IMPROVING BANANA AND OIL PALM SEEDLINGS GROWTH USING INDIGENOUS NITROGEN FIXING AND PHOSPHATE SOLUBILIZING BACTERIA

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ABSTRACT

Oil palm is the biggest commodity crop while banana is the second largest fruit crop planted in Malaysia. Both are highly nutrient-demanding crops that requires a large amount of fertilizer. This present an opportunity to find alternative source of nutrient that is much cheaper than the imported inorganic fertilizer for oil palm and banana plantation. Hence, the objective of this study was to develop a newly formulated biofertilizer incorporating locally isolated indigenous nitrogen fixing bacteria (NFB) and phosphate solubilising bacteria (PSB) into oil palm empty fruit bunch (EFB) compost as alternative nutrient source for banana and oil palm to improve their growth and nutrient uptake. Three NFB and PSB strains (*Enterobacter cloacae*, *Burkholderia cepacia* and *Serratia marcescens*) were successfully isolated from the root of oil palm and banana to be formulated as biofertilizer for evaluation on oil palm and banana seedlings. The formulated biofertilizer showed high viable cell count of NFB and PSB inoculants in the carrier (fine EFB compost) i.e 2.45 x 10^8 cfu/g to 4.31 x 10^9 cfu (colony forming unit)/g carrier was recorded at the second day after inoculation. Biofertilizer formulation containing *Enterobacter cloacae* showed promising and consistent effect on the growth of oil palm and banana seedlings including increased plant height (13.7% for oil palm and 15.6% for banana), improved total dry biomass production (27.4% and 33.8% respectively) and high total nutrient uptake (nitrogen (N) 30.6-48.1%, phosphorous (P) 27.2-33.1%, potassium (K) 30.5-37.7%, magnesium (Mg) 48.8%, sulfur (S) 18.1-28.5% and boron (B) 24.5%). *Enterobacter cloaceae* also showed good root colonization ability as shown from the Field emission scanning electron microscopy analysis. The use of nutrient-rich EFB compost (3.06% N, 0.37% P, 4.74% K, 3.32% calcium (Ca), 0.79% Mg, 0.07% S, 70.3 mg/kg B, 102.1 mg/kg copper (Cu), 6600.9 mg/kg iron (Fe), 160.2 mg/kg zinc (Zn), 455.2 mg/kg manganese (Mn) and 41.4% total carbon, C) as carrier for biofertilizer was able to improve the soil properties notably soil pH, organic C, total N, total P, available P and exchangeable Mg. As a conclusion, the application of locally isolated NFB and PSB together with EFB as carrier (as well as source of nutrient) was successful to improve the growth of oil palm and banana seedlings together with the soil properties. Biofertilizer formulation containing *Enterobacter cloacae* showed higher performance relative to the other formulations, hence recommended to be applied as soil mixture or directly applied into the planting hole of oil palm and banana seedlings during transplanting to the field to encourage the growth and nutrient uptake during early planting phase.

ABSTRAK

Kelapa sawit merupakan tanaman komoditi terbesar manakala pisang merupakan tanaman buah-buahan yang kedua terbesar di Malaysia. Kedua-dua tanaman ini memerlukan input nutrien yang tinggi. Ini membuka peluang untuk mendapatkan sumber nutrien alternatif yang lebih murah daripada baja inorganik import untuk ladang kelapa sawit dan pisang. Dengan ini, objektif kajian ini adalah untuk membangunkan formulasi biobaja baru daripada gabungan bakteria pengikat nitrogen (NFB) dan bakteria pelarut fosfat (PSB) ke dalam kompos tandan kosong sawit (EFB) sebagai sumber nutrien alternatif kepada kelapa sawit dan pisang demi menggalakkan pertumbuhan dan pengambilan nutrien. Tiga jenis NFB dan PSB, iaitu *Enterobacter cloacae*, *Burkholderia cepacia* dan *Serratia marcescens* berjaya diasingkan daripada akar kelapa sawit dan pisang untuk diformulasikan sebagai biobaja untuk penilaian kepada anak benih kelapa sawit dan pisang. Biobaja ini mencatatkan jumlah sel hidup inokulan NFB dan PSB yang tinggi dalam pembawa (kompos EFB halus) sebanyak 2.45 x 10^8 hingga 4.31 x 10^9 cfu (unit pembentuk koloni)/g pembawa. Formulasi biobaja yang mengandungi *Enterobacter cloacae* menunjukkan prestasi yang menyakinkan dan konsisten kepada anak benih kelapa sawit dan pisang dapat meningkatkan ketinggian sebanyak 13.7% dan 15.6% masing-masing serta meningkatkan jumlah berat kering sebanyak 27.4% dan 33.8% masing-masing. Formulasi *Enterobacter cloacae* ini juga menggalakkan jumlah pengambilan nutrien oleh anak benih kelapa sawit dan pisang pada kepekatan 30.6-48.1% (nitrogen, N), 27.2-33.1% (fosforus, P), 30.5-37.7% (potassium, K), 48.8% (magnesium, Mg), 18.1-28.5% (sulfur, S) dan 24.5% (boron, B). Analisa Mikroskopi pancaran medan imbasan elektron juga menunjukkan *Enterobacter cloaceae* mempunyai keupayaan kolonisasi akar yang baik. Kompos EFB yang kaya dengan nutrien (3.06% N, 0.37% P, 4.74% K, 3.32% kalsium (Ca), 0.79% Mg, 0.07% S, 70.3 mg/kg B, 102.1 mg/kg kuprum (Cu), 6600.9 mg/kg besi (Fe), 160.2 mg/kg zink (Zn), 455.2 mg/kg Mangan (Mn) dan 41.4% jumlah karbon, C) digunakan sebagai pembawa dalam biobaja turut meningkatkan sifat-sifat tanah terutamanya pH, jumlah organik C, jumlah N, jumlah P, jumlah P tersedia dan Mg tertukar-ganti. Sebagai kesimpulan, aplikasi pencilan bakteria NFB dan PSB dari punca tempatan beserta EFB sebagai bahan pembawa (juga sebagai sumber nutrien) telah berjaya meningkatkan pertumbuhan anak benih kelapa sawit dan pisang beserta kandungan tanah. Formulasi biobaja mengandungi *Enterobacter cloaceae* yang menunjukkan kesan yang lebih tinggi berbanding formulasi baja lain adalah disyorkan untuk digunakan sebagai campuran tanah atau ditaburkan terus ke dalam lubang tanam anak benih kelapa sawit dan pisang semasa penanaman di ladang bagi menggalakkan pertumbuhan dan pengambilan nutrien semasa fasa awal penanaman.

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LIST OF ABBREVIATIONS

ACC	-	1-aminocyclopropane-1-carboxylate
ANOVA	-	Analysis of variance
ARA	-	Acetylene reduction assays
AS	-	Ammonium sulphate (AS)
Av. P	-	Available phosphorous
BNF	-	Biological nitrogen fixation
BNF	-	Burk's nitrogen free
C	-	Carbon
C/N ratio	-	Carbon : nitrogen ratio
CEC	-	Cation exchange capacity
CPO	-	Crude palm oil
CRD	-	Complete Randomised Design
DMRT	-	Duncan's Multiple Range Test
EFB	-	Empty fruit bunches
EM	-	Effective microbe
EX	-	Exchangeable
FESEM	-	Field Emission Scanning Electron Microscope
FESEM	-	Field Emission Scanning Electron Microscope
GML	-	Ground Magnesium Limestone
Ha	-	Hectares
IAA	-	Indole acetic acid
IRRI	-	International Rice Research Institute
K	-	Potassium
Kies	-	Kieserite
LCC	-	Leguminous cover crops
MC	-	Moisture content

Mg	-	Magnesium
MOP	-	Muriate of Potash
MPOB	-	Malaysia Palm Oil Board
MRP	-	Mussoorie Rock Phosphate
N	-	Nitrogen
NA	-	Nutrient agar
NB	-	Nutrient broth
NBRIP	-	National Botanical Research Institute's Phosphate
NCBI	-	National Centre for Biotechnology Information
NFB	-	Nitrogen fixing bacteria
OC	-	Organic carbon
OM	-	Organic matter
P	-	Phosphorous
PGPR	-	Plant growth promoting rhizobacteria
PKV	-	Pikovskaya
POME	-	Palm oil mill effluent
PR	-	Phosphate Rock
PSB	-	Phosphate solubilizing bacteria
SAS	-	Statistical Analysis System
SEM	-	Scanning Electron Microscope
SI	-	Solubilization Index
SRF	-	Slow release fertilizer
TC	-	Total carbon

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CHAPTER 1

INTRODUCTION

1.1 Background

Palm oil is one of the most important edible oil in terms of world production that contributed about 35% of the total vegetable oils production in 2012/2013. Malaysia and Indonesia are the largest world palm oil producers with market percentage of 39% and 48%, respectively in 2010/2011. Oil palm planted area in Malaysia was drastically increased about 1.2 million hectares from 3.9 million hectares in 2004 to 5.1 million hectares (ha) in 2012 where Peninsular Malaysia remained as the largest planted area with 2.6 million ha, followed by Sabah with 1.4 million ha and Sarawak about 1.1 million ha in year 2010 (Malaysia Agribusiness Directory 2013/2014, 2013).

Oil palm plantation in Malaysia is managed by government agencies, private companies and smallholders. The Federal Land Development Authority of Malaysia (FELDA) is among the largest oil palm plantation agency in Malaysia with total oil palm planted area of 13% of the total oil palm planted area in Malaysia in year 2013. Other government oil palm plantation agencies, independent smallholder estates and private companies contributed about 11%, 14% and 62%, respectively of the total oil palm planted area in Malaysia (Malaysia Palm Oil Board, 2015).

Banana is one of the most important fruit crops in Malaysia with the estimated planted area around 33,495 ha in 2013. It is the second largest planted fruit crop in Malaysia after durian (88,641 ha) (Malaysia Agribusiness Directory 2013/2014, 2013). Under the New Key Economy Area of Agricultural (NKEA Agriculture) programme, banana was identified as one of the high-value non-seasonal tropical fruit crops. Approximately 9,000 ha of oil palm plantation land owned by a large government linked company (GLC) have been identified for intercropping of banana during oil palm replanting period under the entry point project number 7 (EPP 7) which is "Upgrading capabilities to produce fruit and vegetables for premium markets" (Economy Transfer Programme, 2013).

Fertilizer is the major input in agriculture to sustain plant growth and achieving high yield production. Oil palm and banana are among the highest nutrient demanding crops as compared to other commodity and fruit crops. Typical nutrient requirement for oil palm (136 palms/ha) are as follows (per hectare per year); 129 kg of nitrogen (4.5 kg/palm of Ammonium Sulphate), 109 kg of phosphorous (P_2O_5) (2.5 kg/palm of Rock Phosphate) and 367 kg of potassium (K_2O) (4.5 kg/palm of Muriate of Potash) (Izwanizam *et al*., 2013). Banana (1600 trees/ha) however, requires a much higher nutrient input than oil palm with 566 kg of nitrogen, 566 kg of phosphorous (P_2O_5) and 592 kg of potassium (K_2O) per hectare for two years (Department of Agriculture Malaysia, 2009).

The fertilizer consumption of oil palm in Malaysia in year 2010/2011 was about 374,000 tonnes of nitrogen (N), 179,000 tonnes of phosphorous (P_2O_5) and 989,000 tonnes of potassium (K_2O) which is 78.2% of the total fertilizer usage in Malaysia (Heffer, 2013). Banana recorded the highest fertilizer consumption fruit crops of Malaysia in 2008 with the estimated consumption of 6,425 tonnes of nitrogen, 6,250 tonnes of phosphorous and 10,190 tonnes of potassium (Sabri, 2009).

Nitrogen and phosphorous are the two major macronutrients which is very important for all the crops especially during early planting for vegetative growth.

Nitrogen is the most important element for banana plant growth where its deficiency could occur even on the very fertile soil and often observed under the poor rooting condition. Phosphorous uptake in banana is very rapid in the short and longer periods of planting (between 2-5 months after planting) notably for vegetative growth under tropics weather condition (Lahav and Turner, 1989).

From an industrial point of view, minimization of fertilizer cost is desirable, as this would ultimately reduce the overall operational cost for oil palm and banana plantations. For example, the estimated fertilizer cost for oil palm production in Felda Agricultural Services Sdn. Bhd. (FASSB) was about 34.4% of the total operation cost or RM 50.38/ton of fresh fruit bunch in year 2011 (FASSB, 2012). Fertilization cost in oil palm and banana plantation was increased due to fluctuation in fertilizer price in world market. The average imported fertilizer price in Malaysia has increased about 87.0% from RM 739/ton in year 2005 to RM 1,383/ton in 2013. Phosphorous fertilizer recorded the highest price increase at 135.4% followed by nitrogen fertilizer and potassium fertilizer at 107.8% and 61.6% respectively (Malaysia Agribusiness Directory 2013/2014, 2013). Therefore, an alternative source of fertilizer is important to reduce the impact of high fertilizer cost to ensure the competitiveness of banana and oil palm industry in Malaysia. In view of this, the use of oil palm biomass which is cheap and present in abundance is a feasible and commercially viable approach. One example is the effort by Felda Global Ventures (FGV) to recycle the oil palm empty fruit bunch, EFB (obtained from oil palm mills) in producing EFB compost, which can act as organic fertilizer to substitute the role of synthetic chemicals in satisfying some part of the nutrient requirement for oil palm and banana. Nevertheless, the application of EFB compost in the estate do pose some limitations such as its bulky nature, high moisture content (50-60%) and large volumes required to fulfill the plant requirement due to their low nutrient contents (1.34 % N, 0.67 % P, 1.96 % K in dried weight; Kavitha *et al.,* 2013) as compared to inorganic fertilizer. Currently, FASSB has produced fortifier EFB compost by incorporating the EFB with inorganic fertilizers such as Di-ammonium phosphate, Christmas Island Rock Phosphate, Muriate of Potash and Kieserite to increase the nutrient content of the compost. This fortifier EFB compost showed comparable plant growth performance, nutrient uptake and dry biomass production for the newly

planted oil palm as compared to the effect from the use of conventional fertilizer. The fortifier EFB not just able to save the fertilizer cost by reducing the nutrients application concentration by 30%, but also maintaining the adequate nutrient requirement for the plants (FASSB, 2013).

Nevertheless, there is always the possibility of further reducing the dependency on these N and P-based inorganic fertilizer. One of the approaches that can be taken is by the utilization of nitrogen fixing bacteria (NFB) and phosphate solubilising bacteria (PSB) to fix the atmospheric nitrogen and solubilized the insoluble phosphate in the soil to supply nitrogen and phosphorous for oil palm and banana. Both NFB and PSB inoculation to the banana and oil palm plants has been shown to have a synergistic effect in promoting faster plant growth (Rodriguez-Romero *et al.*, 2005, Mia *et al.*, 2009 and Shamsudin, 1994). Based on this, it is highly potential to incorporate NFB and PSB into EFB compost to produce an effective biofertilizer that shall act as an alternative basal fertilizer for oil palm and banana plantation. The biofertilizer is possible to be applied into the planting hole during planting to supply the nitrogen and phosphorous for the plant and promote vigorous plant growth in early stage of planting.

1.2 Problem Statement

Banana and oil palm are two nutrient-demanding crops that require a large amount of fertilizer input during both vegetative and reproductive stage. However, current high price of imported fertilizer in the world market (up to 87.1% increment from RM 739/ tonne in 2005 to RM 1383/ tonne in 2013) has resulted in the drastic increased of the production cost in oil palm and banana plantation. Therefore, an alternative source of organic-based fertilizer is needed for oil palm and banana plants to reduce the impact of high fertilizer price to ensure the competitiveness of oil palm and banana industry in world market. The oil palm empty fruit bunch (EFB)

compost which consisted of high nutrient content of potassium (1.8% K), nitrogen (2.2% N) and phosphorus (0.8% P) and magnesium (1.1% Mg)) has great potential to be utilized as fertilizer for oil palm and banana (Pupathy and Radziah, 2013). This is expected to substantially reduce the use of the relatively more expensive imported inorganic chemicals as source for fertilizer. Apart from this, the application of N-fixing and P-solubilizing bacteria into the EFB compost can also further reduce the dependency on inorganic N and P fertilizers.

1.3 Aim and Objectives

To evaluate the effectiveness of a newly formulated biofertilizer, by incorporating indigenous nitrogen fixing and phosphate solubilizing bacteria into oil palm empty fruit bunch (EFB) compost, as carrier for an alternative nutrient source to improve the growth and nutrient uptake of banana and oil palm seedling.

1.4 Scopes of Study

1. To isolate and screen the newly isolated indigenous nitrogen fixing and phosphate solubilizing bacteria from banana and oil palm root for its nitrogen fixing capacity, phosphate solubilizing activity, fastest growing strain and most viable strain in mixed culture.

2. To produce EFB compost from oil palm empty fruit bunches with palm oil mill effluent (POME) as the nutrient rich carrier for the attachment of nitrogen fixing and phosphate solubilizing bacteria.

3. To formulate biofertilizer with newly isolated indigenous nitrogen fixing and phosphate solubilizing bacteria into EFB compost as carrier.

4. To evaluate the effectiveness of biofertilizer formulation on banana and oil palm seedlings growth, biomass production and total nutrient uptake by the banana and oil palm seedling.

1.5 Research Significance

1. Evaluation on the potential of oil palm empty fruit bunch (EFB) compost inoculated with indigenous newly isolated nitrogen fixing and phosphate solubilizing bacteria as an effective nutrient source for banana and oil palm seedling.

2. Development of a new biofertilizer with an immediate commercial-viability to be used by Felda Agricultural Services Sdn Bhd (FASSB), as the R&D commercialization company of Felda Global Ventures Holdings Berhad i.e. the biggest oil palm plantation owner in Malaysia.

3. Reduction on the time period required for the growth of banana and oil palm seedlings through the application of the newly formulated biofertilizer that resulted in improved plant growth and nutrient uptake by the plants.

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