UNIVERSITI PUTRA MALAYSIA

GanoCare™ FERTILIZATION EFFECTS ON GROWTH AND SUPPRESSION OF BASAL STEM ROT DISEASE OF OIL PALM SEEDLINGS

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By

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

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DEDICATION

This thesis is dedicated to Ayah, Mama, Ika, Aci & Azman.

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

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February 2016

Chairman : **Professor Mohamed Hanafi Musa, PhD**

Institute : **Tropical Agriculture**

The oil palm industry is on alert for the basal stem rot (BSR) disease. The BSR which is caused by *Ganoderma boninense*, is the major disease of oil palm and is a serious threat to the palm oil production in Malaysia. One of the ideal solutions to manage BSR disease is through supplementation of nutrients. With no definitive cure at the present, thus this study introduced a new fertilizer technology called GanoCare™, as an effort to suppress BSR incidence in oil palm. The GanoCare™ fertilizer was produced from powdered oil palm empty fruit bunches (EFB) incorporated with beneficial elements. The optimum levels of GanoCare™ from two different application frequencies for the growth of oil palm was determined in a six-month nursery trial, and subsequently extended in the field trial for 21 months. All growth and physiological parameters evaluated were positively correlated with GanoCare™ levels up to a certain level. Two rates at 10 g/palm for every month application and 30 g/palm for every three months application were identified as optimum levels for the growth of oil palm seedlings. In the field, the optimum levels were at 100 g/palm for every month application and 300 g/palm for every three months application. The effect of optimized rate of GanoCare™ 1 on growth, physiological and BSR disease suppression were assessed in the nursery stage using sitting (bait) technique, in which the treatments added were CT (control), T1 (pre-treatment with 10 g/month), T2 (pre-treatment with 30 g/three months), T3 (pre-treatment and continuous treatment with 10 g/month) and T4 (pre-treatment and continuous treatment with 30 g/three months). A follow up in the field stage was carried out for 21 months to test on severity of *Ganoderma* using baiting technique under natural condition. The treatments were similar as in the nursery stage, however with some alterations to suit the condition of the experiment. In nursery, the application of GanoCare™ 1 significantly enhanced the height, girth, frond count, total leaf area, chlorophyll content, photosynthesis rate, fresh and dry weight of oil palm seedlings when compared to the control. Despite some modifications done to the treatments, the results from the field trial validate the pathological results from the nursery, in which both experiments similarly showed that application of GanoCare™ was able to suppress the incidence of BSR disease compared to the control seedlings. For both studies in nursery and field, the disease reduction was higher for seedlings given pre-

treatment and continuous treatment (T3 and T4) compared to the seedlings that were pre-treated only (T1 and T2), suggesting that continuous application is required to provide maximum protection from *Ganoderma* disease, and that pre-treatment alone was not sufficient to allow the development of a strong defense system in the seedlings. Combination of pre-treatment and continuous treatment with 30 g of GanoCare™ at three-month intervals (T4) was the most effective treatment in both nursery and field by reducing the BSR disease by 77.78 and 82.36%, respectively.

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

KESAN PEMBAJAAN GanoCare™ KE ATAS PERTUMBUHAN DAN PENYEKATAN PENYAKIT REPUT PANGKAL BATANG ANAK KELAPA SAWIT

Oleh

NUR AKMAL BINTI REBITANIM

Februari 2016

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Industri kelapa sawit kini sedang dikejutkan dengan penyakit reput pangkal batang (BSR). Penyakit BSR yang disebabkan oleh *Ganoderma boninense*, adalah penyakit utama kelapa sawit dan merupakan satu ancaman serius kepada pengeluaran minyak sawit di Malaysia. Salah satu penyelesaian yang berpotensi untuk mengawal penyakit BSR adalah melalui rawatan nutrien. Disebabkan tiada langkah berkesan pada masa ini, maka kajian ini memperkenalkan teknologi baja baru yang dikenali sebagai GanoCare™, sebagai usaha untuk mengurangkan kejadian BSR kelapa sawit. Baja GanoCare™ dihasilkan daripada serbuk tandan buah kosong sawit (EFB) yang digabungkan bersama unsur-unsur yang berfaedah. Tahap optimum GanoCare™ dari dua frekuensi aplikasi yang berlainan bagi menilai pertumbuhan kelapa sawit telah dijalankan di tapak semaian selama 6 bulan, yang kemudiannya dilanjutkan ke kajian lapangan selama 21 bulan. Kesemua penilaian ke atas parameter pertumbuhan dan fisiologi menujukkan perhubungan positif dengan tahap aplikasi GanoCare™ sehingga ke suatu tahap. Dua kadar iaitu 10 g/pokok bagi aplikasi setiap bulan dan 30 g/pokok bagi aplikasi setiap tiga bulan telah dikenal pasti sebagai tahap optimum untuk pertumbuhan anak pokok kelapa sawit. Untuk kajian lapangan, tahap optimum ialah 100 g/pokok bagi aplikasi setiap bulan dan 300 g/pokok bagi aplikasi setiap tiga bulan. Kesan kadar optimum GanoCare™ 1 pada pertumbuhan, fisiologi dan penyekatan penyakit BSR dinilai di peringkat tapak semaian menggunakan teknik duduk (umpan), di mana rawatan yang diberikan adalah CT (kawalan), T1 (pra-rawatan dengan 10 g/bulan), T2 (pra-rawatan dengan 30 g/tiga bulan), T3 (pra-rawatan dan rawatan berterusan dengan 10 g/bulan) dan T4 (pra-rawatan dan rawatan berterusan dengan 30 g/tiga bulan). Susulan seterusnya dijalankan di peringkat lapangan selama 21 bulan untuk menguji keterukan serangan *Ganoderma* dengan menggunakan teknik umpan di bawah keadaan semula jadi. Rawatan adalah sama seperti di peringkat tapak semaian, bagaimanapun dengan beberapa pengubahsuaian untuk disesuaikan dengan keadaan eksperimen. Di tapak semaian, aplikasi GanoCare™ 1 dengan berkesannya meningkatkan tinggi, lilitan, kiraan daun, jumlah permukaan daun, kandungan klorofil, kadar fotosintesis, berat basah dan kering anak pokok kelapa sawit jika dibandingkan

dengan pokok kawalan. Keputusan daripada percubaan lapangan mengesahkan keputusan patologi dari tapak semaian, di mana kedua-dua eksperimen menunjukkan bahawa penggunaan GanoCare™ dapat mengurangkan kejadian penyakit BSR berbanding dengan benih kawalan. Untuk kedua-dua kajian di tapak semaian dan lapangan, pengurangan penyakit adalah lebih tinggi untuk benih yang telah diberikan pra-rawatan dan rawatan berterusan (T3 dan T4) berbanding dengan benih yang diberi pra-rawatan sahaja (T1 dan T2), dan ini menunjukkan bahawa aplikasi berterusan diperlukan bagi memberi perlindungan maksimum daripada penyakit *Ganoderma*, dan pra-rawatan sahaja tidak mencukupi untuk membolehkan pembangunan sistem pertahanan yang kuat dalam benih. Kombinasi pra-rawatan dan rawatan berterusan dengan 30 g GanoCare™ setiap tiga bulan (T4) adalah rawatan yang paling berkesan untuk kedua-dua tapak semaian dan lapangan dengan masing-masing dapat mengurangkan penyakit BSR sebanyak 77.78 dan 82.36%.

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I certify that a Thesis Examination Committee has met on 17 February 2016 to conduct the final examination of Nur Akmal binti Rebitanim on her thesis entitled "GanoCare^{TM} Fertilization Effects on Growth and Suppression of Basal Stem Rot Disease of 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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%	Percent
$	Dollars
°C	Degree celcius
AMF	Arbuscular mycorrhiza fungi
ANOVA	Analysis of variance
AUDPC	Area under the disease progress curve
B	Boron
BSR	Basal stem rot
C	Carbon
Ca	Calcium
$CaNO_3$	Calcium nitrate
Cl	Chlorine
cm	Centimeter
Co	Cobalt
CO_2	Carbon dioxide
CRD	Completely Randomized Design
Cu	Copper
DI	Disease incidence
DR	Disease reduction
DS	Disease severity
DSIB	Disease severity of bole index
DSIF	Disease severity of foliar index
DSIR	Disease severity of root index
EB	Endophytic bacteria
EFB	Empty fruit bunch
Fe	Iron
FELCRA	Federal Land Consolidation and Rehabilitation Authority
FELDA	Felda Land Development Authority
FFB	Fresh fruit bunch
FPSSB	FELCRA Plantation Services Sdn. Bhd.
g	Gram
GSM	*Ganoderma* selective medium
H	Hydrogen
H_2O_2	Hydrogen peroxide
ha	Hectare
ISR	Induced systemic resistance
K	Potassium
K_2O	Potassium oxide
kg	Kilogram
LSD	Least significant difference
m	Meter
MARDI	Malaysian Agricultural Research and Development Institute
MEA	Malt extract agar
Mg	Magnesium
mg/L	Milligram per liter
MgO	Magnesium oxide
mL	Milliliter
mm	Millimeter

Mn	Manganese
Mo	Molybdenum
MPOB	Malaysian Palm Oil Board
N	Nitrogen
Nm	Nanometer
O	Oxygen
P	Phosphorus
P_2O_5	Phosphorus pentoxide
PAL	Phenylalanine ammonia-lyase
PDA	Potato dextrose agar
pH	Hydrogen potential
POD	Peroxidase
ppm	Part per million
PPO	Polyphenol oxidase
RCBD	Randomized complete block design
RNA	Ribonucleic acid
RWB	Rubber wood block
S	Sulphur
SFS	Severity of foliar symptoms
Si	Silicon
SiO_2	Silicon dioxide
TE	Trace element
TEM	Transmission electron microscopy
™	Trademark
UPM	Universiti Putra Malaysia
v/v	Volume to volume
Zn	Zinc

CHAPTER 1

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) was cultivated as early as 3000 B.C in Egypt (Friedel, 1987). It is a perennial crop that exists in the equatorial tropics of Africa, America and South-East Asia (Hartley, 1988). From growing wild, oil palm was developed into an important agriculture crop and has been planted in all tropical parts of the world, especially in South East Asia countries, such as Malaysia, Thailand and Indonesia (Shuit et al., 2009). After Indonesia, Malaysia is the second major producer and exporter in oil palm production, with both countries accounted for nearly 90% of the total world crude oil production (Carter et al., 2007; Sharma, 2007). In developing countries, such as Malaysia and Indonesia, oil palm industry is one of the important tools to alleviate poverty. Oil palm is said as the "golden crop of Malaysia" since it plays important key role in the Malaysian economy and generates profitable export earnings for this country (Bivi et al., 2010).

Oil palm is cultivated mostly for its oil in which the global demand for palm oil is tremendously increase for the last few decades. During the Industrial Revolution in Europe, oil palm was largely cultivated due to massive demands in manufacturing of lubricants and soap (Hardon, 1995). Presently, palm oil is commonly used as edible vegetable oil for human consumption and also a significant source of biodiesel and biogas. The billion dollar oil palm industry brings huge profitability more than any other vegetable oil, such as corn oil, linseed oil, sunflower oil, soybean oil and canola oil (Carrere, 2001). In 2010, about 47.9% of the world palm oil production was contributed by Malaysia, with export revenue earnings from oil palm products of more than RM 60 billion (Simeh and Fairuz, 2009; Abas et al., 2011). In 2011, oil palm produced 31.3% of global oils and fats, with only 5.3% of global land used for cultivation, showing that this crop brings high productivity with little land requirement (Mielke, 2012).

Nonetheless, oil palm is vulnerable to various pests and diseases, among which is the basal stem rot (BSR) disease. The BSR disease is caused by white rot fungus called *Ganoderma boninense*, which this pathogen has brought a massive damage to the oil palm plantation in Pacific Islands and Papua New Guinea, and in Southeast Asia, particularly in Indonesia and Malaysia (Ariffin, 1990; Flood and Hasan, 2004; Pilotti, 2005). On coastal soils of Malaysia, palm losses raised as high as 85% by the time palms were replanted at 25 years. About 31 to 67% of disease incidence has caused up to 46% yield reduction in Malaysia (Singh, 1991). It was estimated that only 1% disease incidence can bring a loss of up to 38 million US$ annually to Indonesian industry (Flood and Hasan, 2004). In Malaysia, the BSR incidence reported was 3.71% and total area impacted was 59,148 hectares, with the most severe areas affected particularly in Perak, Johor and Sabah (Idris et al., 2011).

Among the early signs of BSR disease are leaves yellowing and spear rotting. The fronds are usually folded like an arrow, thus restricting the oil palms' ability to photosynthesize (Idris et al., 2000). The foliar symptoms of BSR will only emerged after substantial portion of the bole is infected. The internal xylem vessels which are vital to the oil palm's transport systems usually deteriorate, thus inhibiting the access of water and plant nourishment. Originally, BSR disease was reported to only attacks matured palms over 25 years old, however the disease symptoms have been detected to infect younger palms (Turner, 1981; Khairudin, 1993; Ariffin et al., 2000; Susanto et al., 2005). Unfortunately, to date, no definitive solution is available to cure this disease.

A variety of methods are currently being practiced as an integrated approach to manage BSR disease. Development of oil palm progenies resistant to *Ganoderma* may offer the most effective method and long term solution for controlling BSR disease. Even though breeding programs for lightly resistant progenies to *Ganoderma* are actively being undertaken by few research organizations, including Malaysian Palm Oil Board (MPOB), however tolerant oil palm progenies are yet unavailable due to the genetic diversity of the fungus. Utilization of fungicides is an important component in the BSR management. Fungicides, such as carbendazim, cycloheximide and drazoxolone are reported to be strongly inhibitory to the fungus (Ramasamy, 1972; Jollands, 1983; Khairudin, 1990). However, application of fungicides only assist to slow the infection of *Ganoderma* and arise of public alertness on the effect of fungicides leads to detrimental effects of a non-target organisms and environmental issues, such as ground water contamination (Sathya et al., 2010; Sundram, 2012).

This eventually resulted to a more environmental friendly practice, which is the use of antagonistic microbes as biological control agents (Sundram, 2005). Several promising antagonists, such as *Trichoderma* spp. (Ilias, 2000; Sundram, 2005), *Penicillium* spp. (Dharmaputra et al., 1989), *Aspergillus* (Shukla and Uniyal, 1989), *Pseudomonas* spp. and *Bacillus* spp. (Susanto et al., 2005) have demonstrated potential efficacy against *G. boninense*. Incidence of BSR disease on four months old oil palm seedlings was reduced when treated with *Trichoderma* spp. as aggregates on paddy grain (Zakaria et al., 2010). In addition, treatment with conidial suspension of *Trichoderma harzianum* (isolate FA 1132) suppressed the incidence of BSR in oil palm (Nur Ain Izzati and Abdullah, 2008). Even so, biological approach itself cannot stop the emergence of *G. boninense* in oil palm.

Owing to these limitations, developing another alternative which is cost effective and more environmentally safe by improvement of the oil palm defense system, through addition of required mineral nutrients becomes an imperative solution. Nutrient supplementation to the soil as fertilizer is known to affect the susceptibility of plants towards some diseases caused by fungal pathogens (Veresoglou et al., 2013). In most cases, a balanced of mineral nutrient added to the soil in the form of fertilizer improves the plants' ability to disease resistance (Usherwood, 1980; Fageria, 2009). This approach may not necessarily be a cure for the BSR disease but it could arrest the spread of disease in the oil palm. A reduction in BSR incidence was observed when oil palm seedlings were supplemented with Cu, Ca and combination of Cu and Ca compared to the control seedlings (Nur Sabrina et al., 2012). This ideal solution to delay BSR disease development in oil palm is through manipulation of lignin by plant nutrient management as it assists in enhancing the defense mechanism in plants (Paterson et al., 2009).

Therefore, the present study was undertaken to investigate the effect of GanoCare™ in suppressing BSR disease in oil palm. The GanoCare™ (consisted of GanoCare™ 1 and GanoCare™ 2) is a new fertilizer technology, formulated to prevent BSR disease in oil palm. The fertilizer was derived by combining powdered empty fruit bunches (EFB) with beneficial element. The effect of GanoCare™ 1 on vegetative growth, physiological parameters and suppression of BSR disease of oil palm seedlings was investigated through the nursery trials using sitting technique. Most research studies have been limited in the nursery without validation assessment from the field trial. Thus, in this study, the scope was broaden by carrying out the GanoCare™ 1 treatments in the field stage based on seedling baiting technique.

Thus, the study was carried out with these objectives:

1. To determine the optimum levels of GanoCare™ from two different application frequencies for both nursery and field trials.
2. To investigate the effect of optimized rates of GanoCare™ 1 on the performance of oil palm seedlings in the nursery trial.
3. To investigate the effect of GanoCare™ 1 on BSR disease suppression of oil palm seedlings in the nursery trial using sitting (bait) technique.
4. To evaluate the effect of GanoCare™ 1 on BSR disease suppression of oil palm seedlings in the field trial using baiting technique.

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