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EFFECTS OF EMPTY FRUIT BUNCH BIOCHAR AND NITROGEN-FIXING BACTERIA ON SOIL QUALITY AND GROWTH OF SWEET CORN

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By

DIYAR KAREEM ABDULRAHMAN

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

April 2015

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DEDICATION

This research is dedicated to all the scientists, researchers and environmentalists who are soldiering to keep the sanctity of our Earth's atmosphere preserved. For my great supervisor who cared so much about my work and who intelligence, support and guidance played a vital role in completing this study. For my beloved father (Kareem Abdulrahman Qadir) and mother (Amira Ali Raza) whom I wish could be here to see me become Master of Science, degree. To my dearest and beloved wife Ngin Aziz Mohammed and finally, to my beloved country Kurdistan- Iraq.

Abstract of Thesis Presented to the Senate of Universiti Putra Malaysia in Fulfillment of the Requirement for the Degree of Master of Science.

EFFECTS OF EMPTY FRUIT BUNCH BIOCHAR AND NITROGEN-FIXING BACTERIA ON SOIL QUALITY AND GROWTH OF SWEET CORN

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April 2015

Chairman : Associate Professor Radziah Othman, PhD

Faculty : Agriculture

There has been an increasing interest in biochar research as soil amendments to improve soil properties leading to improve most of plant growth in a sustainable agriculture. Tropical soil is generally unfertile unit, low organic matter, plant nutrients, acidic and low microorganisms that affect crop production. Addition of biochar such as empty fruit bunch (EFB) could improve the soil fertility. Laboratory and glasshouse studies were conducted with the following objectives, i) to determine the effect of soil sterilization, EFB biochar and N_2-fixing bacteria *Stenotrophomonas sp.* (Sb16) on indigenous soil microbial population, enzyme activity and soil chemical properties, and ii) to determine the effect of EFB biochar and N_2-fixing bacteria Sb16 on growth and nutrient uptake of sweet corn. Five rates of EFB biochar (0, 0.25, 0.5, 0.75 and 1%) were applied to sterilized and non-sterilized soil either with or without N_2-fixing bacteria Sb16 and incubated for 40 days under laboratory condition. The factorial study was arranged in a Complete Randomized Design (CRD) with 3 replications. Microbial population, enzyme activity and chemical properties of soil were determined at the end of incubation. Sweet corn was grown in pots containing 6 kg soil and applied with the 5 levels of EFB biochar (0, 5, 10, 15 and 20 t/ha) either with or without bacteria Sb16. Plants were sampled at tasseling stage and analyzed for dry biomass, chlorophyll content and nutrient uptake. Soil samples were analyzed for soil biochemical and microbiological properties. Results of laboratory study showed that application of EFB biochar at 0.5% without inoculation and 0.25% with N_2-fixing bacteria Sb16 in both soils significantly increased population of soil bacteria, fungi, actinomycetes and N_2-fixing bacteria (NFB), enzymes (urease, acid phosphatase and fluorescein diacetate hydrolysis (FDA) activity), and soil chemical properties (pH, organic C, total N, available P and exchangeable K, Ca and Mg. A glasshouse experiment showed that application of EFB biochar at 5 t/ha and N_2-fixing bacteria Sb16 significantly ($P<0.05$) improved growth of corn (shoot and root biomass, root length, root volume, plant height and leaf chlorophyll content, nutrient uptake), soil microbial populations, FDA and selected soil chemical properties. The EFB biochar at 5 t/ha and N_2-fixing bacteria Sb16 stimulated soil quality and plant growth. Addition of high EFB rates (15 and 20

t/ha) to soil negatively affected all the observed parameters. Addition of EFB biochar to soil with N_2-fixing bacteria may be an alternative solution in improving nutrients, enzymes and diversity of microorganisms in soil and thus led to improve plant growth. The studies showed that application of EFB biochar at 5 t/ha or 0.25% with N_2-fixing bacteria Sb16 and 10 t/ha or 0.5% without bacteria inoculated improved corn growth and the quality of soil for sustainable corn production.

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

KESAN TANDAN KOSONG BIOCAR (EFB) DAN BAKTERI A PENGIKAT NITROGEN TERHADAP KUALITI TANAH DAN PERTUMBUHAN JAGUNG MANIS.

Oleh

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Terdapat peningkatan terhadap kajian biocar sebagai pembaik-pulih tanah untuk mengingkatkan ciri-ciri tanah ke arah meningkatkan pertumbuhan tanaman lestari. Ciri biokimia tanah seperti aktiviti enzim mempunyai pernanan utama dalam kitaran nutrien dan boleh dianggap sebagai pengukur kualiti tanah. Tanah tropika mempunyai kandungan yang rendah dalam bahan organik, nutrient tumbuhan, kebolehan pertukaran kation (CEC), pH, larut lesap yang tinggi, dan kekurangan mikroorganisma hidup yang memberi kesan terhadap hasil tanaman. Kajian makmal dan rumah kaca telah dijalankan berdasarkan objektif berikut, i) Untuk mengenalpasti kesan tanah steril, tandan kosong biocar (EFB) dan bakteria pengikat nitrogen *Stenotrophomonas sp.* Sb16 terhadap populasi asal mikroorganisma tanah, aktiviti enzim dan ciri-ciri kimia tanah, dan ii) Untuk mengenalpasti kesan tandan kosong biocar (EFB) dan bakteria pengikat nitrogen Sb16 terhadap pertumbuhan dan penyerapan nutrient oleh jagung manis. Lima kadar tandan kosong kelapa sawit biocar (EFB) (0, 0.25, 0.5, 0.75 and 1 %) telah digunakan untuk yang steril dan tidak steril dengan samaada mengandungi atau tidak mengandungi bakteria pengikat nitrogen Sb16 dan inkubasi selama 40 hari di bawah keadaan makmal. Kajian faktorial adalan mengikut rekabentuk rawak lengkap (CRD) dengan tiga replikasi. Populasi mikrob, aktiviti enzim dan ciri-ciri kimia telah dikenalpasti diakhir proses inkubasi. Jagung manis telah ditanam dalam 6 kg tanah/pasu dan ditambah dengan lima kadar tandan kosong biocar (EFB) (0, 5, 10, 15, dan 20 t/ha) samaada mengandungi atau tidak mengandungi bakteria Sb16. Tanaman dan tanah telah diambil sampel pada peringkat teasseling dan telah dianalisis untuk berat kering biojisim, pengambilan nutrien, biokimia tanah dan ciri-ciri mikrobiologi. Hasil kajian makmal menunjukkan penggunaan tandan kosong biocar (EFB) pada 0.5% tanpa inokulasi dan 0.25% dengan bakteria pengikat nitrogen Sb16 dalam kedua dua tanah dengan nyata menginggikan populasi bakteria tanah, kulat, aktinomiset, dan bakteria pengikat nitrogen (NFB), enzim (urea, asid fosfotas, aktiviti hidrolisis fluoresin diasetat (FDA) aktiviti, dan ciri- ciri kimia tanah (pH, C organic, jumlah N, P tersedia dan pertukaran K, Ca dan Mg). Kajian rumah kaca menunjukkan bahawa penggunaan biocar EFB pada 5 t/ha dan bakteria pengikat Sb16 dengan nyata (P

<0.05) meningkatkan pertumbuhan jagung dengan biojisim pucuk dan akar, panjang akar, jumlah akar, tinggi pokok dan kandungan klorofil daun), pengambilan nutrient, populasi mikrob tanah, FDA dan ciri-ciri kimia tanah yang dipilih. Biocar EFB pada 5 t/ha dan bakteria pengikat nitrogen Sb16 merangsang kualiti tanah dan pertumbuhan pokok. Penambahan kadar biocar EFB yang tinggi (15 dan 20 t/ha) kepada tanah tidak memberi kesan kepada semua parameter yang diperhatikan. Penambahan biocar EFB kepada tanah dan bakteria pengikat nitrogen mungkin menjadi penyelesaian alternatif dalam meningkatkan nutrien, enzim dan kepelbagaian mikroorganisma tanah dan seterusnya menjadi tunjang untuk meningkatkan pertumbuhan tanaman. Kajian menunjukkan penggunaan biocar EFB pada 10 t/ha or 0.5% tanpa bakteria Sb16 dan kadar yang rendah 5 t/ha or 0.25% dengan bakteria pengikat nitrogen menigkatkan pertumbuhan jagung dan kualiti tanah untuk penghasilan jagung yang mampan.

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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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LIST OF ABBREVIATIONS

A.A	Actinobacteria Isolation Agar
AAS	Atomic Absorption Spectrophotometer
ANOVA	Analysis of Variance
BET	Brunauer, Emmett and Teller
BNF	Biological Nitrogen Fixation
CEC	Cation Exchange Capacity
CFU	Colony Forming Unit
C:N	Carbon to Nitrogen Ratio
CRBD	Completely Randomized Block Design
DNA	Deoxy Ribonucleic Acid
EFB	Empty Fruit Bunch
FDA	Fluorescein Diacetate Hydrolysis
GLM	General Linear Model
MUB	Modified Universal Buffer
NA	Nutrient Agar
NFB	N_2- Fixing Bacteria
NFB	N- Free Broth
OD	Optical Density
OM	Organic Matter
PAWC	Plant Available Water Content
P-NPP	Aldisodium P- nitrophenyl Phosphate Tetrahydrate
RBSA	Rose Bengal Isolation Agar
SAS	Statistical Analysis System
SDW	Sterile Distilled Water
SOC	Soil Organic Carbon
THAM BUFFER	Tris (hydroxyl methyl) Amino Methan
TSP	Triple Super Phospha

CHAPTER 1

INTRODUCTION

Tropical soils are mostly infertile soil that affect soil quality and plant growth. The main characteristics of tropical soils are acidic soils and have low fertility (Shamshuddin and Fauziah, 2010). Aluminium (Al) and manganese (Mn) is toxic elements to plants, while Ca and Mg often are deficient in acidic soil. In addition, this soil has low pH, cation exchange capacity (CEC), poor water holding capacity, low microbial population, poor soil enzyme activity and high leaching of basic cations that are essential for soil properties and plant growth (Aini and Vimala, 2002). The amount of N present in acidic soil is extremely small to provide the productivity of agricultural systems and responsible for the decomposition of organic matter. The beneficial soil free living N_2 fixing bacteria that supply some nutrients in a form that plants can absorb easily from the soil and improve plant growth can die of nutrient deficiency in tropical soil (Topoliantz, 2005).

In tropical soil, the size of most crops is restricted and yield is low. Plants suffering from element toxicity and poor nutrient will show a restricting root system, which may affect the capacity for mineral nutrient acquisition and resulting in reducing plant growth (Rout et al., 2001). These acidic soils are constraints to crop production. Optimum growth of sweet corn occurs when essential nutrients are available, but growth will not satisfy when sweet corn requires a continuous supply of chemical properties in soil (Fageria and Baliger, 2008). In Malaysia, the production of corn is unsatisfactory and its imports for a amounting of feed to millions of Ringgit (USDA, 2014). To meet these requirements, yield corn production should be increased by way of using proper fertilizer and other inputs.

However, this soil infertility can be ameliorated effectively by applying liming material, organic matter, bio-fertilizer, biochar and other amendments (Topoliantz, 2005). In order to control infertile soil, soil microbial activity and soil pH need to be substantially improved. Application of oil palm waste into soil can be an alternative to improve soil fertility. Empty fruit bunch (EFB) has been turned to EFB biochar roughly 20 tonnes/day by a Nasmeck company in Malaysia (USDA, 2014). EFB biochar is produced from the burning of oil palm EFB at high temperatures under low oxygen conditions with the intention to sequester carbon, mitigate CO_2 emission and at the same time to be used as a soil amendment (Norazlina et al., 2014).

A few researches have been conducted to produce biochar from oil palm EFB and used as soil amendments for its role as a nutrient source for crop production (Nair and Lawson, 2012). Sahara and Lim (2000) showed that application of biochar increased soil mineral N, pH, exchangeable K, Ca, Mg, soil moisture and organic matter leading to improved plant growth. Biochar has been known to affect the soil chemical, biological and enzyme activities and plant growth (Bailey et al., 2010; Lehmann et al., 2011). The ameliorating effect of biochars on acidic soil was assumed to be consistent with their composition and properties which depend on the biomass feedstock type and pyrolytic conditions.

Addition of biochar with inoculation of beneficial microbes can be important for improving soil fertility and plant physiological activity through the transfer of nutrients into forms that can be uptake easily from the soil (Arnoldus et al., 2011; Jin, 2010; Blackwell et al., 2009). Inoculation of rice plant with *Stenotrophomonas sp* strain (Sb16), a free living N_2 fixing bacteria has improved rice growth isolated from rice crop (*Oryza sativa* L.) (Radziah et al., 2013). In Malaysian soil, Studies showed that the inoculation significantly increased plant growth, yield and nutrient uptake of rice and can supplement about 40% of total N to the rice (Naher et al., 2011). Total world biological nitrogen fixation is estimated at 139-172 million t/year, which is three times higher than industrially fixed nitrogen and contributes significantly to biological nitrogen fixation in agriculture and natural N cycle (Ishizuka, 1992).

Previous studies showed that biochar addition, affected bacterial population due to its effect on soil pH, temperature, nutrients, water, oxygen, and metabolic compounds (Jeffery et al., 2011). Infertile soil can be ameliorated effectively by applying liming material, organic matter and beneficial microorganisms. Little information is available on the potential of biochars with free living N_2-fixing bacteria and their interactions to reduce soil acidity. Studies suggest that crop yields and soil field capacity can increase as a result of applying biochar as an amendment to the soil (Christopher et al., 2012). Insufficient information is available on the application of EFB biochar with N_2-fixing bacteria on plant growth and soil quality. The following studies were conducted with the following objectives;

1- To determine the effect of soil sterilization, EFB biochar and N_2-fixing bacteria *Stenotrophomonas sp*. (Sb16) on indigenous soil microbial population, enzyme activity and soil chemical properties.

2- To determine the effect of EFB biochar and N_2-fixing bacteria on growth and nutrient uptake of sweet corn.

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