



**UNIVERSITI PUTRA MALAYSIA**

***DEVELOPMENT OF BIOORGANIC FERTILIZER CONTAINING *Amphinema GanoEF2* AND EVALUATION OF ITS EFFECTIVENESS IN PROMOTING OIL PALM GROWTH AND SUPPRESSING *Ganoderma boninense****

**SITI HAIRUNNISA BINTI MOHD KAMEL**

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UNIVERSITI PUTRA MALAYSIA  
BERILMU BERBAKTI

**DEVELOPMENT OF BIOORGANIC FERTILIZER CONTAINING *Amphinema*  
*Gano*EF2 AND EVALUATION OF ITS EFFECTIVENESS IN PROMOTING OIL  
PALM GROWTH AND SUPPRESSING *Ganoderma boninense***

By

**SITI HAIRUNNISA BINTI MOHD KAMEL**

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

**June 2014**

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## DEDICATION

Special dedication to:

**My Dear Husband & My Little Princess,**

*Mohd Siraj Munir Yaacob  
Nur Airis Rayyani Mohd Siraj Munir*

**My Mother,**

*Puan Naimah Ayob*

**To Dearly Missed My Late Father,**

*Mohd Kamel Ibrahim*

**My Family & Family in Law**

*For motivating  
For understanding  
For everything*

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 FERTILIZER CONTAINING *Amphinema*  
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**June 2014**

**Chairman : Rosimah Nulit, PhD**  
**Faculty : Science**

The oil palm, *Elaeis guineensis* is the most important crop in Malaysia which producing an average palm oil yields approximately 4 tonnes per hectare per year. Basal stem rot (BSR) disease which is caused by *Ganoderma boninense* is the most serious disease that infected oil palm. Recently, the control of BSR has been focused on the use of biocontrol agent such endophytic fungi. Therefore, the objectives of this study were; 1) to formulate bioorganic fertilizer containing *Amphinema* GanoEF2, 2) to evaluate the effectiveness of bioorganic fertilizer containing *Amphinema* GanoEF2 in enhancing growth of oil palm seedlings, and 3) to determine the efficacy of bioorganic fertilizer containing *Amphinema* GanoEF2 for controlling *Ganoderma* disease in oil palm seedlings. *Amphinema* GanoEF2 was isolated from symptomless root of oil palm by Malaysian Palm Oil Board (MPOB) and was found to have an antagonistic activity against *G. boninense*. Two types of bioorganic fertilizers; empty fruit bunch (EFB) and Real Strong bioorganic fertilizer (RSBF) were incorporated into powder formulation containing *Amphinema* GanoEF2. The products were prepared in five different ratios of vermiculite containing *Amphinema* GanoEF2 to bioorganic fertilizers (EFB/RSBF) (10:50 g, 20:40 g, 30:30 g, 40:20 g and 50:10 g). The shelf life of *Amphinema* GanoEF2 and efficacy towards *G. boninense* were determined by *in vitro* study during eight months of storage. Ratio of 30 g of vermiculite *Amphinema* GanoEF2 to 30 g of EFB and 50 g of vermiculite *Amphinema* GanoEF2 to 10 g of RSBF recorded the highest fungal colonies of *Amphinema* GanoEF2 among other ratios with the percentage inhibition of radial growth (PIRG) against *G. boninense* recorded at 50% and 52%, respectively. The chosen ratios were used to prepare the product and were evaluated in nursery trials for oil palm growth and controlling BSR disease.

The application of bioorganic EFB *Amphinema* GanoEF2 (T2) and bioorganic RSBF *Amphinema* GanoEF2 (T3) on oil palm seedlings has significantly increased the plant height, frond length, number of frond, number of leaflets, stem diameter, leaf area index and chlorophyll content as compared with untreated seedlings (T1). The total biomass of T2 (109.15 g) and T3 (112.03 g) seedlings have a significant difference compared with T1 seedlings after eight months of treatments with the value of relative growth rate (RGR) for T1, T2 and T3 seedlings at 1.44 g/month, 1.49 g/month and 1.52 g/month, respectively. The efficacy of bioorganic EFB *Amphinema* GanoEF2 (T3) and bioorganic RSBF *Amphinema* GanoEF2 (T4) to control *Ganoderma* disease were evaluated and found that T3 and T4 seedlings recorded a significant reduction of BSR disease. The percentage of disease incidence (DI) for T3 and T4 seedlings were only 50% and 40%, respectively, compared with T1 seedlings which recorded 80% of DI. The disease severity index of foliar, root and bole (DSIF, DSIR, DSIB), dead seedlings (DS) and area under disease progress curve (AUDPC) also showed significant difference between treated seedlings (T3 and T4) with untreated seedlings (T2). Both bioorganic EFB *Amphinema* GanoEF2 and bioorganic RSBF *Amphinema* GanoEF2 were enhanced the growth of oil palm seedlings and were capable in reducing the BSR infection.

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

**PENGHASILAN BAJA BIOORGANIK YANG MENGANDUNGI *Amphinema*  
GanoEF2 DAN PENILAIAN KEBERKESANANNYA DALAM  
MENINGKATKAN PERTUMBUHAN KELAPA SAWIT DAN MERENCAT  
*Ganoderma boninense***

Oleh

**SITI HAIRUNNISA BINTI MOHD KAMEL**

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**Pengerusi : Rosimah Nulit, PhD**  
**Fakulti : Sains**

Kelapa sawit, *Elaeis guineensis* merupakan tanaman paling penting di Malaysia yang menghasilkan purata hasil minyak sawit lebih kurang 4 tan per hektar setiap tahun. Penyakit reput pangkal batang (RPB) yang disebabkan oleh *Ganoderma boninense* merupakan penyakit paling serius yang menjangkiti kelapa sawit. Kebelakangan ini, kawalan RPB telah diberi tumpuan pada penggunaan agen kawalan biologi seperti kulat endofitik. Oleh itu, objektif kajian ini ialah; 1) untuk membuat formulasi baja bioorganik yang mengandungi *Amphinema* GanoEF2, 2) untuk menilai keberkesanan baja bioorganik yang mengandungi *Amphinema* GanoEF2 dalam meningkatkan pertumbuhan anak sawit, dan 3) untuk menentukan keberkesanan baja bioorganik yang mengandungi *Amphinema* GanoEF2 untuk mengawal penyakit *Ganoderma* pada anak sawit. *Amphinema* GanoEF2 telah diisolasi dari akar sawit yang tidak mempunyai simptom oleh Malaysian Palm Oil Board (MPOB) dan didapati mempunyai aktiviti antagonistik terhadap *G. boninense*. Dua jenis baja bioorganik; tandan kosong buah (EFB) dan baja bioorganik Real Strong (RSBF) telah dimasukkan ke dalam formulasi serbuk yang mengandungi *Amphinema* GanoEF2. Produk tersebut telah disediakan dalam lima nisbah vermikulat yang mengandungi *Amphinema* GanoEF2 kepada baja bioorganik (EFB/RSBF) yang berbeza (10:50 g, 20:40 g, 30:30 g, 40:20 g and 50:10 g). Jangka hayat *Amphinema* GanoEF2 dan keberkesanan terhadap *G. boninense* telah ditentukan dengan ujikaji *in vitro* semasa lapan bulan dalam penyimpanan. Nisbah 30 g vermikulat *Amphinema* GanoEF2 kepada 30 g EFB dan 50 g vermikulat *Amphinema* GanoEF2 kepada 10 g RSBF mencatatkan koloni kulat *Amphinema* GanoEF2 tertinggi berbanding nisbah-nisbah yang lain dengan

peratusan perencatan pertumbuhan radial (PIRG) terhadap *G. boninense* direkodkan masing-masing pada 50% dan 52%. Nisbah yang dipilih digunakan untuk menyediakan produk dan akan dinilai pada pertumbuhan anak sawit dan mengawal penyakit RPB dalam percubaan nurseri. Penggunaan bioorganik EFB *Amphinema* GanoEF2 (T2) dan bioorganik RSBF *Amphinema* GanoEF2 (T3) pada anak sawit telah meningkatkan tinggi pokok, panjang pelepah, bilangan pelepah, bilangan daun pada pelepah, diameter batang, indeks luas daun dan kandungan klorofil secara signifikan berbanding anak sawit yang tidak dirawat (T1). Jumlah biojisim anak sawit T2 (109.15 g) dan T3 (112.03 g) mempunyai perbezaan signifikan berbanding anak sawit T1 selepas lapan bulan rawatan dengan nilai kadar pertumbuhan relatif (RGR) untuk anak sawit T1, T2 dan T3 masing-masing pada 1.44 g/bulan, 1.49 g/bulan dan 1.52 g/bulan. Keberkesanan bioorganik EFB *Amphinema* GanoEF2 (T3) dan bioorganik RSBF *Amphinema* GanoEF2 (T4) untuk mengawal penyakit *Ganoderma* telah dinilai dan didapati anak sawit T3 dan T4 mencatatkan penurunan penyakit RPB yang signifikan. Peratusan insiden penyakit (DI) untuk anak sawit T3 dan T4 masing-masing hanya 50% dan 40% berbanding anak sawit T1 yang mencatatkan 80% DI. Indeks keterukan penyakit pada foliar, akar dan batang (DSIF, DSIR, DSIB), anak sawit mati (DS) dan kawasan di bawah lengkungan perkembangan penyakit (AUDPC) juga menunjukkan perbezaan yang signifikan di antara anak sawit yang dirawat (T3 dan T4) dengan anak sawit yang tidak dirawat (T2). Kedua-dua bioorganik EFB *Amphinema* GanoEF2 and bioorganik RSBF *Amphinema* GanoEF2 telah meningkatkan pertumbuhan anak sawit dan berkeupayaan dalam menurunkan jangkitan RPB.



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I certify that a Thesis Examination Committee has met on 27 June 2014 to conduct the final examination of Siti Hairunnisa Binti Mohd Kamel on her thesis entitled “Development of Bioorganic Fertilizer Containing *Amphinema* GanoEF2 and Evaluation of Its Effectiveness in Promoting Oil Palm Growth and Suppressing *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 1988. The Committee recommends that the student be awarded the Master of Science.

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## LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AMF	Arbuscular mycorrhizal fungi
AUDPC	Area Under Disease Progress Curve
BSR	Basal stem rot
CFU	Colony forming unit
CRD	Completely randomized design
DI	Disease incidence
DS	Dead seedling
DSI	Disease severity index
DSBI	Disease severity bole index
DSFI	Disease severity foliar index
DSRI	Disease severity root index
EFB	Empty fruit bunches
GSM	<i>Ganoderma</i> Selective Medium
ISR	Induce Systemic Resistance
MEA	Malt Extract Agar
PDA	Potato Dextrose Agar
PGPF	Plant growth promoting fungi
PIRG	Percentage inhibition of radial growth
spp.	Species
RBA	Rose Bengal Agar
RGR	Relative growth rate
rpm	Rate per million
RSBF	Real Strong bioorganic fertilizer

## CHAPTER 1

### INTRODUCTION

The oil palm (*Elaeis guineensis*), is the most important crop in Malaysia. Oil palm industry is one of the largest industries in Malaysia which producing an average palm oil yield approximately 4 tonnes per hectare per year. In year 2012, the oil palm planted area was increased to 5.08 million hectares compared with 2011 which was 5.00 million hectares (MPOB, 2012). In any crop production, the maximum production is obtained by keeping the plants healthy. In Malaysia, the oil palm is blessed by being largely disease free but suffering from one major disease which is basal stem rot (BSR) disease which is caused by *Ganoderma* Karst. BSR disease become the main killer of oil palm and therefore gives the bad impact to the economic growth of Malaysian oil palm industry.

Since the BSR disease caused severe losses of oil palm production, controlling it is an important factor. Physical control method of BSR does not prove any satisfactory. The method is actually to avoid infection to the new crop rather than cure the infected palms. The available technique to control the disease is by chemical control by using the fungicidal treatment but this control practice found to be ineffective in controlling BSR diseased as the failure to deliver the fungicide to the diseased margin and within the diseased lesion. Palm cultivation industry relies heavily on the use of chemicals to control disease control BSR. But the use of the chemical control gave bad impact on the environment. Environment will be polluted when the excessive release of chemicals from the use of chemical control. Moreover, the use of chemical control also requires a very high cost and this is a loss because the results are still unsatisfactory.

To overcome this problem, the use of biological control agents is the best approach. Several endophytic microorganisms, *Trichoderma* Pers. (Shukla and Uniyal, 1989; Wijesekera *et al.*, 1996; Nur Ain Izzati and Abdullah, 2008; Shamala *et al.*, 2008) and *Penicillium* Link. (Dharmaputra *et al.*, 1989) have been shown to have antagonistic activity against *G. boninense in vitro*. Oil palm roots are usually infected by arbuscular mycorrhizal fungi (AMF) (Nadarajah, 1980). This offers the prospects of using AMF as a biological control agent against *Ganoderma*. Previous study by Yow and Nasir (2001) reported that young oil palms pre-inoculated with AMF and planted next to diseased stumps remained free of BSR disease for at least 3 years, whereas non-inoculated palms were mostly infected. The used of endophytic bacteria, *Burkholderia* Yabuuchi *et al.* and *Pseudomonas* Migula (Bivi *et al.*, 2010; Zaiton *et al.*, 2008; Zaiton and Idris, 2007) and endophytic fungus, *Hendersonia* (Idris *et al.*, 2010; Nurrashyeda *et al.*, 2011) also gave positive result as the they are able to inhibit the growth of pathogen.

Endophytic microorganisms are a relatively new field of study in biological disease control. From ecological aspect, it enhances the natural complexity and diversity of the plant environment, providing greater biological balance and stability. The use of endophytic microorganisms should be preferable to other biological control agents as they are internal colonizers, and therefore capable to compete within the vascular systems, inhibiting *Ganoderma* from both nutrients and space for its proliferation. Some of endophytic microorganisms such as *Serratia* Bizio, *Burkholderia*., *Pseudomonas*., *Bacillus* Cohn, and *Fusarium* Link. have been found able to induce system resistance in plants (Kloepper *et al.*, 1992; Dorwoth and Callan, 1996). .

Endophytic fungi are defined as an organism that lives in association with plants for most, if not all, of its life cycle and lives within the intercellular spaces of plants. This microbe is an important biological control agent due to its ability to colonize intra and intercellular spaces of plants to restrain the invasion of pathogen and minimize the colonization of pathogen to the plant area (Kloepper *et al.*, 1999) and thus indirectly reduced the risk of being infected. Endophytic fungus, *Amphinema* GanoEF2 was isolated from symptomless root of oil palm seedlings and was found to have antagonistic activity against *Ganoderma boninense* (Idris *et al.*, 2010). The fungus was manipulated as a biological control agent to control BSR disease in oil palm seedlings. In this study, the *Amphinema* GanoEF2 was formulated in powder formulation and incorporated with bioorganic fertilizer to enhance its ability in promoting the vegetative growth of oil palm seedlings and controlling BSR disease. The use of this bioorganic fertilizer containing *Amphinema* GanoEF2 is highly practical, eco-friendly and low cost technology. In this way these bioorganic fertilizer may control the BSR disease and also help to reduce the use of chemical fertilizers and protecting the environment from heavy metals.

This study focused on promoting the use of biological control agents for controlling BSR disease in oil palm at nursery or field levels with the following objectives:

1. To formulate bioorganic fertilizer containing *Amphinema* GanoEF2.
2. To evaluate the effectiveness of bioorganic fertilizer containing *Amphinema* GanoEF2 in enhancing the growth of oil palm seedlings.
3. To determine the efficacy of bioorganic fertilizer containing *Amphinema* GanoEF2 for controlling *Ganoderma* disease in oil palm seedlings.



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