furthermore, the growing cost of pesticides, particularly in less-affluent regions of the world, and consumer demand for pesticide-free food has led to a search for substitutes for these products. There are also a number of fastidious diseases for which chemical solutions are few, ineffective, or nonexistent.

For the past 10 years, many studies have been done with emphasis on fundamental research to form a rational basis for devising control methods. Some control over BSR are in the physical form, such as agronomic practices, surgery, soil mounding and sanitation in existing planting and at replanting. In the light of these problems, any effort to reduce its uses is necessary. Hence, the use of biological control such as biofertilizers and other microbial products were applied.

In order to reduce the usage of chemical fertilizer, the application of microbials such as fungi, bacteria, actinomycetes and mycorrhiza as biocontrol agents is now being studied to control the BSR disease. A relatively new field of study in biological disease control is endophytic microorganisms, which includes endophytic bacteria, endophytic actinomycetes and endophytic fungi. From an ecological point of view, endophytic microorganisms provide greater biological balance and stability. The use of these endophytic microorganisms therefore should be preferable to other biological control agents as they are internal colonizers, and capable to compete within the vascular systems, inhibiting *Ganoderma* from both nutrients and space for its proliferation (Zaiton *et al*., 2006).

Biological control is being considered as an alternative method to reduce the usage of chemicals in agriculture. Biological control is the reduction of inoculums density or disease-producing activities of a pathogen in its active or dormant state by one or more microorganisms. Despite considerable effort in the area of biological control, few practical applications have become established in agriculture for the control of plant diseases. A relatively new field of study in biological disease control is endophytic microorganisms, specifically, endophytic fungus. Biological control is achieved by competition, hyperparasitism, induced resistance and hypovirulence. Common biocontrol agents include *Trichoderma*, *Aspergillus* and *Penicillium*.

The usage of mechanical and chemical treatments to control and suppress BSR disease in oil palm has yet to be proven satisfactorily. The use of Bioorganic Fertilizer (BF) at oil palm plantations is one of the methods to reduce the cost and is hoped to reduce the usage of chemical fertilizer application. In this study, two types of BF were used; bioorganic Empty Fruit Bunch (EFB) and Real Strong Bioorganic Fertilizer (RSBF). EFB is one of the by-products of oil palm produced in large quantities throughout the year and during the replanting process. Composting process of EFB converts the essentially organic in nature waste, into humus that is suitable for crop production. The bunch consists of moisture and solids (Thambirajah *et al*., 1995). RSBF, the bioorganic fertilizer contains plant-based organic matter and formulated to allow easy and balanced plant uptake of nutrient. Essentially it is made up of 65% organic matter, 30% of chemicals and 5% Zeolite (www.allcosmos.com, 2010).

Thus, biological control is used as an alternative to control the BSR disease, and this research was focus on the usage of a biological control agent, the endophytic fungus *Phlebia* GanoEF3, for controlling *Ganoderma* disease infection in the field, and act as a growth promoter of oil palm seedlings.

Therefore, the objectives of this study are:

1. To develop powder formulations of endophytic *Phlebia* GanoEF3 by incorporating organic and inorganic fertilizer as a carrier.

2. To study the potential of Bioorganic containing endophytic *Phlebia* GanoEF3 as a biological fertilizer for oil palm and enhance the growth of oil palm seedlings.

3. To study the capability of Bioorganic containing endophytic *Phlebia* GanoEF3 to suppress BSR disease on oil palm seedling.

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