UNIVERSITI PUTRA MALAYSIA

EFFECTS OF Leclercia adecarboxylata INCORPORTED WITH AGRICULTURE WASTE MATERIALS ON GROWTH OF COCOA (Theobroma cacao L.) SEEDLINGS

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By

NURFADZILAH BINTI MADIAN

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

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

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July 2019

Chair : Tan Geok Hun, PhD
Faculty : Agriculture

Cocoa industry in Malaysia has reached the crisis of production where the production and acreage on planting are declining drastically. The total production of cocoa in 2000 was 70,231 tons and continues to decline to 1,723 tons in 2016. This industry has started to decline since 1990's due to the outbreak of disease, unstable price, and relatively high cost of fertilizer. Therefore, Malaysia Cocoa Board is trying hard in minimizing the outbreak of disease and reducing the cost of fertilizer since the major contribution to the cost of production come from the cost of fertilizer. Hence, there is a potential value by emerging the technologies of biofertilizer since the cost production is much cheaper and more environmental friendly. The study was aimed to develop and produce a biofertilizer known as bioenhancer and evaluate the potential value of selected bacteria incorporated with selected carrier materials on the growth response of cocoa seedlings. This study consists of three stages which are isolation and screening of the potential bacteria based on *in-vitro* and *in-vivo* studies, viability determination of *Leclercia adecarboxylate* in the carrier materials and efficacy of bioenhancer in cocoa seedling growth performance. In the first stage, there are 256 isolates (140 of rhizospheric isolates and 116 endophytic isolates) of sixteen healthy cocoa root samples from eight different locations were obtained and further screening was done to identify the most beneficial bacteria with several abilities such as ability to fix nitrogen, solubilize phosphorus and potassium, and ability to produce plant hormone (IAA). This evaluation was done through *in-vitro* study in Laboratory TG 3 (JTP), Universiti Putra Malaysia and Microbiology laboratory in Cocoa Research and Development Centre, Jengka, Pahang. Further *in-vivo* study was done in glasshouse nursery at CRDC Jengka to identify highly potential bacteria to be used in the bioenhancer. Seven potential isolates were adopted and based on the result, isolate was identified as *Leclercia adecarboxylata.* This bacteria shows higher effect on cocoa seedling growth performance. The next stage was evaluation of bacteria viability in the selected carrier materials. The result showed that combination of cocoa pod husk (CPH)

and rice husk char (RHC) or single material of rice husk char (RHC) with 1.2% of bacteria solution showed higher colony forming unit after sixth month of applications. The last stage of the study showed that the application of 1.2% of beneficial bacteria with 30 g of CPH and RHC promotes the cocoa seedlings growth rate by 56.05% (plant girth) and 73.15%(plant height) while, applications of 1.2% of beneficial bacteria with 45g of CPH and RHC increase leaves, stem, root weight by 38.66%, 75.35%, and 50.40% respectively as compared with normal chemical fertilizer applications. This result indicated that bacteria incorporated with selected carrier material improve cocoa seedling growth and biomass. The outcome of this study would reduce the cost of production for cocoa plantation and at the same time, helps in utilizing the waste product from agriculture sector.

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

KESAN GABUNGAN *Leclercia adecarboxylata* BERSAMA HASIL SISA PERTANIAN PADA PERTUMBUHAN ANAK POKOK KOKO (*Theobroma cacao*. L)

Oleh

NURFADZILAH BINTI MADIAN

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Industri koko di Malaysia telah dilanda krisis pengeluaran hasil dimana jumlah pengeluaran dan penanaman telah menurun secara drastik. Jumlah keseluruhan pengeluaran biji koko kering pada tahun 2000 adalah 70,231 tan dan terus menurun kepada 1,723 tan pada 2016. Prestasi industri ini mula mengalami penurunan sejak tahun 1990 disebabkan oleh wabak penyakit, harga yang tidak stabil dan harga baja kimia yang tinggi. Justeru itu, Lembaga Koko Malaysia berusaha untuk meminimumkan wabak penyakit dan menurunkan kos pembelian baja dimana baja merupakan penyumbang kos yang terbesar didalam kos pengeluaran. Oleh itu, terdapat potensi yang besar dalam penggunaan biobaja dimana kos pengeluarannya adalah lebih murah dan mesra alam. Kajian ini bertujuan untuk membangun dan menghasilkan biobaja (baja penggalak) serta mengkaji potensi bakteria terpilih yang digabungkan bersama hasil sisa pertanian pada tindak balas pertumbuhan anak pokok koko. Kajian ini terdiri daripada tiga fasa iaitu pemencilan dan penyaringan bakteria berpotensi melalui kajian kultur dan kajian didalam nurseri, kajian kebolehupayaan bakteria terpilih yang digabungkan bersama hasil sisa pertanian untuk hidup dan keberkesanan baja penggalak pada tumbesaran anak pokok koko. Pada fasa kajian yang pertama, terdapat 256 pencilan (140 pencilan Rhizosfera dan 116 pencilan endosfera) dari 16 sample akar pokok koko yang sihat dan diambil dari lapan lokasi berbeza di seluruh Malaysia. Pencilan tersebut seterusnya disaring untuk mencari pencilan bakteria terbaik dimana mampu untuk mengikat nitrogen, melarut fosforus dan kalium, dan mampu untuk menghasilkan hormon penggalak akar (IAA). Fasa pertama kajian ini dijalankan di dalam makmal penyelidikan TG3 (JTP), Universiti Putra Malaysia dan Makmal Mikrobiologi di Pusat Penyelidikan dan Pembangunan Koko Jengka, Pahang (PPPK, Jengka). Bagi mengenalpasti bakteria yang memberikan kesan paling baik, kajian aplikasi terhadap anak pokok koko turut dijalankan di dalam rumah kaca di PPPK Jengka. Tujuh pencilan bakteria dipilih dan berdasarkan kepada data yang direkodkan, bakteria *Leclercia adecarboxylata* telah dikenalpasti sebagai

bakteria yang menunjukkan kesan terbaik terhadap pembesaran anak pokok koko. Fasa kedua kajian ini adalah mengkaji kebolehupayaan bakteria untuk hidup setelah digabungkan bersama bahan pembawa. Data yang telah diambil menunjukkan bahawa rawatan menggunakan gabungan kulit koko dan arang sekam padi dan rawatan menggunakan arang sekam padi sahaja dengan gabungan 1.2% larutan bakteria menunjukkan kadar kebolehupayaan hidup yang tinggi selepas 6 bulan disimpan. Fasa terakhir di dalam kajian ini menunjukkan bahawa aplikasi 1.2% larutan bakteria bersama 30 g kulit koko dan arang sekam padi meningkatkan kadar tumbesaran anak pokok koko sebanyak 56.0% (ukurlilit pokok) dan 73.15% (tinggi pokok) manakala rawatan menggunakan 1.2% larutan bakteria bersama 45 g kulit koko dan arang sekam padi meningkatkan berat basah dan kering bagi daun, batang dan akar sebanyak 38.66%, 75.35% dan 50.40% jika dibandingkan dengan rawatan kontrol menggunakan baja NPK. Ini menunjukkan bakteria yang digabungkan dengan bahan pembawa terpilih mampu meningkatkan tumbesaran dan biojisim pada anak pokok koko. Kajian ini boleh membantu dalam mengurangkan kos pengeluaran dan membantu dalam menguruskan sisa sektor perladangan dan seterusnya membantu dalam meningkatkan kadar tumbesaran anak pokok koko.

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

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I hereby confirm that:

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LIST OF ABBREVIATIONS

(NH4)2SO4	Ammonium Sulphate
°c	Degree Celsius
µg	Microgram
ACC	1-Aminocyclopropane-1-Carboxylate
ANOVA	Analysis of Variance
BNF	Biological Nitrogen Fixation
C	Carbon
Ca	Calcium
Ca(H2PO4)2	Calcium Dihydrogen Phosphate
Ca3(PO4)2	Tricalcium Phosphate
CaCl2	Calcium Chloride
CaCO3	Calcium Carbonate
CEC	Cation Exchange Capacity
Cfu	Colony Forming Unit
cm	Centimetre
CPH	Cocoa Pod Husk
CRD	Completely Randomized Design
CRDC	Cocoa Research and Development Centre
Cu	Copper
CuSO4.5H2O	Copper Sulphate Pentahydrate
E	East
FAO	Food And Agriculture Organization
FeCl3	Iron (III) Chloride
FeCl3.6H2O	Iron (III) Chloride Hexahydrate
FeSO4.7H2O	Iron (II) Sulphate Heptahydrate
g	Gram
H3BO3	Boric Acid
Ha	Hectare
IAA	Indole Acetic Acid
K	Potassium
K2HPO4	Dipotassium phosphate
KCl	Potassium Chloride
Kg	Kilogram
kN	Kilo Newton
KOH	Potassium Hydroxide
L	Litre
LB	Luria Bertani
LSD	Fisher's Least Significant Design
M	Molar
MCB	Malaysian Cocoa Board
mg	Milligram
Mg	Magnesium
MgSO4.7H2O	Magnesium Sulfate Heptahydrate
ml	Millilitre
mL/L	Millilitre/Litre
mm	Millimetre
MnSO4.H20	Ferrous Sulphate Monohydrate

N	Nitrogen
N	North
N2	Nitrogen Gas
NA	Nutrient Agar
NaCl	Natrium Chloride
NaMO4	Sodium Molybdate
NaOH	Sodium Hydroxide
Nfb	N-Free Solid Malate Medium
NH4+	Ammonia
nm	Nanometre
NO3-	Nitrate
O	Oxygen
P	Phosphorus
PGPR	Plant Growth Promoting Rhizobacteria
PSB	Phosphate Solubilizing Bacteria
RCBD	Randomized Completely Block Design
RNA	Ribonucleic Acid
RHC	Rice Husk Char
RPM	Revolution Per Minute
T	Tonne
TE	Trace Elements
w/w	Weight To Weight
ZnSO4.7H2O	Zinc Sulphate Heptahydrate

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CHAPTER 1

INTRODUCTION

Cocoa industry in Malaysia has undergone challenging phase recently. The industry has started to decline since 1990's due to the outbreak of disease and relatively high cost of productions especially high cost of fertilizer (Malaysian Cocoa Board, 2010). In cocoa plantation, the cost of production for 1 ha of cocoa plantation was RM 6,120 and the cost of fertilizer was more than 50% from cost of production (Nurfadzilah and Rozita, 2016).

In Malaysia, inorganic fertilizer accounted for more than 90 percent of the fertilizer used (Sabri, 2009). Unfortunately, prolonged use of mineral fertilizers cause leaching and run-off of nutrients, especially in nitrogen and phosphorus which lead to environmental problems (Adesemoye and Kloepper, 2009). Several reports showed that the continuous use of inorganic fertilizer will generate environmental problems. Over 50% of nitrogen application was lost from agricultural systems through evaporation, trace gases or leaching (Adesemoye and Kloepper, 2009). Similarly for Phosphorus, where up to 90% is precipitated by metal complexes in the soil and leads to Phosphorus pollution (Park et al., 2011). In the past few decades, the application of mineral fertilizer has increased tremendously. Based on the statistics from FAO (Food and Agriculture Organization), world demand for N, P and K fertilizer is increased to 7.3% from 186,895 thousand tonnes in 2014 to 200,522 thousand tonnes in 2018. Organic or biofertilizer was used as an alternative replacement to reduce the effect of mineral fertilizer usage. This type of fertilizer is a low-cost technology, eco-friendly and harmless too environment with the benefit of supplementing nutrients.

Biofertilizer is an organic fertilizer which contains living microorganism which, when applied to the soil, or root surface of the plants, it helps in promoting growth by increasing the availability of major nutrient to the plants (Bhattacharjee and Dey, 2014). Naturally, biofertilizer helps in activating the microorganism in the soil thus increase soil fertility and protect it during drought, avoid from soil diseases and stimulate plant growth (Abbasniayzare et al., 2012). The development of biofertilizer requires carrier materials to be mixed with microorganisms. Specific microorganisms with specific abilities and good carrier materials will contribute to the successfulness of biofertilizer. In 2016, the acreage of cocoa is about 17,367 ha, which contributes about 1,723 tonnes of dried cocoa beans. Each tonne will represent about 250 kg of dry cocoa pod husks (Malaysian Cocoa Board, 2017). Therefore, the amount of dry cocoa pod husks produced is about 439.25 tonnes. Cocoa pod husk is rich in K, which contains about 2,768.0 ± 51.97mg/100g (Vriesmann et al., 2011). Therefore, it has an opportunity to develop the potential use of cocoa pod husk as organic material as well as to use it as a carrier material in developing biofertilizer.

Another surplus from rice processing mills was rice husk char. According to Theeba et al., (2012), approximately 1,200 tonnes/mill/year of rice husk char were found in rice mills. This material was unutilized causing environmental pollutions. Fortunately, this waste material can be used as soil amendments, where it rich in plant nutrient (Tang et al., 2013) and improve physical and chemical properties when applied to the fields (Mishra et al., 2017). Because of the priority to reduce the chemical fertilizer and reuse the waste from the agriculture industry, special focus has to be given in producing biofertilizer or bioenhancer. This alternative would reduce the inorganic fertilizer dependency thus increase the yield and productivity of cocoa growth.

1.1 Objectives of the study

The strength of this study is based on the usage of selected soil microorganisms and the function of a carrier material. Hence, this study was aimed to develop and produce a biofertilizer known as bioenhancer to help in increasing the plant growth of cocoa seedlings. In the view of importance selected microorganism as beneficial in the enhancement of crop growth and mobilization of nutrient to the crops, this study was undertaken with the following objectives: -

i. To isolate and screen the potential bacteria based on in-vitro and in-vivo studies.
ii. To determine and quantify the viability of selected bacteria in the carrier materials.
iii. To investigate the effects of bioenhancer on the growth performance of cocoa seedling.

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