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DEVELOPMENT AND EVALUATION OF BIO-FORMULATION FOR GROWTH AND SUPPRESSION OF WHITE ROOT ROT DISEASE IN RUBBER SEEDLINGS

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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July 2020

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White root rot (WRR) disease caused by *Rigidoporus microporus* is a serious problem of rubber trees. Fungicides are used to control WRR disease, but they cause fungicide resistance. Moreover, fungicides effectiveness depends on early detection of disease, while use of biocontrol agents would be an effective approach to control WRR disease. Silicon (Si) is a beneficial element that increases disease resistance, but it is mostly present in insoluble forms. Silicate solubilizing bacteria (SSB) are biocontrol agents that can solubilize insoluble silicates. Arbuscular mycorrhizal fungi (AMF) are known to improve nutritional status of plants. This study evaluated SSB isolate from rubber rhizosphere and AMF (*Glomus mosseae*) with Si for growth promotion and WRR disease suppression in rubber seedlings, and then evaluated the efficacy of peat based bio-formulation of SSB and AMF with Si for WRR disease suppression. A laboratory study was conducted to isolate and characterize SSB isolates and then screened for growth promotion of rubber seedlings in a glasshouse study. Two glasshouse studies were conducted using selected UPMSSB7 isolate from isolation study and AMF with Si for growth promotion and WRR disease suppression. The peat based bio-formulation of UPMSSB7 and AMF with Si was developed to evaluate its shelf life during 24 weeks of storage. A glasshouse study was conducted to evaluate bio-formulation of UPMSSB7, AMF with Si for WRR disease suppression compared with fungicide. The results from *in vitro* study revealed that UPMSSB7 (*Enterobacter* sp.) had the highest solubilization of silicate (11.55 mg L^{-1}) in liquid assay at 10 DAI and inhibition of *R. microporus* (57.24%). The glasshouse study revealed that UPMSSB7 with Si had the highest growth parameters of rubber seedlings. Thus, UPMSSB7 was selected for subsequent studies. The growth promotion study revealed that co-inoculation (T5) had the highest stem height (104.8 cm), leaf area (3510.7 cm^2), shoot and root dry weight (29.22 g and 29.20 g, respectively). The WRR disease suppression study revealed that co-inoculation (T5) had the lowest disease incidence (DI, 16%), disease severity of foliar (DSF, 14%) and root rot symptoms (DRS, 12%). The bio-

formulation study revealed that bio-formulation of UPMSSB7 and AMF with Si (T4) had the highest shelf life of bio-formulation during 24 weeks of storage. The glasshouse study revealed that bio-formulation of UPMSSB7 and AMF with Si (T4) reduced WRR disease as effectively as fungicide and had the lowest DI, DSF and DSR (18%, 17% and 16%, respectively). It was concluded that bio-formulation could be used as an effective sustainable approach to suppress WRR disease in rubber seedlings at nursery stage to reduce the use of fungicides.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PEMBANGUNAN DAN PENILAIAN BIOFORMULASI UNTUK PERTUMBUHAN DAN PERENCATAN PENYAKIT REPUT AKAR PUTIHPADA ANAK POKOK GETAH

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Penyakit reput akar putih (WRR) yang disebabkan oleh *Rigidoporus microporus* adalah masalah serius pada pokok getah di seluruh dunia. Racun kulat digunakan untuk mengawal penyakit WRR, tetapi menyebabkan ketahanan terhadap racun kulat. Lebih-lebih lagi, keberkesanan racun kulat bergantung pada pengesanan awal penyakit, sementara penggunaan agen biokontrol akan menjadi pendekatan yang efektif untuk mengendalikan penyakit WRR. Silikon (Si) adalah unsur bermanfaat yang meningkatkan daya tahan penyakit, tetapi kebanyakannya terdapat dalam bentuk yang tidak larut. Bakteria larut silikat (SSB) adalah agen biokontrol yang dapat melarutkan silikat tidak larut. Kulat mikoriza arbuskular (AMF) diketahui dapat meningkatkan status pemakanan tumbuhan. Kajian ini dinilai SSB mengasingkan dari rizosfera getah dan AMF (*Glomus mosseae*) dengan Si untuk peningkatan pertumbuhan dan mengawal penyakit WRR pada anak pokok getah, dan kemudian menilai keberkesanannya bio-formulasi yang berasaskan gambut dengan SSB dan AMF dengan Si untuk mengawal penyakit WRR. Kajian makmal dilakukan untuk mengasingkan dan menciri SSB pencildan kemudian disaring untuk meningkatkan pertumbuhan anak pokok getah dalam kajian rumah kaca. Dua kajian rumah kaca dilakukan menggunakan pencil UPMSSB7 yang dipilih daripada kajian pengasingan dan AMF dengan Si untuk meningkatkan pertumbuhan dan perencatan penyakit WRR. Bioformulasi berasaskan gambut dengan UPMSSB7 dan AMF dengan Si dibuat untuk menilai jangka hayatnya selama 24 minggu penyimpanan. Satu kajian rumah kaca dilakukan untuk menilai bioformulasi UPMSSB7, AMF dengan Si untuk perencatan penyakit WRR dibandingkan dengan racun kulat. Keputusan daripada kajian *in vitro* mendedahkan bahawa UPMSSB7 (*Enterobacter* sp.) mempunyai keterlarutan silikat paling tinggi (11.55 mg L^{-1}) dalam ujian cecair selepas 10 DAI dan perencatan *R. microporus* (57.24%). Kajian rumah kaca mendedahkan bahawa UPMSSB7 dengan Si mempunyai paling tinggi parameter pertumbuhan anak pokok getah. Oleh itu, UPMSSB7 dipilih untuk kajian seterusnya. Kajianpeningkatan

pertumbuhan mendedahkan bahawa ko-inokulasi (T5) mempunyai paling tinggi ketinggian batang (104.8 cm), luas permukaan daun (3510.7 cm^2), berat kering pokok dan akar (29.22 g dan 29.20 g, masing-masing). Kajian perencatan penyakit WRR menunjukkan bahawa ko-inokulasi (T5) mempunyai paling rendah insiden penyakit (DI, 16%), penyakit keterukan daun (DSF, 14%) dan simptom reput akar (DRS, 12%). Kajian bio-formulasi menunjukkan bahawa bio-formulasi UPMSSB7 dan AMF dengan Si (T4) mempunyai paling tinggi jangka hayat bio-formulasi selama 24 minggu penyimpanan. Hasil daripada kajian rumah kaca menunjukkan bahawa bioformulasi UPMSSB7 dan AMF dengan Si (T4) mengurangkan penyakit WRR sama efektif dengan racun kulat dan mempunyai DI, DSF dan DSR (18%, 17% dan 16%, masing-masing). Disimpulkan bahawa bioformulasi boleh digunakan sebagai pendekatan mampan yang berkesan untuk mengawal penyakit WRR pada anak pokok getah pada peringkat nurseri bagi mengurangkan penggunaan racun kulat.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. 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) were adhered to.

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LIST OF ABBREVIATIONS

AID	Autoclaved Induced Digestion
AMF	Arbuscular Mycorrhizal Fungi
ANOVA	Analysis of Variance
AUDPC	Area Under Disease Progress Curve
CAS	Chrome Azurol Sulphonate
CFU	Colony Forming Units
CMC	Carboxy Methyl Cellulose
CRD	Completely Randomized Design
DI	Disease Incidence
DR	Disease Reduction
DSF	Disease Severity of Foliar Symptoms
DSR	Disease Severity of Root Rot Symptoms
IAA	Indole-3-Acetic Acid
ISR	Induced Systemic Resistance
L-tryp	L-tryptophan
MEA	Malt Extract Agar
PDA	Potato Dextrose Agar
PGPB	Plant Growth Promoting Bacteria
PGPR	Plant Growth Promoting Rhizobacteria
PIRG	Percentage Inhibition of Radial Growth
RCBD	Randomized Complete Block Design
rRNA	Ribosomal Ribonucleic acid
rpm	Rotation per minute
RISDA	Rubber Industry Smallholders Development Authority

RWB	Rubber Wood Block
SAS	Statistical Analysis Software
SEM	Scanning Electron Microscopy
SPAD	Soil Plant Analysis Development: Chlorophyll Content
SSB	Silicate Solubilizing Bacteria
sp.	Species (singular)
spp.	Species (plural)
TSB	Tryptic Soy Broth
WRR	White Root Rot

CHAPTER 1

INTRODUCTION

1.1 Introduction

Rubber tree (*Hevea brasiliensis* Muell. Arg.) is a second most important industrial crop after oil palm in Malaysia (Sharib and Halog, 2017). Rubber tree has major economic importance because of its latex production, which is primary source of natural rubber (Venkatachalam *et al.,* 2013). It is grown extensively in South-East Asia, for production of natural rubber and, increasingly, timber (Evans and Turnball, 2004). Malaysia is currently the 5th largest producer of natural rubber in the world (Aziz, 2019).

The disease problems, increase in oil palm plantation area, shortage of skilled tappers and poor adoption of modern technologies among the smallholders are important factors responsible for decline in rubber production in Malaysia (Noordin, 2013). The white root rot (WRR) disease is the most destructive disease of rubber trees in all rubber growing countries, such as Malaysia, Thailand, Indonesia, Philippines and Sri Lanka as well as Africa (Goh *et al.*, 2018; Mohammed *et al.*, 2014). *Rigidoporus microporus* is causative fungal pathogen of WRR disease (Ogbebor *et al.*, 2010).

R. microporus is controlled by chemical fungicides, which cause environmental hazards along with harmful effects on soil microorganisms (Soytong *et al.*, 2005). Besides being expensive, chemicals may also result in the fungicide resistance (Jayasinghe, 2010). Moreover, the above ground foliar symptoms of WRR disease appear when affected rubber trees are beyond treatment. Therefore, fungicides are only applied on affected trees and effectiveness of the chemicals depends on early and reliable detection of *R. microporus*. In this context, the use of biological control agents to suppress WRR disease could be effective, economical and eco-friendly (Boukaew *et al.*, 2017). Several antagonistic agents, *Streptomyces* sp. (Nakaew *et al.*, 2015), *Trichoderma* spp. (Jayasuriya and Thennakoon, 2007), *Chaetomium* spp. and *Aspergillus niger* (Kaewchai and Soytong, 2010) have been reported to be effective in suppressing WRR disease in rubber trees both *in vivo* and *in vitro* studies.

Silicon (Si) is a beneficial element, which improves the growth of many plants and also provides resistance to plants against diseases (Francois *et al.*, 2005). In earth's crust, Si is the second most common element. But, most of Si is bound with recalcitrant silicate minerals and only a small amount of Si is available for plants (Struyf *et al.*, 2010). In soil, the insoluble form of Si can only be converted into the soluble form by biological activities of microorganisms. Silica (SiO_2) is compounds containing both Si and oxygen while silicate refers to Si containing crystalline or amorphous compounds, such as calcium silicate (Epstein, 2009).

Silicate solubilizing bacteria (SSB) improve plant growth and enhance plant defense mechanism against phytopathogens (Naureen *et al.*, 2015b). The SSB have been reported to solubilize the insoluble Si (Kang *et al.*, 2017) and improve growth of oil palm (Santi *et al.*, 2018), rice (Lee *et al.*, 2019) and sugarcane (Phonde and Banerjee, 2015). The SSB have also been shown to protect plants against rice sheath blight fungal disease in rice (Ng *et al.*, 2016). The SSB could suppress phytopathogens through polymerization of silicates in the soil (Vijayapriya and Muthukkaruppan, 2010).

Arbuscular mycorrhizal fungi (AMF) have been reported to stimulate growth of many plants, such as rubber (Sosa-Rodriguez *et al.*, 2013), maize (Mustafa *et al.*, 2010) and oil palm (Sundram and Idris, 2009). The AMF protect the plants against root rot disease in banana (Declerck *et al.*, 2002) and basal stem rot disease in oil palms (Rini, 2001). The AMF enhanced plant growth by solubilizing nutrients in the soil, especially phosphorus (Parkash and Aggarwal, 2011).

Combined application of plant growth promoting rhizobacetria (PGPR) and AMF enhanced plant growth and reduced root rot disease in chickpea plants (Akhtar and Siddiqui, 2008). Combined inoculation of PGPR (*Pseudomonas fluorescens*) with AMF (*Glomus mosseae*) inhibited fungal pathogen activity and promoted plant growth (Behn, 2008). The N_2-fixing bacteria (*Azotobacter chroococcum*) and AMF (*G. mosseae*) improved the growth of pomegranate compared to single microbial agent (Aseri *et al.*, 2008). Application of SSB (*Bacillus edaphicus*) and calcium silicate significantly increased cane yields in sugarcane (Brindavathy *et al.*, 2012). However, co-inoculation of SSB and AMF with Si to suppress WRR disease in rubber has not been explored. Hence, this study was carried out to evaluate the effects of co-inoculation of SSB isolate and AMF (*Glomus mosseae*) with Si on plant growth promotion and WRR disease suppression in rubber seedlings. This study was also designed to develop and evaluate the efficacy of bio-formulation of SSB isolate and AMF (*Glomus mosseae*) with Si for WRR disease suppression in rubber seedlings as compared to fungicide.

1.2 Objectives of study

The objectives of this study were:-

1. To isolate and characterize silicate solubilizing bacteria (SSB) from rubber rhizosphere for plant growth promotion and antagonistic potential against *R. microporus* under *in vitro* and to screen SSB for growth promotion in rubber seedlings under glasshouse conditions,
2. To evaluate co-inoculation of selected SSB and AMF (*Glomus mosseae*) with Si for plant growth promotion and white root rot disease suppression in rubber seedlings,

3. To develop bio-formulation of selected SSB, AMF and Si using peat moss and to evaluate the shelf life and properties of formulation during 6 month of storage duration, and
4. To evaluate bio-formulation for plant growth promotion and white root rot disease suppression in rubber seedlings in comparison with fungicide.

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BIODATA OF STUDENT

The student was born on 1st January 1989 at Bahawalpur, Punjab, Pakistan. He completed his primary education from Govt. Elementary School, Channi Goth (Bahawalpur). He completed his secondary education from Govt. Higher Secondary School, Channi Goth (Bahawalpur).

He received his Bachelor's Degree of Agriculture (Agronomy) from The Islamia University, Bahawalpur (Pakistan) in July, 2011. Then, he obtained his Master's Degree of Agriculture (Agronomy) in Sept. 2013 from University of Agriculture, Faisalabad (Pakistan). His Master's Degree was funded by USAID Merit Scholarship from the Higher Education Commission (HEC), Pakistan.

He joined Fatima Sugar Mills Limited, Muzaffargarh (Pakistan) as a cane development officer in Nov. 2013 till Feb. 2015. He then joined Govt. Secondary School, Chak Loharan (Bahawalpur) as secondary school educator (Biology) in March 2015 till Feb. 2017.

In Feb. 2017, he joined Bahauddin Zakariya University (BZU), Multan (Pakistan) as a lecturer and also got overseas scholarship to pursue PhD under BZU faculty development program through the Prime Minister of Pakistan.

In March 2017, he started his PhD program under the supervision of Prof. Dr. Radziah Othman. After her retirement, he was then put under supervision of Associate Professor Dr. Mohd Yusoff bin Abd Samad, Department of Land Management, Faculty of Agriculture, University of Putra Malaysia.

He has publications in international journals. He joined many conferences/congresses for oral and poster presentations held in Malaysia.

LIST OF PUBLICATIONS

Journal papers

Imran Shabbir, Mohd Yusoff Abd Samad, Radziah Othman, Mui-Yun Wong, Zulkefly Sulaiman, Noraini Md Jaafar and Syed Asad Hussain Bukhari (2020). White root rot disease suppression in rubber plant with microbial co-inoculants and silicon addition. *Rhizosphere* 15, p.100221. https://doi.org/10.1016/j.rhisph.2020.100221

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Imran Shabbir, Radziah Othman, Mohd Yusoff Abd Samad, Mui-Yun Wong, Zulkefly Sulaiman, and Noraini Md Jaafar (2018). Characterization of silicate solubilizing bacteria from rubber plantation for growth promotion and antagonistic properties against *Rigidoporus microporus* pathogen. 10th International Symposium on Plant-Soil Interactions at Low pH. Faculty of Agriculture, Universiti Putra Malaysia. June 25-28, 2018. Palm Garden Hotel IOI Resort Putrajaya, Malaysia. (Poster Presentation)