UNIVERSITI PUTRA MALAYSIA

SUPPRESSION OF LIGNOHEMICELLULOSIC DEGRADATION CAUSED BY Ganoderma boninense IN OIL PALM DURING BASAL STEM ROT ESTABLISHMENT USING BENZOIC ACID

ROZI FERNANDA

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By

ROZI FERNANDA

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

"Special dedication to my late father; Nazaruddin bin Mansyur, mother; Netti Hernawati, brother and sisters; Rahmat Hidayat, Lidya Widya, Nurul Nadia, Jerli Yanita for their endless love, prayers, motivation, continuous support, and encouragement throughout my study"

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

The palm oil industry is one of the most successful industries in the world in terms of agricultural crops towards economic growth and development in its producing countries. Indonesia and Malaysia have become the main producers of palm oil dominated approximately 85% of the total world production of palm oil. However, a decline in the production of this oil has been observed that occurred due to the presence of white-rot fungus *Ganoderma boninense* the causal pathogen of basal stem rot (BSR) disease causing the degradation in the lower part of the trunk. *G. boninense* degrades the major components of wood in the lower trunk makes a loss of its support to the palm, which eventually caused the palm to collapse. Inefficacy in controlling this disease could be the lack of understanding of how the pathogen establishes itself in the host and degrade the structural components of oil palm trees. One of the potential compounds that can be used to suppress the growth of *G. boninense* is benzoic acid (BA). BA belong to aromatic carboxylic acid group that has antifungal effect against many pathogenic fungi. Therefore, this study aimed to understand a step-by-step mechanism of structural degradation of oil palm by *G. boninense* and the effect of BA on suppression of *G. boninense* growth and enzymatic activities while maintaining the structural components of oil palm wood (OPW). The antifungal activity of BA at different concentrations against the growth and enzymatic activities of *G. boninense* were tested using OPW as the substrate. Further, the use of BA to strengthen the lignin structure in oil palm seedlings was evaluated. BA at a concentration above 5 mM successfully inhibited the growth of *G. boninense* in *in-vitro* study. The mechanism of pathogen suppression by BA was related to the disruption and depolarization of fungal cell membrane lead to the loss in membrane permeability and integrity and finally caused the alteration in morphological, anatomical, and ultracellular structure of fungal mycelia. The study on wood degradation by *G. boninense* in response to BA treatment showed that *G. boninense* simultaneously degrade all the components of OPW. A significant (*P* < 0.05) reduction in wood weight loss and wood chemical component was observed in the BA-treated woodblock at 5mM and above. BA significantly reduced the degradation of OPW depending on its concentration. Microscopic observation of wood inoculated with *G. boninense* showed that the severe damage in wood structure of untreated wood block in compare with treated wood block showing least damage in the wood structure. This was due to the suppression of the ligno-hemicellulolytic activity of *G. boninense* by BA. The observation at the nursery level showed that during the disease establishment, *G. boninense* colonized the plant's root tissue 10 to 14 days after inoculation, while BA-treated seedlings showed the slow disease progression that colonized the root tissues in 7 to 8 weeks after inoculation. BA affect the morphological, anatomical, and cellular structure of *G. boninense* unable them to produce lignohemicellulolytic enzymes for degradation of wood lignohemicellulose. In addition, BA induced the activity of defense-related enzymes that increase lignin biosynthesis in the oil palm seedlings. These findings could be useful in understanding the mechanism of *G. boninense* infection in OPW and contribute to the development of sustainable control of BSR disease.

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

PENINDASAN DEGRADASI LIGNOHEMISELULOSA DISEBABKAN OLEH *Ganoderma boninense* PADA KELAPA SAWIT SEMASA FASA PENUBUHAN PENYAKIT REPUT PANGKAL BATANG MENGGUNAKAN ASID BENZOIK

Oleh

ROZI FERNANDA

April 2022

Pengerusi : Prof. Madya. Khairulmazmi Ahmad, PhD
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Industri minyak sawit adalah salah satu industri yang paling berjaya di dunia dari segi tanaman pertanian ke arah pertumbuhan ekonomi dan pembangunan di negara pengeluar. Indonesia dan Malaysia adalah pengeluar terbesar minyak sawit yang mendominasi kira-kira 85% daripada jumlah pengeluaran minyak sawit dunia. Walau bagaimanapun, terdapat penurunan dalam pengeluaran minyak berlaku disebabkan oleh kulat *Ganoderma boninense,* patogen penyakit reput pangkal batang (BSR) yang menyebabkan degradasi di bahagian bawah batang sawit. *G. boninense* mendegredasi komponen utama kayu yang membuat kehilangan sokongan kepada pokok sawit yang akhirnya menyebabkan pokok sawit tumbang. Ketidakberkesanan dalam mengawal penyakit ini disebabkan oleh kekurangan pemahaman mekanisma tentang bagaimana patogen ini menjangkiti pokok sawit dan penguraian komponen binaan struktur pokok kelapa sawit. Asid benzoik dapat digunakan sebagai salah satu antikulat untuk menindas pertumbuhan *G. boninense*. Asid benzoik tergolong dalam kumpulan asid karboksilik yang mempunyai kesan antikulat terhadap banyak patogen. Oleh itu, kajian ini bertujuan untuk memahami mekanisma penguraian struktur lignohemiselulosa kelapa sawit oleh *G. boninense* dan kesan asid benzoik pada kepekatan optimum untuk menindas aktiviti enzim dan pertumbuhan patogen ini sambil mengekalkan komponen binaan struktur batang kelapa sawit. Aktiviti antikulat dari asid benzoik pada kepekatan yang berbeza terhadap pertumbuhan dan aktiviti enzimasi *G. boninense* telah diuji dengan menggunakan kayu kelapa sawit sebagai substrat. Keberkesanan asid benzoik dalam mengukuhkan struktur lignin di dalam anak benih kelapa sawit juga dinilai. Asid benzoik pada kepekatan di atas 5 mM berjaya menghalang pertumbuhan *G. boninense* dalam kajian in-vitro. Mekanisma penindasan pertumbuhan patogen oleh asid benzoik adalah

berkaitan dengan gangguan dan depolarisasi membran sel kulat yang mengakibatkan kehilangan kebolehtelapan dan integriti membran yang akhirnya menyebabkan perubahan dalam struktur morfologi, anatomi, dan ultraselular miselia kulat. Kajian mengenai degradasi kayu sebagai tindak balas kepada rawatan asid benzoik menunjukkan bahawa *G. boninense* memusnahkan semua komponen kayu kelapa sawit secara serentak. Pengurangan yang ketara ($P \leq 0.05$) dalam penurunan berat kayu dan komponen kimia kayu diperhatikan pada kayu yang dirawat oleh asid benzoik pada 5 mM dan ke atas. Asid benzoik dengan ketara berupaya mengurangkan degradasi kayu kelapa sawit bergantung kepada kepekatannya. Pemerhatian mikroskopik kayu setelah degradasi *G. boninense* menunjukkan bahawa kerosakan teruk dalam struktur kayu yang tidak dirawat di dalam perbandingan dengan kayu yang dirawat menunjukkan kerosakan paling rendah terhadap struktur kayu. Ini disebabkan oleh penindasan aktiviti lignohemiselulolitik *G. boninense* oleh asid benzoik. Pemerhatian di peringkat nurseri menunjukkan bahawa semasa perkembangan penyakit, *G. boninense* menakluki tisu akar tumbuhan pada 10 hingga 14 hari selepas inokulasi, sementara anak benih yang dirawat dengan asid benzoik menunjukkan perkembangan penyakit yang perlahan dalam menjangkiti tisu akar dalam tempoh 7 hingga 8 minggu selepas inokulasi. Asid benzoik menyebabkan kerosakan struktur morfologi, anatomi, dan selular *G. boninense* sehingga tidak berupaya menghasilkan enzim lignohemiselulolitik untuk degradasi komponen kayu sawit. Tambahan, asid benzoik mendorong aktiviti enzim perumah berkaitan sistem pertahanan dengan meningkatkan aktiviti biosintesis lignin pada anak pokok kelapa sawit. Penemuan ini bermanafaat dalam memahami mekanisma jangkitan *G. boninense* dan pertahanan pada kayu kelapa sawit seterusnya menyumbang kepada pembangunan kawalan mampan penyakit reput pangkal batang sawit.

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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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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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LIST OF ABBREVIATIONS

Ø	Diameter
%	Percentage
ANOVA	Analysis of variance
AUDPC	Area under disease progress curve
BA	Benzoic acid
BSR	Basal stem rot
BSA	Basal serum albumin
cm	Centimeter
CMC	Carboxymethyl cellulose
DAI	Day after inoculation
DNS	Dinitrosalicylic acid
DMP	2.6-dimethoxyphenol
DS	Disease severity
DR	Disease reduction
FT-IR	Fourier transform infrared
g	Gram
h	Hours
HR-TEM	High-resolution transmission electron microscope
L	Liter
LiP	Lignin peroxidase
MAI	Month after inoculation
MEA	Malt extract agar
M	Molar
mM	Millimolar

mm	Millimeter
mg	Milligram
Min	Minute
MnP	Manganese peroxidase
MPOB	Malaysian Palm Oil Board
OD	Optical density
OP	Oil palm
OPT	Oil Palm trunk
OPW	Oil palm wood
PAL	Phenyl ammonia-lyase
PDA	Potato dextrose agar
PIDG	Percentage inhibition of diameter growth
POD	Peroxidase
PPO	Polyphenol oxidase
RCBD	Randomized Complete Block Design
rpm	Rotations per minute
SA	Salicylic acid
SEM	Scanning electron microscope
WAI	Week after inoculation
WRF	White rot fungi

CHAPTER 1

INTRODUCTION

1.1 Background

Oil palm (*Elaeis guineensis*) is the most productive vegetable oil-producing crop in the world. It is one of the important contributors to economic development of Malaysia and Indonesia as the major producers of palm oil. Indonesia palm oil responsible for 60% of global production followed by Malaysia at 25% (USDA, 2019). Production of oil palm (OP) increases in Indonesia by 65.9% annually (Purnomo et al., 2020). In 2018, OP has significantly contributed to the Gross Domestic Product (GDP) of Malaysia agricultural sector at 37.9% (Uzir, 2019). To fulfil global needs of this oil, as the main producer, Indonesia expand the land for OP cultivation every year (Purnomo et al., 2020).

Having an significant role in economic growth and development and rapidly increasing global demand for palm oil, the palm oil industry faced some constraints (Susila and Supriono, 2001). The major constraint in OP production is the presence of diseases in many oil palm cultivation (Basiron and Weng, 2004). Having disease and unproductive plants can reduce the yield and productivity of OP that lead to significant economic loss to the palm oil industry (Assis et al., 2016). There are many diseases caused by different types of organism such as insect pests, bacteria, and fungi have been identified to cause the disease in OP plantations. Basal stem rot (BSR) disease caused by basidiomycetes fungus, *Ganoderma boninense* is considered as the most serious disease (Wong et al., 2012) that reduces the production of palm oil (Flood et al., 2010 ; Rakib et al., 2014).

The BSR is reported to cause an economic loss of up to USD 365 million per annum in Malaysia (Seman, 2018). It has been estimated the BSR disease caused a yield loss of 68.73% of obtainable yield per year from all the infected palm (Kamu et al., 2021). The infection rate of BSR has been increased from 1.5% in 1995 to 7.4% in 2017 (Idris et al., 2019). In 2020, the total oil palm planted area infected by *G. boninense* estimated around 443,430 ha in Malaysia (Bharudin et al., 2022). In one of the plantation in North Sumatera BSR devastated thousands of hectares of the palm tree caused 31 to 67% loss of tree per hectare in 25 years oil palm plantation (Riyanto et al., 2020). Estimated the loss at USD 256 million for every 1% incidence in Indonesia (Darmono, 2011). These yield losses were estimated not only from the dead tree but also from the disease infected tree (Kannan et al., 2017). Fruit bunch yield is continuously reduced until the fungus kills the tree (Olaniyi & Szulczyk, 2020).

1.2 Problem statement

The BSR disease caused by *G. boninense* is the most destructive disease of OP industry in Southeast Asia (Idris 1999; Susanto et al., 2005; Wong et al., 2012). BSR has already resulted in a significant loss of income in Malaysian and Indonesian OP industry. To control the *G. boninense* in the plantation, clean clearing, chemical, and biological control methods, and improving disease resistance have been practiced. However, none of those was promising. Some of the chemicals that are applied by trunk injection require technical skills and intensive equipment for their use in the plantation (Lam and Chiu, 1993; Kalidas, 2012).

G. boninense causes degradation in oil palm wood (OPW) by their enzymatic activities leads to a reduction in plant yield and productivity. The degradation of lignohemicellulose components by enzymatic activity of *G. boninense* are associated with BSR infection of OP yet this aspect has been overlooked. The lignin component is destroyed by the fungus, allowing it to attack the cellulose and cause the OP to collapse because it can no longer support the weight of the oil palm. Inefficacy in controlling this devastating disease might be due to the lack of understanding on pathogen establisment in the host and its role in wood component degradation. A recent study reported that phenolic compounds has successfully suppressed the lignin-degrading enzyme produced by the pathogen (Surendran et al., 2018b). Amongst the tested phenolic compounds, benzoic acid (BA) was the most potent inhibitor (Surendran et al., 2017). Plant produce BA naturally in respons to environmental stresses (Williams et al., 2003). BA has an antifungal activity towards many pathogens. The mechanism of pathogen suppression by BA is related with the disruption in fungal cellular structure that damaged the organelles and affect the membrane function such as cell septation during cell division (Ganapathy et al., 2021; Nahela et al., 2021). Knowing its ability as an inhibitor in fungal growth, the use of BA could be one of the potential methods for controlling pathogenic fungi. Therefore, this study was designed to understand the fundamental role of *G. boninense* in wood degradation causing BSR and patterns of wood degradation in BSR epidemiology. Additionally, the emphasis is given on the use of BA as prevention of lignohemicellulose degradation caused by *G. boninense* as a potential compound for BSR suppression.

1.3 Hypothesis

BA could be used as an antifungal compound to control BSR disease by understanding the mechanism of lignohemicellulose degradation due to *G. boninense* establishment.

1.4 Research objectives

The general objective is to understand the mechanism of lignohemicellulose degradation due to *G. boninense* establishment and evaluate the use of BA for BSR suppression. The specific objectives of the study are as follow:

1. To determine the role of BA in altering the morphology and cellular structure of *G. boninense* and to evaluate the patterns of wood degradation by *G. boninense* and alterations due to BA treatment in OPW blocks.

2. To determine the changes in wood chemical components and enzymatic activities of *G.boninense* during lignohemicellulosic degradation of OPW blocks in response to BA treatment.

3. To determine the potential use of BA in strengthening the OPW lignin structure and suppression of *G. boninense* in oil palm seedlings during BSR establishment.

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