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POTENTIAL OF Trichoderma harzianum AND Bacillus cereus AS BIOCONTROL OF Ganoderma boninense IN OIL PALM

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By

GHAZALA SAAD AHMED ABUFANA

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

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in Fulfillment of the Requirements for the Degree of Master of Science

POTENTIAL Of *Trichoderma harzianum* AND *Bacillus cereus* AS BIOCONTROL OF *Ganoderma boninense* IN OIL PALM

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August 2016

Chairman: Nusaibah binti Syd Ali, PhD

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Sustainability of oil palm industry is crucial for sustaining Malaysia's gross domestic product (GDP) by agricultural sector. However, without positioning efforts in eliminating or controlling *Ganoderma* disease encountered by this commodity crop, sustainability is not attainable. Thus, primary emphasis on developing early control or preventative measures that will contribute to a sustainable environment as well as cater this catastrophic is crucial. Therefore, this study was designed with the objectives: i)To investigate *Trichoderma harzianum* and *Bacillus cereus* antagonistic bio-inoculant potential in inhibiting *Ganoderma boninense* growth and ii)To determine the effects on vegetative growth and suppression of *G. boninense* infection in oil palm seedlings pre-inoculated with *T. harzianum* and *B. cereus* consortium. In order to achieve the objectives set for the present study, cultures of UPM29 (*T. harzianum*) and UPM15 (*B. cereus*) were isolated from rhizosphere of oil palm from plantation in a preliminary study. In this current study, all microbial isolates were subjected to morphological and molecular identification and screened for its antagonistic activity against *G. boninense in vitro*. The antagonistic activities were evaluated via dual culture test, culture filtrate assay, mycelial growth test, chitinase and β-glucanase assay. All the experiments above were repeated three times using completely randomized design (CRD) with eight replications. Nonetheless, nursery trial was conducted at Ladang 15, Faculty of Agriculture, UPM for a duration of 24 weeks on four months old oil palm seedlings (D×P) that were artificially inoculated with pathogenic (UPM13) *G. boninense* based on a newly developed artificial inoculation method known as dip, place and drench (DPD). Eight treatments with six individual replicates were designed for this nursery trial and conducted as randomized completely block design (RCBD). In addition, preparation of biological control agent (BCA) inoculants, the spore suspension were adjusted to a concentration of 6.0×10^7 conidia mL^{-1} for fungal isolate and 1×10^{12} CFU for bacterium. Disease assessments were analyzed based on disease severity (DS) of foliar, roots and disease reduction (DR). As a result, UPM15 and UPM29 demonstrated significant antagonistic activities against *G. boninense* growth by exhibiting a PIRG value of 72.9% and 95.7% via culture filtrate test and hyphae malformation and

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shrinkage in mycelial growth test respectively and exhibited ability in producing lignocellulolytic enzymes; chitinase and β-glucanase. In the nursery trial conducted, single application of *B. cereus* was found to be the most effective treatment in suppressing *Ganoderma* disease of oil palm with DR rate of 94.8% followed by single application of *T. harzianum* (79.0%). In addition, single application of both BCAs pointed out that *B. cereus* (13.3 g) was the BCA accountable in contributing an increase in the root dry weight and *T. harzianum* for the top dry weight (14.1 g). However, seedlings treated with the mixture of BCAs gave the highest dry root weight of 17.4 g compared to all other treatments. Hence, based on the results of this present study, UPM15 strain (*B. cereus*) may be an excellent option of BCA to be applied in the commercial oil palm nurseries prior to field transfer and tested in the fields where high incidence of *Ganoderma* disease were recorded for disease suppression.

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

POTENSI *Trichoderma harzianum* DAN *Bacillus cereus* SEBAGAI KAWALAN BIOLOGI *Ganoderma boninense* PADA SAWIT

Oleh

GHAZALA SAAD AHMED ABUFANA

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Kemampanan industri sawit adalah penting bagi mengekalkan keluaran dalam negara kasar (KDNK) Malaysia dalam sektor pertanian. Walau bagaimanapun, tanpa meletakkan usaha dalam menghapuskan atau mengawal penyakit *Ganoderma* pada tanaman komoditi ini, kemampanan tidak boleh dicapai. Justeru itu, fokus utama dalam membangunkan langkah-langkah kawalan awal atau pencegahan yang turut menyumbang kepada persekitaran yang mampan selain menangani penyakit ini adalah penting. Oleh itu, objektif-objektif kajian ini adalah: i) untuk mengkaji potensi antagonistik isolat UPM29 (*Trichoderma harzianum*) dan isolat UPM15 (*Bacillus cereus*) sebagai bio-inokulan dalam merencatkan pertumbuhan isolat UPM13 (*Ganoderma boninense*) dan ii) untuk mengkaji kesan pra-inokulasi calon-calon agen kawalan biologi; *T. harzianum* dan *B. cereus* terhadap pertumbuhan dan jangkitan *G. boninense* pada anak sawit. Oleh itu, bagi mencapai objektif-objektif kajian yang telah ditetapkan, isolat UPM29 (*T. harzianum*) dan UPM15 (*B. cereus*) yang telah berjaya diasingkan daripada tisu akar pokok sawit dalam satu kajian awal digunakan selepas menjalankan pencirian serta identifikasi secara morfologi dan molikul dalam kajian ini. Selain itu, UPM29 (*T. harzianum*) dan UPM15 (*B. cereus*) turut disaring sebagai agen kawalan biologi terhadap UPM13 (*G. boninense*) secara *in vitro.* Aktiviti-aktiviti antagonis secara *in vitro* dinilai melalui ujian dwi-kultur, asai tapisan kultur, ujian pertumbuhan miselium, asai enzim chitinase and β-glucanase. Kesemua eksperimen di atas diulang sebanyak tiga kali menggunakan rekabentuk rawak yang lengkap (CRD) dengan lapan replikasi. Selain itu, ujian nurseri telah dijalankan di Ladang 15, Fakulti Pertanian, UPM selama 24 minggu menggunakan anak sawit (D × P) yang berusia empat bulan yang diinokulasi secara artifisial dengan kulat patogenik UPM13 (*G. boninense*) berdasarkan satu kaedah inokulasi artifisial yang baru dibangunkan dan dikenali sebagai celup, letak dan siram (DPD). Bagi ujian nurseri ini, lapan rawatan dengan enam replikasi telah direka dan dijalankan dalam rekabentuk blok lengkap yang rawak (RCBD). Di samping itu, bagi penyediaan inokulum agen kawalan biologi (BCA), kepekatan spora kulat UPM29 diselaraskan kepada 6.0×10^7 konidia mL^{-1} dan 1×10^{12} CFU untuk bakteria UPM15. Penilaian penyakit *Ganoderma* pula dilakukan

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berdasarkan simptom keterukan penyakit (DS) pada daun, akar dan peratus pengurangan penyakit (DR). Berdasarkan kepada keputusan kajian ini, UPM15 dan UPM29 telah menunjukkan aktiviti-aktiviti antagonis yang signifikan terhadap pertumbuhan *G. boninense* dengan mempamerkan nilai PIRG ,72.9% dan 95.7% dalam asai tapisan kultur dan malformasi serta pengecutan dalam pertumbuhan hifa melalui ujian pertumbuhan miselium masing-masing dan juga mempamerkan keupayaan untuk menghasilkan enzim lignosellulolitik; chitinase dan β-glucanase. Di dalam ujian nurseri yang dijalankan, aplikasi inokulum *B. cereus* secara tunggal merupakan rawatan yang paling efektif dalam merencatkan penyakit *Ganoderma* pada anak sawit dengan kadar DR 94.8% dan ini diikuti dengan aplikasi tunggal *T. harzianum* (79.0%). Aplikasi tunggal kedua-dua BCA telah mempamerkan bahawa *B. cereus* (13.3 g) bertanggungjawab dalam menyumbang kepada peningkatan berat kering akar dan *T. harzianum* sebagai penyumbang berat kering bahagian atas pokok (14.1 g). Oleh itu, berdasarkan pada keputusan kajian ini, strain UPM15 (*B. cereus*) mungkin merupakan pilihan BCA yang terbaik untuk diaplikasikan di nurseri komersil sawit sebelum pemindahan anak sawit ke ladang dilakukan dan juga diuji di dalam ladang yang mempunyai insiden penyakit *Ganoderma* yang tinggi bagi kajian perencatan penyakit.

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I certify that a Thesis Examination Committee has met on 29 August 2016 to conduct the final examination of Ghazala Saad Ahmed Abufana on his thesis entitled "Potential of *Trichoderma harzianum* and *Bacillus cereus* as Biocontrol of *Ganoderma boninense* in Oil Palm" 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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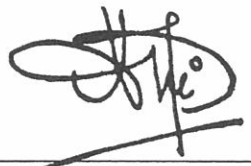

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AUDPC	Area Under Disease Progress Curve
CFU	Colony forming unit
cm	Centimeter
CRD	Completely randomized design
°C	Degree celsius
DNA	Deoxyribonucleic acid
DI	Disease Incidence
DR	Disease reduction
g	Gram
h	Hour
kg	Kilogram
µl	Microliter
mL	Millititer
%	Percentage
NA	Nutrient agar
NB	Nutrient broth
PDA	Potato dextrose agar
PDB	Potato dextrose broth
PIRG	Percentage inhibition of radial growth
PCR	Polymerase chain reaction

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CHAPTER 1

INTRODUCTION

The oil palm (*Elaeis guineensis* Jacq.) is a monoecious plant belonging to the genus *Elaeis* of the Aracaceae family. The oil palm is believed to be originated from the tropical rainforest belt of West Africa and introduced into Southeast Asia by the British colonial administration in the early 1870s. To date, oil palm is widely cultivated in Malaysia as the major cash crop (Corley and Tinker, 2003). Malaysia is the world's largest exporter of palm oil which having exported 18 million tonnes of palm oil products in 2011 (MPOB, 2012). China, Pakistan, the European Union, India and the United States are the primary importers of Malaysian palm oil products (MPOB, 2012). Therefore, oil palm has been designated as the nation's most valuable commodity crop as it has been contributing to the robust developing economy of Malaysia since 1985 to the present with the oil palm sector ranked as the fourth largest contributor to Malaysia's economy, with contribution of RM53 billion in gross national income accounts (Malaysia Palm Oil Council, 2013). In addition, this economically high yielding palm is now one of the world's major edible oil and a significant precursor of biofuel (http://www.fas.usda.gov/psdonline/psdHome.aspx).

Despite the enormous economic contribution of oil palm to Malaysia, it is facing a devastating threat of a serious plant disease, notably *Ganoderma* disease (Nusaibah *et al*., 2011; Naher *et al*., 2012). *Ganoderma boninense* and *Ganoderma zonatum* species were documented as the deadly causal pathogens of this disease (Rakib *et al.,* 2014). More severely, in plantations where oil palm stumps were left in the ground at replanting, more serious palm losses due to *Ganoderma* disease has been observed (up to 25% occurred within 7 years) (Subagio and Foster, 2003). Subagio and Foster (2003) also recorded an average decline of the yield of the fresh fruit bunch (FFB) of 0.16 t ha^{-1} for every palm loss, and when the stand had declined by 50% the average FFB yield reduction was 35%.

In order to achieve a sustainable palm oil production, effective techniques for controlling basal stem rot (BSR) disease must be developed and adopted by oil palm planters. Synthetic chemicals are being used as the main method for controlling *Ganoderma* diseases despite the high cost and environmental hazards associated with the application of chemicals. However, awareness and research on the residual effects of pesticides on human health through food and contamination of the environment has awakened the research on the hazards of synthetic chemicals used in plantations. Therefore, demands from consumers and government authorities have increased throughout the world to reduce the use of synthetic pesticides. Hence, there is an urgent need to develop alternative control practices that will be more affordable, sustainable and ecologically friendly.

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Recently, biological control has been reported as an alternative strategy to control crop disease and reduce damage caused by plant pathogens (Wang *et al*., 2000; Gerhardson *et al*., 2002). The effective microbes such as bacteria, fungi, actinomycetes and mycorrhiza have been reported as biological control agents of different diseases (Wang *et al*., 2000). These microbes inhibit or parasitize pathogens by excreting antibiotics in combination with extracellular cell wall-degrading enzymes, competition for nutrients (i.e.: iron, nitrogen or carbon) in colonization sites, and stimulation of plant resistance mechanisms and development (Howell, 2003).

These EM exhibiting strong antifungal activities are an alternative strategy for *Ganoderma* disease management, apart from being ecologically conscious and environmentally friendly. Furthermore, previous studies have shown positive outcomes in controlling *Ganoderma* disease via application of biological control agents (Zaiton *et al*., 2008, Izzati and Abdullah, 2008; Sundram *et al*., 2014).

To date, the interest and focus are on improving the effectiveness of these biological agents to strengthen their ability in plant disease management and control by using the strategy of combining different beneficial microorganisms together in a consortium. In this current study, the consortium of *Trichoderma harzianum* and *Bacillus cereus* was studied in an association with oil palm rhizosphere and its effect in controlling *Ganoderma* disease. *In vitro* and *in vivo* experiments were conducted to evaluate antagonistic abilities of the microorganism(s); singularly and in combination.

The following objectives were set to conduct the study:

1. To investigate *T. harzianum* and *B. cereus* antagonistic bio-inoculant potential in inhibiting *G. boninense* growth.
2. To determine the effects on vegetative growth and suppression of *G. boninense* infection in oil palm seedlings pre-inoculated with *T. harzianum* and *B. cereus* consortium.

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