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SOIL SCIENCE

EFFECTS OF BIOCHAR AND *STENOTROPHOMONAS MALTOPHILIA* (SB16) ON SOIL PROPERTIES AND GROWTH OF SWEET CORN

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ABSTRACT

Laboratory and glasshouse studies were conducted in the Faculty of Agriculture, University Putra Malaysia during in the year 2014 to determine the effect of empty fruit bunch biochar and nitrogen-fixing bacteria *Stenotrophomonas* sp. (Sb16) on soil microbial populations, enzymes, mineral composition and growth of sweet corn. Five rates of biochar (0, 0.25, 0.5, 0.75 and 1%) were applied to sterilized and non-sterilized soil either with or without bacteria Sb16 and incubated for 40 days under laboratory condition. The treatment was arranged in a complete randomized design with three replications. Sweet corn was grown in pots containing 6 kg soil and applied with five levels of biochar (0, 5, 10, 15 and 20 t/ha) either with or without bacteria Sb16. The factorial experiment was organized in a randomized complete block design, with five replications. Results of laboratory study showed that application of biochar at 0.5 percent without inoculation and 0.25 percent with bacteria Sb16 in both soils significantly increased population of soil bacteria, fungi, actinomycetes and N_2-fixing bacteria, enzymes (urease, acid phosphatase and fluorescein diacetate hydrolysis activity), and soil chemical properties (pH, organic C, total N, available P and exchangeable K, Ca and Mg. Glasshouse experiment showed that application of biochar at 5 tons per hectare with bacteria inoculated significantly ($P<0.05$) improved growth of corn (shoot and root biomass, root length, root volume, plant height and leaf chlorophyll content). The biochar (5 t/ha) and bacteria (Sb16) stimulated soil quality and growth of sweet corn. Addition of high rates of biochar to soil negatively affected all observed parameters. Addition of 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.

Keywords: ***Zea mays rugosa; Zea mays saccharata;*** **sweet corn; EFB biochar; N_2-fixing bacteria; *Stenotrophomonas* sp; corn growth; Malaysia.**

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INTRODUCTION

Sustainable agriculture provides environmental and economic impacts on conserving natural resources and protecting the environment. New term of biochar is naturally occurring in some of the highly fertile soils in the world and gives large crop yields and enhances soil fertility despite the fact that surrounding soils are infertile. However, infertile soil can be ameliorated effectively by applying liming material, organic matter, bio-fertilizer, biochar or other amendments. In order to control infertile soil, soil properties 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 tons/day by a Nasmeck company in Malaysia (28). A farmer will gain a profit from biochar, when biochar amendment replaces agricultural lime or the profit of crop production given with biochar application as a substitute for lime, on the other hand, getting a benefit will depend on the price of biochar (33, 35).

Countries in different parts of the world have a long history with using biochar in sustainable agricultural soils. Biochar was produced by ancient, indigenous human habitation; these areas

still remain highly fertile soils despite centuries of leaching from heavy tropical rains, due to the incorporation of large amounts of biochar into these soils (7, 14). Studies should that chemical changes in the soil are central to the darkening of these soils stimulated soil physicochemical properties, microbial and enzymes activity and thus causing in soil quality and yield production (13, 37). Biochar has the potential to offer multiple environmental benefits where they do not only contribute to carbon storage, but at the same time act as a soil amendment (11, 19). Biochar is applied to soil as intention to improve a range of soil physical properties including; pore-size distribution, total porosity, soil density, water holding capacity and soil moisture content (2, 36). Biochar is becoming a popular alternative to organic amendments that are being applied to soils to increase soil nutrient (20, 23). The addition of biochar to highly leached and infertile soils has shown to give an almost immediate increase in the availability of basic cations (14, 33), and a significant improvement in crop yields (21). Agricultural wastes are important in soil agro-ecosystems as these are able to provide plant nutrients such as C, N, K, P, Ca, and Mg. Comprehending interactions between biochar and application conditions, soil texture, organic matter (OM), macronutrients and soil pH will be a main factor in deciding long-term influences of biochar application on soils.

Enzymes are proteins produced by soil microbial community that allow to involve in innumerable reactions to proceed at faster rates by reducing the energy and soil organic matter as well as nutrient cycling (22, 34). Soil enzymes, such as urease, phosphatase and fluorescein diacetate hydrolysis activity (FDA) play an important role in decomposition of organic matter, nutrient cycling for microbial activity and plant nutrient uptake (19). However, enough information is not available on the short-and longer-term influences of biochar on soil enzyme activities.

Biochar has potential to absorb organic and inorganic molecules in a wide range, may provide a mechanism to protect these enzymes (21, 37), but in general, there is a little information exist of the possible impacts of biochar on soil enzymes. Biochar has been reported as a possible means to improve soil nutrient and biological activity (43), enhancing soil microbial population (17). Biochar can amend soil habitat due to interactions with soil mineral, organic matter and microbial oxidation (10, 30). However, the relationships between chemical and physical properties of biochar and their influences on soil microbial activity and probable concomitant impacts on soil processes are poorly understood.The potential benefits of using biochar for agricultural soils and crop production have received significant attention from researchers in recent years. Biochar can be applied as means as a soil amendment to enhance soil fertility and crop production in a wide range of soils (3, 33). A pot experiment (20) recorded that addition of biochar increased biomass of rice and cowpea. These findings may relate to level of nutrient in organic soil amendment. The effect of biochar application on sweet corn and soybeans also resulted in the greatest dry root biomass weight in mineral soil (36). Also in a four year field study, yields of maize grain increased upto 140 percent on a Colombian savanna Oxisol (23). Increased nutrient retention by biochar may be the most important factor for increasing crop yields on infertile acid soils (1, 11).

From the limited information available, no optimum range or type of biochar application has been produced to improve plant productivity. Biochar from many sources of biomass can be produced through the pyrolysis process in the absence of oxygen and high temperature. Different types of biochar have different characteristics depending on the feedstock and pyrolysis conditions. All biochar production is not equal or unique. Quite the contrary, different types of biochar havewidely different physical, chemical and biological specifications that may have an important effect on the opportunities of application in the field (44).In Malaysia, there are two potential biomasses from industrial wastes that can be used as biochar; oil palm empty fruit bunches and rice husks (RH). It is reported that around 20 million tonnes of EFB was produced annually during the processing in the mills and number is expected to increase

greatly by the year. In Malaysia, the chemical characterization of EFB biochar showed that total C, pH, CEC and other substantial amounts of micro-nutrients are mostly higher than other types of biochar (27). Biochar may be added to soils with the intention to improve soil functions and plant growth as well as appreciable carbon sequestration value. In addition, the conversion of EFB biomass to biochar can be an alternative to the sustainable management of the industrial waste. However, there is very limited information on the properties of commercial EFB biochar produced in Malaysia to be used as soil amendments in remediating contaminated soil. Malaysian soils are mostly acidic or infertile soil that affects soil quality and plant growth. Acid soils are limiting crop production in 30-40% of the world's arable land and are a major growth-limiting factor for plants in many parts of the world (34).Tropical soils are most common where high precipitation, free drainage favors leaching, biological production of acids and the low population of indigenous microorganisms (34).Tropical soil can be ameliorated effectively by applying liming material, organic matter, bio-fertilizer, biochar and other amendments (41). Other than biochar, bio-fertilizer or beneficial inoculation, such as N_2-fixing bacteria can be used to ameliorate soil fertility by fixing N and transferring nutrients in soil (15, 25). Several crops such as rice, wheat and maize need 20 to 40 kg soil N per hectare to satisfy the N requirements for each tonne of grain produced (31). To face such large demand, farmers must apply inorganic N fertilizers that have negative environmental effects or rely on beneficial microbes such as biological nitrogen fixation (BNF) and the input of organic wastes, such as manure or biochar. Two types of nitrogen fixing bacteria are known: free-living (non-symbiotic) bacteria, such as cyanobacteria and Azotobacter with cereal crops (wheat, rice and corn) and symbiotic bacteria associated with leguminous plants (9, 12). Several of soil bacteria responsible or capable for transforming atmospheric N_2 into ammonium (NH_4), which is a form of N that can be used directly by plants. Nitrogen cycling in natural ecosystems relies by N_2-fixing bacteria for agricultural production. N_2-fixing bacteria produce nitrogen, which is much more effective and less costly to improve plant growth. However, free-living N_2 bacteria in the soil may provide substantial amounts of nitrogen (0 to 60 kg N/ha/ year) (7, 17). This could be important in organically amended soils, which typically have a lower proportion of nitrogen in available forms.

EFB biochar can be applied with free-living N_2 fixing bacteria to improve soil fertility, microbial activity and plant growth. Therefore, this study was conducted to investigate the effect of soil sterilization, oil palm EFB biochar as a soil amendment and N_2-fixing bacteria *Stenotrophomonas sp.* Sb16 on soil enzyme activity, indigenous microbial population, chemical properties and growth of sweet corn.

MATERIALS AND METHODS

Soil and EFB biochar preparation: A Laboratory and glasshouse experiments were carred out in the faculty of Agriculture, University Putra Malaysia (UPM) during the year 2014. The soil samples were collected randomly from UPM farm top soil (0 - 15 cm depth). Soil samples were air-dried for five days, ground and sieved through (2.00 mm) mesh for laboratory and (4.00mm) mesh for glasshouse study. The EFB biochar was made from an empty fruit bunch of oil palm which was provided by Nasmech Sdn. Bhd., Selangor. This biomass was gone through the pyrolysis process between 350-450 ^{0}C to produce EFB biochar. The soil and EFB biochar were analyzed for their chemical characteristics and the results are shown in Table 1.

Preparation of free- living N_2-fixing bacteria: The bacterial culture of *Stenotrophomonas sp.* (Sb16) was used for soil inoculation (30). The bacteria were obtained from Faculty of Agriculture, UPM. The strain was sub-cultured in 100 ml Erlenmeyer flask with Jensen's N-free broth and shaken continuously for 36 h (100 rpm at 28 ^{0}C) (30), until reaching approximately 10 ^{8} (cfu / ml).

Laboratory experiment: Five rates of oil palm EFB biochar (0, 0.25, 0.5, 0.75 and 1%) were applied to sterilized and non-sterilized soil. Each

conical flask contained 150 g of sterilized or non-sterilized soil with different levels of EFB biochar (0, 0.375, 0.75, 1.125 and 1.5 g) were mixed properly. Bacterial treatments in both soils were inoculated with one ml 10^8 (cfu) N_2-fixing bacteria Sb16. The flasks were covered with aluminum foil and incubated for 40 days at room temperature. Factorial study was conducted using a completely randomized design with three replications. The soil was analyzed for microbial populations, enzyme activity and chemical properties.

Glasshouse experiment: The soil and EFB biochar used in this study was sieved (2.00 mm) before mixing them thoroughly. Six kilogram of the soil was mixed thoroughly with EFB biochar at five rates (0, 5, 10, 15 and 20 t / ha) and placed in drained pots, either in the presence or absence of N_2-fixing bacteria (Sb16). The soil and EFB biochar mixture in pots were incubated for 20 days to interact with soil before planting with corn. Uniformly sized grain corn seeds were used. Five seeds were planted into the soil at 5.0 cm below. One ml 10^8 (cfu/ml) of N_2-fixing bacteria (Sb16) was applied to each seed of bacterial treatments. All pots were watered daily. When the seeds had germinated, only one seedling in each pot was left to grow. Then, the recommended rate of N, P and K fertilizers for corn cultivation was applied uniformly in all the treatments after two weeks of sowing in the form of urea (60 kg / ha), triple superphosphate (TSP) (60 kg / ha) and muriate of potash (MOP) (90 kg / ha), respectively. The treatments were arranged in RCBD, with five replications. The corn plants were harvested at tasseling stage and the parameters of tops and roots were recorded before they were oven.

Chemical analysis of soil and EFB biochar: The pH soil and EFB biochar were determined using the Beckman Digital pH meter in a 1:2.5 (w/v) for soil and 1:10 (w/v) for EFB biochar: water ratio (27). Determination of organic C was done on the LECO CR-412 carbon Analyzer using combustion method (25).Total N was determined according to Kjehdahl Method (6). Available phosphorus was found using Bray II Method (4) and analyzed by Auto Analyzer (Lachat instruments, Quik Chem® FIA+ 8000 series). Determination of CEC, K, Ca and Mg in soil was determined by using a leaching method (5, 16) and analyzed by an atomic absorption spectrophotometer (AAS) (Perkin Elmer, 5100 PC), but for total elements in biochar was done by digesting EFB biochar technique (27).

Measurement of leaf chlorophyll content from a hand-help SPAD-502 Meter: Absolute chlorophyll concentration measurements by SPAD meter makes simple, rapid, and non-destructive measurements provide a relative indication of leaf chlorophyll concentration compared to the extraction methods. Sweet corn leaf tissue for these measurements was determined by taken three times for each leaf and average calculated at tasseling stage.

Determination of plant growth parameters

The plant height was first measured before harvesting. The plant height was measured by a standard ruler from the growth media surface to tip of the main stem. The measurement of plant height was expressed as centimeter (cm). Five plants were harvested from each treatment and separated into root and shoot. Combination of stem and leaves part was considered as part of shoot. After separation of the plant parts, it was dried individually under sunlight for one day and then oven dried at 65 ^{0}C for 48 h. The shoot and root dry part was recorded by using digital balance (QC 35EDES- Sartorius- Germany). The dry weight of the root and shoot were presented as gram (g). Root volume was also determined using water cylinder for counting the volume of each root (8).

Root scanning: After harvesting the plants from the pots these were enclosed in a plastic bag immediately to prevent the dehydration, washed carefully with tap water and separated into shoot and root to the root growth. After root being washed, the root was prepared for the determination of root length by scanning using a root scanner (Model Epsom Expression 1680) which is connected to a computer program Win RHIZO 2007. To do this test, fine electrical wire

of three different diameters was cut. Scan the segments and tooks as many scans as possible with a total lengths and of segments with different diameters. Finally root length was calculated by WinRHIZO against actual root length.

Soil microbial populations: Soil microbial community (bacteria, fungi, actinomycetes and N_2-fixing bacteria) were determined using 10 g of fresh soil following the dilution plate technique (28). A 100 µl of sample at selected dilutions was transferred onto Nutrient agar (NA) for bacteria, Rose Bengal Streptomycin Agar (RBSA) for fungi (24), Actinobacteria isolation agar (A.A) for actinomycetes and N_2-free media for NFB. The colonies were counted as colony forming unit (cfu) dry soil^{-1}, and then transformed to log^{10} values for statistical analysis.

Determination of soil enzyme activity: Soil samples were air-dried and sieved through 2 mm. Soil phosphatase activity was determined as described earlier (39). Soil urease activity was determined as described by Tabatabai and Bremner (40). Fluorescein diacetate hydrolysis Assay (FDA) was conducted for measuring the enzyme activity of microbial populations and can provide an estimate of overall microbial activity in an environmental sample (27).

Statistical analysis: The data were recorded and analyzed using analysis of variance 2 way (ANOVA) by Statistical Analysis System (SAS) version 9.3 for Windows. The significant difference of treatment means was checked by the Tukey's General Linear Model Test (GLM) at the 5 percent level.

RESULTS AND DISCUSION

Chemical characterization of EFB biochar and soil

The chemical composition of soil and EFB biochar are listed in Table 1. Soil is acidic soil, while the oil palm EFB biochar is alkaline pH (pH 9.39) and it was abundantly higher than soil.The EFB biochar had almost 26 times higher of total carbon content, 8 times of CEC and 16 times of value total N than soil sample. EFB biochar was found to contain substantial amounts of nutrients with high concentration of K, Ca and Mg.

Table 1. The chemical analysis of soil and EFB biochar

Properties	Soil	EFB biochar
pH	4.6	9.39
Carbon %	2.01	52
Total N %	0.1	1.58
Available P (mg / kg)	34	--
Total P %	--	0.22
Exch K (cmol $_{(+)}$ / kg)	0.2	--
Total K %	--	4.9
Exch Ca (cmol $_{(+)}$ / kg)	23.	--
Total Ca %	--	0.11
Exch Mg (cmol $_{(+)}$ / kg)	0.8	--
Total Mg %	--	0.14
CEC (cmol $_{(+)}$ / kg)	8.1	63.2

Laboratory study

Effects of soil sterilization, EFB biochar and N_2-fixing bacteria (Sb16) on soil microbial populations: Indigenous soil bacteria, fungi, actinomycetes and N_2-fixing bacteria significantly ($P<0.05$) increased and associated with EFB biochar and N_2-fixing bacteria Sb16 application in sterilized and non-sterilized soil (Fig. 1). EFB biochar amended soil was observed to stimulate the soil microbial community. In sterilized soil, addition of EFB biochar at 0.25% positively improved soil bacteria, fungi and NFB populations, while higher actinomycetes were found at 0.5%. All EFB biochar treatments were better than no biochar rate (control). In non-sterilized soil, EFB biochar at 0.25% positively responded soil fungi activity, while the others were found at 0.5%. Application of EFB biochar at 0.5% was given the highest populations of microbes, but combining N_2-

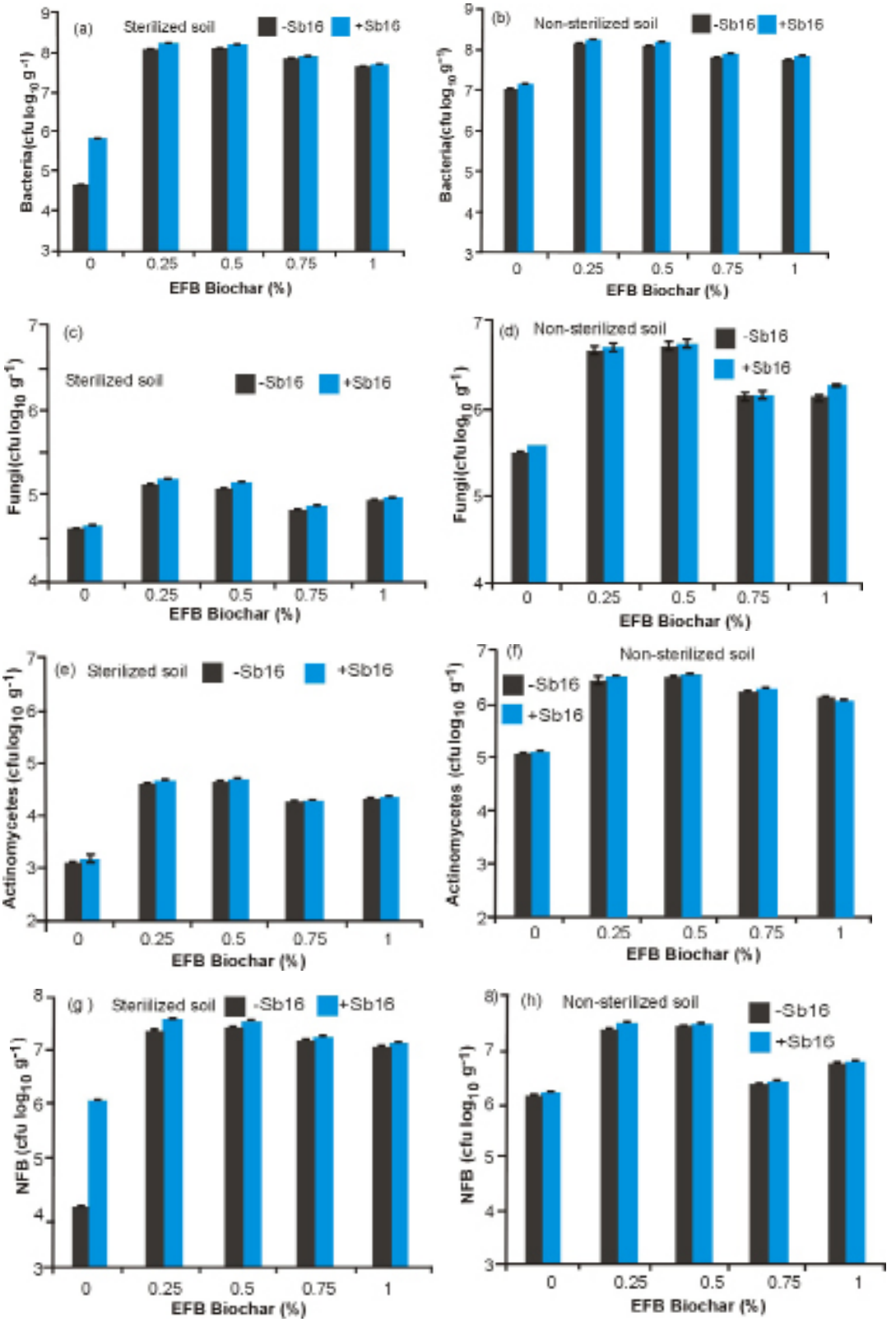

Fig. 1. Effect of soil sterilization, EFB biochar and N_2-fixing bacteria (Sb16) on soil bacteria, fungi, actinomycetes and N_2-fixing bacterial populations.
Vertical bars represent S.E. of means followed by the same letter or not significantly differnet at $P \leq 0.05$.

Sterilized soil	Bacteria	Fungi	Actinomycetes	N_2-fixing bacteria
EFB Biochar	0.0001*	0.0001*	0.0001*	0.0001*
Sb16	0.0001*	0.0001*	0.0014*	0.0001*
EFB Biochar*Sb16	0.0001*	0.1722^{ns}	0.4958^{ns}	0.0001*
Non-sterilized soil	**Bacteria**	**Fungi**	**Actinomycetes**	**N_2-fixing bacteria**
EFB Biochar	0.0001*	0.0001*	0.0001*	0.0001*
Sb16	0.0001*	0.0001*	0.0001*	0.0001*
EFB Biochar*Sb16	0.0008*	0.0039*	0.0003*	0.0200*

Means in each column for each variable do not significantly differ according to Tukey's test at 5%.

fixing bacteria Sb16 with oil palm biochar at 0.25% significantly improved soil microbial activities. Soil bacterial inoculation was better than non-inoculated. There was no interaction between the factors of soil fungi and actinomycetes in sterilized soil. The oil palm biochar at 0.25% and N_2-fixing bacteria Sb16 significantly enhanced soil microbial community, except the actinomycetes population responded more positively to EFB biochar at 0.5% and bacteria Sb16 in both soils. Lower microbial population was decreased with increasing EFB biochar rate. All EFB biochar treatments with and without bacteria Sb16 showed more effective than no biochar amended soil. Application of EFB biochar at 0.75% and 1% with and without bacteria inoculated adversely affected soil microbial activity. Enhancement population of microbes could be due to increase of pH and available nutrient in soil, which affected by EFB biochar and bacterial inoculation. Biochar may also have a suitable habitat to protect beneficial microbes from predators in soil. Some previous scientists (38, 39) were found that addition of biochar may contain growth promoting compounds and essential nutrition for microbial growth. Biochar has high surface area and porosity that enables it to retain nutrients and also provide a suitable habitat for beneficial microorganisms to flourish (43).The abundance bacteria in biochar amended soils might be attributed to the properties and characteristics of EFB biochar itself and soil. Rachel and Randey (30) reported that biochar is providing a suitable habitat, where indigenous microorganisms may escape from predators, as well as providing substrates to meet many of their diverse carbon, energy, and nutrient demands. EFB biochar is alkaline nature (pH 9.39) and has an important role to increase microbial activity in acidic soils by improving soil pH.

Effects of soil sterilization, EFB biochar and N_2-fixing bacteria (Sb16) on soil enzyme activity

EFB biochar and N_2-fixing bacteria Sb16 significantly ($P<0.05$) affected soil urease, phosphatase and fluorescein diacetate hydrolysis (FDA) activity in both soils (Fig. 2). There was no significant difference between oil palm biochar and N_2-fixing bacteria on soil phosphatase and FDA in both soils. Among all treatments, 0.5% EFB biochar resulted in higher value of enzymes activity in sterilized and non-sterilized soil. All EFB biochar rates proved to be more effective than no biochar rates (control). Organic amendment at 0.25% and N_2-fixing bacteria Sb16 differentially affected soil urease activity in sterilized and non-sterilized soil. The potential activity of EFB biochar at 0.5% and N_2-fixing bacteria Sb16 significantly improved soil phosphatase and FDA in both soils. Compared to the control, urease activity was higher in sterilized soil than non-sterilized, while soil phosphatase and FDA were better in non-autoclaved soil. The results showed that selected enzyme in soil significantly declined when EFB bochar rate increased. The N_2-fixing bacteria Sb16 decreased the high amount of EFB biochar to lower rate. Lower rate of EFB biochar with inoculation was sufficient to improve soil urease, phosphatase and FDA activity in soil. Organic carbon rich and free living N_2-fixing bacteria significantly impacted soil enzyme activity and were higher in non-sterilized soil than sterilized soil.

The research on the impacts of organic amendment in activities of soil enzymes is still scarce, although an influential number of articles have appeared lately. Biochar has the capacity to absorb a wide range of organic and inorganic molecules may provide a mechanism to protect enzyme's activity (22). Application of biochar amendment

positively affected soil enzyme activities may be due to high pH, surface area, pore size distribution, and charge properties (17). Krull *et al.* (18) reported that when the organisms require for P increased, application of P in soil improved acid phosphatase activity. Organic carbon having abundant microorganisms can influence enzyme activity in soil and activity of N_2-fixing bacteria to improve N in soil that be a reason to enhance soil enzymes activity. When the chemical P fertilizer supply exceeded organism demand for P, the acid phosphatase activity was inhibited by P fertilizer. Asai *et al.* (1) reported that application of biochar was stimulated soil microbes which are widely produced some enzymes. Soil enzymes may positively influence soil quality and it is very important to understand the probable roles of soil enzymes in order to maintain soil health and its fertility management in ecosystems. The nutrients of soil are very important for plant growth which improved with EFB biochar and N_2-fixing bacteria application.

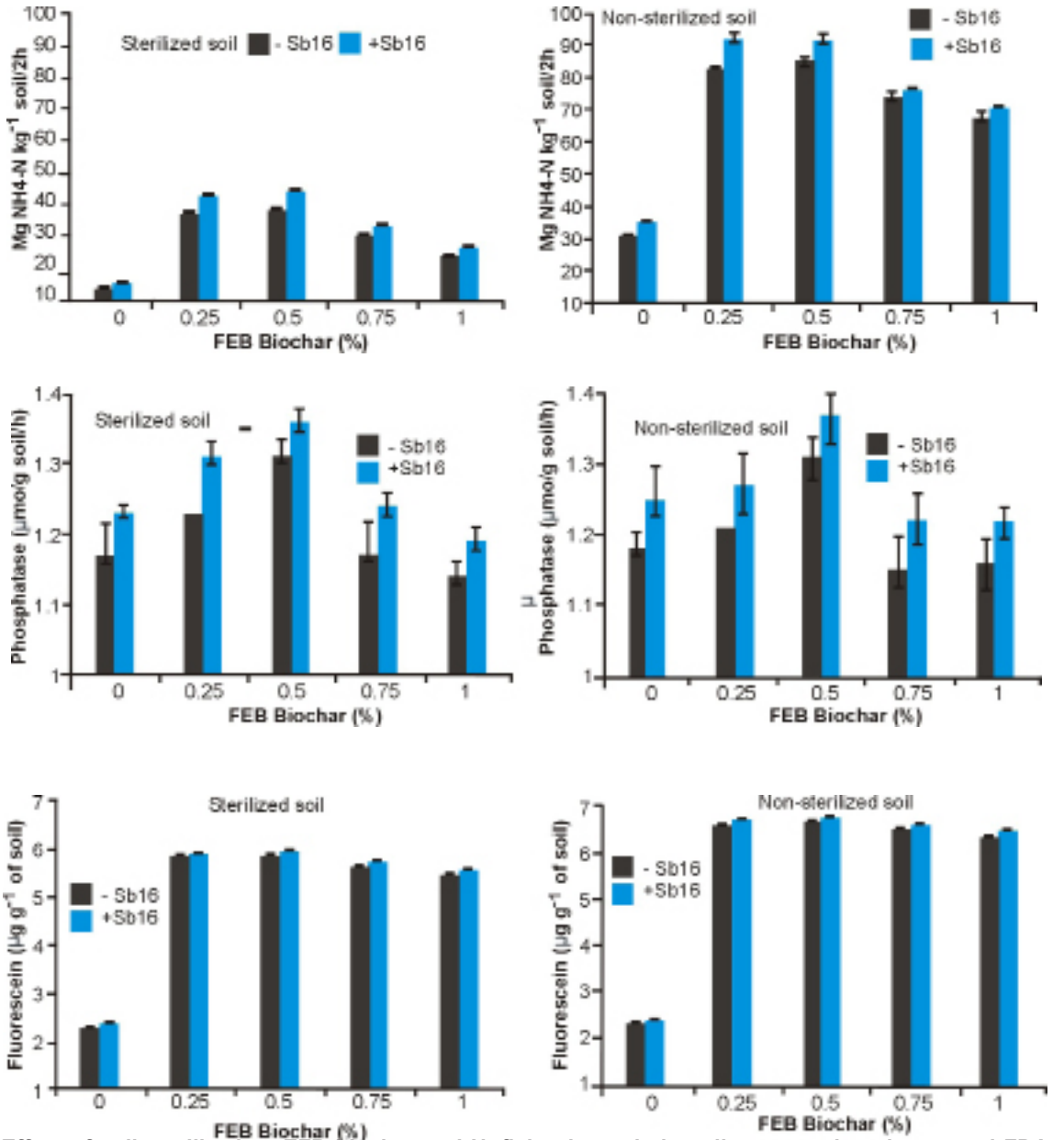

Fig. 2. Effect of soil sterilization, EFB biochar and N_2-fixing bacteria in soil urease, phosphataseand FDA activity. Vertical bars represent S.E. of means followed by the same letter are not significantly different at ($P \leq 0.05$).

Sterilized soil	Urease	Phosphatase	FDA
EFB Biochar	0.0001*	0.0001*	0.0001*
Sb16	0.0001*	0.0001*	0.0001*
EFB Biochar*Sb16	0.0004*	0.8275^{ns}	0.9987^{ns}
Sterilized soil	**Urease**	**Phosphatase**	**FDA**
EFB Biochar	0.0001*	0.0001*	0.0001*
Sb16	0.0001*	0.0001*	0.0001*
EFB Biochar*Sb16	0.0304*	0.9905^{ns}	0.7258^{ns}

Means in each column for each variable are not significantly different according to Tukey 's test at 5%.

Effects of soil sterilization, EFB biochar and N_2-fixing bacteria (Sb16) on soil chemical properties

Application of oil palm biochar and N_2-fixing bacteria Sb16 in sterilized and non-sterilized soil significantly improved soil chemical properties (Table 2 and 3). Among all EFB biochar rates, 0.25% showed the highest value of exchangeable P and potassium K in sterilized soil, while the other chemical properties observed at 0.5% EFB biochar. Higher value of organic C and P was found at 0.25% EFB biochar in non-sterilized soil, while the others showed at 0.5%. There were no significant differences between oil palm biochar and N_2-fixing bacteria Sb16 of soil pH, organic C, total N and exchangeable K in sterilized soil and also no significant difference between bacteria Sb16 and EFB biochar on pH and P in non-sterilized soil. Bacterial inoculation was better than non-inoculated. The EFB biochar amendment at 0.25% and 0.5% and N_2-fixing bacteria Sb16 showed the highest value of soil chemical properties in both soils. Alkaline EFB biochar at 0.5% and bacteria Sb16 slightly improved soil pH in both soils. Soil pH was better in non-sterilized soil than sterilized soil. Higher soil pH (pH 5.8) was recorded at 0.5% EFB biochar and N_2-fixing bacteria Sb16 in non-sterilized soil (Table 3), while the lowest (pH 4.5) was observed at 0% EFB biochar rate in sterilized soil (Table 2). Applying of Alkaline EFB biochar the decreased soil acidity. Similar finding was noted by Martin (24) where improved soil pH with application of alkaline biochar.

Highest value of organic C was observed at 0.5% EFB biochar in non-sterilized soil, while the lowest was found at zero percent EFB biochar in sterilized soil. Addition of EFB biochar at 0.25% and bacteria Sb16 showed the highest value in both soils (2.78 and 3.19%). Organic C was higher in non-sterilized soil than sterilized soil. High total carbon (52% C) in EFB biochar can affect soil organic C in soils. Soil organic C increased may be explained by the carbon and energy substrates provided by biochar itself or remains in the soil as humus and dead microbial cells may also directly increase the soil organic C pool (27, 29).

EFB biochar amendment at 0.25% and N_2-fixing bacteria Sb16 application resulted in the highest total N in non-sterilized soil (0.22%) (Table 3). Combination of EFB biochar with bacteria Sb16 was more effective for total N in sterilized soil than non-sterilized soil. The increase of EFB biochar rate with or without bacteria Sb16 adversely affected the total N than that found at other treatments. Release of excess nutrient elements from EFB biochar and activity of N_2-fixing bacteria could be a reason to increase N input in soil. The initial increase in total N content could be due to the macronutrients abundant in the biochar, fixing N by beneficial bacteria and mineralization of organic N in the biochar which released ammonium-N as one of the degradation products (9, 10, 23). The high mineral N content in soil may be affected by soil pH and activity of bacteria Sb16 to convert atmospheric N to ammonium into soil. The light fraction organic matter in the soil and microbial biomass could have resulted in better nutrient release from EFB biochar.

Addition of EFB biochar at 0.5% resulted in higher P than other biochar rates in both soils. Lower value of soil P was observed at 0.25% EFB biochar and bacteria Sb16 in both soils. However, soil available P was higher in sterilized soil than non-sterilized which soil may be due to increase of phosphate solubilizing bacteria population to solubilize P in soil or decomposing of chemical properties in EFB biochar. Gaskin *et al.* (13) indicated that soluble PO_4^{-3} increased due to the abundant macronutrients in biochar and mineralization of soil organic P. This was similar to the exchangeable potassium K. The increase could be due to the high cation contents in EFB biochar. The unamended soil (control) showed no changes of amount K during the incubation study. There was a slight increase of K in soil treated with bacteria Sb16 alone. Application of EFB biochar at 0.25% and N_2-fixing bacteria Sb16 gave the highest K (0.90 cmol (+) /kg) in non-sterilized soil (Table 3). This increase of soil K may be due to the presence of K in EFB biochar. Application of oil palm biochar with and without N_2-fixing bacteria Sb16 significantly enhanced chemical and biological properties and enzyme activity in an acidic soil. EFB biochar may have potential for enhancing soil quality upto a certain limit as beneficial soil amendment but at higher rates its contamination can affect harm for the soil properties.

Table 2. Effect of EFB biochar and N_2-fixing bacteria Sb16 in sterilized soil on soil chemical properties.

EFB biochar (%)	pH	C%	N%	P(mg/kg)	K (com (+)/kg-1)
0 – Sb16	4.5 ± 0.03	1.87 ± 0.01	0.004 ± 0.01	28 ± 0.91 g	0.17 ± 0.01
0 + Sb16	4.7 ± 0.03	1.92 ± 0.01	0.006 ± 0.01	31 ± 0.93 g	0.20 ± 0.01
0.25 – Sb16	5.5 ± 0.03	2.71 ± 0.01	0.06 ± 0.01	81 ± 0.90 c	0.80 ± 0.01
0.25 + Sb16	5.6 ± 0.03	2.78 ± 0.01	0.09 ± 0.01	96 ± 0.57 a	0.85 ± 0.01
0.5 – Sb16	5.6 ± 0.03	2.73 ± 0.02	0.08 ± 0.01	85 ± 0.57 c	0.75 ± 0.02
0.5 + Sb16	5.7 ± 0.03	2.75 ± 0.02	0.11 ± 0.01	91 ± 0.96 b	0.81 ± 0.01
0.75 – Sb16	5.4 ± 0.03	2.2 ± 0.01	0.06 ± 0.01	60 ± 0.98 e	0.65 ± 0.01
0.75 + Sb16	5.5 ± 0.03	2.23 ± 0.02	0.08 ± 0.01	69 ± 0.95 d	0.69 ± 0.01
1 – Sb16	5.3 ± 0.03	1.96 ± 0.01	0.04 ± 0.01	50 ± 0.97 f	0.44 ± 0.02
1 + Sb16	5.4 ± 0.03	2.01 ± 0.01	0.06 ± 0.01	56 ± 0.95 e	0.47 ± 0.01
Biochar	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
Sb16	0.0001*	0.0001*	0.0001*	0.0001*	0.0006*
Biochar*Sb16	0.2781^{ns}	0.2124^{ns}	0.2390^{ns}	0.0133*	0.8252^{ns}

Means in each column for each variable do not significantly differ according to Tukey 's test at 5%.

Table 3. Effect of EFB biochar and N_2-fixing bacteria Sb16 in non-sterilized soil on soil chemical properties.

EFB biochar (%)	pH	C%	N%	P(mg/kg)	K (com (+)/kg-1)
0 – Sb16	4.6 ± 0.06	2.10 ± 0.01	0.11 ± 0.01	34 ± 1.15 g	0.21 ± 0.01 f
0 + Sb16	4.8 ± 0.09	2.12 ± 0.01	0.12 ± 0.01	38 ± 1.00 g	0.22 ± 0.01 f
0.25 – Sb16	5.6 ± 0.03	3.11 ± 0.02	0.17 ± 0.01	68 ± 1.15 c	0.82 ± 0.01 c
0.25 + Sb16	5.7 ± 0.06	3.19 ± 0.01	0.22 ± 0.01	73 ± 0.60 a	0.90 ± 0.01 a
0.5 – Sb16	5.7 ± 0.03	3.13 ± 0.01	0.19 ± 0.01	70 ± 0.60 c	0.85 ± 0.01 b
0.5 + Sb16	5.8 ± 0.09	3.17 ± 0.01	0.21 ± 0.01	80 ± 0.07 b	0.87 ± 0.01 b
0.75 – Sb16	5.4 ± 0.09	2.91 ± 0.01	0.16 ± 0.01	56 ± 1.15 e	0.72 ± 0.01 d
0.75 + Sb16	5.6 ± 0.09	2.95 ± 0.01	0.17 ± 0.01	63 ± 2.30 d	0.74 ± 0.01 d
1 – Sb16	5.4 ± 0.09	3.03 ± 0.02	0.13 ± 0.01	50 ± 2.10 f	0.65 ± 0.01 e
1 + Sb16	5.5 ± 0.06	3.05 ± 0.01	0.14 ± 0.01	58 ± 1.15 e	0.67 ± 0.01 e
Biochar	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
Sb16	0.0001*	0.0001*	0.0001*	0.0001*	0.0002*
Biochar*Sb16	0.0884^{ns}	0.0480*	0.0343*	0.2548^{ns}	0.0007*

Means in each column for each variable do not significantly differ according to Tukey 's test at 5%.

Glasshouse study

Effects of N_2-fixing bacteria Sb16 and EFB biochar on growth of sweet corn: The study showed that application of N_2-fixing bacteria Sb16 and EFB biochar significantly improved growth of corn plants (as indicated by root and shoot biomass, root length, root volume, plant height and leaf chlorophyll content) and nutrient uptake (Fig. 3). Application of EFB biochar with or without bacteria Sb16 resulted in better plant growth than without EFB biochar and bacterial inoculation. EFB biochar with N_2-fixing bacteria Sb16 significantly ($P<0.05$) increased corn growth at tasseling stage. All EFB biochar levels showed a relatively larger increase in growth compared to without EFB biochar (control). Among all treatments EFB biochar, 5 t/ha showed highest (48.5 g/plant) of shoot biomass, while the lowest (41.2 g/plant) was observed at 20 t/ha EFB biochar. Root development was affected by application of EFB biochar at 5 and 10 t/ha. The results showed significant improvement in plant height and leaf chlorophyll content due to addition of EFB biochar at 10 t/ha compared to other EFB biochar rates. Application of N_2-fixing bacteria slightly improved growth of corn than non-inoculated. Higher weight (61.4 g/plant) of shoot biomass was found at 5 t/ha EFB biochar and N_2-fixing bacteria Sb16, while the lowest value (20.6 g/plant) was observed at 0 t/ha EFB biochar (control). Root biomass increased two folds with EFB biochar 5 t/ha and bacterial inoculation compared to control. Root length and root volume showed better growth at 5 t/ha EFB biochar and bacteria Sb16 than other treatments. Similarly, leaf chlorophyll or leaf greenness and plant height in corn significantly improved by EFB biochar at 5 t/ha and bacteria Sb16 application. Leaf greenness increased 144 percent times compared to unamended soil (control). Increase levels of EFB biochar with and without bacteria Sb16 negatively affected growth of corn. EFB biochar which is alkaline product can improve soil pH and CEC and thus improve soil nutrient and plant growth. EFB biochar provides a conducive environment for beneficial microbes, especially the activity of N_2-fixing bacteria to increase total N in a form that can plant absorb easily from the soil. Lower rate of EFB biochar i.e. 5 t/ha could possibly provide adequate nutrient and suitable conditions for microbes to enhance nutrient in soil and plant growth.

Biochar has been known to have the ability to amend soil properties, leading to impact beneficially on plant growth and nutrient uptake (1 , 2). Application of biochar improved soil acidity, pore structure, surface area, essential nutrients and changes of microbial populations, thus increasing crop productivity. By contrast, EFB biochar application with bacterial inoculation resulted in a strong positive rate-dependent impact on plant growth in tropical soils. Leaf greenness is widely known that is an essential parameter for plant status, such as, photosynthetic potential, N uptake and plant productivity. Solubilization and porosity of ash-biochar may control the release of soluble nutrients and available to absorb by plants (8). The biochar may catalyses the breakdown of organic matter by supplying microbial habitat carbon substrate and nutrients. Biochar affected microbial activity by improving the physical and chemical in soil (36). Application of biochar to poor fertile soil has been found to provide longer-lasting improvements in soil fertility (44). Biochar positively affected the soil nutrient availability in two common ways: nutrient addition and nutrient retention. The ash in biochar contains plant nutrients, mostly are macronutrients and micronutrients (11). Biochar retains, attracts and holds nutrients of soil directly via negative charge that evolves on its surfaces, and this negative charge can buffer acidity in the soil or exchange by soil and plant roots (42). Several groups of microorganisms recognized in biochar were able to regulate plant growth through nutrient cycling (32). Enzymes and more compounds were promoted characteristics in biochar and could also enhance plant growth. Combining EFB biochar and N, P as well as K fertilizer possibly provide adequate nutrient with bacterial plants to increase nutrient in soil and corn growth. Application of biochar generally increased the root hairs and effective root surface areas beyond

common root absorption zones causing in higher nutrient transfer beneficial for plant production and nutrient uptake (41). Lower rate of EFB biochar and bacteria Sb16 may release adequate nutrient into soil and can be easily taken up for plant growth by beneficial organism. However, research is needed in order to better understand how the addition of biochar and inoculation improve soil quality and plant growth before it becomes a common practice. There are many problems that are directly or indirectly linked to the application of biochar on agricultural system. Oil palm EFB biochar with or without bacterial inoculation improved growth of sweet corn, nutrient uptake and soil properties. Addition of EFB biochar to soil at higher levels adversely affected plant growth and soil properties. Addition of EFB biochar may be an alternative solution in enhancing the quality in acid soil. Inoculation of bacteria Sb16 not directly aids to enhance the soil condition conducive for the release of nutrients from EFB biochar. This finding supported the earlier reports on positive influence of biochar in stimulating soil fertility and plant growth (30). The incorporation of oil palm biochar and N_2-fixing bacteria can induce ameliorating changes to chemical and biological properties and enzyme activity of acidic soils and improve crop production. Future research needs to evaluate the effect of other beneficial microbes such as arbuscular mycorrhizal fungi (AM) or plant growth promoting rhizobacteria (PGPR) applied with EFB biochar in acidic soils under field conditions.

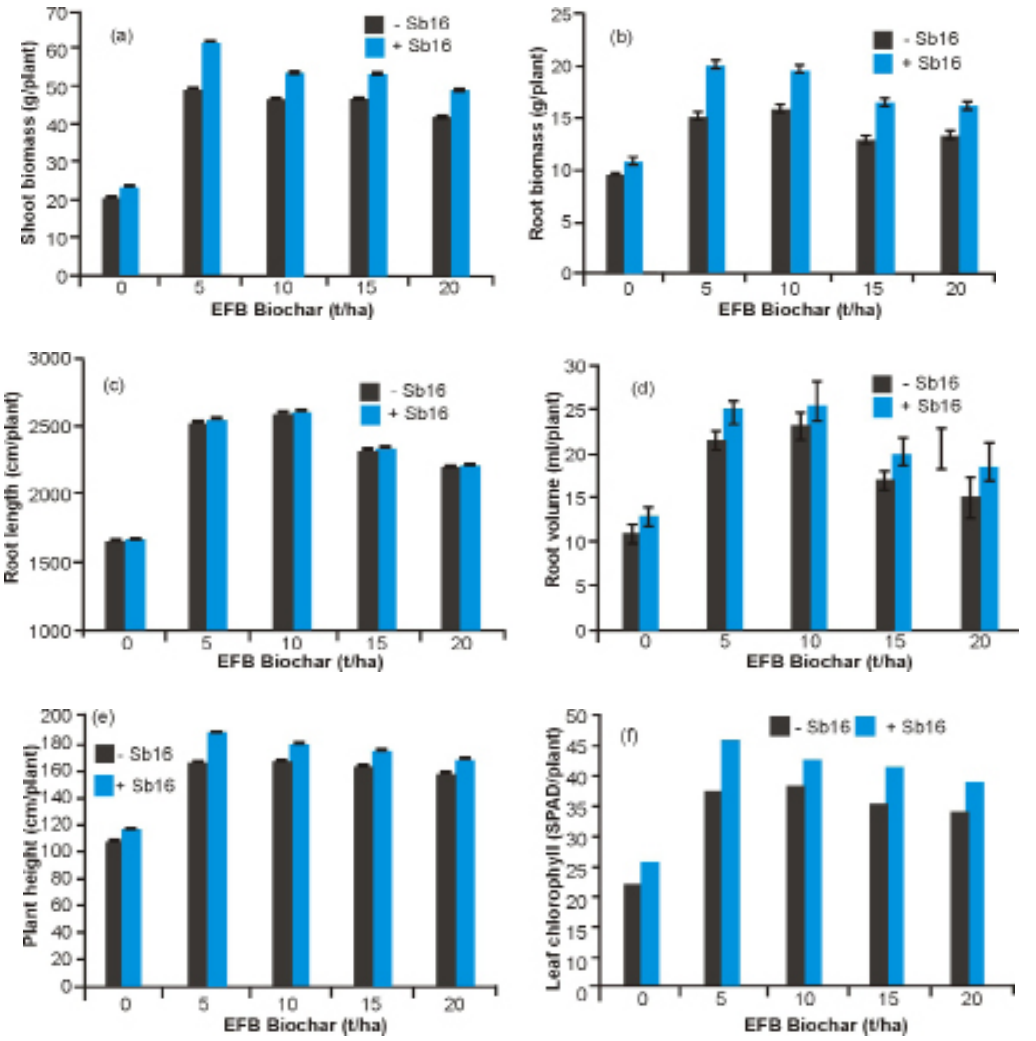

Fig. 3. Effect of EFB biochar and N_2-fixing bacteria Sb16 on shoot biomass (a), root biomass (b), root length (c), root volume (d), plant height (e) and leaf chlorophyll content (f).

CONCLUSION

The results of study concluded that EFB biochar made from agricultural waste oil palm EFB at 5 t/ha or 0.25% with N_2-fixing bacteria practically performed better as a soil amendment. Bacterial soil inoculation improved chemical and biological properties, enzyme activity and growth of corn than non-inoculated. Addition of high amendment rates to soil adversely affected plant growth and soil properties. Special attention should be paid to processing conditions and types of biochar applied. Premixed biochar with soil and N_2-fixing bacteria look necessary for stabilization of the soil and better growth of corn. It is interesting to note that lower rate of EFB biochar 5 t/ha with N_2-fixing bacteria promoted highest plant growth. Smaller additions and longer mixing times are strongly recommended. The incorporation of oil palm biochar with N_2-fixing bacteria can induce ameliorating changes to chemical and biological properties and enzyme activity of acidic soils and improve plant production. The suitable rate of EFB biochar application affordable by farmers is 10 t/ha without inoculation or 5 t/ha with bacterial inoculation.

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