UNIVERSITI PUTRA MALAYSIA

INDUCED RESISTANCE OF OIL PALM SEEDLINGS AGAINST BASAL STEM ROT USING CALCIUM, COPPER AND SALICYLIC ACID

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By

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The oil palm (*Elaeis guineensis*) tree, which belongs to the Palma family, is one of the most versatile crops in tropical countries. Oil palm was the major commodity inside the Malaysian agriculture sector. Malaysia is presently the world's leading exporter of oil palm and it was second only to soybean as the major source of vegetable oil. However, a soil fungus pathogen, *Ganoderma boninense*, which causes basal stem rot (BSR) in the oil palm's trunk, destroys thousands of hectares of plantations in Southeast Asia every year. Thus far there was no conclusive method for early detection of BSR in order to control the disease from spreading in the plantation.

Plant nutrient and hormone are vital variables and modifying these as a control method may prevent *Ganoderma* attack. The limited nutrients and hormone of plants can trigger lignin degradation and conversely ensuring they are available for oil palm may limit the rot. These nutrients could conceivably be supplied by foliar and soil application with a view to control the disease in addition to them being used as fertilizers. Fertilizers are added which hold some of these minerals, but the point here is to control the disease and also improve the growth of the oil palm. The objective of the present work is to study calcium (Ca), copper (Cu) and salicylic acid (SA) application in oil palm and to determine their potential as fertilizers to control against *Ganoderma boninense in vitro* and *in vivo*.

The *in vitro* effects of single and combined application of Ca, Cu and SA were evaluated on growth and sporulation of *G. boninense*. In poison medium test, T7- (Ca+Cu+SA) showed effective control of *G. boninense in-vitro* with EC_{50} and EC_{90} values of 1500+150+150 ppm and 2000+200+200 ppm, respectively. However, in dipping test, T7- (Ca+Cu+SA) indicates effective control of *G. boninense* at low concentration, 500+50+50 ppm as shown by EC_{90} analysis. Pre-treatment of *Ganoderma*-infected rubber wood block with 500 ppm Ca+50 ppm Cu+50 ppm SA reduced the number (20.14%) and (0.8 g) weight of basidiocarp compared to the control. This was followed by a significant reduction in weight loss of the *Ganoderma*-infected rubber wood block (41.85%) suggesting the inhibition of the degradative enzymatic activity of the fungus. The mixture of Ca, Cu and SA had potential to suppress growth of *G. boninense* under *in vitro* condition.

The effect of Ca, Cu and SA further tested in a glass house for about 9 months for their efficacy in controlling BSR in oil palm seedlings through foliar and soil drenching by control of water application. As tested in oil palm seedlings inoculated with *G. boninense*, both of them, alone and in mixture suppressed *G. boninense* compared to the control. Not only they delayed the onset of BSR symptoms, but also promoted the growth of the seedlings. The application of Ca, Cu and SA together reduced disease incidence most effectively and was recorded reduction at 81.0% and followed by BSR disease incidence reduced for mixture of Ca and SA; a mixture of Cu and SA; and Ca alone respectively with a 76.1% reduction. In addition, treatment with Ca+Cu+SA increased the levels of the defense related enzymes phenolics, peroxidase, lignin and hydrogen peroxide in oil palm seedling tissues. Plant growth and antibiosis, plant nutrient and hormone enhanced the resistance in plants through the induction of defense enzymes in the oil palm seedlings.

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ARUHAN RINTANGAN ANAK BENIH KELAPA SAWIT TERHADAP REPUT PANGKAL BATANG MENGGUNAKAN KALSIUM, KUPRUM DAN ASID SALISILIK

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Pokok kelapa sawit (*Elaeis guineensis*), yang tergolong dalam famili Palma adalah salah satu tanaman yang paling versatil di negara-negara tropika. Kelapa sawit merupakan komoditi utama di dalam sektor pertanian Malaysia. Kini, Malaysia adalah pengekspot terkemuka di dunia minyak sawit dan ia adalah kedua selepas kacang soya sebagai sumber utama minyak sayuran. Walau bagaimanapun, patogen kulat tanah, *Ganoderma boninense*, yang menyebabkan reput pangkal pada batang pokok kelapa sawit, memusnahkan beribu-ribu hektar ladang di Asia Tenggara setiap tahun. Setakat ini tidak ada kaedah muktamad untuk pengesanan awal BSR untuk mengawal penyakit daripada merebak.

Nutrien dan hormon tumbuhan adalah pembolehubah penting dan mengubahsuai ianya sebagai kaedah kawalan boleh menghalang serangan *Ganoderma*. Mengehadkan nutrien dan hormon tumbuhan boleh mencetuskan degradasi lignin dan sebaliknya memastikan ianya sedia ada untuk kelapa sawit boleh mengehadkan pereputan. Nutrien tersebut boleh digunakan untuk dibekalkan pada foliar dan tanah dengan tujuan untuk mengawal penyakit itu di samping digunakan sebagai baja. Baja mengandungi beberapa mineral bagi mengawal penyakit dan juga untuk meningkatkan pertumbuhan kelapa sawit. Objektif penyelidikan ini adalah untuk mengkaji aplikasi kalsium (Ca), kuprum (Cu) dan asid salisilik (SA) kepada anak benih kelapa sawit dan menentukan potensi mereka sebagai baja untuk mengawal jangkitan *G. boninense* secara *in vitro* dan *in vivo*.

Kesan *in vitro* tunggal dan campuran aplikasi Ca, Cu dan dan SA telah dinilai pada tumbesaran dan sporulasi *G. boninense*. Pada ujian media racun, T7- (Ca+Cu+SA) menunjukkan kawalan berkesan terhadap *G. boninense* secara *in vitro* dengan nilai EC_{50} dan EC_{90} pada 1500+150+150 ppm dan 2000+200+200 ppm, masing-masing. Walau bagaimanapun, pada ujian pencelupan, T7- (Ca+Cu+SA) menunjukkan kawalan yang berkesan terhadap *G. boninense* pada kepekatan rendah, 500+50+50 ppm seperti ditunjukkan pada analisis EC_{90}. Pra-rawatan pada blok kayu getah yang dijangkiti *Ganoderma* pada 500 ppm Ca+50 ppm Cu+50 ppm SA mengurangkan bilangan (20.14%), dan (0.8 g) berat basidiokap berbanding kawalan. Ini diikuti oleh pengurangan yang ketara dalam penurunan berat pada blok kayu getah yang dijangkiti *Ganoderma* (41.85%) menunjukkan perencatan aktiviti enzim degradasi daripada kulat. Campuran Ca, Cu dan SA mempunyai potensi untuk menghalang pertumbuhan *G. boninense* secara *in vitro.*

Kesan Ca, Cu dan SA diuji lagi dalam rumah kaca selama 9 bulan untuk keberkesanan mereka bagi mengawal RPB pada anak benih kelapa sawit melalui aplikasi pada foliar dan tanah dengan kawalan penyiraman air. Seperti yang diuji pada benih kelapa sawit yang disuntik dengan *G. boninense*, kedua-dua mereka, bersendirian dan campuran menghalang *G. boninense* berbanding kawalan. Bukan sahaja mereka melambatkan kemunculan simptom RPB tetapi juga menggalakkan pertumbuhan benih. Aplikasi Ca, Cu dan SA bersama-sama mengurangkan insiden penyakit paling berkesan dan merekod pengurangan 81.0% dan diikuti oleh insiden penyakit RPB dikurangkan bagi campuran Ca dan SA; campuran Cu dan SA; dan Ca sahaja masing-masing dengan penurunan 76.1%. Di samping itu, rawatan dengan Ca+Cu+SA meningkatkan tahap enzim yang berkaitan ketahanan seperti phenolik, peroxidase, lignin, dan hidrogen peroksida dalam tisu anak benih kelapa sawit. Pertumbuhan pokok dan antibiosis, nutrien tumbuhan dan hormon meningkatkan rintangan dalam tumbuhan melalui induksi enzim pertahanan dalam anak benih kelapa sawit.

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophometer
ANOVA	Analysis of Variance
BSR	Basal Stem Rot
CRD	Completely Randomized Design
CWA	Cell Wall Apposition
CWDE	Cell Wall Degrading Enzymes
DI	Disease Incidence
DR	Disease Reduction
DSI	Disease Severity Index
EC_{50}	Effective Concentration 50%
EC_{90}	Effective Concentration 90%
ER	Epidemic Rate
E	Effective
LE	Least Effective
HR	Hypersensitive Reaction
IAA	Indole-3-Acetic Acid
MAI	Month After Inoculation
MEA	Malt Extract Agar
µL	Micro Liter
µm	Micrometer
mM	Milimolar
M	Molar
MIT	Methylisothiocyanate

MPOB	Malaysian Palm Oil Board
nm	Nanometer
NE	Not Effective
PAL	Phenylalanine Ammonia Lyase
PDA	Potato Dextrose Agar
PIRG	Percentage Inhibition of Radial Growth
POD	Peroxidase
ppm	Parts per million
PR	Pathogenesis Related
psi	Per square inch
RCBD	Randomised Completely Block Design
SA	Salicylic Acid
SAS	Statistical Analysis Software
SEM	Scanning Electron Microscopy
spp	Species
TEM	Transmission Electron Microscope
TPC	Total Phenolics Content
v/v	Volume/ volume
WAI	Week After Inoculation
WPL	Weight Percent Loss

CHAPTER 1

GENERAL INTRODUCTION

Oil palm (*Elaeis guineensis*) is the most significant commodity crop in the Malaysian agricultural sector. The rapid increase in area planted with oil palm from 300,000 ha in 1970 to 5.0 million ha in 2011 (Sime Darby, 2012) shows the economic importance of this crop. According to Sime Darby (2012), the export earnings from oil palm products reached about RM80.41 billion in 2011.

However, a soil borne fungus identified as *Ganoderma boninense*, which induced basal stem rot (BSR) disease in the oil palm's trunk, ruins thousands of hectares of plantations in Southeast Asia almost every year. A direct loss of oil palm trees when the disease causing infected palm trunks to break at the base. About (30 to 70%) losses of oil palm occurred from 4.85 million hectares of the total oil palm area, due to BSR disease which have an adverse effect on the oil palm industry. This disease is conceived to be the most serious oil palm disease in Malaysia and other parts of South East Asia (Susanto, 2009).

In Peninsular Malaysia, most of the oil palm estates discover this disease in the second or the third replanting. About 90% of the estates in West Malaysia have been infested with *G. boninense* (Khairuddin and Chong, 2008). In Sabah and Sarawak, most of the estates are in their first cycle of planting, allowing for alternative hypotheses for the lower relative incidence of pathogen attacks in these regions. Thus, *G. boninense* has a significant effect on the lifetime of affected trees and adverse effect on yield (Corley and Tinker, 2003).

The *G. boninense* was found to infect 4 to 5 years old palms more drastically compare than 1 to 2 years old (Ariffin *et al.*, 2000). The symptoms of oil palm disease are withering, yellowing of fronds followed by necrosis on one side of older fronds. Necrosis occurring in the lower leaves extend progressively to younger leaves of the crown. When the foliar symptoms were observed, at least one-half of the basal stem has been killed by the fungus. Young palms can take 6 months to 2 years to die after the first symptoms, but mature palms take 2 to 3 years.

Contact with living palm roots with colonized debris within the soil has been believed as a primary infection of palms by *Ganoderma* species (Idris *et al.*, 2002). Secondary spread of inoculum was assumed to be in contact with roots and roots of living palms (De Oliveira *et al.*, 2005). Palms between 7 to 15 years old are also infected, beside, the very old palms and this is the peak age of fruit production and effort should be made to control disease spread out and losses of trees. During its earliest point of entry very little information about the fungus was realized.

Unfortunately, the external symptoms become visible when the disease is too far in advanced. Thus, BSR disease seems a silent time bomb to oil palm trees.

Field controls of BSR by contact chemicals have not been very successful even *in vitro* efficacy of fungicides have been reported against *G. boninense* (Soepena *et al*., 2000). In addition, drenching of fungicides failed to control effectively (Chung, 1990). Control by physical methods such as clean clearing and tree surgery has had but transient effects, although there is testify that BSR can be dealt if all the disease inoculum is removed before planting or replanting the crop. Besides, biological control of *Ganoderma* involves the use of antigonistic fungus, e.g. *Trichoderma* sp. and the use effective micro-organisms (Illias and Abdullah, 1999; Sariah, 2003; Siti Muslimah *et al*., 2010). The results have been variable. To date, no commercial application of biocontrol agent is practiced. Therefore, treatment such as nutrient and plant hormone should be applied in the selected concentration at the seedling stage in order to make them resistant towards BSR disease. Thus, it is important to develop disease control methods that are good compatible with biological control, like using agents that elicit natural inducible plant defences at low concentrations.

The nutritional status of a plant has a major impact on disease susceptibility, and this has been responsible for suppressing a variety of diseases (Engelhard, 1989). Previous studies tested calcium nitrate suppressed BSR symptoms on clonal materials (Sariah and Zakaria, 2000) and it is found that copper played a significant role in organic and conventional systems for battling some fungal diseases. *Ganoderma* wilts disease caused by *G. applanatum* and *G. lucidum* in coconut trees has been controlled by copper-based fungicides (Nambiar *et al*., 1992). Lately, salicylic acid a naturally occurring plant hormone had attention after it was found, it can induce resistance to pathogens and abiotic stress tolerance in plants (Gautam and Singh 2009; Pieterse *et al*., 2009; Ramirez *et al*., 2009).

Calcium, magnesium, copper, carbon, salicylic acid and nitrogen are decisive variables for plants and modifying these as a control method may keep *Ganoderma* attack. These nutrients and plant hormone could conceivably be added by soil application to control the disease at the same time utilized as fertilizers. Applied fertilizers comprise some of these minerals to control diseases and to improve the development of the oil palm. The existing literature is silent on controlling *G. boninense* although the uses of soil amendments have been suggested by Sariah and Zakaria (2000). Due to the increasing economic impact of this disease in Malaysia, effective and viable management strategies need to be established. In this research, first, an experiment about in *vitro* and block study, followed by glass house trials and eventually access the plant secondary metabolites. Hence the hypothesis of this study is; nutrients and plant hormone have the potential to suppress BSR development in oil palm seedlings.

This research is conducted with the following objectives:

1. To assess *in vitro* effect of salicylic acid, calcium and copper ions on growth and sporulation of *Ganoderma boninense*.
2. To investigate the induction of systemic acquired resistance (SAR) of oil palm seedlings after being treated with calcium, copper ions and salicylic acid.
3. To assess the plant secondary metabolites in oil palm seedlings after being treated with calcium, copper ions and salicylic acid.

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