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Assessing Phosphorus Uptake, Soil Phosphorus Availability and the Growth of Maize (*Zea mays. L*) upon Biochar Application at Different Rates in Tropical Acid Soil

Ahmad Khairi Bin Mat Napi

F16A0006

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DECLARATION

I hereby declare that the work embodied in here is the result of my own research except for the excerpt as cited in the references.

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Signature

Student's Name : AHMAD KHAIRI BIN MAT NAPI

Matric No : F16A0006

Date : 02 / 01 / 2020

Verified by :

____

Supervisor Signature

Supervisor's Name :

Stamp :

Date :

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Assessing The Growth, Phosphorus Uptake, And Soil Phosphorus Availability Of Maize (*Zea mays. L*) Upon Biochar Application At Different Rates In Tropical Acid Soil

ABSTRACT

Phosphorous deficiency is considered to be one of the most frequently occuring limitations in the productivity of crops grown in tropical and subtropical ecological regions where P can strongly fixed to the soil through adsorption and precipitation thereby reducing its bioavailability to the plants. Biochar could be used to improve soil chemical properties and minimize P fixation in acid soils because these organic amendments have high affinity for Al and Fe. Therefore, this study aimed to characterize the soil samples and biochar used and determine the soil P availability, total P uptake and phosphorous use efficiancy of maize (*Zea mays L.*) to biochar application.. A field experiment was conducted in Agro Techno Park at Universiti Malaysia Kelantan. Treatments with biochar showed significant increase in the soil pH, and significant reduction of exchangeable aluminium and iron in the soils with biochar compared to treatments with soil only and soil with chemical fertilizers only. There was also significant increase in the phosphorus uptake and dry matter production (leaves, stems, and roots) of *Zea mays* L. in treatments amended with biochar. This was due to reduction of soil exchangeable aluminium and iron concentrations, thus reduced the aluminium toxicity in the root zone, and increased the phosphorus availability in the soil. As conclusion, biochar can be used to improve the P availability, phosphorus uptake and dry matter production of *Zea mays* L. cultivated in tropical acid soil by reducing the soil phosphorus fixation.

Keywords: *Soil phosphorus fixation, rice husk biochar, zea mays L., phosphorus uptake, dry matter production*

Menilai Pertumbuhan, Pengambilan Fosforus, Dan Ketersediaan Fosforus Tanah Dalam Penanaman Jagung (*Zea mays. L*) Menggunakan Biochar Pada Kadar yang Berbeza Dalam Tanah Asid Tropika

ABSTRAK

Kekurangan fosforus dianggap sebagai salah satu batasan yang paling kerap terjadi dalam produktiviti tanaman yang ditanam di kawasan ekologi tropika dan subtropika di mana P dapat ditekan dengan kuat ke tanah melalui penjerapan dan pemendakan sehingga mengurangkan ketersediaan semulajadi ke tanaman. Biochar boleh digunakan untuk memperbaiki sifat kimia tanah dan meminimumkan penumpukan P pada tanah asid kerana perubahan organik ini mempunyai pertalian yang tinggi untuk Al dan Fe. Oleh itu, kajian ini bertujuan untuk mengenalpasti sampel tanah dan biochar yang digunakan dan menentukan ketersediaan tanah P, pengambilan P dan penggunaan khasiat fosforus jagung (*Zea mays L.*) menggunakan biochar. Percubaan lapangan dijalankan di Agro Techno Park di Universiti Malaysia Kelantan. Rawatan dengan biochar menunjukkan peningkatan yang ketara dalam pH tanah, dan pengurangan ketara aluminium dan besi yang boleh ditukar dalam tanah dengan biochar berbanding dengan rawatan dengan tanah sahaja dan tanah dengan baja kimia sahaja. Terdapat juga peningkatan ketara dalam pengambilan fosforus dan pengeluaran bahan kering (daun, batang, dan akar) *Zea mays L.* dalam rawatan yang dipinda dengan biochar. Ini disebabkan oleh pengurangan aluminium dan kepekatan besi dalam tanah, dengan itu mengurangkan ketoksikan aluminium di zon akar, dan meningkatkan ketersediaan fosforus di dalam tanah. Sebagai kesimpulan, biochar boleh digunakan untuk meningkatkan ketersediaan P, pengambilan fosforus dan pengeluaran bahan kering *Zea mays L.* yang ditanam dalam tanah asid tropika dengan mengurangkan fosforus fiksasi tanah.

Kata kunci: Penetapan fosforus tanah, biochar sekam padi, *Zea mays L.*, pengambilan fosforus, pengeluaran bahan kering

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LIST OF ABBREVIATION AND SYMBOLS

N	Nitrogen
P	Phosphorus
K	Potassium
Al	Aluminium
Fe	Iron
OH	Hydroxide
OM	Organic Matter
C	Carbon
Si	Silicon
Mn	Manganese
H	Hydrogen
Ca	Calcium
Mg	Magnesium
Na	Sodium
Zn	Zinc
Cu	Copper
HPO_4^-, HPO_4^{2-}	Orthophosphate
NaOH	Sodium hydroxide
HCl	Hydrochloric acid
H_2SO_4	Sulphuric acid

$C_6H_8O_6$	Ascorbic acid
$Al_2(MoO_4)_3$	Ammonium molybdate
KH_2PO_4	Potassium dihydrogen phosphate
pH	Potential of hydrogen
EC	Electrical Conductivity
CIRP	Christmas Island Rock Phosphate
MOP	Muriate of Potash
ATP	Adenosine triphosphate
DNA	Deoxyribonuclease acid
DAS	Day after Sowing
FAO	Food and Agriculture Organization Of United Nation
MARDI	Malaysian Agricultural Research and Development Institute
SPSS	Statistical Package for Social Science
ANOVA	Analysis of Variance

CHAPTER 1

INTRODUCTION

1.1 Research Background

1.1.1 Background

Phosphorus (P) is important in few processes in plants such as respiration, photosynthesis, cell division and cell enlargement, energy storage and transplantation. It is worried in various key plant functions, which include photosynthesis, transformation of starches and sugar, energy transfer, nutrient movement within the plant and transfer of genetic traits from one era to the subsequent (Energy, Phosphorus, & Adp, 1999). One of the most common limitations in productivity is the lack of phosphorus. Synthesis of proteins and chlorophyll is also influenced by phosphorus. It is part of the essential compound needed for an important life process. Plant propagation and vegetative growth can be enhanced by the use of phosphorus. The presence of P for corn will improve crop yields (Chen, 1994). Improving the formation and growth of early roots (Hajabbasi & Schumacher, 1994) as well as improving the quality of plants, and needed for seed formation (IPNI, 1999) is influenced by stage P.

The high rainfall and weather, especially tropical soils (ultisols and oxisols) lead to the loss of base cation. In such a soil, acidic cations like Al and Fe dominate, and depend on soil pH, they can set inorganic phosphorus (P) (Adnan, 2003). Typically, phosphorus is found in plants in pH between slightly acidic to neutral, ie between 6 - 7. Under this range, P is due to active forms of al and Fe oxide and hydroxide, while at higher pH (> 7), P is less likely to be due to rain with calcium. Therefore, the use of ordinary P fertilizer such as rocks phosphate is required to weave Al and Fe ions to maintain sufficient supply of P in acidic soils (Rahman, Gikonyo, Silek, Goh, & Soltangheisi, 2014) . However, this approach does not work as it is not economical. Excess or disproportionate use of fertilizers is not environmentally friendly as it causes eutrophication and ammonia volatilization. Organic amendments are now used to reduce P soil pressures to restore soil fertility problems. Additional organic amendments were used to improve availability of bioavails especially P and soil chemical properties through web site absorption P in the tropics, (Ohno and Amirbahma, 2010). Organic amendments can enhanced groundwater retention and carbon sequestration, soil fertility and crop productivity. (Galinato, Yoder, & Granatstein, 2011)

Among the most widely grown cereals in world is maize (*Zea mays L.*). After wheat and rice, maize is the third highest in the world and is the most important grain (Anon, 2000). Maize can be grown on various agro-climate zones and its suitability to a variety of environments can not be compared to other crops. Its are grown from 58 ° N to 40 ° S, from below sea level to height greater than 300 meters and in an area of 250 mm to more than 5000 mm of rain each year, with the cycle expanding between 3 and 13 months . Corn is a staple food for humans and is widely used in many developing countries such as Asia, Africa and America. Corn has a high nutritional value and these grains provide a large proportion of the rural zones around 50 and 70% of protein and calorie intake (Serna-Saldivar et al., 2008) . Maize contains about 72% starch, 4% lipid and 10% protein, providing energy density of about 365 kcal / 100 g.

Biochar is a result of pyrolysis of biomass in a depleted oxygen environment. It contains a permeable carbonace structure and different utilitarian gatherings (Lehmann, 2009). Natural substances and inorganic salts, for example, humic and fluvic materials and are accessible N, P and K, can fill in as manures and acclimatized by plants and microorganisms. Biochar benefits the environment through nutrient pollution, improved soil properties, climate change mitigation, waste management, and energy production (Lehmann and Joseph, 2015). Soil aggregation and water holding capacity can be increased with the use of biochar (Obia et al., 2016). Therefore, soil cation exchange capacity will increase(Amin & Eissa, 2017), thus nutrients against leaching also increases and makes some of them more available to plants e.g., N, P and K (Amin, 2016).

1.1.2 Problem Statement

In the productivity of plants growing in tropical and subtropical ecology, one of the most common limitations is phosphorus deficiency (Ramaeker et al., 2010) where P is very strong in the soil through adsorption and rainfall, reducing plant bioavailability (DoVale & Fritsche-Net, 2013). Becoming an essential compiler for organic compounds in plants, plant growth will be reduced with a shortage of P(Shrestha, Amgain, & Aryal, 2016). One of the major constraints to maintaining optimal crop production is low soil concentration of P (Cui et al., 2011) and at the initial stage growth, its availability is very important (Shrestha et al., 2016). Chemical properties of the soil can be repaired and the accumulation of P on acid soil can be minimized by the use of biochar because the organic conversion has a high correlation to Al and Fe (Ch'ng et al., 2014). However, there is little study focusing on the use of rice husk biochar in improving the growth of maize, phosphorous uptake and soil phosphorus availability in tropical countries. Apart from that, lack of awareness among farmers related to the application of rice husk biochar in improving the growth of maize, and phosphorous uptake in tropical countries encourage the benefits to be discover in the future. However, biochar is used to increase the use of P from inorganic and organic P fertilizers was focused on the very limited number of studies (Shen et al., 2016). Recently, Gul and Whalen (2016) also underscored the lack of research on the efficiency of use of P although there is data on the intake of P by various crops under the biochar amendment.

1.1.3 Research Question

If P in acid soils is less available because the are fixed by large amount of Al and Fe, it be possible that biochar potential can improve P efficiancy?

1.1.4 Objectives

The objectives of this study were to:

1) Characterize the soil samples and biochar used
2) Determine the growth parameters, soil P availability and total P uptake of maize (*Zea mays L.*) upon biochar application.

1.1.5 Scope of Study

This study focuses on a way to asses the growth of maize plant, P uptake and soil available P of maize (*Zea mays L.*) upon biochar application at different rates in tropical acid soil.

1.1.6 Significance of Study

This work may not only contribute to the reduction of P fertilizers inputs into the soil in Jeli but also paves better means of adding value to agro-industrial wastes such as biochar to avoid environmental pollution. This study would also reduce the depending of farmer toward the chemical fertilizers.

CHAPTER 2

LITERATURE REVIEW

2.1 Phoshphorus Cycle In Soil – Plant Cycle

2.1.1 Precipitition

Iron (Fe) and Aluminum (Al) phosphate minerals control precipitate at acidic pH and at neutral to pH calcium (Ca) alkali and magnesium (Mg) phosphate mineral precipitation. Crystallinity and mineral sorts discover control of the stability of phosphate minerals in the soil (Bornø, Müller-Stöver, and Liu, 2018).

2.1.2 Absorption And Desorption

Adsorption or desorption of P ion occurs on the surface of Fe and Al (hydrous) oxide and on the edge of clay minerals. pH, cation exchange and competition between different anions are influenced by adsorption or desorption processes for exchange sites. P's adsorption increased following an increase in oxide charges (hydrous) due to reduced pH. Along these lines, soil properties, for example, pH, surface, cation exchange capacity (CEC), anion exchange capacity (AEC), soil organic matter (SOM) and mineralogy control P are in various soil types (Bornø, Müller-Stöver, & Liu, 2018)

2.1.3 Mineralization

Through the process of mineralization, like N and organic P is converted into inorganic phosphate. Inorganic phosphate immobilization, on the other hand, is a mineralization reaction. During immobilization, microorganisms transform their organic form into organic phosphates, which are then incorporated into their living cells. The process of phosphours immobilization and mineralization occur simultaneously in the soil. The temperature, moisture and aeration are factors that affect P mineralization (Silva et al., 2000) .

2.1.4 Immobalization

Immobilized inorganic phosphates, on the contrary, are re-mineralization reactions. Microorganisms transformed inorganic forms into organic phosphates during immobalization, which are then incorporated into their living cells. Immobilization occurs when the plant is present in the form of P is consumed by microbes, making P a form of organic P which is not present in the plant. When the microbes die, microbial P will become available from time to time (Hyland et al., 2005)

2.1.5 Important of Phosphorus in Plant

In crop production, the main element is phosphorus (P). Growth of roots, flowering, seedling and seedling can be increased by phosphorus. Phosphorus is an important component of DNA of all living thingsIt is also a component of RNA, a compound that reads the genetic code of DNA to build proteins and other compounds essential to plant structure, seed production and genetic transfer. The structure of both DNA and RNA is linked together by phosphorus bonds. Phosphorus is the main component of adenosine triphosphate (ATP) which is involved in most biochemical processes in plants and allows them to produce nutrients from the soil. During photosynthesis, the form of ATP has phosphorus in its structure, and the process from the early growth of the seed through the formation of cereals and mature. Cell development and DNA formation are influenced by phosphorus. Inadequate P in the soil can lead to reduced flower development, low seed quality, reduced plant yields and delayed crop maturity. Therefore, phosphorus is important for the overall health and spirit of the plant. Growth of roots, increased stalks and stem strength, better seed production and flower formation and so forth have been stimulated by phosphorus (Freeman, 2019).

2.1.6 Present Concern With Soil Phosphorus

Phosphorus deficiency due to insoluble organic P by Al and Fe makes acidic soil (Adnan, 2003). This response contributes to less plant availability. Information on the chemical form P is the basis for understanding the soil dynamics P and its interactions in acidic soil. This is necessary for P management in agriculture. Organic soil, pH, and cation exchanged and soluble Al, Fe, and Ca were influenced the soil P availability(Smithson, 1999). In plants, usually phosphorus at soil pH 6 and 7. P deficiency increases in most crops when the soil pH is low. The amount of conventional and large amounts of organic fertilizers and lime unlike phosphate rocks and Triple Superphosphate (TSP) is used to weave Al and Fe ions.

2.2 Phosphorus Pools

Land P pools can be widely conceptualized as P solutions, sorbed P, P minerals, and organic P. P quantities absorbed into the surface of Fe and Al oxides and calcium carbonates by electrostatic and covalent bonds are called sorbed P. Mineral P consists of solid compounds P. Various specific surface areas and organizational structures (from amorphous to crystals). This compound (eg apatites) may be due to the process of pedology or product P reactions that are added to the soil (eg in calcium phosphate from superphosphate) (Moody, Speirs, Scott, & Mason, 2013). Various organic P compounds such as phytates that are present in plant waste and organic matter are protected by organic P. All these P pools are in equilibrium with orthophosphate in soil solution by the process of absorption of desorption (in P state), dissolution (in mineral P case), and drill-fillers (in the case of organic P). The solubility of iron and manganese bound under the condition of a variable aerobic-anaerobic soil may be affected by the oxidation reduction process, but it is not considered further in this work. P solution is the main source of P supply for plants, mainly through the process of propagation, which depends on many factors including the gradient of P concentration between the root surface and the bulk solution, the supply rate P to P as solution solution, volumetric groundwater content, soil P accumulation (changes in the quantity of soil P to change the solution of P) and water filtration in clay soil (tortuosity factor)(Moody et al., 2013).

2.3 Factors Affecting Phosphorus Availability

The soil organic matter content and fertilizer placement, can significantly affect P in the harvesting season between the factors affecting the availability of phosphorus (Stiles, 2019).

2.3.1 Fertiliser Placement

Steel plots close to seed (by appeal) can increase their availability on crops because P is relatively unpeelable in the soil profile. Efficient use of P can be reduced if the placement outside the root zone (such as broadcasting) unless the root grows extensively into the place it is placed. Ensuring that P is placed close to the root, while avoiding combustion of the seeds, it is necessary to increase the P availability in the crops throughout the season (Stiles, 2019).

2.3.2 Organic Matter

Different mechanisms in the extraction of phosphorus can be increased depending on the level of organic matter. Phosphorus, in the form of organic phosphates, can form complexes with organic matter. From time to time, P attached to organic matter can be transferred by organic anion from soil settlement, effectively increasing the availability of P to crop roots for recruitment. Organic materials (especially humus) may also attach and interfere with the binder P, making P more used to harvest by plant roots, rather than bonded with other nutrients such as Al and Fe through strong chemical bonds. Finally, P, which has been bound in soil organic matter, can be mined throughout the season, and will act as a P source available for plant extraction (Stiles, 2019).

2.4 Common Management Of Phosphorus Fixation Problem

2.4.1 Liming

Phosphorus will react with aluminium and iron when the soil is too acidic. So that, it can make unsuitable to plants. While the phosphorus will react with calcium if the soil is too alkaline also becomes inaccessible. Phosphorus is most accessible to plants when soil is at a 'Goldilocks' zone of acidity. There are approaches to make more phosphorus accessible to plants. For instance, soil acidity will decrease when lime (calcium hydroxide) were added. This is a typical practice. Liming is a bread-and-butter device for agriculture (Margenot, 2018) However, liming can impact different ways by which phosphorus may end up accessible to plants. Enzym , called phosphatases, are likewise known to impact the measure of phosphorus accessible to plants. Biochars produced at high temperatures tend to be alkaline(Novak et al., 2007). Applications of alkaline biochars will increase soil pH through a liming effect(Schulz & Glaser, 2012), consequently enhancing soil P availability.

2.4.2 Phosphorus Fertilizer Supply

Tropical soils generally have deficiency in available P, high P fixation and strong soil acidity, due to high weathering and leaching loss, which together constrain soil P fertility and plant productivity . To keep up yield generation, c.a. 15 million tons of essential P are connected all around to P-constrained farming area each year(Wang et al., 2012), but Acquisition by plants rarely exceeds 20% of the amount of P fertilizer used (Friesen et al., 1997), mainly due to strong P fixation in soils. Phosphorus fertiliser is mostly gotten from mined rock phosphate which is non-renewable resource . Current models recommend that provisions of rock phosphate might be exhausted before the finish of this century, as worldwide interest for P will keep on expanding throughout the following 50 years, because of population pressure(Streube et al., 2012). In order to increase P availability in soils and enhance sustainable P use, then it is necessary to focus efforts on seeking P fertiliser replacements and, also on improving the properties of oils, thus rendering stable P available to plants.

2.4.3 Organic Amendment

To overcome soil fertility problem, organic amendments such as Oxisols and Ultisols are now in use. In the tropics, the addition of organic amendments has been used to improve soil chemical properties and the availability of bio nutrients, especially P through minimizing absorption sites P (Ohno & Amirbahman, 2010). Groundwater retention, soil fertility and crop productivity, and absorption of C can be increased with organic alteration capabilities (Galinato et al. 2011). Organic amendments come from various sources including green manure, plant waste, industrial waste, household waste and animal waste are provided in large quantities for such purposes. In order for soil organic matter to be maintained, organic changes are encouraged to be used to improve aeration function, soil biology and reduce compaction, moisture retention, pollution attenuation and increasing nutrient supply (Girmay et al., 2008). However, there are many benefits associated with using land for organic changes depending on the quantity and nature of organic waste.

2.5 Biochar

Biochar is a carbon-rich strong created by biomass warming with almost no oxygen (Ding et al., 2016) and shows permeable carbon structure, numerous utilitarian gatherings, and aromatic surfaces. The biochar generation process is basically made out of moderate pyrolysis, gasification, hydrothermal utilization, and lightning. Biochar created from pyrolysis of biomass can modify soil physical properties (DeLuca, 2016). The chemical and physical properties of biochar depend on pyrolysis temperature and process parameters affecting the physical and chemical properties of biochar such as furnace temperature and residence time, as well as on foodstuffs (Joseph et al., 2010). Nitrogen, carbon, hydrogen and some lower nutrient elements, such as K, Ca, Na, and Mg are biochar element composition (Zhang, Voroney, & Price, 2015) . Biochar has a high amount of area, high stability and high specific surfaces in functional groups. The temperature of pyrolysis and the feed material affects the properties of biochar (Cantrell et al., 2012).The range of pyrolysis temperature from 200 - 800 ◦ C (Hossain et al., 2011). Usually, pyrolysis temperature increases from 300 to 800 ° C will increase carbon content, while hydrogen and nitrogen levels decrease. By increasing pyrolysis temperature, specific surface area and microscope formation in biochar can be increased. The very porous structure of biochar can contain a number of materials that can be executed and such as fluvic (Lin et al., 2012). In addition, its molecular structure shows high levels of chemical stability and microbes(Cheng et al., 2008). Biochar has a high specific surface areas and some polar or nonpolar materials, which have strong affinity for inorganic ions such as phosphate ions, nitrates and heavy metals (Schmidt et al., 2015).

2.6 Potential Of Applying Biochar In The Soil Phosphorus – Fixing Soil

The properties of soil microbes can also be enhanced by biochar as well as physical properties and chemical properties. The combination of biochar with soil has been demonstrated by many studies which can improve water retention and aggregation, reduce bulk density, improve soil structure, and enhance porosity (Baiamonte et al., 2015). Soil electrical conductivity increased by 124.6% and cation exchange capacity increased by 20% (Laird et al., 2010) and reduced soil acidity by 31.9% (Oguntunde et al., 2004) when biochar were applied. The benefits of biochar addition to agricultural land can be highlighted through recent surveys (Glaser et al., 2002;Warnock et al., 2007). Among them is promotion of plant growth(Chan et al., 2008 ; Hossain et al., 2010), heavy metals bioavailability can be limited (Park et al., 2011), reduced N2O soil clearance (Kammann, Linsel, Gößling, & Koyro, 2011), and reduced nutrient-soluble solubility, which in turn reduces fertilizer requirements (Liang et al., 2006;D. Laird et al., 2010). Because biochar is a joint product of bioenergy production and contributes to carbon sequestration purposes, while simultaneously increasing yields and reducing fertilizer use, biochar is considered a win-win solution to meet global environmental challenges (Laird, 2008) .

2.7 Mechanism Of Biochar In Reducing Soil Phosphorus – Fixation

2.7.1 Chelation Of Al And Fe

P solubility of soil can be changed by biochar through several mechanisms. The precipitation P can be affected by biochar by changing the soil pH and therefore the ionic ionic interaction power with Al^{3+}, Fe^{3+}, and Ca^{2+}; or by absorbing organic molecules that typically act as chelates (such as phenolic acids, complex proteins and carbohydrates) of metal ions that otherwise precipitate P. Hydrophobic or charged biochar is more effective in absorbing organic molecules and organo-biochar or organo-mineral-biochar complexes that are produced over time, leading to better solubility, retention and availability. Soil microorganisms affect the release of soil P through the solubilization process. For example, (Suksabye, Pimthong, Dhurakit, Mekvichitsaeng, & Thiravetyan, 2016) reported that PO_4^{3-} solubilizing bacteria Pseudomonas aeruginosa and Bacillus subtilis were effective in solving large Ca^3 (PO_4) solubility 2.77. Promoted bacterial growth that was appropriate to produce P solubilization in the presence of biochar may affect inorganic P bioavailability.

2.7.2 Increase Soil pH

Biochars produced at high temperatures tend to be alkali (Nova et al., 2009; Peng et al., 2011). The use of alkaline biochar through the liming effect can increase soil pH (Glaser et al., 2002), so the availability of soil P also increases . The soil pH can be converted by biochar amendment depending on the soil type or biochar used for soil. In short, the application of alkaline biochar can enhanced the acidified soil pH and subsequently affects the bioavailability of nutrients (Raboin et al., 2016;R. Zhang et al., 2017). Therefore, as a land conversion, biochar can cause liming effect to neutralize soil acidity, and improve soil quality (Kookana et al., 2011) by increasing the availability of basic soil nutrients(Glaser et al., 2002). On the other hand, the application of acidic or neutral biochar to alkaline soils may reduce soil pH and may affect the solubility of soil nutrients such as P and trace elements. (Laghari et al., 2015).

2.7.3 Direct Phosphorus Supply

Increase in plant availability P with the use of biochar in agriculture is often reported (Gao & DeLuca, 2018; Gul, Whalen, Thomas, Sachdeva, & Deng, 2015). The use of biochar to the soil can directly or indirectly affect the soil P dynamic through various mechanisms including: 1) Changing the soil pH (Xu, Sun, Shao, & Chang, 2014); 2) stimulate the formation of organo complexes or change the adsorption / desorption equilibrium P (H DeLuca, 2016; Soinne, Hovi, Tammeorg, & Turtola, 2014); 3) change the solubility of P by the activities of influencingmicrobial enzymes (Gao, Hoffman-Krull, & DeLuca, 2017), mycorrhizal associations. The process of mineralization involving soil organisms can produce phosphorus in organic form. Biochar can change the activity and abundance of this microbial with the availability of P. Phosphatase is an enzyme that can hydrolyze organic compounds P and convert them into other forms of organic P, assimilated by plants.

CHAPTER 3

METHODOLOGY

3.1 Preparation And Soil Sampling

The soil samples were taken at 0-20 cm from an uncultivated land in Agro Techno Park Universiti Malaysia Kelantan Jeli Campus. A total of 5 sacks of soil samples were taken within a 20m x 20m randomly. The soil were air-dried, ground and sieve to pass through a 2-mm sieve, respectively for laboratory analysis.

3.2 Soil Analysis

Before the field experiment was carried out, the soil were analyzed for bulk density, soil texture, soil pH, total organic matter, total carbon, soil extractable K, Mg, Ca, Na, Cu, Fe, Zn, soil electrical conductivity (EC), and soil available P.

3.2.1 Bulk Density Determination

To determine the soil bulk density, coring method was used (Dixon & Wisniewski, 1995). First, corings were hammered into the soil to desired soil depth and then removed carefully from the soil. The excess soil was trimmed. The corings together with the soils were weighed and was put into the oven for oven dried at 105 ^{o}C until constant weighed attained. The bulk density was determined by the equation described by Dixon and Wisniewski (1995) as follows.

Bulk density (g cm^{-3}) = Dry soil weight (g) / Soil voulme (cm^3)

3.2.2 Soil Texture Determination

According to (Bouyoucos, 1962), the soil texture determination was carried out by using hydrometer method. A 50 g of soil was placed in a blender cup. A 4 M NaOH was dropped wisely to adjust the pH until becomes 10. Then, distilled water was poured into the blender cup until it is within 10 cm of the top rim. Next, the blender cup was placed on stirring machine and mix for 15 minutes. Soil sample was transfered into 1 L of measuring cylinder after 15 minutes stirring. The distilled water was added into a measuring cylinder up to a volume of 1130 mL.. The soil suspension was stirred for 40 seconds using stirring rod. After that, a hydrometer will be placed into the suspension and the meniscus ib the hydrometer stem was recorded and then hydrometer was removed and rinsed. The soil suspension was stirred again and the second reading of the hydrometer was recorded. The expected result was equivalent to the amount of silt and clay in grams of the soil sample. The same steps were repeated and the third reading and the temperature was taken after 2 hours period. The calculations of the soil tecture are as follow (Bouyoucos, 1962).

Percentage of sand + silt + clay = 100%

For 40 seconds reading :

Percentage of silt + clay = (a/50) × 100% = w

Percentage of sand = (100-w) % = x

After 2 hours reading :

Percentage of clay = (b/50) × 100% = y

By difference : Percentage of silt = w – y = z

3.2.3 Soil pH And Soil Electrical Conductivity (EC)

To determine the soil pH and EC, potentiometer method was used. Digital pH and EC meter were used to measure soil pH and EC , a ration of 1:2:5 (soil and distilled water suspension) was used in this method (Peech, 1965). A 12.5 mL of distilled water together with 5 g of air-dried soil were add in beaker at ratio of 1:2:5 and this produce was repeat for 3 samples. The samples were shaken at 180 rpm using a shaker for 15 minutes. After that, the samples were left overnight for 24 hours before using a digital pH meter for pH determination and EC meter for EC determination.

3.2.4 Soil Total Organic Matter And Total Carbon (C) Determination

To determine the total organic matter and total C in this study, combustion method was used (Tan, 2003). The air-dried sample was placed in an oven and was lefted for 24 hours at 60 ^{o}C. The sample was cooled down using desiccator. First, the initial weight of the crucible was taken. Then, the weight of the crucible was weighed with the addition of 5 g of the soil sample. After that, the sample was ashed at 300 ^{o}C in the muffle furnace for an hour and the temperature will increase to 550 ^{o}C. The ashing process was continued for another 8 hours. Lastly, the sample was allowed to cool before inspection. The weight of the sample in the porcelain after ash was calculated. The total OM and C was calculated using the following calculation (Tan, 2003).

$$\text{Total OM} = \frac{\text{Initial weight of soil sample (g)} - \text{final weight of sample (g)}}{\text{Initial weight of soil sample (g)}} \times 100\% = X$$

Total C = X × 0.58

3.2.5 Soil Exchangeable Acidity and Aluminium

The soile exchangeable acidity and Al were determined by using titration method (Rowel, 1994). A 10 g of soil and 30 mL of 1 M KCI were placed into a beaker and left overnight for 24 hours. The sample was filtered after 24 hours with Whatman Filter Paper No. 2 into 100 mL volumetric flask after and was made up to the mark. Then, 50 mL of the soil extract was pipetted into 250 mL connical flask. Five drops of phenolphthalein were added as indicator. The solution was titrated against 0.01M NaOH until the appearence of pink colour. This measure the soil exchangeable acidity. The solution will the once again titrate against 0.01 M HCI until the solution become colourless and this measure the soil exchangeable Al. The exchangeable acidity and soil exchangeable Al was calculated by using the following calculation (Rowel, 1994).

$$\text{Exchangeable acidity (cmol kg}^{-1}\text{)} = \frac{0.2 \times \text{Titrate volume of 0.01 M NaOH} \times 10}{\text{soil mass (g)}}$$

$$\text{Exchangeable Al (cmol kg}^{-1}\text{)} = \frac{0.2 \times \text{Titrate volume of 0.01 M HCl} \times 10}{\text{soil mass (g)}}$$

3.2.6 Soil Extractable K, Ca, Mg, Na, Cu, Zn And Fe Determination

Soil extractable K, Ca, Mg, Na, Cu, Zn and Fe were exctracted by Mehlich No. 1 Double Acid Method (Mehlich, 1953). A 10 g of soil sample was weighed and placed into a 250 mL conical flask. Then, a 40 mL of the extraction reagent was added and the solution was shaken for about 10 minutes on a reciprocal shaker. After that, the supernatant was filtered into another beaker using Whatman Filter Paper No. 2, and the extract was aspirated into AAS and the reading was recorded. The soil exchangeable cations will be calculated using the equation below (Mehlich, 1953) :

$$\text{Soil exchangeable cation (ppm)} = \text{ASS reading (ppm)} \times \frac{\text{Volume of extractant (mL)}}{\text{Weight of soil sample (g)}}$$

3.2.7 Soil Available P Determinantion

Mehlich No. 1 Double Acid Method was used to extract the soil available P (Mehlich, 1953). A 0.8 mL of concentrated HCL and 1.35 mL of concentrated H_2SO_4 will pipet into 1000 mL volumetric flask and the volume will made up to volume. A 5 g of sample was weighed and placed into a 50 mL beaker. After that, a 25 mL of the extraction reagent was added and then the solution was shaken on reciprocal shaker for 10 minutes. Then, supernatant was filtered into plastic vials using Whatman Filter Paper No. 2 and the P extract was collected. The solution was analyzed by the molybdenum blue method (Murphy & Riley, 1962) and the developed blue colour was analyzed by UV spectrophotometer at 882 nm wavelength.

3.3 Experimental Design And Treatments

A field experiment was conducted in Agro Techno Park at University Malaysia Kelantan Jeli Campus. A total 18 beds were planted with 12 plants for every bed. The test crop used in this filed experiment was the *Zea mays L*. As the cultivation of *Zea mays L*. was done in this field experiment, each of the beds were supplied with N, P, and K fertilizer to ensure the optimum growth of the plants. The fertilizers were applied to ensure the optimum growth of the plants are Urea (46%), Christmas Island Rock Phosphate (CIRP) (30% P_2 O_5) and Muriate of Potash (MOP) (60% K_2O) and each of them were applied at 60 kg N ha^{-1} (130 kg N ha^{-1} Urea), 60 kg P_2O_5 ha^{-1} (200 kg CIRP ha^{-1}) and 40 kg K_2O ha^{-1} (67 kg K_2O ha^{-1}) respectively based on the recommendation by MARDI. However, the application rate of the commercial rice husk biochar was different for each treatment (Table 3.1). These fertilizers were applied on 10^{th} day of sowing (DAS) and 28^{th} DAS by equal splits. The experimental design used in this study was randomised complete block design (RCBD) with 3 replications.

Table 3.1 : List of treatments to be evaluated in field experiment

Treatment	Description
T_0	Soil only (No Fertilizer)
T_1	Soil + 130 kg Urea ha^{-1} + 200 kg CIRP ha^{-1} + 67 kg MOP ha^{-1}
T_2	Soil + 130 kg Urea ha^{-1} + 200 kg CIRP ha^{-1} + 67 kg MOP ha^{-1} + 5 t ha^{-1} biochar
T_3	Soil + 130 kg Urea ha^{-1} + 200 kg CIRP ha^{-1} + 67 kg MOP ha^{-1} + 10 t ha^{-1} biochar
T_4	Soil + 130 kg Urea ha^{-1} + 200 kg CIRP ha^{-1} + 67 kg MOP ha^{-1} + 15 t ha^{-1} biochar
T_5	Soil + 130 kg Urea ha^{-1} + 200 kg CIRP ha^{-1} + 67 kg MOP ha^{-1} + 20 t ha^{-1} biochar

3.4 Post-Treatment Soil Analysis

The soil sample was collected during the tasseling stage, which is approximately 55^{th} day. The soil samples were collected, air-dried, crushed and sieved using 2 mm sieve. After that, the soil samples were analysed for pH, soil EC, soil total C, soil total organic matter, soil total N, soil available P, soil exchangeable Al, and soil exchangeable acidity using the same procedure described previously.

3.4.1 Plant Tissue Analysis

The Maize plant in the field experiment was harvested and partitioned into leaves, stem and root separated at 55^{th} day for plant tissue analysis. Single dry ashing method was used for plant tissue analysis in order to extract the total P in plant tissues (root, stem, and leaves) (Tan, 2003). The concentration of P in roots, stems, and leaves was multiplied by respective dry weight to obtain the amount of P uptake by plants.

3.4.2 Plant Growth Parameters Of Maize (*zea mays. L*)

Plant height and number of leaves were measured at 55th day at the tasseling stage.

3.5 Statistic Analysis

The data in this study was analysed using Statistical Package for social Science (SPSS 21.0). The Analysis of Variance (ANOVA) was used to detect the treatment effects while Turkey's test will be used to separate the treatment means at $p \leq 0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Selected Physico – Chemical Properties of Soil Samples

Table 4.1 shows the selected physico-chemical properties of the soil sample prior to the pot experiment. The soil was a loamy sand and had a low pH which is 4.44. The soil also contained relative high concentration of Al and Fe. This was due to the low soil pH. Phosphorus fixation occurs due to sorption and desorption process in the soil. The C:N and C:P ratios of the soil were 10.69 and 8.18, respectively. The soil available P was 0.17. In the productivity of plants growing in tropical and subtropical ecology, one of the most common limitations is phosphorus deficiency(Ramaekers, Remans, Rao, Blair, & Vanderleyden, 2010) where P is very strong in the soil through adsorption and rainfall, reducing plant bioavailability (DoVale & Fritsche-Neto, 2013). Low P availability in soils was due to sorption and desorption process in the soil. This process occurs due to the large amount of sesquioxide elements such as aluminium (Al) and iron (Fe) in the soil that chelate P tightly thus inhibit the P for plant uptake. This process usually occur in the soil with low pH as it contain more cations such as H^{+} , Al^{3+}, Fe^{2+}, and which favor the strong binding with P ions (Moazed, Hoseini, A.A., & Abbasi, 2010).

Table 4.1 : Selected physico – chemical properties of soil samples

Property	Value Obtained
Bulk Density (g cm^{-3})	1.37
Soil Texture	Sand : 86% Clay : 8% Silt : 6% Loamy Sand
pH (water)	4.44
EC (dS m^{-1})	65.33
Total Organic Matter (%)	2.39
Total Carbon (%)	1.39
Total N (%)	0.13
Available P (ppm)	0.17
C/N Ratio	10.69
C/P Ratio	8.18
Exchangeable Al ($cmol_c kg^{-1}$)	0.07
Exchangeable Acidity ($cmol_c kg^{-1}$)	0.64
Exchangeable K (ppm)	77.76
Exchangeable Ca (ppm)	45.39
Exchangeable Mg (ppm)	44.36
Exchangeable Na (ppm)	3.88
Exchangeable Fe (ppm)	198.94
Exchangeable Cu (ppm)	1.24
Exchangeable Zn (ppm)	3.76

4.2 The Effect of Treatment on Selected Physico – Chemical Properties of Soil after Field experiment

The selected physico – chemical properties of soil after the pot experiment are shown in Table 4.2. The soil treated with mixture of chemical fertilizer and biochar (T2-T5) showed significant increase in soil pH compared to the soil only (T0). Several authors observed, soil that applied with biochar can increase the soil pH (D. A. Laird, 2008). Soil pH increased because of the is the precipitation of exchangeable and soluble Al and Fe as insoluble Al and Fe hydroxides (Ch'ng, Ahmed, & Nik, 2014). The soil treated with mixture of chemical fertilizer and biochar (T2-T5) and also soil with chemical fertilizer only (T1) showed significant increase in soil EC compared to the soil only (T0). Biochar applications can improve soil ECs by releasing biochar (cation and anion) nutrients into the soil solution, which is accessible for plant uptake (Shah & Shah, 2018). Treatment with biochar also significantly reduced acidity and Al compared to soil samples by using biochar. This was due to the chelation of Al and other cation in the soil (Yong , 2012). The total orgranic matter and total C in the table 4.2 also shows the significant increase compared to the soil only(T0) and soil with chemical fertilizer (T1). Notably, as a pyrolused product, rapid microbial degradation protected the biochar and is able to securely sequester carbon,(Lehmann, Gaunt, & Rondon, 2006). Other study also stated that decomposition of native will reduce by biochar and added soil organic carbon by sorbing organic compounds (Arif et al., 2017). Soil total N that applied with biochar were increased compared to the soil only(T0) and soil with chemical fertilizer (T1). Soil N contents will increase because of the process mineralization could exceed immobalization when chemical N fertilizer was integrated with biochar (Arif et al., 2017) The soil available P treated with biochar (T2-T5) and chemical fertilizer (T1) significantly increased compare to soil only (T0). The findings of other studies shows soil P availability can be improve when biochar were applied(Cui et al., 2011). This is due to the high P content on the surface of the biochar (Zhai et al., 2015) or the more suitable soil conditions associated with the addition of biochar, which stimulate microbial activity (Anderson et al., 2011) . Table 4.2 also presented the highest potassium(K) in treatment with biochar compared to the soil only (T0) and soil with chemical fertilizer (T1). Nutrients (P and K) availability in soil will increased with the use of biochar as reported in the study (Prendergast-Miller, Duvall, & Sohi, 2014). Other research reports that benefit NPK from both organic and inorganic nutrient sources in nutrient poor soils are provided by biochar (Widowati & Asnah, 2014;Ali, Arif, Jan, Khan, & Jones, 2015)

Table 4.2: Selected physico – chemical properties of soil samples after field experiment

Treatment	pH	EC (dS m^{-1})	Exchangeable Acidity ($cmol_c kg^{-1}$)	Exchangeable Aluminium ($cmol_c kg^{-1}$)	OM (%)	TC (%)	Total N (%)	Available P (ppm)	K
T0	5.24±0.13b	66.67±8.82b	0.46±0.06a	0.06±0.10a	3.91±0.07b	2.27±0.04a	0.13±0.04b	0.14±0.22b	57.16±15.36a
T1	6.08±0.10a	116.67±14.53a	0.55±0.17a	0.06±0.03a	3.82±0.25b	2.22±0.15a	0.14±0.04b	0.23±0.22a	51.36±4.29a
T2	6.66±0.08a	110.00±5.77a	0.46±0.07a	0.06±0.02a	4.12±0.24a	2.39±0.14a	0.18±0.03a	0.50±0.24a	84.19±16.51a
T3	6.80±0.10a	93.33±8.82a	0.48±0.08a	0.06±0.05a	4.19±0.11a	2.43±0.07a	0.24±0.05a	0.27±0.98a	82.47±20.88a
T4	6.50±0.06a	93.33±21.86a	0.51±0.09a	0.04±0.02a	4.71±0.28a	2.73±0.16a	0.27±0.04a	0.28±0.11a	85.57±11.86a
T5	6.64±0.05a	113.33±8.82a	0.45±0.13a	0.04±0.00a	4.30±0.39a	2.50±0.23a	0.26±0.07a	0.51±0.13a	95.40±18.72a

Mean between columns with different letter(s) indicate significant difference between treatments by Turkey's test at $p \leq 0.05$.

4.3 The Effect of Treatment on plants after Field experiment

Figure 4.1 shows the effect of treatments on number of leaves in *Zea mays* L. after field experiment. Based on the graph, it can be observed T5(Soil + 130 kg Urea ha^{-1} + 200 kg CIRP ha^{-1} + 67 kg MOP ha^{-1} + 20 t ha^{-1} biochar) shows the highest number of leaves in the field experiment. On the other side, it can be observed, T0 (soil only) shows the lowest number of leaves in the field experiment. Figure 4.2 represents the effect of treatments on plants height in *Zea mays. L* after field experiment. Treatments with chemical fertilizer (130 kg Urea ha^{-1} + 200 kg CIRP ha^{-1} + 67 kg MOP ha^{-1}) and biochar show significant difference on plant height and the graph shows T3 (Soil + 130 kg Urea ha^{-1} + 200 kg CIRP ha^{-1} + 67 kg MOP ha^{-1} + 10 t ha^{-1} biochar) has the highest among the treatment. Biochar application also response the positive plant growth that observed in the study (Schulz & Glaser, 2012 ;Obia, Mulder, Martinsen, Cornelissen, & Børresen, 2016). Other study also stated that corn growth was enhanced by the addition of biochar (Tanure et al., 2019). This is due to improved soil fertility, improved soil structure, efficient use of nutrients and water holding capacity (Oguntunde, Fosu, Ajayi, & Van De Giesen, 2004; Asai et al., 2009). Phosphate binding to free cations can be reduced when biochar is used, and P release is used for further plant growth (Haoye Zhang, Liu, & Liu, 2016)

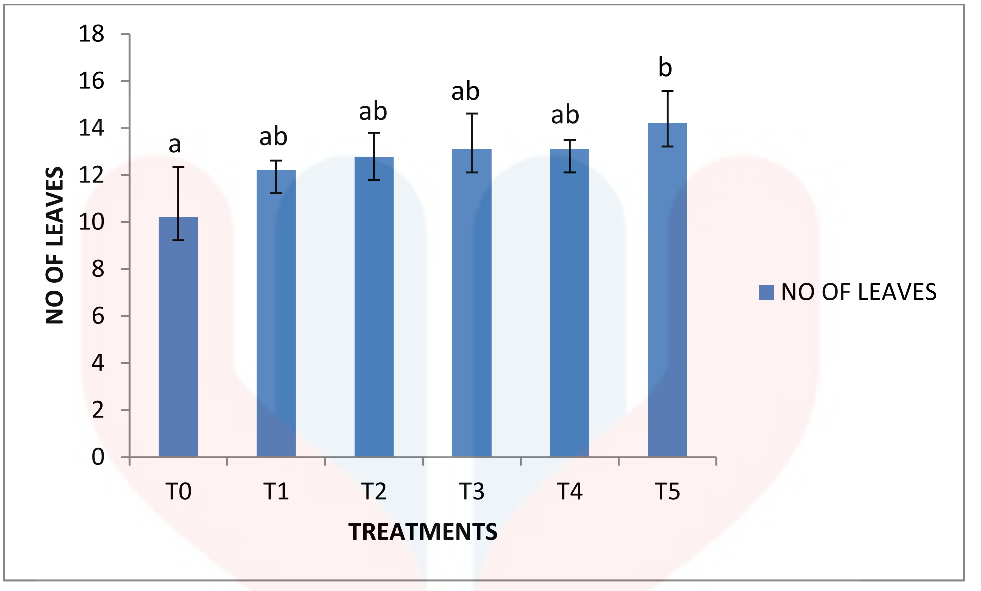

Figure 4.1: Effect of treatments on number of leaves in *Zea mays* L. after field experiment.

Mean between columns with different letter(s) indicate significant difference between treatments by Turkey's test at p≤0.05.

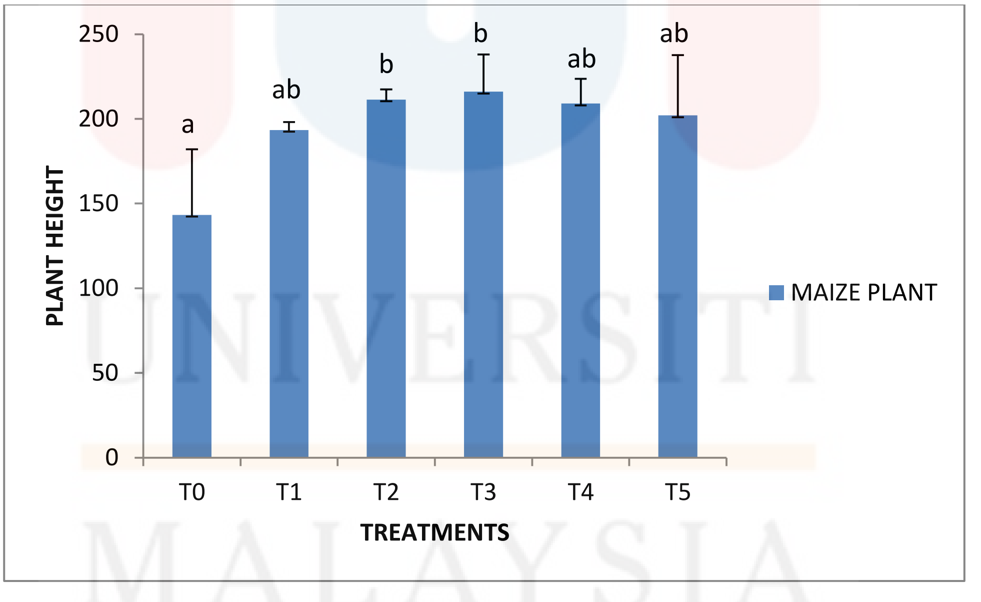

Figure 4.2: Effect of treatments on plant height in *Zea mays L.* after field experiment.

Mean between columns with different letter(s) indicate significant difference between treatments by Turkey's test at p≤0.05.

The dry mass of each part (roots, stems, leaves) of *Zea mays* L. were illustrated by Figures 4.3. The treatment with chemical fertilizer and biochar (T2-T5) showed a significant increase in the dry mass production of leaves, stems and roots. These findings are consistent with the results obtained in several studies (Ch'ng *et. al.*, 2015; Mokolobate & Haynes, 2003).

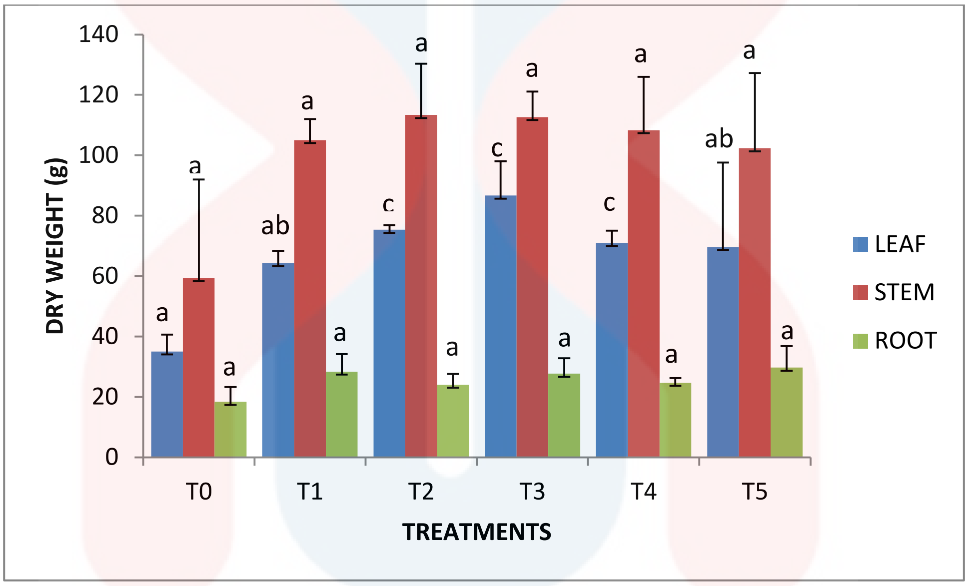

Figure 4.3 : Effect of treatments on dry weight of leaves, stems and roots in *Zea mays L.* after field experiment

Mean between columns with different letter(s) indicate significant difference between treatments by Turkey's test at $p \leq 0.05$.

There is no significant different among treatment on total P (roots, stem, leaves) that presented in Figure 4.4. But in figure 4.5, there is significant different on P uptake(leaves, stem, roots) compared to the soil only(T0) . This indicates that there was an increase in the available P in the soil. Increasing the soil pH and reduction of exchangeable Al in the soil also contributed to the increase of P uptake. The supply of organic material by biochar is said to aid the increase in microorganism efficiency by providing them with suitable condition. The soil microorganism helps in the soil mineralization process by converting organic P into an avilable form of phosphophate namely orthophosphate for plant uptake (Hylan, et al.,2005). The irrespective to microbial inoculants, P, K and Ca absorption to maize plant increased by the application of biochar (Rafique et al., 2020). Apart from mineralization process, another factor contributed to the increase in plant tissues and P uptake after application of biochar in T2-T5 was the chelation of Al and Fe which released the available P for plant uptake. In acidic soil, most of the P are fixed by Al and Fe which also known as sesquioxides by forming a strong bond between them.

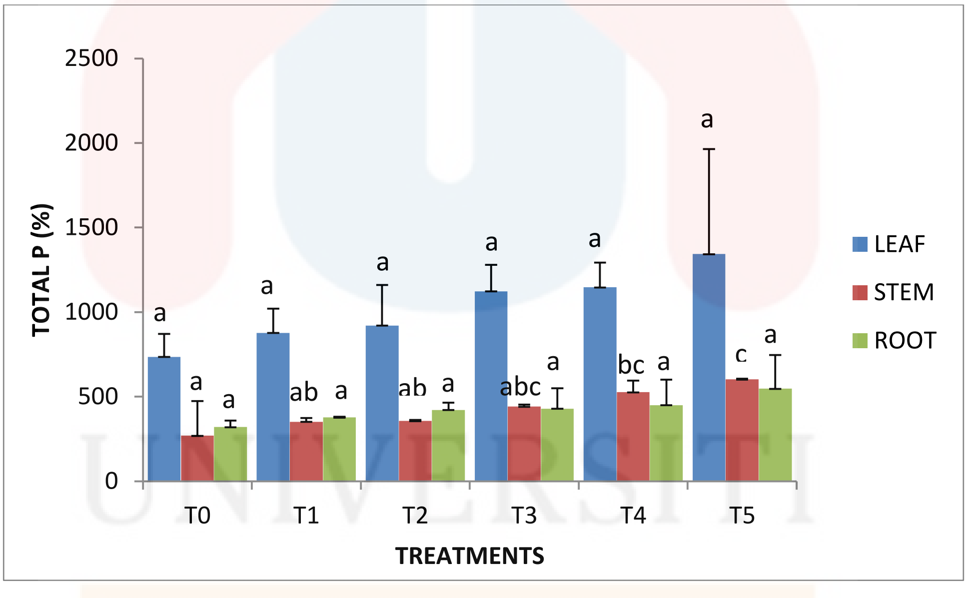

Figure 4.4: Effect of treatments on total phosphorus in *Zea mays L.* after field experiment.

Mean between columns with different letter(s) indicate significant difference between treatments by Turkey's test at p≤0.05.

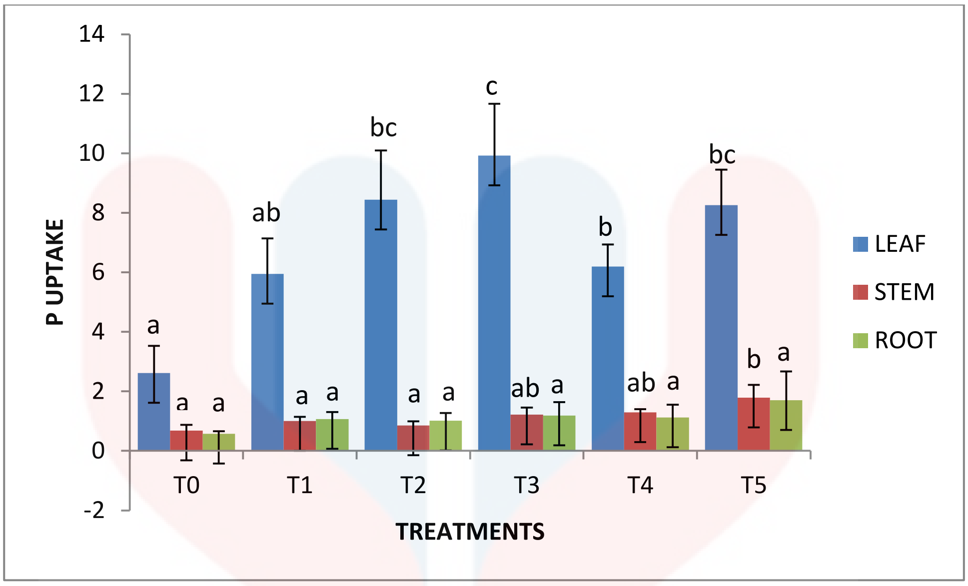

Figure 4.5: Effect of treatments on phosphorus uptake by *Zea mays L.* after field experiment.

Mean between columns with different letter(s) indicate significant difference between treatments by Turkey's test at $p \leq 0.05$.

The observation of the roots development indicated that the soil treated with chemical fertilizer and biochar had a better root growth compared to the soil only (T0) and soil with chemical fertilizer only (T1)(Figure 4.6). Eventhough T1 was supplied with NPK, the chemical fertilizer alone couldn't support the supply of nutrient to the soil. This statement is proven as several studies suggest that organic source of P are more effective that the inorganic ones (Adriano, Gutiérrez, Dendooven, & Salvador-Figueroa, 2012; Ch'Ng, Ahmed, & Majid, 2016)). Supplying biochar can release nutrients slowly according to the quantity that is needed by plant. Besides, the alleviation of Al toxicity at the root zone as a result of an increase of soil pH (Table 4.2) after the application of biochar allowed a greater volume of soil for root elongation.

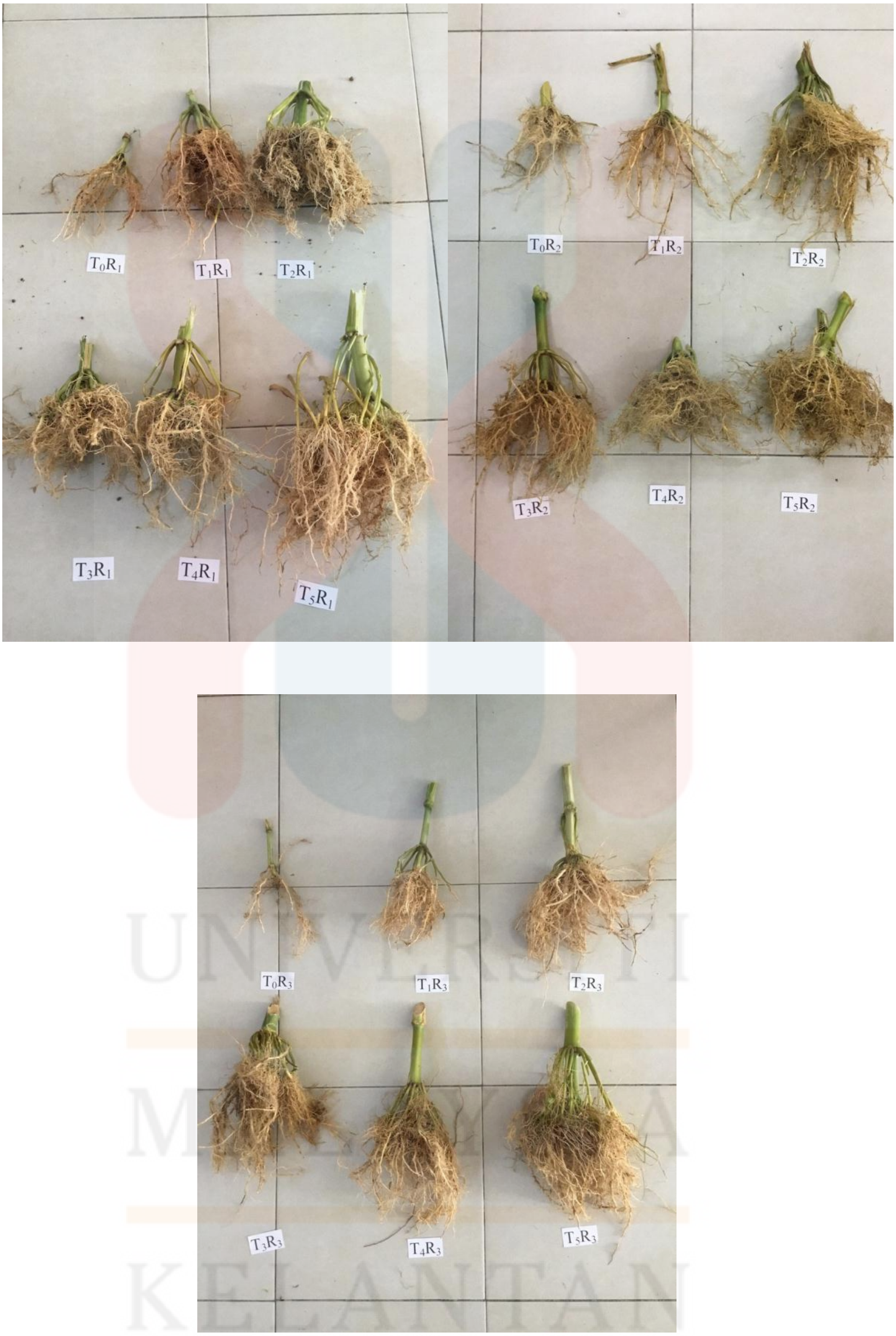

Figure 4.6: Effect of treatments on the root growth of *Zea mays L.*

CHAPTER 5

CONCLUSION AND RECOMMENDATION

Rice husk biochar can be used to improve the growth, total P and P uptake of *Zea mays L.* cultivated in tropical acid soil by enhancing the soil P acidity. This is proven as treatment with biochar significantly increased the soil pH, EC, reduced exchangeable Al and acidity in the soil. Besides, the treatments of biochar with chemical fertilizer also increased in the dry mass production, total P and P uptake in maize compared to soil only (T0) and soil with chemical fertilizer only (T1). Apart from that, the differences between the inorganic and the combination of both organic and inorganic treatments showed that supplying chemical fertilizer alone was not enough to supply nutrients and overcome the P fixation problem in acidic soil. It is concluded that, supplying organic amendment have high tendency to reduce the soil exchangeable Al, increased soil pH and P availability, reconstruct the chemical properties of soil by supplying organic materials, thus reducing the amount of phosphate fertilizer applied to the soil and increase soil P availability. As recommendation, this study can be further evaluated in the field for at least 3 cycles to confirm the findings.

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APPENDICES

Figure A.1 : Maize plot in agro techno park UMK

Figure A.2 :The ground sample of maize

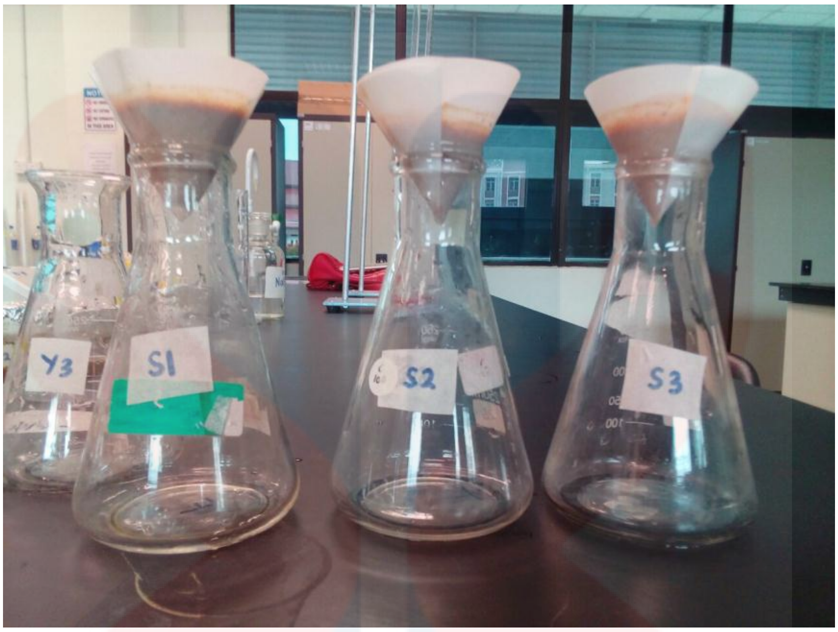

Figure A.3 : Filtering the sample soil sample

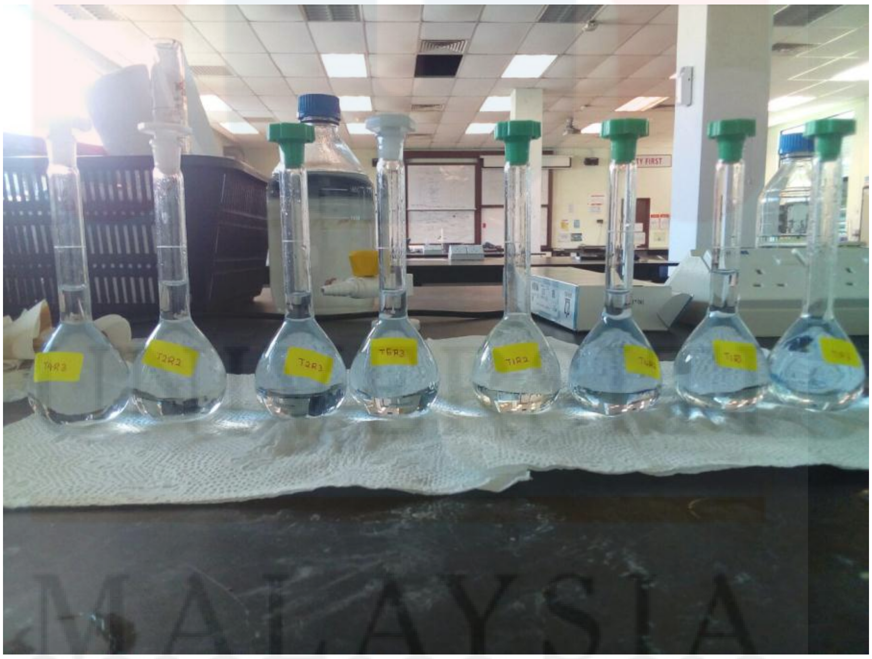

Figure A.4 : Development of blue colour during phosphorus determination

Figure A.5 : Distillation using Kjeldhal machine

Figure A.6 : Measure the EC of the soil