EVALUATION OF SILICON AND SALICYLIC ACID FOR CONTROLLING BASAL STEM ROT DISEASE ON OIL PALM (*Elaeis guineensis* Jacq.) CAUSED BY *Ganoderma boninense*

By

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Basal stem rot (BSR) disease, caused by *Ganoderma boninense,* is the most destructive disease that causes decay of the root system before ultimately rotting the lower stem of the oil palm (*Elaies guineensis*) which reduces the weight of fruit bunches. Repeated use of fungicides to control the disease develops fungal resistance and it is costly and environmentally unfriendly. The objectives of this study were to evaluate *in vitro* antifungal activity of salicylic acid (SA) and silicon (Si) in inhibiting mycelial growth of *boninense* and to evaluate the *in vivo* efficacy of Si treatment on the growth and oil palm seedlings resistance to *G. boninense*. For *in vitro* evaluation, 50, 100, 150, 200 and 250 mg/L of salicylic acid (SA), silicon dioxide (SiO_2) and pure silicon (Si) were added individually to sterilized potato dextrose agar (PDA) to investigate the *in vitro* efficacy on the mycelial growth of *Ganoderma boninense* under laboratory conditions. The highest percentage inhibition of radial growth (PIRG) was observed for Si treatment followed by silicon dioxide (SiO_2). Si treatment showed the highest percentage, 100% growth inhibition of *boninense* at concentration of 200 and 250 mg/L. Si can inhibit the mycelial growth of *boninense* significantly ($p<0.05$) at a lower concentration compared to SA and SiO_2. The treatment also showed a significant difference compared to the other two treatments as even at a concentration of 100 mg/L, the Si treatment inhibited the mycelial growth of *boninense* better than the rest of the other treatments tested. There was a significant difference ($p<0.05$) in almost all concentrations tested for both Si and SiO_2 except for PIRG at 200 and 250 mg/L which did not show a significant difference. The half maximal effective concentration (EC_{50}) that inhibited mycelial growth of *boninense* was 68.57 mg/L, while for SiO_2 and SA were 273.95 mg/L and 381.33 mg/L, respectively. For *in vivo* evaluation in the greenhouse, oil palm seedlings were pre-treated with 50, 100, 150, 200 and 250 mg/L of silicon (Si) respectively before being inoculated with *Ganoderma boninense* using the Dip, Place, and

Drench (DPD) method and observed for five consecutive months. The group of inoculated seedlings that received no Si treatments were labelled as untreated control seedlings (UTC). Seedlings treated with Si showed a significant ($p<0.05$) reduction in disease severity against BSR where the concentrations of 150, 200 and 250 mg/L showed the lowest severity in leaf symptoms which were 7.36%, 6.49% and 4.05%, respectively with healthier and green leaves while the seedlings without Si showed the highest severity of leaf symptoms with multiple leaves turning yellow and dying. At five months after inoculation, examination of internal bole tissues of oil palm seedlings treated with Si at a concentration of 250 mg/L was the most effective in suppressing BSR and recorded a 3.0% mean percentage disease severity compared to other treatments as well as the untreated infected seedlings that showed 35.0% mean percentage disease severity. Si treatment plays an important role in keeping *boninense* infection below threshold by restricting the pathogen's ability to penetrate host tissues.

Keywords: Basal stem rot disease, *Ganoderma boninense,* oil palm, salicylic acid, silicon

SDG: GOAL 2: Zero Hunger, GOAL 9: Industry, Innovation and Infrastructure, GOAL 15: Life on Land.

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

PENILAIAN RAWATAN SILIKON DAN ASID SALISILIK UNTUK MENGAWAL PENYAKIT REPUT PANGKAL BATANG (BSR) POKOK KELAPA SAWIT DISEBABKAN OLEH *GANODERMA BONINENSE*

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Penyakit reput pangkal batang (BSR) yang disebabkan oleh *Ganoderma boninense* ialah salah satu penyakit kelapa sawit yang paling serius kerana ia menyebabkan pereputan progresif sistem akar pokok palma sebelum akhirnya mereputkan pangkal batang kelapa sawit (*Elaies guineensis*), sekaligus menjejaskan kuantiti hasil tanaman kelapa sawit. Penggunaan racun kulat secara berulang kali dalam mengawal penyakit tersebut membina ketahanan kulat dan ia juga mahal dan tidak mesra alam sekitar. Tujuan penyelidikan ini adalah untuk menilai aktiviti antikulat *in vitro* asid salisilik (SA) dan silikon (Si) dalam menyekat pertumbuhan miselium *boninense* dan juga menilai kesan silikon terhadap pertumbuhan dan ketahanan anak pokok kelapa sawit terhadap *G. Boninense* secara *in vivo*. Bagi penilaian *in vitro*, 50, 100, 150, 200 dan 250 mg/L asid salisilik (SA), silicon dioksida (SiO_2) dan silikon tulen (Si) telah ditambah kepada agar dekstros kentang (PDA) yang telah distreril untuk mengkaji kesannya terhadap pertumbuhan miselium *Ganoderma boninense* dalam keadaan makmal. Peratusan tertinggi perencatan pertumbuhan jejari miselium (PIRG) telah dicatatkan untuk Si diikuti oleh silikon dioksida (SiO_2). Si menunjukkan peratusan tertinggi, 100% perencatan pertumbuhan miselium *boninense* pada kepekatan 200 dan 250 mg/L. Si mampu merencatkan pertumbuhan miselium *boninense* dengan signifikan ($p<0.05$) pada kepekatan yang lebih rendah berbanding SA dan SiO_2, di mana pada kepekatan hanya 100 mg/L, ia sudah mampu untuk merencatkan pertumbuhan miselium *boninense* dengan lebih berkesan berbanding kesemua kepekatan yang diuji dalam kajian ini. Kesemua kepekatan Si dan SiO_2 yang telah diuji mempunyai perbezaan yang signifikan ($p<0.05$) antara satu sama lain kecuali untuk PIRG pada 200 dan 250 mg/L yang tidak menunjukkan sebarang perbezaan signifikan. Kepekatan efektif (EC_{50}) silikon yang boleh menghalang pertumbuhan miselium ialah 68.57 mg/L manakala bagi silikon dioksida dan asid salisilik masing-masing ialah

273.95 mg/L dan 381.33 mg/L. Bagi penilaian *in vivo* di rumah kaca pula, anak pokok kelapa sawit telah diberikan rawatan Si pada kepekatan 50, 100, 150, 200 dan 250 mg/L terlebih dahulu sebelum diinokulasi dengan *Ganoderma boninense* menggunakan teknik Dip, Place and Drench (DPD) dan pemerhatian telah dilakukan selama lima bulan berturut-turut. Kumpulan anak pokok yang telah diinokulasi tetapi tidak diberikan sebarang rawatan Si telah dilabel sebagai kumpulan anak pokok tidak terawat (UTC). Anak pokok yang menerima Si menunjukkan pengurangan keterukan pereputan pangkal batang yang signifikan ($p<0.05$) di mana kepekatan 150, 200 dan 250 mg/L menunjukkan keterukan simptom daun paling rendah, iaitu 7.36%, 6.49% dan 4.05% dengan daun yang hijau dan sihat manakala anak pokok yang tidak menerima Si menunjukan keterukan simptom daun paling tinggi dengan banyak daun yang kekuningan dan mati. Selepas lima bulan proses inokulasi selesai, pemeriksaan terhadap tisu pangkal batang anak pokok telah menunjukkan bahawa anak pokok yang menerima Si pada kepekatan 250 mg/L merupakan rawatan yang paling efektif bagi mengurangkan keterukan BSR dan mencatatkan 3.0% purata peratusan keterukan pangkal batang berbanding kumpulan kepekatan yang lain termasuk kumpulan anak pokok yang tidak dirawat yang mempunyai 35.0% purata peratusan keterukan pangkal batang. Si telah terbukti memainkan peranan penting dalam memastikan jangkitan *boninense* berada dalam keadaan terkawal dengan mengawal kebolehan patogen tersebut untuk menyerang tisu perumah.

Kata kunci: Penyakit reput pangkal batang, *Ganoderma boninense,* kelapa sawit, salisilik asid, silikon

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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 Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

˚C	degree Celcius
%	percent
ANOVA	Analysis of Variance
BLAST	Basic Local Alignment Search Tool
bp	Base pairs
BSR	Basal stem rot
Ca^{2+}	Calcium ion
cm	centimetres
Cu^{2+}	Copper ion
DNA	Deoxyribonucleic acid
DMRT	Duncan's Multiple Range test
DPD	Dip, Place and Drench
DS	Disease severity
EC_{50}	Half maximal effective concentration
ELISA	Enzyme-Linked Immunosorbent Assay
FELCRA	Federal Land Consolidation and Rehabilitation Authority
FELDA	Federal Land Development Authority
FFB	Fresh fruit bunches
g	gram
g/L	gram per litre
HR	Hypersensitivity response
ITS	Internal transcribed spacer
kg	kilogram
MEGA	Molecular Evolutionary Genetics Analysis

min	minutes
mg/L	milligram per litre
mL	millilitres
mm	millimetres
MPOB	Malaysian Palm Oil Board
NCBI	National Center for Biotechnology Information
P	Phosphorus
PAL	Phenylalanine ammonia-lyase
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PDB	Potato dextrose broth
PIRG	Percentage inhibition of radial mycelial growth
ppm	parts per million
psi	pounds per square inch
RCBD	Randomised complete block design
RISDA	Rubber Industry Smallholders Development Authority
S	Sulphur
s	seconds
SAR	Systemic acquired resistance
SAS	Statistical Analytical System
SD	Standard deviation
SEM	Scanning Electron Microscopy
Si	Silicon
SiO_2	Silicon dioxide
TBE	Tris-Borate-EDTA
UPM	Universiti Putra Malaysia

USA	United States of America
UTC	Untreated control group
UV	Ultraviolet ray
V	Volt
w/v	weight in volume

CHAPTER 1

INTRODUCTION

Oil palm (*Elaeis guineensis*) has become one of the most significant oil-producing crops in the world. It is considered as the most effective oil-producing crop in the world, since to generate one tonne of oil, only 0.26 hectares of land are required. Therefore, oil palm trees are valuable to the countries in which they can grow well. The oil palm has become the most valuable agricultural commodity in Malaysia and it is essential for the country's economic growth since the oil palm thrives in the Malaysian climate (Mohd Fahmi *et al.*, 2015). In 2016, Malaysia and Indonesia became the world's largest exporter of palm oil, exporting about 90% of the 43.76-million-ton palm oil exports (Ahmad Kushairi, 2017). Other top oil palm producers include Thailand, Nigeria, Papua New Guinea, Colombia, and Ecuador, that total up to 19 million hectares or roughly 0.36% of the oil palm cultivation areas of all agricultural land in the world (Maluin *et al*., 2020). With these large amounts of production and export, any yield loss caused by oil palm diseases would affect the country's total annual yield per year. However, the spread of some diseases is inevitable, and action must be taken to control them as soon as possible.

The productivity of palm oil production can be impacted by the infection of fungi. As the fungi infect oil palm trees, they may develop a disease, commonly known as wood rot disease. This disease can be categorized further according to the site of infection, and according to Rees (2009), among the most common diseases attacking oil palm is basal stem rot (BSR). Other than that, diseases including upper stem rot caused by *Ganoderma* spp. (common in Asia) as well as vascular wilt that is commonly caused by *Fusarium oxysporum* pathogen present growth and oil yield reduction issues for oil palm growers (Riyadi, 2021).

Basal stem rot (BSR), caused by *Ganoderma boninense*, is a white rot fungus of hardwoods which damages oil palm crops. It is a disease that causes a gradual deterioration of the tree root system, before finally rotting the basal stem of the oil palm. The pathogen of the disease usually attacks older palms, however due to expansion of oil palm cultivation land, the disease incidence of BSR was also rapidly increased and detected at younger oil palms. Unfortunately, the yield loss from BSR may exceed up to 80% since the infected oil palms typically collapse before the harvest period of the fruit bunches. According to Arif *et al*. (2011), the loss of revenue caused by this pathogenic fungus was roughly calculated to be around RM1.5 billion each year. The yield losses did not come only from the reduction of oil palm stands, but the disease also reduced the weight and the quantity of fruit bunches per infected oil palm tree. Since the damage caused by the disease is huge, oil palm planters rank BSR as the most serious disease problem and place improved BSR control as a top priority.

BSR disease incidence can be reduced if the mycelial growth or the spread of the spores of *boninense* can be inhibited. The application of salicylic acid can help in suppressing the mycelial growth of *boninense*, hence slowing the spread of the fungus on oil palm trees. Salicylic acid is a natural, common substance that is generated by plants as a defense mechanism against infections, in response to the plant's immune system signal. It is a crucial element for plant resistance to pathogen attacks and plays a significant part in modulating the plant responses to several abiotic stresses (Jing *et al.*, 2007; Wu *et al.*, 2008). It may enhance the plant defense mechanism towards *boninense* attacks and thus increase the plant's tolerance against the disease.

Meanwhile, for silicon application, chemicals are one of the most important trace elements found in plants that are beneficial in the plant immune's system against pathogen attacks. Many studies have shown that among all nutrients taken by plants at a micro level, silicon uptake is regularly present at a level comparable to macronutrients. Research evidence has also concluded that sufficient uptake of silicon (Si) improves the ability of agricultural crops, particularly rice, to tolerate numerous biotic and abiotic stresses (Ma and Takahashi, 2002).

Research Problem

Repeated use of fungicides to control the disease develops fungal resistance and is costly and environmentally unfriendly.

Hypothesis

The salicylic acid and silicon application may enhance oil palm resistance in response to *boninense*.

Objectives

1. To evaluate *in vitro* antifungal activity of salicylic acid (SA) and silicon (Si) in suppressing mycelial growth of *Ganoderma boninense*.

2. To investigate *in vivo* efficacy of the proposed treatments to suppress *Ganoderma boninense* causing basal stem rot on oil palm.

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