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PRELIMINARY SCREENING FOR POTENTIAL INHIBITORS FOR *Ganoderma boninense*

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THIS DISSERTATION IS SUBMITTED AS PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE WITH HONOURS

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DECLARATION

I hereby declare that this dissertation is the result of my own work except for quotations of citations which have been fully acknowledged.

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ABSTRACT

Basal stem rot which is caused by *Ganoderma boninense* is the most serious disease faced by oil palm *Elaies guineensis* in Malaysia. The study was conducted to investigate any potential antimicrobial properties in *E. guineensis* roots after elicitation by UV radiation and any antagonist activity of *Aspergillus niger* against *Ganoderma boninense*. *In vitro* antagonistic activity assays by using dual culture technique was done to test the antagonistic activity of *Aspergillus niger* to *Ganoderma boninense*. The result did not show any antagonism of *Aspergillus niger* against *Ganoderma boninense*. Twenty oil palm seedlings roots were used and 140g of oil palm roots were elicited by UV light with 254nm. The plant material from elicited oil palm root with different concentration (0.86 mg/ µl, 0.83 mg/ µl, 0.96 mg/ µl and 0.85 mg/ µl) was extracted. The paper disc diffusion method was used to screen antimicrobial activity of the extracts. The acetone extracts were loaded on each paper disc (6mm) and air dried to evaporate the acetone. Then, the tested microorganism is *Aspergillus niger*. The result showed no inhibition zone around the discs. This indicates that there was no antimicrobial compound present in the crude extracts. Insufficient concentration of any potential antimicrobial compound from the crude extracts caused the failure of crude extracts to show a promising antimicrobial activity. The study did not show any antagonistic activity of *Aspergillus niger* against *Ganoderma boninense* and no antimicrobial compound was present in the crude extract.

ABSTRAK

Penyakit reput pangkal disebabkan oleh *Ganoderma boninense* adalah penyakit yang paling serious dihadapi oleh kelapa sawit di Malaysia. Kajian ini telah dijalankan untuk mengkaji sebarang kehadiran kompound antimikrob pada akar kelapa sawit selepas dirawat oleh UV dan belajar aktiviti antagonistik kulat *Aspergillus niger* terhadap *Ganoderma boninense*. Aktivi *in vitro* antagonistik telah dijalankan dengan menggunakan teknik *dual culture*. Tiada kesan antagonistik ditunjukkan di antara *Aspergillus niger* terhadap *Ganoderma boninense*. Sebanyak 20 anak pokok kelapa sawit telah digunakan dan 140g akar kelapa sawit telah dirawat dengan UV. Ekstraksi daripada akar kelapa sawit yang menerime rawatan UV menghasilkan kepekatan berlainan iaitu 0.86 mg/ μl, 0.83 mg/ μl, 0.96 mg/ μl and 0.85 mg/ μl. Kaedah penyebaran disk kertas telah digunakan untuk menguji aktiviti antimikrob pada ekstrak mentah. Ekstrak mentah dengan kepekatan tersebut telah diserapkan ke dalam setiap disk kertas. Mikroorganisma yang diuji ialah *Aspergillus niger*. Keputusan aktiviti antimkrob menunjukkan tiada kehadiran zon rencatan di sekeliling disk kertas. Ini menunjukkan tiada sebarang kompound antimikrob tidak terkandung dalam ekstrak mentah. Kepekatan kompound antimikrob ekstrak yang terlalu rendah menyebabkan ekstrak mentah tidak dapat menunjukkan aktiviti antimikrob. Kajian ini gagal menunjukkan sebarang aktiviti antagonistik di antara *Aspergillus niger* terhadap *Ganoderma boninense* dan tiada kehadiran sebarang kompound antimikrob dalam ekstrak mentah.

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ABBREVIATIONS

BSR	Basal Stem Rot
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
GSM	*Ganoderma* Selective Media
PIRG	Percentage Inhibition Radial Growth
Spp.	Species

UNITS

cm	centimeter
g	Gram
Kg	Kilogram
μl	microliter
°C	Celsius Degree

CHAPTER 1

INTRODUCTION

1.1 Research Background

Oil palm (*Elaies guineensis* jacq.) is truly " a golden crop of Malaysia" since it generates profitable export earning for the country and produces numerous primary products which include processed palm oil, palm kernel oil, palm kernel cake and oleochemicals as well as vast array of uses and products to market nowadays (Dahlan, 2000). Oil palm planted area in Malaysia has been expanding from 2,692,286 hectares in 1995 to more than 3.5 millions hectares in 2004. However, similar to the cultivation of others crops, oil palms are plagued with disease (Chung, 2005).

The basal stem rot (BSR) disease caused by *Ganoderma boninense* is considered the most serious disease faced by oil palm in Malaysia (Benjamin, 1995). Oil palm has an economic life span of 25-30 years (Dahlan, 2000). Basal stem rot can kill more than 80 percent of stands by the time they are half-way through normal economic life. For many years, basal stem rot was considered to infect older palms over 25 to 30 years due to senescence factor although incidence of the disease in young palms had also been periodically recorded. When palms are replanted after coconuts and oil palms during the mid-1950s in the Far East, the disease began to affect large number of palms of 10 to 15 years after planting with symptoms also being observed in much younger palms far more frequently than previous (Turner, 1981). The incidence increase rapidly and the disease level can reach 40 to 50 percent (Gurmit, 1991). High incidence of BSR resulted in economic losses due to no yield from dead palms and significantly reduced weight and number of fruit bunches (Turner, 1981).

There is currently no effective cure for *Ganoderma* infection in an existing stand. Preventive and ameliorative treatments which are commonly carried out show various degrees of effectiveness. The use of a mycorrhizal product was tested on newly planted palms on deep peat but its effect on controlling the disease is not conclusive. Soil mounding where the palm bole and adjoining root mass from diseased palms are removed away from new planting point by an excavator have also been tried. Soil mounded palms normally start to develop new roots about six to eight months after soil mounding. With a reduction of about 20% fallen palms and full fruits bunches yield increase of about 50%, the practice of soil mounding *Ganoderma* infected palms is cost-effective. However, soil mounding is more preferable to carry out during dry season and the cost and time of soil mounding far exceeds removing the diseased palm (Lim, 2005).

There are some management practices such as clean clearing which involves methods of disposing old stands to reduce inoculums. Two basic systems are involved in clean clearing. In the first system, the palms to be felled are poisoned, root pruned and then pushed over by a bulldozer then followed by root raking to remove root discs and hole tissues. The second is palms are felled by an excavator without poisoning, boles extracted and the trunks shredded into smaller pieces and all tissues completely burnt when dry. However, disease level is still sufficiently high despite clearing old stands (Hartley, 1967).

Crop rotation is also often practiced to control soil-borne diseases but perennial crops like oil palms, the long cropping cycle and the limited choice of economically viable short-term alternative makes crop rotation not a suitable solution. A study where soybean were planted in *Ganorderma* infected ex-oil palm area in 1974, incidence of BSR showed no difference when compared to direct oil palm to oil palm replant (Gurmit , 1991). Chemical control is also applied to control *Ganoderma*. There are a number of fungicides which are strongly inhibitory towards *Ganoderma spp.* especially organo-mercury formulations and injection of potassium hydroxyquinoline sulphate has been tried. However, attempts by trunk injection have failed due to difficulty on precise placement of the fungicide (Turner, 1981). The systemic fungicides which include tridemorph, carboxin, triadimefon, triadimenol, flutriafol, propiconazole and difenocozole are also applied to control BSR but these fungicides have not shown promising results (Gurmit, 1991).

Control of Basal Stem Rot has involved cultural techniques, mechanical and chemical control. The failure to control the disease because *Ganoderma* has various resting stages such as melanised mycelium, basidiospore and pseudosclerotia. Biological control and planting resistant material may be alternatives. Present studies conducted at Indonesian Oil Palm Research Institute showed that disease incidence was lower in a field treated with biological agents than in untreated fields. Biological agents (*Trichoderma harzianum* and *Gliocladium viride*) were found to significantly reduce BSR incidence as shown in Table 1.0

Table 1.0: The effect of biocontrol agents on Basal Stem Rot incidence

Treatment	Disease incidence (%)
Control	18.06
Trichoderma harzianum	0.00
Gliocladium viride	0.00
Bacillus sp.	9.72
Trichoderma harzianum + *Gliocladium viride* + *Bacillus sp.*	8.33

Source : Susanto *et al.*, 2005

To identify the possibility of oil palm resistance against *Ganoderma boninense* in certain circumstances need further investigation in detailed studies. However, if resistance in oil palm against *Ganoderma* is possible, it may contribute to tackling the problem of *Ganoderma*. Therefore, it is necessary to determine if any antimicrobial compounds are present in *Elaies guineensis*.

1.2 Objectives

The study was conducted with the following objectives:

(i) To investigate any potential antimicrobial properties in *Elaies guineensis* roots after abiotic elicitation.

(ii) To verify antagonism activity of *Aspergillus niger* against *Ganoderma boninense*.

CHAPTER 2

LITERATURE REVIEW

2.1 The Oil Palm

Elaeis guineensis Jacq. which is commonly known as oil palm is the most important species in the genus Elaeis which belongs to the family of Palmae. The oil palm is an erect monoecious plant that produces separate male and female inflorescences. In the past, oil palm was wind and insect pollinated. Nowadays, assisted pollination is a standard management practice in plantations. Harvesting commences about 24 to 30 months after planting and each palm can produce between eight to 15 fresh fruit bunches (FFB) per year weighing about 15 to 25 kg each, depending on the planting material and age of the palm. Each FFB contains about 1000 to 1300 fruitlets; each fruitlet consists of a fibrous meoscarp layer, the endocarp (shell) which contains the kernel. Present day, planting materials are capable of producing 39 tonnes of FFB per ha and 8.6 tonnes of palm oil and actual yields from good commercial plantings are about 30 tonnes FFB per ha with 5.0 to 6.0 tonnes oil. At the national level, the average FFB yield in 2001 was 19.14 tonnes while palm oil productivity was 3.66 tonnes per ha (Teoh, 2002).

With rapid expansion of oil palm cultivation recent years especially in Sarawak and Sabah, the incidence of pests such as leaf-eating caterpillars, rhinocerous beetles, termites, rats and *Ganoderma* Basal Stem Rot (BSR) have also increased. BSR caused by *Ganoderma boninense* is a major disease of oil palm especially those planted on peat and coastal soils (Lim, 2005). The disease is a limitation to production and can become catastrophic at times when the palms are reaching their most productive period (Sariah *et al*, 1994).

2.2 *Ganoderma boninense*

Ganoderma boninense is from the order Aphyllophares (Sumbali, 2005). *Ganoderma* species cause root and stem rot disease on a range of palms and perennial beverage crops in the humid tropics. *Ganoderma boninense* is a particularly important pathogen of oil palm in south-east Asia which causes basal stem rot disease. The earliest visible symptoms occur on foliage. Generally, the first foliage symptom is the presence of excessive spear leaves which are normally produced at a rate of 2 to 3 per month. The foliage is also much paler green in color than healthy palm (Turner, 1981). By the time foliar symptoms appear, it is usually found that at least one-half of the cross-section area at the stem base has been killed by the pathogen. This severely restricts the supply of water and nutrients to the aerial part, so the external symptoms are wilting and malnutrition (Hillocks *et al*, 1997). The disease produces a dry rot of internal tissues. Characteristic internal symptoms are light brown rot of both stem and bole with irregularly shaped dark bands which contains swollen chlamydospore-like hyphal cells.

At the edge of the infected area is an irregular yellow zone which appears as a result of defense mechanism of the palm. Roots of infected palms are very friable and their internal tissues become very dry and powdery. Cortical tissue is brown and the stele is become black (Hartley, 1967).

Ganoderma fructifications develop either at the stem base or occasionally on infected roots close to the palm (Turner, 1981). Sporophores usually develop as the decay advances (Gurmit Singh, 1991). The time of sporophore appearance depends on the internal rotting extending to the stem periphery, so there is a wide variation in palm age when *Ganorderma* is seen. The sporophores first appear as small white buttons of tissues on the leaf bases or on the stem. These develop rapidly into familiar bracket shape. The mature sporophores are vary in shape, size and colour. The upper surface of the sporophores varies from light to dark brown with a white margin. The under surface is white and perforated by numerous pores (Turner, 1981).

There are a numbers of factors that cause basal stem rot on oil palm. High incidence of basal stem rot always happens when replanting oil palm from coconut and oil palm. *Ganoderma* is a facultative parasite capable of living as a saprophyte on rotting stumps and roots (Hillocks *et al*, 1997). When a suitable host like oil palm becomes available it will colonize it and establish a parasitic relationship. Where oil palm are replanted from oil palm and coconut, there will be a build-up of *Ganoderma* inoculum on stumps and trunk tissues. Replanted oil palm will be infected by root contact with stumps (Turner, 1981). The attached palm roots will ultimately become colonized by *Ganoderma* as well as spread of the disease within a field is mainly through root contact between healthy palm and diseased palm (Miller, 1999). Environmental factors also have been suggested as influencing the incidence of basal stem rot. Basal stem rot on inland soils is not as high as in coastal areas. The coastal areas are mainly clays, silty clays or clay loams. These are heavy soils with poor internal drainage and a high water retention capacity. The water table during rainy season is usually high. Therefore, high moisture condition in soils will attract *Ganoderma* to attack oil palms (Turner, 1981). Soil pH and conductivity can also affect BSR. Incidence has been observed to be lower on acid sulphate soils and on recent marine soils where the salt content is high (Gurmit, 1991).

2.3 Antimicrobial Compounds

Higher plants are routinely exposed to microorganisms, both above and below the ground. Fortunately, only a handful of them cause diseases. The microbe fails to establish itself due to lack of activation of pathogenicity functions or highly effective plant defense mechanisms. For microbes infected plants, evidence of an intense host-pathogen interaction is present and eventually result in restriction of the pathogen. In this case, host tissue often display activated defense functions that produce antimicrobial compounds, enzymes and structural reinforcement that may limit pathogen growth (Mert-Türk, 2002).

Antimicrobial compounds from plants are broadly classified into two categories: phytoanticipins and phytoalexins (Mansfield, 1994). Phytoanticipins such as jasmonic acid, avenacins, phenolics and terpenoids are low molecular weight and pre-infectional plant metabolites which are normally present in concentrations high enough to inhibit most microbes. In other plant species, the concentration of the antimicrobial substances normally may be low but may increase enormously after infection in order to combat attack by microorganisms (Grayer *et al*, 1994). Phytoalexins are defined as low molecular weight, antimicrobial compounds that are synthesized and accumulated in plants after exposure to microorganism and abiotic agents. Phytoalexins represent one component of a battery of induced defense mechanisms used by plants including lytic enzymes such as chitinase and glucanases, oxidizing agents, cell wall lignification and a number of pathogenesis-related (PR) proteins and transcripts of unknown functions (Mert-Türk, 2002).

Information and understanding of the molecular interaction between the causing agents and either susceptible or tolerant varieties including the basic physiology of palm-pathogen interactions is little due to factors of the long life cycle and the difficultness to perform studies. Studies involving the treating of coconut calli with chitosan have been done to identify and characterize both signal transduction intermediates and genes products involved in biochemical and molecular defense against pathogens. By using RNA differential display, the result indicated that some gene products related to receptors which activate certain defense mechanism such as strengthen of cell wall and regulation of oxidative burst. These results support the notion that elicitation of palm tissues cultivated *in vitro* constitutes a suitable alternative to characterize further the interactions between this tropical crop and its associated pathogens (American Society of Plant Biologist, 2006).

2.4 Biological control of soil-borne Plant Pathogen by Antagonistic Fungi

With respect to biological control, antagonism is the interaction of a biocontrol agent with a plant pathogen. Mechanisms of antagonism are classified into 3 categories (Baudoin, 1988):

a) Antibiosis

Antibiosis is the inhibition or destruction of one organism by a metabolic product of another. These products may be relatively small toxic molecules, volatiles or lytic enzymes (Reuveni, 1995). It may reduce or prevent germination of fungal propagules, invoke lysis or inhibit growth after germination. A study conducted by Howell 1979 showed that *Pseudomonas fluorescens* produced antibiotic pyrrolnitrin to reduce *Rhizoctonia solani* on cotton seedlings.

b) Hyperparasitism

Hyperparasitism and predation, the parasitism or predation of one organism by or on another (Baudoin, 1988). An example of parasitism in soil is extensive attack of *Rhizopus oryzae* by *Syncephalis california*. The parasitic did not destroy the mycelium but caused hyphae to swell and significantly reduced sporulation.

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