PHYSIOLOGICAL AND HISTOPATHOLOGICAL ANALYSES OF SILICON-MEDIATED RESISTANCE ON *Rigidoporus microporus* IN RUBBER ROOTSTOCK SEEDLINGS (*Hevea brasiliensis*)

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2022

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by

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Thesis submitted in fulfilment of the requirements
for the degree of
Doctor of Philosophy

September 2022

ACKNOWLEDGEMENT

In the name of Allah S.W.T. the Beneficent and the Compassionate. I would like to express my deepest gratitude to Allah S.W.T. the Almighty for His guidance and blessing in my journey to complete my Ph.D. thesis.

First of all, I would like to thank my supervisor, Dr. Nik Mohd Izham Mohamed Nor, for his time, guidance, advices, great effort, support, enthusiasm, and sharing of his expertise to complete my study. Not to be forgotten, my co-supervisor, Professor Dr. Latiffah Zakaria, Associate Professor Dr. Siti Nordahliawate Mohd Sidique, and Dr. Murnita Mohmad Mahyudin, for their valuable guidance throughout my study. I am also grateful to the Malaysian Rubber Board for funding my study fees and allowances. I am also indebted to the Ministry of Higher Education Malaysia (Fundamental Research Grant Scheme: FRGS/1/2020/WAB04/USM/02/2), and all Malaysians for directly and indirectly funding my education up to this level.

My special and sincere appreciation goes to my families and in-laws, especially my beloved wife (Nor Suhada), parents (Shaikh Abd Hadi and Jamilah), and children (Aiman, Anis, Anas, Aidan) for their endless support, prayers, encouragement, and sacrifices in ensuring the success of my study. I am grateful to the staff of the Malaysian Rubber Board and School of Biological Sciences, USM, especially Dr. Safiah, Mr. Aizat, Mrs. Khairita, Mr. Arif, Mr. Khairuddin, Mrs. Santhini, and Mrs. Shafawati for their assistance throughout my study. I would also like to thank my laboratory colleagues in 117, Dr. Nurul Farizah, Dr. Paul, Haslinda, Emier, Husna and Aisyah as well as labmates for their help, guidance, and support. To all people I have mentioned and those not mentioned here, may Allah S.W.T. give you the best in return. InsyaAllah. Thank You.

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LIST OF SYMBOLS

α	Alpha
p	p-value
℃	Degree celsius
cm	Centimetre
kg	Kilogram
g	Gram
L	Litre
M	Molar
min	Minute
h	Hour
µl	Microlitre
µg	Microgram
ml	Millilitre
mL L^{-1}	Millilitre per litre
mm	Millimetre
mM	Millimolar
µM	Micrometre
n	Total number
rpm	Rate per minute
%	Percent
/	Or
MYR	Malaysian Ringgit
™	Trademark
©	Copyright
×	Times
=	Equal
±	Plus minus
<	is less than
>	is greater than
≤	is less than or equal to
ppm	Part per million
µg	Micro gram
pH	Potential of hydrogen
ha	Hectare

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AUDPC	Area Under Disease Progress Curve
BHD	Berhad
Ca_2SiO_4	Calcium silicate
CRD	Completely Randomized Design
DAMP	Damage-associated molecular pattern
DR	Disease Reduction
DSF	Disease Severity Foliar
DSR	Disease Severity Root
ED	Effective Dose
EDX	Energy Dispersive X-Ray Analysis
FAA	Formalin Acetic Acid
GDP	Gross Domestic Product
HCl	Hydrocloric acid
IBN	Internal Bark Necrosis
MAMP	Microbe-associated molecular pattern
MANOVA	Multivariate analysis of variance
MRB	Malaysian Rubber Board
MTI	Microbial triggered immunity
NaOH	Sodium hydroxide
Na_2O_3Si	Sodium meta-silicate
Na_2SiO_3.9H2O	Sodium silicate nanohydrate
H_4SiO_4	Ortosilicic acid
PB	Prang Besar
PDA	Potato Dextrose Agar
PIRG	Percentage Inhibition of Radial Growth
PPFD	Photosynthetic Photon Flux Density
PRR	Pathogen recognition receptor
RCBD	Randomized Complete Block Design
RRIM	Rubber Research Institute of Malaysia
SE	Standard Error
SDN	Sendirian
SiO_3^{2-}	Silicic anion
$Si[OH]_4$	Monosilicic acid
SPSB	Stesen Penyelidikan Sungai Buloh
SPSS	Statistical Package for the Social Sciences
TPC	Total Phenolic Compounds
USM	Universiti Sains Malaysia
WRD	White root disease

ANALISIS FISIOLOGI DAN HISTOPATOLOGI KERINTANGAN SILIKON TERHADAP *Rigidoporus microporus* DALAM ANAK BENIH POKOK GETAH PENANTI (*Hevea brasiliensis*)

ABSTRAK

Penyakit akar putih adalah penyakit akar yang paling teruk yang disebabkan oleh kulat bawaan tanah, terutamanya *Rigidoporus microporus*. Patogen ini merosakkan sistem akar, dan akhirnya membunuh pokok getah, *Hevea brasiliensis* Muell. Arg. Penggunaan racun kulat kimia (propiconazole) secara berkala telah menyebabkan masalah pencemaran alam sekitar, berbahaya kepada pengguna dan mahal. Sebagai pilihan, penggunaan baja bersepadu silikon telah terbukti berjaya dalam menguruskan penyakit pada padi, epal, pokok berangan dan banyak lagi. Kajian ini bertujuan untuk mengkaji kemungkinan menggunakan silikon terlarut untuk mengawal kejadian penyakit akar putih. Kajian kebolehubahan dan ujian penghasilan enzim lakase pada sembilan pencilan *R. microporus* dari stok kultur dilakukan untuk memilih tiga pencilan yang menunjukkan tahap kevirulenan yang bererti. Dalam ujian kepatogenan, pencilan *R. microporus* terpilih kemudian diinokulasi pada anak benih pokok getah penanti untuk memilih pencilan yang paling virulen. Pencilan Ayer Molek (AM) menunjukkan tahap virulen yang paling tinggi dalam kalangan pencilan *R. microporus* dan telah digunakan dalam eksperimen selanjutnya sebagai sumber utama patogen. Sebagai rawatan alternatif untuk menggantikan racun kulat propiconazole, kajian telah dijalankan untuk menentukan kesan pelbagai jenis aplikasi silikon terlarut (asid silisik, natrium meta-silikat, natrium silikat, dan kalsium silikat) ke atas pertumbuhan *R. microporus* dalam kedua-dua kajian *in-vitro* dan *in-vivo*. Perbezaan dalam pengumpulan silikon telah disebabkan oleh kemampuan penyerapan silikon oleh akar. Oleh itu, keberkesanan silikon terlarut telah diperhatikan pada dua jenis anak benih pokok getah penanti dari Kumpulan 1 (RRIM 2002) dan Kumpulan 2 (RRIM 2024). Aspek yang paling penting dalam penyelidikan ini adalah memanfaatkan sepenuhnya peranan silikon dalam memberikan toleransi pada anak benih pokok getah penanti terhadap jangkitan penyakit akar putih. Kesan perencatan langsung dari silikon terlarut diuji secara *in-vitro*, dan hasilnya menunjukkan bahawa silikon terlarut mempunyai kesan yang berkaitan dengan dos perencatan pada pertumbuhan *R. microporus* pada kepekatan serendah 10 ppm. Asid silisik dan natrium meta-silikat dipilih untuk kajian rumah tumbuhan berdasarkan perencatan pertumbuhan miselium *R. microporus* yang optimum pada kepekatan 5000 ppm. Penggunaan asid silisik lebih berkesan pada anak benih pokok getah penanti klon RRIM 2002, manakala natrium meta-silikat menunjukkan hasil yang memberangsangkan pada anak benih pokok getah penanti klon RRIM 2024 berdasarkan nilai data fisiologi yang lebih tinggi (kandungan klorofil relatif, kadar fotosintesis, konduktansi stomata dan kadar transpirasi) dicatatkan setelah sembilan bulan diinokulasi. Penemuan ini dapat memberi petunjuk untuk memilih anak benih pokok getah penanti yang lebih tahan terhadap penyakit akar putih untuk pengeluaran klon getah yang berkualiti. Menariknya, penggunaan 200 mL asid silisik dan natrium meta-silikat pada kadar kepekatan 5000 ppm sebanyak dua kali sebulan melalui curahan tanah telah secara signifikan mengurangkan keparahan penyakit daun (DSF), keparahan penyakit akar (DSR), kawasan di bawah kemajuan penyakit (AUDPC), pengkolonian patogen dan pengurangan penyakit (DR). Sebagai tambahan, pengumpulan silikon dan metabolit sekunder dalam bentuk Jumlah Sebatian Fenolik (TPC) meningkat dengan ketara pada akar anak benih pokok getah penanti yang diinokulasi setelah menggunakan silikon terlarut. Pemerhatian menggunakan mikroskop kompaun cahaya di bawah analisis histopatologi menunjukkan pengurangan kewujudan hifa *R. microporus*, pengumpulan TPC yang lebih tinggi, serta dinding sel korteks dan xilem yang lebih tebal dalam tisu akar anak benih pokok getah penanti yang dirawat dengan silikon terlarut. Penyiasatan mikroskopi elektron imbasan (SEM) pada akar anak benih pokok getah penanti yang diinokulasi mendapati kehadiran hifa *R. microporus* di dalam akar anak benih pokok getah penanti yang tidak dirawat dengan silikon terlarut dan anak benih pokok getah penanti yang hanya dirawat dengan propiconazole. Sebaliknya, tidak ada hifa *R. microporus* yang terdapat di dalam akar anak benih pokok getah penanti kawalan dan anak benih getah penanti yang dirawat dengan silikon terlarut. Analisis unsur menggunakan EDX menunjukkan jumlah kehadiran silikon yang rendah pada akar yang tidak dirawat dengan silikon terlarut, sementara jumlah silikon yang tinggi terdapat pada akar yang dirawat dengan silikon terlarut. Hasil ini menunjukkan bahawa penggunaan asid silisik dan natrium meta-silikat pada anak benih pokok getah penanti semasa dijangkiti *R. microporus* telah meningkatkan parameter fisiologi serta kandungan silikon dan TPC dalam tisu akar anak benih pokok getah. Hal ini menunjukkan bahawa kedua-dua silikon terlarut ini mempunyai kesan perencatan tidak langsung terhadap jangkitan *R. microporus* terhadap anak benih pokok getah penanti. Oleh itu, silikon terlarut boleh dianggap sebagai rawatan alternatif terhadap penyakit akar putih pada anak benih pokok getah penanti yang menawarkan rawatan mesra alam dan keberkesanan kos berbanding racun kulat konvensional.

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ABSTRACT

White root disease (WRD) is the most severe root disease caused by a soil-borne fungus, mainly *Rigidoporus microporus*. This pathogen destroys the root system, leading to the death of the rubber tree, *Hevea brasiliensis* Muell. Arg. The periodical application of chemical fungicides (propiconazole) to control this pathogen has caused environmental pollution issues, hazardous to users, and costly. As an option, the application of silicon integrated fertiliser has proven to be successful in managing diseases on rice, apple, chestnut tree and many else. This study aimed to explore the possibility of using soluble silicon to control the incidence of WRD. The variability study and laccase enzyme production test on nine *R. microporus* isolates from the culture stock were carried out to select three isolates that showed a significant virulence level. In a pathogenicity test, selected isolates of *R. microporus* were then inoculated on rubber rootstock seedlings to choose the most virulent isolate. Ayer Molek (AM) isolate was indicated to be the most virulent isolate of *R. microporus* and had been used in further experiments as a primary source of the pathogen. As an alternative element to propiconazole fungicides, studies had been conducted to determine the effect of different types of soluble silicon (silicic acid, sodium meta-silicate, sodium silicate, and calcium silicate) application on *R. microporus* growth in both in-vitro and in-vivo study. The differences in silicon accumulation had been attributed to the silicon absorbing ability of the roots. Thus, the efficacy of soluble silicon was observed on two types of rubber rootstock seedlings from Group 1 (RRIM

2002) and Group 2 (RRIM 2024). The most crucial aspect in this research is to make full use of the role of Si in conferring tolerance in rubber rootstock seedlings against infection of WRD. The direct inhibitory effect of soluble silicon was tested in-vitro, and results showed that they had an inhibitory dose-related impact on *R. microporus* growth at concentrations as low as 10 ppm. Silicic acid and sodium meta-silicate were selected for plant house study based on the optimal inhibition growth of *R. microporus* mycelial at the concentration of 5000 ppm. The application of silicic acid was more effective on rubber rootstock seedlings of clone RRIM 2002, whereas sodium meta-silicate showed promising results on rubber rootstock seedlings of clone RRIM 2024 based on the higher values of physiological data (relative chlorophyll content, photosynthesis rate, stomatal conductance, and transpiration rate) recorded after nine months inoculation. This finding could give a direction to select the rubber rootstock seedlings which is more resistant to WRD for quality rubber clones' production. Remarkably, the application of 200 mL silicic acid and sodium meta-silicate at the concentration rate of 5000 ppm twice a month as a soil drenching had significantly reduced the disease severity of foliar (DSF), disease severity of root (DSR), area under disease progress (AUDPC), pathogen colonisation and disease reduction (DR). In addition, the accumulation of silicon and the secondary metabolite in the form of Total Phenolic Compounds (TPC) was significantly increased in the roots of inoculated rubber rootstock seedlings after applying soluble silicon. The observation using a compound light microscope under histopathological analyses showed the reduction of *R. microporus* hyphae existence, higher accumulation of TPC as well as thicker cell wall of cortex and xylem in the root tissues of rubber rootstock seedlings supplemented with soluble silicon. Scanning electron microscopy (SEM) investigation on inoculated rubber rootstock seedlings' roots found the presence of *R.*

microporus hyphae inside the roots of untreated rubber rootstock seedlings with soluble silicon and rubber rootstock seedlings treated only with propiconazole. On the contrary, no *R. microporus* hyphae were found inside the roots of control rubber rootstock seedlings and rubber rootstock seedlings treated with soluble silicon. The elemental analysis using EDX demonstrated a low amount of silicon presence in untreated root with soluble silicon, while high amounts of silicon were found in treated root with soluble silicon. These results indicated that silicic acid and sodium meta-silica application to rubber rootstock seedlings under *R. microporus* infectious conditions increase physiological parameters as well as silicon content and TPC in rubber rootstock seedlings' root tissue. This can suggest that both soluble silicon have an indirect inhibitory effect on *R. microporus* infection of rubber rootstock seedlings. Therefore, soluble silicon can be considered an alternative treatment against WRD in rubber rootstock seedlings which offers an environmentally friendly and cost-effective treatment compared to conventional fungicide.

CHAPTER 1

GENERAL INTRODUCTION

Rubber trees (*Hevea brasiliensis*), native to South America's Amazon Valley, were brought to Asia through India in the latter half of the 19th century. They are currently grown in 20 countries around the world, including Malaysia, to manufacture natural rubber and wood panels. In Malaysia, rubber tree plantations occupied approximately 1.06 million hectares, with gross natural rubber output estimated to be 514,700 tonnes in 2020 (Malaysian Rubber Board, 2021). Rubber trees are grown for their latex production, which is used to make products like tyres, tubes, and hoses.

Rubber trees, like every other crop, are constantly threatened by biotic and abiotic stresses such as floods, droughts, and microbial diseases. White root disease (WRD) is a common plant disease caused by a soil-borne fungus, primarily *Rigidoporus microporus* (Polyporales, Basidiomycota), that destroys the root system of *H. brasiliensis* Muell. Arg and eventually kill the plants (Nandris et al., 1987a; Chaiharn et al., 2019; Go et al., 2021). According to Nandris et al. (1987a), *Rigidoporus microporus* (Sw.) Overeem (syn. *Rigidoporus lignosus* (Klotzsch) Imazeki) is a wood invasion fungus or wood degrader that causes damage to the woody tissue of over 100 different trees types, with the rubber tree suffering enormous losses in plantation.

WRD is classified as a devastating disease of the rubber tree globally, including in Malaysia (Liyanage et al., 1977; Jayasuriya & Thennakoon, 2007; Goh et al., 2018; Chaiharn et al., 2019). Every year, WRD affects a substantial portion of rubber plantations, resulting in a severe economic deficit (Siddiqui et al., 2017).

According to Holiday (1980), about 18% of the 700 hectares of rubber planting areas in Malaysia were seriously affected by WRD infection. Furthermore, the most recent study, conducted in 2012 by the Malaysian Rubber Board, showed that the occurrence of white root disease in 1,065,630 hectares of Malaysian total rubber plantations are 10 - 15% of Peninsular Malaysia, 20 - 30% of Sabah, and 9 - 20% of Sarawak (Atan, 2015). WRD infection may result in significant tree death and, in extreme cases, destroy an entire stand (Guyot & Flori, 2002). Such tree loss will significantly decrease the tree stand, resulting in reduced rubber yields, increased wind damage facilitated by the clearance caused by tree loss, and increased development costs due to tapper re-tasking in such areas. Additionally, older rubber plantations have recorded yield losses of up to 50% (Ogbebor et al., 2013a). This disease causes significant financial loss, especially to rubber smallholders (Soytong & Kaewchai, 2014).

White root disease can affect rubber trees of any age or clone. The disease can invade all plant life stages, from seedlings to mature trees (Wattanasilakorn et al., 2017). It was more widespread during the first and fourth years after planting. The noticeable symptoms of WRD were yellowing leaves on a single branch, a few branches, or the entire canopy, depending on the magnitude of the disease, and eventually, the tree died. It is also demonstrated that the pathogen can infect the roots through rhizomorphs emerging from stumps or infected woody debris left in the field and contacting the infected debris (Nandris et al., 1987b; Guyot & Flori, 2002). The fungus may decompose woody structures and spread to plant roots by spreading the hyphae to the bark surface (Farhana et al., 2017). The white root disease spreads quickly and infects neighbouring plants, especially in rubber plantation areas with poor sanitation and drainage. The causal pathogen may also be found on dying plants, branches/twigs, decomposing tree stumps, and rotting leaves with fruiting bodies.

The selection of rubber rootstock seedlings and systematic breeding are crucial for producing healthy rubber clones to overcome the limitations. To date, there are no rubber clones resistant to the infection of *R. microporus* (Kelaniyangoda et al., 2013; Farhana et al., 2017). For the best performance in rubber tree seedlings formation, research indicates that the choice of rootstock clone becomes essential for increasing the productivity of the plantation (Vieira et al., 2016). The rootstock can positively affect the scion, and the scion can also have a positive effect on the rootstock, which eventually affects plant growth (Carr, 2012; Daud et al., 2012; Yao et al., 2017). Unselected seedlings as rootstocks are not recommended because they will affect plant growth and reduce the dry rubber yield (Malaysian Rubber Board, 2009b). Healthy rubber trees are susceptible to infection by free rhizomorphs that grow on stumps and woody debris embedded in the soil, or by direct root contact with an infected neighbouring tree. *R. microporus* develops rhizomorphs at deep soil roots and infects adjacent tree roots. The attacked plants sometimes die, reducing the latex production of a rubber plantation (Geiger et al., 1986a; Nandris et al., 1987a; Nandris et al., 1988). Therefore, selecting rootstock seedlings that are more resistant against WRD has become very important.

Nutrient uptake depends on the rubber root (Ogbebor et al., 2015). In addition, the grafted clones benefit from a good root system vigour and soil anchorage provided by the taproot of the seedlings used as rootstocks (Masson & Monteuuis, 2017). It has been found that silicon (Si) uptake in the leaf depends on the rootstock genotype and not the scion (Coskun et al., 2019). Nevertheless, there was no evidence to suggest that the growth and yield of clones were influenced significantly by rootstock type (Gireesh et al., 2012). There is a standard practice of collecting rubber seeds from the mixed clones rubber smallholder plantations in Malaysia to prepare the rootstock for rubber clones' production. Thus, reliable rubber seeds may be uncertain as a suitable rootstock for grafted clones. On the other hand, it has been mentioned that most of the seeds collected from the rubber smallholder plantation in southern Thailand are from clone RRIM 600, which is known to be susceptible to WRD (Wattanasilakorn et al., 2012). Previous studies claimed some clones to be relatively resistant to WRD than others; however, there is no information on the clone that is entirely resistant to WRD (Holiday, 1980; Peries & Liyanage, 1984; Kelaniyangoda et al., 2013).

Detection of the early stages of infection is difficult because of the insidious nature of the white root rot infection. Trees bearing the visible symptoms of the disease are beyond treatment and recovery (Ogbebor et al., 2013b). According to Ghani (2014), rubber clones were categorized into Group 1 and Group 2 based on the yield data from the Large-Scale Clone Trials. Group 1 clones were selected based on the yield data of five years with the minimum mean yield of 1500 kg/ha/year, whereas Group 2 clones were categorized based on three years of yield data with the minimum mean yield of 1800 kg/ha/year (Nurmi-Rohayu et al., 2015). It has been determined that different types of rubber clones will react differently regarding nutrient uptake and disease resistance (Salisu et al., 2013; Oghenekaro et al., 2016). Therefore, two types of seedlings from clone RRIM 2002 (Group 1) and RRIM 2024 (Group 2) were selected and used in this study to determine the efficacy of these seedlings as a potential rootstock for rubber clones' production, which could overcome the incidence of WRD.

The major limitation of this disease's prevention strategies is that treatments are only performed on diseased plants, and their efficacy depends on the accurate and early identification of pathogens (Mohamed et al., 2014). One of the currently recommended prevention measures is chemical treatment, which aids in eradicating epidemics. Two types of fungicides are used to control the disease, known as bayfidan and bayleton (Gohet et al., 1991; Hoong et al., 1991). The application of propiconazole as a soil drenching was the most effective method to reduce the incidence of white root disease due to its simple and easy treatment required (Hashim & Chew, 1997). However, excessive and uncritical synthetic fungicides have resulted in various environmental ecosystem problems (Ogbebor et al., 2015a). Biological approaches have also been used to manage and control the white root disease in rubber plantations. Various biological methods, including soil sulphur amendment and antagonistic microorganisms such as the species from the *Trichoderma* genus have been applied as an alternative approach of chemical fungicide to control the incidence of WRD in rubber plantations (Satchuthananthavale & Halangoda, 1971; Ismail & Azaldin, 1985; Hashim & Chew, 1997). The sulphur promotes fungal growth antagonistic to fungal root diseases (Omorusi, 2012). Nevertheless, Wicklow (1992) argued that the biocontrol agents could affect microbial communities by competing for nutrient resources, while Knudsen et al. (1997) found the possibility of parasitism reaction or direct production of toxins.

Thus far, no research has been conducted on the potential of soluble silicon (Si) to suppress *R. miroporus* in-vitro and its ability to enhance resistance in *Hevea brasiliensis* in-vivo. Aside from the established antifungal activities, soluble Si has never been studied for antifungal activity against *R. microporus*. Silicon (Si) treatment has significantly affected plant disease protection (Tubana et al., 2016; Elshahawy et al., 2021). Moreover, Si may increase tolerance to diseases and insects by the cell ‘toughness,' making it more difficult for fungi and insects to puncture plant cells

(Carneiro-carvalho et al., 2017). Nonetheless, Malhotra & Kapoor (2019) mentioned that Si is an environmentally sustainable element since it is not harmful to plants. Depending on the implementation process, Si applications can be pollution-free even when used in excess (Etesami & Jeong, 2018).

The development of the solution culture technique facilitated research into the function of Si in plant physiology (Epstein, 2009). Si serves various roles, including enhanced mechanical properties (root penetration, stature, resistance to lodging, and leaf exposure to light), increased growth and yield, salinity resistance, reduced transpiration, and drought resistance (Fauteux et al., 2005). Several studies have shown that Si application will suppress a variety of diseases. Mechanisms include activating plant enzymes and improved resistance caused by amorphous silica deposition or the aggregation of phytotoxic phenolic compounds (Aucique Perez et al., 2014; Bathoova et al., 2021). While Si has been used for centuries to prevent disease in agriculture, its potential use in plant physiology and histology, especially on *H. brasiliensis*, and disease prevention require further understanding and study. Therefore, the current research was designed to see whether soluble Si application to *R. microporus*-infected trees would defeat the disease. Thus, the general aim of the study was to investigate the soluble Si interaction between *R. microporus* and *H. brasiliensis*.

Our specific objectives in this study were as follows;

1) To screen and select *Rigidoporus microporus* isolates from the stock culture based on the virulence levels.

2) To evaluate the direct inhibitory effect of soluble Si on the growth of *Rigidoporus microporus* in-vitro.

3) To investigate the ability of soluble Si in protecting *Hevea brasiliensis* from the pathogenic isolate of *Rigidoporus microporus* through physiological analyses.

4) To determine mechanisms by which soluble Si is mediating a defence response in *Hevea brasiliensis* based on the reaction against *Rigidoporus microporus* through histopathological analyses.

CHAPTER 2

LITERATURE REVIEW

2.1 *Hevea brasiliensis*

2.1.1 An economically important tree of *Hevea brasiliensis*

Para rubber tree (*Hevea brasiliensis* Müll. Arg.) was first discovered in the Amazon basin and is a native plant of Brazil. This tree belongs to the Euphorbiaceae family and can produce natural latex (Hayashi, 2009; Teoh et al., 2011). *H. brasiliensis* is a robust, fast-growing tree with a straight trunk and an open leafy crown. The bark is typically greyish and relatively smooth. The bark of the trunk is where the rubber is extracted. In the wild, the trees can reach heights of over 40 metres and live for more than 100 years. However, due to the growth reduction caused by latex harvesting by tapping, cultivated plants rarely reach beyond 25- 30 m in height (Webster and Paardekooper, 1989). Furthermore, the trees are frequently replanted when the production declines to an uneconomic level after roughly 30 years.

According to Rudall (1987) and Hazir et al. (2020), ‘the latex is tapped by excision of the trunk's external tissue (known as tapping), which contains laticifers.’ The more latex vessels are severed, the more will be the flow of latex. This tree species is a tropical perennial that thrives in humid climates. The optimal conditions for the growth of *H. brasiliensis* are: the temperature is 22 - 30 °C, relative humidity does not exceed 70 to 80 %, and annual rainfall is between 1500 and 3000 mm. Additionally, soil properties have an effect on *H. brasiliensis* growth and productivity. Low latex yield was observed in acidic soil (pH range 4.37 - 4.54) and sandy soil (75.6 - 82.6%).

Higher latex yield depends on higher soil fertility (Akpan et al., 2007). The application of fertiliser at a higher rate was proved to increase the yield of rubber trees (Tiva et al., 2016). However, Salisu et al. (2013) underlined the necessity of providing a sufficient fertiliser rate, particularly to immature rubber trees, because an excessive fertiliser rate might result in plant toxicity and danger. Furthermore, it is essential to apply sufficient fertiliser rates, especially to immature rubber trees, because high fertiliser rates cause additional expenditures for rubber growers (Vrignon-Brenas et al., 2019). Propagation of rubber crops was primarily performed through seeds in the early years (Cardinal et al., 2007). Vegetative propagation via budding became highly popular after 1917. Seeds are currently used mainly in the production of rootstocks.

The nursery operators and rubber industries rely heavily on seeds for rootstock production. Rootstocks will be utilized for bud grafting to create clone plants (Daud et al., 2012). The importance of rootstock as an indicator for the growth and yield of scion has been described in many publications (Combe & Gener, 1977; Abbas & Ginting, 1981; Ng et al., 1981; Daud et al. 2012). Seedlings with a good root system will influence the efficiency of water and nutrient intake from the soil (Bastiah et al., 1996). Unselected seedlings as rootstocks are not suggested since they have very low compatibility values for dry rubber yield (Cardinal et al., 2007). Additionally, clonal rootstock produces relatively uniform latex yield due to its ability to reduce intra-clonal variation (Yao et al., 2017).

Wattanasilakorn et al. (2017) provided a beneficial detailed description of the importance of selecting clonal rootstock tolerant to the root disease. They discovered that clone EIRpsu 5 had the highest photosynthetic efficiency, the highest stomatal conductance, and the least symptom development of white root disease when compared to other clonal rootstocks tested. This finding proved the importance of selecting the viable rootstock for the growth and yield of scion and crucial for reducing the incidence of root rot diseases, particularly white root disease.

H. brasiliensis takes five to seven years to reach maturity on a commercial plantation, and mature trees are 20-30 meters tall with a productive lifespan of 25 to 30 years (Lacote et al., 2004; Mazlan et al., 2019). Numerous plants can produce natural latex, but *H. brasiliensis* is the primary producer. It contributes 99% of the natural latex used in producing natural rubber products on the global market (Nakaew et al., 2015; Krishnan et al., 2019). Since its latex is the primary source of natural rubber, the *H. brasiliensis* tree has been considered economically important (Oghenekaro et al., 2014; Khasim & Omar, 2019).

According to Wongcharoen (2010), natural rubber production has been highest in Asia, Africa, and Latin America. Southeast Asia produces more than 70% of the natural latex, primarily in Thailand, Indonesia, and Malaysia (Rose & Steinbüchel, 2005; Fox & Castella, 2013). In 2020, Thailand produced the highest amount of natural rubber globally, whereas Malaysia was categorised as the sixth largest rubber producer (Malaysian Rubber Board, 2021). Additionally, Malaysia has recorded 1.098 million hectares of rubber plantation area in 2020, with a total production of 515,000 tonnes. According to Sharib & Halog (2017), natural rubber has significantly contributed to Malaysia's economic development, accounting for RM 33.7 billion or 4.69% of the country's Gross Domestic Product (GDP) in 2013. The taxonomic classification of *Hevea brasiliensis* is listed below (CABI, 2021a):

Domain: Eukaryota

Kingdom: Plantae

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Dicotyledonae

Order: Euphorbiales

Family: Euphorbiaceae

Genus: *Hevea*

Species: *Hevea brasiliensis*

2.1.2 Pest and diseases

Many types of pests and diseases attack rubber trees, reducing the yield and becoming a major constraint in natural rubber production. Major pests causing severe infestations on rubber trees have been grouped into insects (e.g: grasshoppers, crickets, termites), molluscs (e.g: snails, slugs) and mammals (mousedeer, wild boars, rats) (Malaysian Rubber Board, 2009c). Termites (e.g: *Coptotermes curvignathus* sp) are an economically important pest of rubber trees and are widely distributed in most rubber-producing countries, including Malaysia (Duong Nguyen et al., 1998; Lee, 2002; Tahiri & Mangue, 2008; Hidayat et al., 2018). The termites cause damage to rubber trees by ingesting the taproot and moving into the trunk (Malaysian Rubber Board, 2009c). Immature rubber trees are killed outright, whereas mature rubber trees can survive for a while until severe winds drive over them.

Rubber trees are susceptible to various diseases caused mainly by a fungal infection. Among the diseases that cause substantial losses in rubber production are *Corynespora* leaf fall, Southern American leaf blight (SALB) disease, abnormal leaf fall, *Colletotrichum* leaf disease, powdery mildew, leaf blight, brown bast, white rot disease, and brown rot disease (Mazlan et al., 2019). These diseases attacked four major parts of the rubber tree: leaf, stem, panel, and root area (Wastie, 1975; Maiden et al., 2017). During rainy seasons, abnormal leaf falls, and gloeosponum leaf spot diseases arise, but powdery mildew and Corynespora leaf fall diseases appear during the dry season, soon after the wintering period (Manju et al., 2015). Abnormal leaf fall caused by *Phytopthora* was reported to cause yield loss of latex about 38 to 56% in India (Krishnan et al., 2019).

In the case of root rot disease, Farid et al. (2009) found the different degree of pathogenic between the white rot disease pathogen, *Rigidoporus microporus* with brown rot disease pathogen, *Phellinus noxius* on 24 months old rubber trees. It was observed that rubber trees were more susceptible to the infection of *R. microporus* compared to *P. noxius*. However, both of these pathogens had showed the disease progress starting with yellowing followed by wilting, defoliation and finally death of the host. Rubber clones in Malaysia are screened for disease resistance before being recommended for large-scale planting using the 'Environmax' planting practices (Chee, 1990; Razar et al., 2021).

2.2 White root disease of rubber

Para rubber is susceptible to a variety of pathogens that cause disease in the root, stem, and leaf systems of the tree. The most devastating diseases in plantations are those that damage the root system (Geiger et al., 1986b; Mohammed et al., 2014). The most critical root pathogens are the basidiomycetes (*Rigidoporus microporus* (Sw.) Overeem., *Phellinus noxius* (Corner) G. Cunn., and *Ganoderma pseudoferreum* (Wakef.) Overeem & B.A. Steinm (Sripathi Rao, 1975). *R.*

microporus is the most harmful among these root pathogens (Mohammed et al., 2014). Tropical rubber plantations suffer from the white-rot disease caused by this invasive pathogen. It also affects cocoa, coffee, tea, coconut, oil palm, Ceylon breadfruit, and Obeche (Begho & Ekpo, 1987; Nandris et al., 1987b; Madushani et al., 2014). It is abundant in plantations but is scarce in natural forests (Onokpise, 2004). H.N Ridley first identified the fungus as a pathogen of *H. brasiliensis* in Malaysia in 1904 (Holiday, 1980).

WRD is threatening rubber tree plantations in South and Southeast Asia and Western Africa, resulting in tree death and significant yield losses (Nandris et al., 1987a; Oghenekaro et al., 2014, 2020; Silva et al., 2014; Syafaah et al., 2020; Wiyono et al., 2020; Go et al., 2021). In contrast, this disease is not severe in South America, where the rubber tree host originated (Oghenekaro et al., 2014; 2020). It was revealed that the annual economic reduction due to white root disease, mainly in smallholder plantations, was approximately 2.1 billion rupiahs (~MYR 63 million) and MYR 716 million in Indonesia and Malaysia, respectively (Prasetyo & Aeny, 2013; Atan, 2015).

Additionally, economic losses in southern Thailand were recorded at around 24,600- 478,930 baht (~ MYR 15,816.98 – MYR 60,796.35) per 0.16 hectare, considering the infection of white root disease on rubber trees as early as one year old until the rubber trees reached the age of 25 years old (Nissapa & Chuenchit, 2011). According to a survey undertaken in some parts of Indonesia, the white-rot fungus has impacted 80,000 hectares of rubber trees (Jayasinghe, 2010). In Nigeria, *R. microporus* kills up to five trees per hectare annually and responsible for 96% of all root diseases in rubber plantations (Omorusi, 2012). Besides, a mean annual incidence of white root disease was reported between 5 - 30% of rubber plantations surveyed in Malaysia which significantly reduces the number of rubber trees stand that can be tapped (Soepena, 1993; Atan, 2015) (Figure 2.1). Moreover, the incidence of WRD caused by *R. microporus* in Malaysia (Peninsular Malaysia, Sabah, and Sarawak) is predicted to reduce the country's annual income by up to 11% (Atan et al., 2017).

Figure 2.1 The sequence of white root disease incidence in Malaysia

2.2.1 The causal agent and symptoms of white root disease of rubber

Rigidoporus microporus is a pathogen of *Hevea brasiliensis* that causes white rot disease (WRD) in numerous tropical crop species (Holiday, 1980; Go et al., 2021). It was initially discovered in 1904 as a pathogen of *H. brasiliensis* at a Singapore botanical garden. *R. microporus* is characterized by an ectotrophic growth habit where the mycelium is visible as white flattened strands of 1-2 mm in thickness, that grow and firmly adhere to the bark of the root (Nandris et al., 1988; Richard & Button, 1996; Andrew et al., 2021).

The disease originates on roots and later spreads to the collar region (Farhana et al., 2017). According to Jayasinghe (2010) and Oghenekaro (2016), foliar symptoms are subsequently initiated with the root system's destruction. Wattanasilakorn et al. (2017), Syafaah et al. (2020), and Go et al. (2021) indicated the foliar symptoms of WRD occur when the leaves turn yellow, wilting, defoliation, and finally, death of the host. Premature flowering and fruit production, branches die back, and white mycelia on roots are common symptoms (Kelaniyangoda et al., 2013; Omorusi et al., 2014; Soytong & Kaewchai, 2014). In the latter stages of the disease bracket, fructifications appear on the collar region of the tree. The above-ground symptoms of the trees indicate that they are mostly beyond treatment and recovery, as the infection spreads quickly and death is imminent (Ismail & Azaldin, 1985; Farid et al., 2009; Omorusi et al., 2014).

For classification, the genus *Rigidoporus* belongs to Kingdom Fungi, Phylum Basidiocomycota, Subphylum Agaricomycotina, Class Agaricomycetes, Subclass Agaricomycetidae, Order Polyporales, Family Meripilaceae and Species *Rigidoporus microporus* (CABI, 2021b).

2.2.2 Infection stages and disease cycle

WRD starts in the roots of dead trees and stumps after being infected by *R. microporus* mycelia or rhizomorphs (Figure 2.2). During the growth phase, the fungus produces white rhizomorphs that may attach to wood debris in the soil and spread over long distances in the absence of wood waste, allowing it to infect healthy trees (Omorusi, 2012; Oghenekaro et al., 2016; Mazlan et al., 2019; Shabbir et al., 2020a). It eventually spreads to the collar region, and foliar symptoms appear only after the roots are destroyed, obstructing water flow and minerals. As a result, the latex flow is halted. The distinctive reddish-brown basidiocarps of *R. microporus* are visible on the stem's collar region (Omorusi, 2012; Ogbebor et al., 2015b; Andrew et al., 2021).

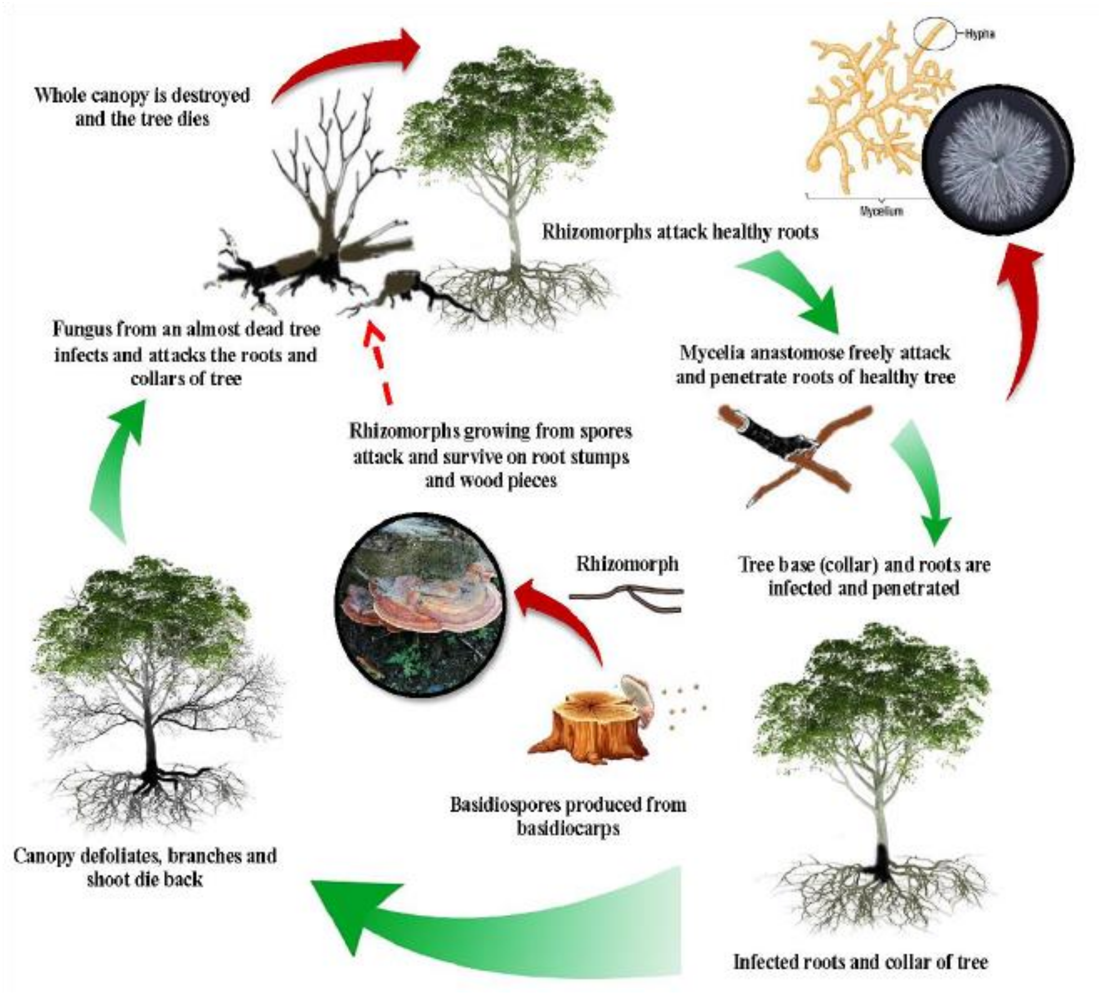

Figure 2.2 The cycle of white root disease (Woraathasin, 2017), as cited in (Nakkanong et al., 2019).

Although the disease is caused by biotic variables (host and pathogen), it is also influenced by abiotic factors such as humidity, temperature, pH, soil porosity, and soil characteristics (Prasetyo et al., 2009). Furthermore, Dalimunthe et al. (2017) discovered that the WRD thrives in environments with high humidity, adequate aeration, and a high organic matter content. In-vitro studies have shown that the media's pH and the type of nitrogen and carbon sources affect the growth and differentiation of fungal mycelial strands (Richard & Button, 1996). The growth of *R. microporus* is aided by neutral pH (it can thrive at pH 6 –7), whereas fungal growth is slowed and suppressed at pH 4 and lower (Ismail & Azaldin, 1985; Go et al., 2021).

When *R. microporus* attacks a rubber tree, it causes the lignified root tissues to deteriorate, resulting in the tree's death (Oghenekaro, 2016). It has been recognised that *R. microporus* is an effective wood decomposer, capable of simultaneous lignin and cellulose breakdown, which is typical of white-rot fungus (Geiger et al., 1989; Nicole & Benhamou, 1991; Oghenekaro et al., 2020). Numerous research on host-pathogen interaction has been conducted, examining various elements of biochemistry, anatomy, histology, and cytology (Nicole et al., 1986a, 1993; Nandris et al., 1987a; Nicole & Benhamou, 1991).

One of the most consistent reactions of the rubber tree in response to *R. microporus* attack is an increase in the number of cell layers under the points of penetration, the walls of some cells thicken significantly, and there is a variable degree of increased peroxidase activity (PA) in infected or newly formed tissues in comparison to healthy tissue (Nicole et al., 1986a). Furthermore, Nandris et al. (1987b) mentioned that phellogen activity causes redundant cell layers at penetration sites during the initial colonisation of the cortical tissues. Some cell walls thicken following suberification or lignification.

The creation of papillae (wall appositions) alters the morphology of the walls in young cork. In addition, tyloses development obstructs vessels in the xylem, and some walls have an additional layer (Nicole et al., 1985). On the other hand, Geiger et al. (1985) and Galliano et al. (1990) discovered that *R. microporus* generates laccase, glycosidases, polyoxidases and manganese peroxidase, which are required for lignin breakdown. Additionally, white-rot fungi destroy the lignin component of plant tissue, allowing them to access the more structured carbohydrate polymers cellulose and hemicellulose (Lynd et al., 2002).

Natural resistance to root pathogen penetration exists in *H. brasiliensis* (Mazlan et al., 2019). According to Nakkanong et al. (2019), when pathogen-infected plant roots congregate in a favourable environment, such as a moist and cool soil environment, the pathogen enters the xylem vessels of the seedling through damping-off, and the pathogens generate hyphae and/or conidia. The root initiates the extraction and deposit of a defence-related chemical in the rhizosphere. On the other hand, the plant recognizes the pathogen via molecular patterns associated with microbes in the basal defence system (MAMPs). MAMP-triggered immunity is triggered when a membrane-bound pattern recognition receptor (PRR) is activated and a plant-derived danger or damage-associated molecular pattern (DAMP) is released (MTI). The leaves and roots of *Arabidopsis* contain MTI. MTI triggers an inducible response to identify pathogenic bacteria, resulting in the creation of secondary metabolic products such as protein. It is regulated by phytohormones such as jasmonic acid, salicylic acid, and ethylene. Most of the time, jasmonic acid is involved in the pathogenesis of biotrophic infections. Salicylic acid is involved in a necrotrophic pathogen attack, whereas ethylene is involved in herbivore attacks.

2.3 Pathogenicity test

Pathogenicity refers to a pathogen's ability to cause disease, reflecting both its genetic component and the host's damage (Bos & Parlevliet, 1995; Casadevall & Pirofski, 1999; Cai et al., 2013). Pathogenicity can also be defined as a complex time-dependent interaction between a host and a pathogen, with each potential variable in a changing environment serving to distinguish the pathogen's host specificity and disease severity (Casadevall & Pirofski, 2003). Pathogens exhibit a wide range of virulence, which refers to how the microbes cause disease (Bos & Parlevliet, 1995; Casadevall & Pirofski, 2009). Although virulence is typically associated with a pathogen's ability to replicate within its host, it can also be influenced by other factors such as environmental conditions. There is typically a correlation between virulence and the pathogen's ability to multiply within the host, but other factors may influence this, such as environmental conditions.

A pathogenicity test can also determine various fungal isolates' host range and virulence. Virulence refers to the degree of damage caused by a pathogen to the host (Bruns et al., 2012). The virulence level also refers to the aggressiveness of the pathogen to incite the disease. The degree of virulence can be influenced by the pathogen, host, various nutrients, and environment (Bodah, 2017; Huber & Haneklaus, 2007), which the environment could influence as the pathogen dispersal is sensitive to either wind or water (Polanco et al., 2014; Velásquez et al., 2018).

The pathogenicity and virulence level may differ depending on the type of plant or variety attacked. Several studies of host range have been conducted for different *R. microporus* isolates such as pathogenicity test on RRIM 600 clone (Kaewchai & Wang, 2009), 10 local clones (PSU1 and PSU2) compared to RRIM 600 and GT 1 (Wattanasilakorn et al., 2012), seven selected clonal rootstocks (EIRpsu 1, EIRpsu 2, EIRpsu 3, EIRpsu 4, EIRpsu 5, EIRpark and EIRrak (Wattanasilakorn et al., 2017), and PB 350 clone (Shabbir et al., 2021). Host range studies provide valuable information for developing control strategies, particularly for biological and chemical applications.

The technique used to determine pathogenicity varies according to the pathogen being tested. For *R. microporus,* artificial inoculation of rubber rootstock seedlings with *R. microporus* methods are applied. For example, in a study by Shabbir et al. (2021) on white root rot disease of rubber caused by *R. microporus,* pre-colonised rubber woodblocks placed in contact with roots were applied for pathogenicity test. The results showed that foliar symptoms appeared on rubber seedlings after 24 weeks of inoculation. The disease severity caused by *R. microporus* was based on the development of internal symptoms in the roots of inoculated rubber seedlings.

2.4 Disease control management

Correct and effective disease management of WRD caused by *R. microporus* is vital to prevent disease outbreaks that can cause significant root damage and production losses. According to Omorusi (2012), root disease control aims to eradicate the sources of infection or inocula during the early phases of tree development to prevent infection of the remaining trees as they mature. Integrated disease management, which involves a combination of different strategies like cultural, biological, and chemical controls, has been suggested as the most effective method to control WRD of rubber (Nakaew et al., 2015; Noran et al., 2015; Monkai et al., 2017; Chaiharn et al., 2019; Shabbir et al., 2021).

2.4.1 Cultural control

Cultural control refers to control methods aimed at disease avoidance through avoiding inoculum introduction, good sanitary practices, and manipulation of cropping patterns (Noran et al., 2015; Ogbebor et al., 2015a). Cultural practises playing a significant role in disease control, particularly those aimed at lowering the inoculum level. According to the Malaysian Rubber Board (2009b), in the mature rubber tree plantation, the isolation trench with 30 cm wide and 60 cm dimensions needs to be constructed all-around the infected tree at half the distances of a neighbouring healthy tree to avoid the infection of WRD.

The methods used to remove old trees from the land when replanting a rubber area determine the residual level of inoculum. Full mechanical clearance, such as uprooting trees, ploughing, and raking the soil to collect and dispose of the rubber roots, will provide the lowest root disease incidence in a replanting area (Soytong & Kaewchai, 2014). However, this procedure is costly and quite difficult for smallholders to adopt.

2.4.2 Biological control

Biological control uses living organisms such as pathogens, predators, and parasites to reduce pest infestations and microbial infection (Stenberg et al., 2021). Controlling plant diseases using biological control could ameliorate high costs, environmental concerns, and health hazards.

Biological control methods tested in-vitro and in-vivo include the use of species of *Hypocrea*, *Trichoderma* spp., *Chaetomium* spp., *Aspergillus niger* and *Streptomyces* isolated from *H. brasiliensis* rhizospheric soil have been investigated and found to be effective as biological agents for suppression of white root disease in *Hevea brasiliensis* caused by *R. microporus* (Jayasuriya & Thennakoon, 2007; Damiri et al., 2014; Ogbebor et al., 2015b; Chaiharn et al., 2019).

Besides, Soytong & Kaewchai (2014) revealed that the bioactive compound from *Chaetomium cupreum* named rotiorinol showed the ability to inhibit 60-80% of *R. microporus* growth at 250 and 500 µg/l with effective dose (ED50) 26 µg/ml. A similar study observed that the stem extracts of kemuning cina were antagonistic against *R. microporus* fungus (Zaini & Halimoon, 2013). Maiden et al. (2017)

revealed the effectiveness of environmentally friendly chitinolytic microorganisms (SPSB 4-4) that can inhibit 91.63% of *R. microporus* growth.

Another study found that using *Cladobotryum semicirculare* inoculant on two different scion-rootstock combinations of rubber clones (PB 350-RRIM 2025 and PB 347 - RRIM 2025) reduced the area under the disease progress curve by 47 to 50%, depending on the severity of white root disease (Goh et al., 2018). *Catharanthus roseus L. (pink)* extracts with the concentration of 2000 µg/mL were found effective to reduce 100% of the disease index (DI) and disease severity (DS) of inoculated rubber rootstock seedlings with *R. microporus* and considered suitable to be used as a bio fungicide for controlling *R. microporus* on rubber crops (Zahari et al., 2019).

Shabbir et al. (2021) suggested that a peat-based bioformulation of *Enterobacter* sp. and an AMF (*Glomus mosseae*) supplemented with Si could be a potential strategy for suppressing WRD and enhancing the growth of rubber rootstock seedlings. However, Chaiharn et al. (2019) mentioned that biological agents were not very effective in large-scale operations, although they have proven effective in combating white root disease in rubber plantations. Therefore, further research and validation in field trials are crucial before biocontrol agents can be promoted commercially.

2.4.3 Chemical control

Chemical application has become critical for effective and rapid plant disease management, as evidenced by many commercial fungicides on the market. In the early year, rubber growers widely adopt the incorporation of sulphur in the planting hole when planting rubber seedlings as a practice to mitigate the incidence of WRD. The increased acidity of the soil from incorporating sulphur may promote the growth of antagonistic microorganisms against *R. microporus* and effectively suppress the growth of *R. microporus* (Ismail & Azaldin, 1985; Liyanage et al., 1977; Omorusi, 2012; Prasetyo & Aeny, 2013). However, Satchuthananthavale & Halangoda (1971) revealed that sulphur supplementation may lose effectiveness after two years, impair plant nutrition, delay the breakdown of major food bases spread throughout the field, and have no influence on disease incidence. It was also discovered that excessive amounts of sulphur to reduce soil pH will damage the early plants' fine roots and cause their deaths (Rodesuchit et al., 2012).

New fungicides, such as triazole compounds, have improved the control of *R. microporus*-caused WRD incidence (Gohet et al., 1991). It was found effective based on the experiments done in-vitro and on a small scale on seedlings or stumps placed in artificially infected soil. The fungicides and sulfur amendments recommended for treating white root rot are propiconazole, hexaconazole and other triazoles (Lam & Chiu, 1993), tridemorph, benomyl and bayfidan (Ogbebor et al., 2015a). The success of fungicide application is higher when an infection is mild; as such, the most effective control is identifying infections at the early stage for effective treatment (Ogbebor et al., 2015a).

According to Rodesuchit et al. (2012), the interval 18 months after planting showed that young rubber stumps in soil amended with triple-superphosphate were infected by the disease and died. In contrast, soil amended with sulfur powder, ammonium sulfate, and urea showed that 92-100% of the budded stumps were not infected, significantly different from the control. Scientists have extensively reported the successful use of chemicals in managing rubber diseases (Jayasuriya et al., 1996; Evueh & Ogbebor, 2008). However, they are expensive and highly toxic to users and the environment (Komárek et al., 2010; Satapute & Kaliwal, 2015; Arena et al., 2017; Suryanto et al., 2017; Chaiharn et al., 2019). Thus, the lack of effective control methods has increased interest in fungus pathobiology research. The alternative environmentally disease management approach shall be considered to mitigate rubber diseases among rubber-producing countries like Thailand, Malaysia, Indonesia, Sri Lanka, Ivory Coast, Cameroon, and Nigeria.

2.5 Silicon

2.5.1 Silicon availability, uptake, and deposition in plant

Silicon (Si) is the second most abundant element in the Earth's crust after oxygen, accounting for approximately 28% of the total, and has wide-ranging implications in plant biology (Epstein, 1994; Luyckx et al., 2017; Tripathi et al., 2020). Despite being the second most abundant element in the earth's crust, Si is not considered or is still being debated as a nutrient for plants, and evidence that Si is essential for higher plants is still lacking (Savvas & Ntatsi, 2015; Luyckx et al., 2017; Wang et al., 2017; Guo-chao et al., 2018; Majumdar & Prakash, 2020).

Although Si is classified as not essential for certain plants, it is still beneficial for many terrestrial plants (Romero et al., 2011; Frew et al., 2018; Islam et al., 2020). Gaur et al. (2020) pointed out the role of silicon in alleviating biotic and abiotic stress. Furthermore, they listed the beneficial impact of silicon in various aspects, including the improvement of morpho-anatomical, physiological, biochemical, and genetic attributes (Figure 2.3). In addition, Majumdar & Prakash (2020) revealed that the uptake of Si by sugarcane (500–700 kg Si ha^{-1}) sometimes surpasses those of the macronutrients (especially N, P, and K). All terrestrial plants contain Si in varying concentrations based on species, ranging from 0.1 to 10% by dry weight (Epstein, 1999; Ma & Takahashi, 2002).