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STIMULATION OF ARBUSCULAR MYCORRHIZAL FUNGI SYMBIOSIS WITH SOURSOP (*Annona muricata* L.) USING RICE HUSK BOCHAR

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By

NUR SAIDAHTUL NADIAH BINTI HARUN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

June 2019

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DEDICATION

This thesis is dedicated to:

My beloved parents
Harun bin Ahamad
Zainah binti Serkawi
-for making me be who I am and encourage me to go on every adventure especially this one-

My dearest siblings
Nur Harlyana
Nur Khairunniza
Nur Izyan Ayuni
Mohd Zharif Fikri
-who did not stop supporting me all the way-

My beautiful friends
-who never let me alone-

My great and helpful supervisor
Dr. Noraini Md Jaafar
-who has been a constant source of knowledge and inspiration-

My dear self
-you've done great and I'm so proud of you-

…THANK YOU ALLAH…

Abstract of thesis is presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

STIMULATION OF ARBUSCULAR MYCORRHIZAL FUNGI SYMBIOSIS WITH SOURSOP (*Annona muricata* L.) USING RICE HUSK BIOCHAR

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Chairman : Noraini Md. Jaafar, PhD
Faculty : Agriculture

Soursop (*Annona muricata* L.) known for its medicinal and health benefits has been increasingly planted in Malaysia. However, there are limited information on soursop cultivation under Malaysian highly weathered soils. Cultivation of soursop can be further explored with incorporation of beneficial microorganism namely arbuscular mycorrhizal fungi (AMF) and organic matter such as rice husk biochar (RHB) as tools in soil management. Thus, local evaluation of AMF sources and RHB application rate on soursop seedlings under Malaysian soil is crucial to fill the knowledge gap. This study seek to stimulate arbuscular mycorrhizal fungi (AMF) symbiosis with soursop (*Annona muricata* L.) using rice husk biochar (RHB) while observing the RHB and AMF effects on soursop seedlings growth, soil physico-chemical as well as biological properties. This was done by growing 2 months old soursop seedlings in 2 separate glasshouse experiments. In the first experiment, 2 factors which are 4 RHB rates (0, 10, 15 and 20 t/ha RHB) with and without AMF inoculation were tested on soursop seedlings for 4 months. The study was arranged in Randomized Complete Block Design (RCBD) and with 4 replications. After 4 months of transplanting, soil treated with RHB and AMF significantly improved mycorrhizal colonisation and sporulation, soursop (*Annona muricata*) plant growth and plant nutrient uptake. Lower rate of RHB with AMF (+AMF + 10 t/ha RHB) promoted plant growth and gave similar effects to that at higher RHB rate (15 t/ha and 20 t/ha RHB) in stimulating AMF development. Soils treated with 10 t/ha RHB either with or without AMF, improved 4.33 % AMF sporulation, 9.30 % plant growth, 2.2 % root development, and 2.57 % plant P uptake compared to control While the first experiment suggested that AMF species inoculated in this study (*Glomus mosseae*) was able to form symbiotic association with soursop at lower application rate (<10 t/ha RHB), further determination on the AMF species compatibility, suitability and host performance at similar RHB rate. Thus, various AMF sources (AMFS 1, AMFS 2 and AMFS 3) in combination were carried out with or without 10 t/ha RHB were evaluated on soursop seedlings under glasshouse conditions. After 2 months of transplanting, all mycorhizal sources either individually (single species) or combination (mixed species) significantly increased AMF sporulation (31%) and improved growth of soursop seedlings compared to non-inoculated control. Mixed AMF species found in AMFS 2 was able to stimulate AMF and plant growth (15.5 % plant height,

0.32 % chlorophyll content as well as bacterial population (0.02%). In conclusion, RHB at lower rate (10 t/ha RHB) was optimal and did not have any detrimental effects to AMF or soursop seedlings. While locally isolated *Glomus moassae* (single species) and commercial inoculums were able to form symbiotic relationship with soursop roots, having more than one AMF species would result in better soursop growth. Inoculating soursop seedlings with AMF at nursery stage up to 6 months old may provide more chances for the plant to establish symbiotic relationship with AMF before transplanted to field.

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

RANGSANGAN SIMBIOSIS KULAT MIKORIZA ARBUSKUL TERHADAP DURIAN BELANDA (*Annona muricata* L.) MENGGUNAKAN BIOCHAR SEKAM PADI

Oleh

NUR SAIDAHTUL NADIAH BINTI HARUN

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Durian belanda (*Annona muricata* L.) yang terkenal dengan faedah perubatan dan kesihatannya telah semakin meluas ditanam di Malaysia. Walau bagaimanapun, maklumat mengenai penanaman durian belanda di tanah terluluhawa Malaysia adalah terhad. Penanaman durian belanda boleh diteroka dengan penggabungan bahan organik dan mikroorganisma berfaedah seperti biochar sekam padi (RHB) dan kulat mikoriza arbuskul (AMF) sebagai kaedah pengurusan tanah. Oleh itu, penilaian tempatan terhadap sumber AMF dan kadar penggunaan RHB pada anak benih durian belanda di tanah Malaysia adalah penting untuk mengisi jurang pengetahuan. Kesan RHB dan AMF pada pertumbuhan anak benih durian belanda, fiziko-kimia tanah serta sifat-sifat biologi telah dinilai oleh pertumbuhan benih durian belanda berusia 2 bulan dalam 2 eksperimen berasingan di rumah kaca. Dalam eksperimen pertama, 2 faktor iaitu 4 kadar RHB (0, 10, 15 dan 20 t/ha RHB) dengan dan tanpa inokulasi AMF diuji pada durian belanda selama 4 bulan. Kajian ini dilakukan menggunakan Reka Bentuk Blok Lengkap Rawak (RCBD) dan 4 replikasi. Selepas 4 bulan proses pindah tanam, tanah dirawat dengan RHB dan AMF meningkatkan pertumbuhan tumbuhan durian belanda (*Annona muricata*), pengambilan nutrien tumbuhan dan pengkolonian mikoriza dan spora mikoriza. Kadar RHB yang lebih rendah dengan AMF (+AMF bersama 10 t/ha RHB) membantu pertumbuhan tumbuhan dan memberikan kesan yang sama dengan RHB yang lebih tinggi (15 t/ha dan 20 t/ha RHB) dalam merangsang pembentukan AMF. Tanah yang dirawat dengan 10 t/ha RHB sama ada secara bersendirian atau kombinasi bersama AMF meningkatkan AMF spora (4.33%), membantu pertumbuhan pokok (9.30%) dan pembentukan akar (2.2%) serta pengambilan tanaman P (2.57%) berbanding tanaman kawalan. Walaupun eksperimen pertama menunjukkan spesies AMF yang diinokulasi dalam kajian ini (*Glomus mosseae*) mampu membentuk persamaan simbiotik dengan durian belanda pada kadar penggunaan yang lebih rendah (<10 t/ha RHB), kajian lanjutan dilaksanakan berkenaan keserasian spesies AMF, kesesuaian dan kelakuan perumah pada kadar RHB yang sama. Oleh itu, pelbagai sumber AMF (AMFS 1, AMFS 2 dan AMFS 3) digabungkan dengan atau tanpa 10 t/ha

RHB dinilai pada benih durian belanda di bawah keadaan rumah kaca. Selepas 2 bulan proses pindah tanam, semua sumber mikoriza sama ada secara individu (spesis tunggal) atau gabungan (spesis campuran) meningkatkan pertumbuhan anak pokok durian belanda berbanding dengan rawatan tanpa inokulasi. Spesis campuran AMF yang ditemui di AMFS 2 dapat merangsang pertumbuhan AMF spora (31%) dan meningkatkan pertumbuhan tanaman (ketinggian pokok (15.5%), kandungan klorofil (0.32%) serta populasi bakteria. Kesimpulannya, RHB pada kadar yang lebih rendah (10 t/ha RHB) adalah kadar yang optimum dan tidak mempunyai sebarang kesan yang merugikan kepada AMF atau anak benih durian belanda. Walaupun *Glomus moassae* (spesies tunggal) dan inokulum komersial dapat membentuk hubungan simbiotik dengan akar durian belanda, mempunyai lebih daripada satu spesies AMF akan menghasilkan pertumbuhan durian belanda yang lebih baik. Inokulasi antara anak pokok durian belanda dengan AMF pada peringkat nurseri sehingga tempoh 6 bulan boleh memberikan lebih banyak peluang untuk tumbuhan menghasilkan hubungan simbiotik dengan AMF sebelum dipindahkan ke lapangan.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

%	Percentage
℃	Celcius
*	significant
et al.	and friends
cfu	colony forming unit
cm	centimeter
cm^2	square centimeter
cm^3	cubic centimeter
g	gram
g/plant	gram per plant
mg/g	miligram per gram
mg/kg	miligram per kilogram
m	meter
mL	mililiter
mm	milimeter
nm	nano meter
ns	not significant
t/ha	ton per hectare
µl	microliter
µm	micrometer
AA	Auto Analyzer
AAS	Atomic Absorption Spectrometer
AMF	Arbuscular Mycorrhizal Fungi
ANOVA	Analysis of Variance
C	Carbon
Ca	Calcium
CO_2	Carbon dioxide
DOA	Department of Agriculture
GAE	Gallic Acid Extraction
HSD	Honest Significant Difference
K	Potassium
MARDI	Malaysian Agriculture Research and Development Institute
Mg	Magnesium
N	Nitrogen
P	Phosphorus
RCBD	Randomized Complete Block Design
RHB	Rice Husk Biochar
SAS	Statistical Analysis System
SPAD	Soil Plant Analysis Development

CHAPTER 1

INTRODUCTION

Annonas such as soursop, apple custards, cherimoya, atemoya and others are economically important in many countries such as Asia, Africa, South, North and Central America. They are widely distributed throughout tropical and subtropical country including Malaysia. Malaysian tropical climate allows various fruits including Annonas to be grown throughout the year. Annonas with stronger consumer demand in Malaysia are sugar apple, cherimoya and soursop.

Soursop (*Annona muricata* L.) is an important tropical fruit with great economic potential. It is a multipurpose plant with acceptable nutritional value as food product, sources of medicinal and industrial products (Southern Centre for Underutilized Crops, 2006). The fruits are usually being eaten fresh or raw, made into juices and act as healing pharmaceutical products while other plant parts are used for other traditional medicinal purposes and has contributed to the planting of soursop with the coverage of 443.99 ha in Malaysia (DOA, 2017). Soursop has bioactive compound such as acetogenine, alkaloids, terpens, flavonoids and oils which can be important in various field such as in agriculture (Pinto *et al.,* 2005).

As a tropical country, Malaysia has been actively cultivating soursop to meet the increasing demand of the fruits and products. However, there are limited informations on soursop cultivation in Malaysia. While soursop's medicinal aspects is increasingly studied and widely acknowledged in Malaysia, the agronomic information of soursop cultivation especially to optimize the growth and bioactive compound in soursop are greatly lacking. Due to its potential, further studies on planting soursop under Malaysian soil and climate are equally important to ensure successful planting and yield production of soursop in Malaysia. However, Malaysian highly weathered soils and limited nutrients availability can be among limiting factors for soursop cultivation in Malaysia. This is seen as a major constraints to optimize soursop cultivation when nutrients such as phosphorus is fixed and not available for plant uptake. Thus, soil management is seen as a method to overcome the concerns for soursop cultivation at both seedlings and field planting in Malaysia by considering the use of beneficial microorganisms as well as soil organic matter.

The arbuscular mycorrhizal fungi (AMF) plays vital role in plant development, plant protection and overcoming soil related problems especially nutrients and water uptake (Coelho *et al.*, 2012). Previous studies have demonstrated the potential of inoculating AMF on soursop (Ojha *et al.,* 2008; Samarao *et al.,* 2011; Syahidah, 2016). Previously Chu *et al.,* (2001) reported that soursop is a mycorrhizal dependent plant. In addition, other works have shown that AMF postitively promote soursop growth in Annonas (Ojha *et al.,* 2008; Samarao *et al.,* 2011). On the other hand, interaction between AMF and organic matter may improve plant nutrient uptake by improving the soil exploration and contributes to stimulate plant growth (Warnock *et al.,* 2007).

The use of organic amendments can promote microbial activities, soil quality and thus may be useful for the growth of many plants. Many studies have been focusing on using biochar as soil amendments (Steiner *et al.*, 2007). Biochar has ability to offer multiple environmental benefits in which they are not only contribute to carbon storage but also as soil amendments (Steinbeiss *et al.*, 2009).

At present, biochar incorporation with beneficial microbes have been shown to help in improving soil fertility and plant physiology. In addition, Christopher *et al.*, (2012) showed that crop yields and soil field capacity can increase due to biochar application. At the same time, these interaction between organic matter stimulating AMF can offer a great potential for sustainable soursop cultivation. In other regions, soursop cultivation has been shown to improve via application of vermicompost and beneficial arbuscular mycorrhizal fungi (AMF).However, the studies related to AMF and biochar on soursop are still limited in Malaysia. Therefore, further understanding in agronomic aspects of soursop cultivation incorporating soil beneficial microorganism and soil amendment is crucial for the soursop growth optimization. Thus, this study is aimed to discover the effects of AMF on growth performance of soursop plant that were planted under Malaysian soils. The glasshouse studies were conducted with the following objectives:

1) to determine the establishment of arbuscular mycorrhizal fungi (AMF) symbiosis with varying rice husk biochar (RHB) application rate and their effects on soursop seedlings, and
2) to compare the establishment of mycorrhizal symbiosis from varying AMF sources and their effects on soursop seedlings.

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LIST OF PUBLICATIONS

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