UNIVERSITI PUTRA MALAYSIA

BIOFERTILIZER FORMULATION COMPRISING PHOSPHATE SOLUBILIZING BACTERIA TO ENHANCE PHOSPHATE AVAILABILITY FOR COCOA GROWTH

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By

MOHD YUSOFF BIN ABD SAMAD

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

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DEDICATION

I dedicate this precious effort, the fruit of my thoughts and study to my affectionate Mother, brothers and sisters, wife Ameza and children Nur Aina, Nur Farhana and Muhammad Firdaus who inspired me to the higher destiny of life.

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

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Chairman : Professor Raja Noor Zaliha Raja Abd Rahman, D.Eng.

Faculty : Biotechnology and Biomolecular Sciences

Production of cocoa can be enhanced by good fertilizer application which normally using inorganic fertilizers. However, prolonged use of inorganic fertilizers poses many environmental problems. The use of microorganisms as biofertilizer with specific functional abilities in the mobilization of nutrients is an alternative to mineral fertilizer for enhancement of soil fertility and crop production in sustainable agriculture. The general objectives of this study were to screen phosphate-solubilizing bacteria (PSB), to formulate the formulation of the bacteria inoculants using cocoa wastes and to study the shelf life of the formulations. Screening of all bacterial isolates from various sources of agricultural soils and wastes were carried out. Twenty-two isolates showed the capability of solubilizing insoluble phosphate by both plate assay and liquid medium techniques. The top three PSB namely Pseudomonas *aeruginosa* strain AGKT1, *Serratia marcescens* strain

AGKT4 and *Bacillus amyloliquefaciens* were chosen for subsequent studies. In preparation of seed culture for use in dry and liquid formulations, optimum concentrations of carbon source, nitrogen source, and density of inoculum were determined using response surface methodology. Carrier materials from cocoa pod husk and cocoa bean shell were found to be superiors as organic carrier-based materials for the selected PSB as compared to peat moss. The shelf life of bioinoculants in the dry and liquid formulations was found to be good as they had a high number of viable cells for up to 6 months with the cfu above 5 $\log_{10}$ cfu/g when stored at both temperatures of 10 ^{o}C and 28 ^{o}C. Application of the dry and liquid formulations on cocoa seedlings showed the good responses to the growth, biomass and chlorophyll content of cocoa seedlings. All the dry formulations significantly increased ($P<0.05$) fresh and dry plant biomass as well as vegetative growth of cocoa seedlings. The three formulations using cocoa bean shell with *Pseudomonas aeruginosa* strain AGKT1, *Serratia marcescens* strain AGKT4 and *Bacillus amyloliquefaciens* resulted in an increase of 86.74%, 114.31%, and 84.35% of total fresh weight of cocoa seedlings, respectively. The dry matter weight of cocoa seedlings also significantly increased at 30.05%, 48.90%, and 19.69% in the formulation using cocoa bean shell with these three PSB as compared to the control. Enhancement of nutrient uptake by the cocoa seedlings and the increase in nutrients in the soil, in particular to phosphorus (P) availability (> 15 ppm) by most dry formulations indicated that the formulations were superiors as bioinoculant preparation.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan Ijazah Doktor Falsafah.

FORMULASI BAJA BIO MENGANDUNGI BAKTERIA PELARUT FOSFAT BAGI MENINGKATKAN KEDAPATAN FOSFAT UNTUK PERTUMBUHAN KOKO

Oleh

MOHD YUSOFF ABD SAMAD

Januari 2012

Pengerusi : Profesor Raja Noor Zaliha Raja Abd Rahman, D.Eng.

Fakulti : Bioteknologi dan Sains Biomolekul

Pengeluaran koko dapat ditingkatkan dengan pemberian baja yang baik yang biasanya mengunnakan baja bukan organic. Namun, penggunaan jangka panjang baja bukan organik menimbulkan pelbagai masalah kepada persekitaran. Penggunaan mikroorganisma sebagai baja biologi dengan kemampuan fungsi-fungsi tertentu dalam mobilisasi nutrien adalah satu alternatif bagi baja mineral bagi meningkatkan kesuburan tanah dan pengeluaran tanaman dalam pertanian yang mampan. Tujuan umum kajian ini adalah untuk memencilkan bakteria pelarut fosfat, untuk merumuskan formulasi inokulan bakteria menggunakan hasil buangan koko dan untuk mengkaji kemampuan hidup bakteria dari formulasi-formula tersebut. Bakteria dipencilkan dari pelbagai sumber tanah pertanian dan bahan buangan. Pemencilan 22 bakteria mampu melarutkan fosfat tidak larut oleh kedua teknik ujian plat asai dan media cecair. Tiga bakteria terbaik dari

Pseudomonas aeruginosa strain AGKT1, *Serratia marcescens* strain AGKT4 dan *Bacillus amyloliquefaciens* telah dipilih dalam kajian selanjutnya. Dalam penyediaan kultura asas untuk digunakan dalam penyediaan formulasi cecair dan kering, kepekatan sumber karbon, nitrogen dan ketumpatan inokulum telah ditentukan dengan mengunakan kaedah tindakbalas permukaan. Bahan pembawa dari kulit buah koko dan kulit biji koko telah didapati sebagai pembawa terbaik sebagai bahan pembawa berasas organik untuk bakteria pelarut fosfat terpilih jika dibandingkan dengan tanah gambut. Daya hidup inokulan dalam formulasi kering dan cecair telah didapati baik kerana mempunyai bilangan sel bernas yang tinggi sehingga 6 bulan dengan unit pembentukan koloni adalah melebihi 5 $\log_{10}$ cfu/g apabila disimpan pada suhu 10 ^{o}C dan 28 ^{o}C. Applikasi formulasi kering dan cecair ke atas anakbenih-anakbenih koko menunjukkan tindakbalas kepada pertumbuhan, biojisim dan kandungan klorofil anakbenih koko. Semua formulasi kering meningkat secara bererti ($P<0.05$) bagi biojisim basah dan kering dan pertumbuhan tampang anakbenih koko. Tiga formulasi menggunakan kulit biji koko dengan penggunaan *Pseudomonas aeruginosa* strain AGKT1, *Serratia marcescens* strain AGKT4 dan *Bacillus amyloliquefaciens* memberikan peningkatan 86.74%, 114.31% dan 84.35% dari jumlah berat basah anakbenih koko, masing-masing. Jumlah berat kering anakbenih koko juga meningkat dengan ketara iaitu sebanyak 30.05%, 48.90% dan 19.69% dalam formulasi menggunakan kulit biji koko dengan tiga bakteria pelarut fosfat ini jika dibanding dengan kawalan. Peningkatan

pengambilan nutrien-nutrien oleh anakbenih koko dan nutrien-nutrien dalam tanah terutamanya kesediaan unsur fosforus (P) (>15 ppm) oleh kebanyakan formulasi kering menunjukkan formulasi-formulasi tersebut adalah yang terbaik sebagai penyediaan bioinokulan.

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I certify that a Thesis Examination Committee has met on **17 January 2012** to conduct the final examination of **Mohd Yusoff Abd Samad** on his thesis entitled "Biofertilizer Formulation Comprising Phosphate Solubilizing Bacteria to Enhance Phosphate Availability for Cocoa Growth" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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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 Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citation which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.

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MOHD YUSOFF ABD SAMAD

Date: 17 January 2012

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