UNIVERSITI PUTRA MALAYSIA

PHENOLIC COMPOUNDS IN ENHANCING PHYSICAL BARRIER AND SUPPRESSING GROWTH OF Ganoderma boninense PER71

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By

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PHENOLIC COMPOUNDS IN ENHANCING PHYSICAL BARRIER AND SUPPRESSING GROWTH OF *Ganoderma boninense* PER71

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Chair : Assoc. Prof. Khairulmazmi Ahmad, PhD
Institute : Plantation Studies

Oil palm (OP) is one of the dominant plantation crops that bring so much abundance to many countries in terms of economic value, quality goods, and food products. However, the production of oil palm is hindered to a great extent, facing a devastating issue which started to decline in the cultivation of palm oil caused by basal stem rot (BSR) disease. *Ganoderma boninense* is the white-rot basidiomycetes, the primary causal pathogen of BSR. This pathogen invades via roots and degrades the lignin and cellulose components. Many approaches are available in controlling BSR, although, there is no effective method to suppress *G. boninense* completely. An alternative way to control the disease is to safeguard the physical barriersand inhibit the production of ligninolytic and hydrolytic enzymes by pathogen. Gallic acid (GA), thymol (THY), propolis (PRO), and carvacrol (CARV) were used to study the effects of phenolic compounds on the growth of *G. boninense* PER71, as well as to determine how well they may suppress the development of ligninolytic and hydrolytic enzymes. These four phenolic compounds with different concentrations were able to inhibit the growth of *G. boninense* PER 71 at different levels. Based on the study, mycelia grown on media containing the phenolic compounds showed greater inhibition at the highest concentration (GA 8 mg/ml, THY 0.25 mg/ml). Significant differences ($p<0.05$) were observed and 94% inhibition was exerted by GA. The mycelial morphology under scanningelectron microscopy (SEM) and high-resolution transmission electron microscopy (HR-TEM) revealed that phenolic compounds have a greater impact on mycelial structure, cell wall and cell membrane. The fungal membrane integrity and permeability tested with a flow cytometer exerted severe damage to the mycelium treated with GA and THY and reported the highest amount of sugar (monosaccharides-glucose) and electrolyte leakage. The ergosterol content present in the *G. boninense* PER71 was very much interrelated with the morphological disruptions. Furthermore, to justify the findings, suppression of hydrolytic and ligninolytic enzymes secreted by *G. boninense* PER71 with the application of phenolic compounds were determined.

The phenolic compounds had shown inhibitory effects and a significant ($p<0.05$) decrease in the secretion of enzymes. Among the phenolic compounds tested, GA was the most effective compound in suppressing the hydrolytic and ligninolytic enzymes followed by THY. The PRO and CARV had some suppression on these enzymes but were not as effective as the other two. The antifungal efficacyof the phenolic compounds during the studies indicated the consistency in eliminating the *G. boninense* PER71. Moreover, the effectual mode of delivery of the phenolic compound (encapsulation of beads) and characterization were performedto validate the structure, quality and release of the phenolic compound via SEM and High Performance Liquid Chromatography (HPLC). The freshly encapsulated beads showed >90% of inhibitory effect on *G. boninense* PER71. This study proposed that GA and THY could be developed further as naturally occurring phenolic compounds and deliver new strategies to eradicate the *G. boninense* and finally could be used to control the BSR disease.

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

SEBATIAN FENOLIK DALAM MENINGKATKAN KERINTANGAN FIZIKAL DAN MENINDAS PERTUMBUHAN *Ganoderma boninense* (PER 71)

Oleh

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Kelapa sawit ialah salah satu tanaman ladang utama yang membawa banyak kemakmuran kepada banyak negara dari segi nilai ekonomi, barangan berkualiti, dan produk makanan. Walau bagaimanapun, pengeluaran kelapa sawit banyak terhalang dan berdepan dengan isu dahsyat yang mula merosotkan pengeluaran sawit oleh penyakit reput pangkal batang (BSR). *Ganoderma boninense* adalah kumpulan basidiomycetes, patogen utama penyakit BSR. Patogen ini menyerang melalui akar dan memusnahkan komponen lignin dan selulosa. Selain itu, terdapat beberapa kaedah sedia ada untuk mengawal BSR, tetapi tiada kaedah yang benar-benar berkesan untuk mengawal *G. boninense* PER71. Salah satu kaedah alternatif untuk mengawal BSR adalah dengan melindungi halangan fizikal dan menghalang pembinaan dan pengeluaran enzim oleh patogen. Oleh itu, penggunaan sebatian fenolik seperti 'Asid Gallic, Thymol, Propolis dan Carvacrol' telah dijalankan terhadap *G. boninense* PER71 untuk menilai potensi mereka di peringkat selular dan memusnahkan penghasilan enzim perosak. Keempat-empat sebatian fenolik dengan kepekatan yang berbeza ini mampu menghalang pertumbuhan *G. boninense* PER71 pada tahap yang berbeza. Selain itu, berdasarkan kajian, miselia yang ditumbuhkan dalam media yang mengandungi sebatian fenolik menunjukkan perencatan yang lebih besar pada kepekatan tertinggi. Berdasarkan statistik, perbezaan ketara ($p<0.05$) telah diperhatikan dan 94% perencatan dilakukan oleh asid gallic. Struktur morfologi miselium di bawah pengimbasan mikroskop elektron (SEM) dan mikroskop electron penghantaran resolusi tinggi (HR-TEM) mendedahkan bahawa sebatian fenolik mempunyai kesan yang lebih besar terhadap struktur miselium, dinding sel dan membran sel. Integriti dan kebolehtelapan membran kulat yang diuji dengan sitometer aliran menyebabkan kerosakan teruk pada miselium yang dirawat dengan GA dan THY dan mencatatkan kebocoran gula (monosakarida- glukosa)dan elektrolit yang paling tinggi. Kandungan ergosterol yang terdapat dalam *G. boninense* PER71 sangat berkait rapat dengan gangguan morfologi. Tambahan pula,untuk mewajarkan penemuan, penindasan enzim hidrolitik dan

ligninolitik yang dirembeskan oleh *G. boninense* PER71 dengan penggunaan sebatian fenolik telah ditentukan. Sebatian fenolik telah menunjukkan kesan penghambatan dan penurunan ketara ($p < 0.05$) pada rembesan enzim. Antara semua sebatian fenolik GA adalah sebatian yang paling berkesan dalam menekan dan mengurangkan enzim hidrolitik dan ligninolitik diikuti oleh THY. Propolis dan carvacrol mempunyai sedikit penindasan pada enzim ini tetapi tidak berkesan seperti dua yang lain. Keberkesanan antikulat sebatian fenolik semasa kajian menunjukkan konsistensi dalam menghapuskan *G. boninense* PER71. Selain itu, kaedah penghantaran berkesan sebatian fenolik (enkapsulasi) dan pencirian telah dilakukan untuk mengesahkan struktur, kualiti dan pelepasan sebatian fenolik melalui SEM dan Kromatografi Cecair Prestasi Tinggi (HPLC). Manik-manik yang disediakan secara segar menunjukkan> 90% kesan penghambatan terhadap *G. boninense*. Kajian ini mencadangkan bahawa GA dan THY mempunyai keupayaan untuk dibangunkan lagi sebagai sebatian fenolik semulajadi dan menyampaikan strategi baharu untuk membasmi *G. boninense* PER71 dan mengawal penyakit BSR dengan lebih berkesan.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment 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

BSR	Basal Stem Rot
WRF	White Rot Fungi
GA	Gallic Acid
THY	Thymol
PRO	Propolis
CARV	Carvacrol
SEM	Scanning Electron Microscopy
HR-TEM	High-Resolution Transmission Electron Microscopy
HPLC	High Performance Liquid Chromatography
PI	Propidium Iodide
DiBAC34	Dibutylbarbituric acid
Lac	Laccase
LiP	Lignin peroxidase
MnP	Manganese Peroxidase
Cl	Cellulase
Xy	Xylanase
Amy	Amylase
CWDE	Cell wall degrading enzyme
PBS	Phosphate buffered saline
LC	Lethal concentration
St	Starch
SA	Sodium alginate
MMT	Montmorillonite
TGA	Thermogravimetric analysis

OP	Oil palm
PIRG	Percentage Inhibition of Radial Growth
ROS	Reactive Oxygen Species

CHAPTER 1

INTRODUCTION

1.1 Background

Oil palm is an important and native plantation crop of Malaysia that upholds the major economic value of the country. It is a native crop of Africa and was introduced in Malaysia in 1870 by the British Colony during their ruling. Oil palm was an ornamental plant during that time, and it has been commercialized in Selangor for plantation in 1870 (Alam et al., 2015). The oil palm brought up Malaysia in achieving the biggest and leading country for oil palm output as it is gaining unbudgeable concession. The production contributed by Malaysia and Indonesia is now about 85% of world production and contributes 30% in world edible oil demand (MPOC, 2020). This demand is dominating the rapeseed, sunflower and soybean which are other oil-bearing crops. Therefore, oil palm is now labelled as the 'Golden Crop' of Malaysia.

According to statistics records, the outrageous production of oil palm was 70.5 million metric tons in 2018 (Global production volume palm oil, 2012- 2020 (Statista, 2020). According to Malaysia Palm Oil Production by year 2020, there was an unexpected decline in growth rate of around 4.81% which is about. This led to a decrement in yield loss annually (MPOB, 2015). This scenario was due to various factors such as climatic conditions, tree ageing, low yield crop and various diseases that influenced the oil palm growth concurrently the production of it. Malaysia is impelled to increase the oil palm production to meet the on-growing world's oil palm demand. This was proved not only by providing sufficient nutrients, implementation of new plantations, usage of high yield varieties, and replantation of old trees with a proper management system but also by more systematic and intensive pest and disease control factors to fill the production gap and produce healthy oil palm to support the demand (Jalani et al., 2002). Despite all many pests and diseases that are hindering the healthy growth of oil palms such as *Oryctes rhinoceros* beetle, bagworm, upper stem rot (USR), basal stem rot (BSR) and many others. To the current statement, palm oil production is enormously affected by the BSR disease (Idris et al., 2010).

Phenolic compounds present in oil palm (*Elaeis guineensis* Jacq.) trunk are essential bioactive compounds found to be potential as an antifungal agent. Studies on wood vinegar as antifungal and anti-termite properties showed positive outcomes. Therefore, naturally occurring phenolic compounds in the oil palm trunk are a good start in defencing pathogens that causes plant diseases and pest attacks (Oramahi et al., 2018). Phenolic compounds from the plants are another point of interchange to increase the antifungal efficacy in oil palm.

1.2 Problem statement

The BSR disease in oil palm is caused by a white-rot fungus *Ganoderma boninense* which is basidiomycetes (Bivi et al., 2016). It was discovered that this fungus posed the greatest destructive threat to countries that grow in oil palm, particularly Malaysia. The most virulent strain of *G. boninense* to Rees et al., (2009). Basal stem rot (BSR) is the most destructive disease and initially was identified by Thompson in 1931 and found to cause a serious impact on the economy of Malaysia's oil palm production (Siddiqui et al., 2021).

The more the disease incidence, the more the structural deformities of oil palm trees such as depletion in the number of bunches, weight of fruits, and the number of fronds to an extent the production can reach the zero percentage yield in the scale (Chong et al., 2012; Rees et al., 2009). During the initial stage of research, it was recorded that only aged plants were infected with *G. boninense* but later the researchers got to know that even seedlings of the early stage were detected with this pathogen (Naidu et al., 2015). The research was proven that the invasion of the pathogen is very devastating and it is due to the contact with the infected roots with healthy roots of oil palm is the mode of infection. Not only that, the dispersal of basidiospores in the field of oil palm is another alternative mode of infection and is highly pathogenic (Rees et al., 2007; Paterson, 2007; Surendran et al., 2017).

Various methods to control and manage the BSR disease *in-vitro* and *in-vivo* were studied. However, it is still insufficient enough to address the disease control effectively. Various disease control methods were introduced to the plantation and the success rate was much constant in helping the oil palm to prolong the life span and increase the yield from infected oil palm. Examples of the control methods carried out are soil mounding (pile of organic matter, inorganic matter, debris and more heaped for protection), usage of Hexaconazole fungicide, clean and clear practices, surgery to excise the diseased part and biological control such as *Trichoderma sp* (Ariffin et al. 2000; Chung 2011; Ferreira et al. 2007). However, some of these methods are expensive, inefficient, developed resistance issues, cause environmental instability due to nitrous oxide production, ability to produce secondary metabolites and toxins and some are only able to control up to 70% of the disease scale (Idriset al., 2004).

Understanding the mechanism of pathogenesis could aid in developing new strategies to control BSR. It is well established that the successful degradation of lignocellulose components (lignin and cellulose) in the plant is the absolute feature of the white-rot fungus (Martinez et al., 2005). Spending more energy in degrading the lignin and gaining more energy to approach the cellulose components for degradation. The rate-limiting step of this infection process is the degradation of lignin (Fernanda et al., 2021). Therefore,

inhibition and suppression of *G. boninense* is the major factor of this rate-limiting step. Degradation of lignin and cellulose simultaneously are patterns of degradation followed by white-rot fungi (Paterson et al., 2009). Moreover, there are two groups of enzymes involved in the degradation of cellular structure which are ligninolytic enzymes and cellulolytic enzymes. These lignocellulolytic enzymes contribute a major role in the disease progression of BSR disease.

To boot, it is advisable to find a substitute or alternative method found in nature such as biomolecule that helps in inhibition and suppression of lignocellulolytic enzymes of *G. boninense* to completely suppress the BSR disease and induce resistance in oil palm. As suggested by Surendran et al. (2018) the biomolecule must be small in size and low molecular weight to travel towards and through the infection area. Not only that, it has to be resistant to auto-oxidation (Surendran et al., 2018).

Phenolic compounds are the compounds that can help to overcome this issue and meet the demand in this globalisation scenario. Phenolic compounds are activated during any environmental conditions such as abiotic and biotic stresses (Surendran et al., 2017; Zahrani et al., 2020). The hydrophobic nature of these compounds assures the preferential partition into lipid membrane and evolves the antifungal efficacy towards the invading pathogen. It is the key element of biological activity due to its molecular structure and is significantly efficient (Pavela et al., 2005). They are bound tightly to the lignin structure and provide protection and strength to the cell wall structure (Hammerschmidt, 2005; Zabka et al., 2009).

1.3 Hypothesis

Phenolic compounds could alter the morphological and cellular structure of *G. boninense* PER71 mycelia and inhibit the production of ligninolytic and hydrolyticenzymes.

1.4 Research objectives

1. To evaluate the potential of phenolic compounds against *G. boninense* PER71 and determine the alterations in the fungal mycelium properties.
2. To evaluate the production and inhibition of ligninolytic and hydrolytic enzymes from *G. boninense* PER71 under the influence of phenolic compounds.
3. To develop and determine the efficacy of encapsulated Gallic acid in suppressing the growth of *Ganoderma boninense* PER71.

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