EFFECT OF BIOCHAR AND *Bradyrhizobium japonicum* INOCULATION ON AMF COLONIZATION, PHOSPHORUS UP TAKE AND BIOLOGIC AL NITROGEN FIXATION (BNF) OF SOYBEAN [*Glycine max* (L.)] AT JIMMA, SOUTH-WEST ETHIOPIA

MSc THESIS

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Effect of Biochar and *Bradyrhizobium japonicum* Inoculation on AMF Colonization, Phosphorus up-take and Biological Nitrogen Fixation (BNF) of Soybean [*Glycine max (*L.)] At Jimma, Southwest Ethiopia

By

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A Thesis

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DEDICATION

This M.Sc. thesis work is dedicated to my Late Father Ato Asfaw Mekonnen

STATEMENT OF AUTHOR

I declare that this piece of work is my own and all sources of materials used for this thesis work have been duly acknowledged. The thesis has been submitted in partial fulfillment of the requirements for the degree of Master of Science at Jimma University and is reserved at the University Library to be made available to users. I solemnly declare that this thesis work is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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BIOGRAPHICAL SKETCH

Eskedar Asfaw Mekonnen was born on September 18, 1982 EC at Masha town, Sheka Zone of Southern Nations, Nationalities, and People's Region. She attended her elementary school from 1988 to 1995 EC and junior secondary schools from 1996 to 1999 EC at Masha High School in Masha. Following the completion of her secondary education, she joined Mizan Teppi University in 2000 EC and graduated with a B.Sc. Degree in Plant Sciences in July, 2002 EC. She was then employed by Teppi Agriculture office where she had served the office as an agronomist for 4 years. In 2007 EC, she joined the graduate studies program of Jimma University College of Agriculture and Veterinary Medicine to pursue her study leading to a Master of Science degree in Agriculture (Agronomy).

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LIST OF ABBREVIATIONS

BNF	Biological Nitrogen Fixation
AMF	Arbuscular Mycorrhizal Fungi
CEC	Cation Exchange Capacity
ATP	Adenosine triphosphate
JARC	Jimma Agriculture Research Center
EC	Electrical conductivity
RCBD	Randomized Complete Block Design
DAP	Diammoniume Phosphate
AC	Arbuscular Colonization
VC	Vesicular Colonization
HC	Hyphal Colonization
TND	Total Nitrogen Difference
LSD	Least significant difference
CV	Coefficient of variation
ANOVA	Analysis of variance
Ndfa	Nitrogen derived from the atmosphere
PVLG	Polyvinyl alcohol-lactic acid-glycerol
FAO	Food and Agriculture Organization
SAS	Statistical Analysis System
IBI	International Biochar Initiative

TABLE OF CONTENTS

TABLE OF CONTENTS *(Cont.d)*

TABLE OF CONTENTS *(Cont.d)*

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF APPENDIX

Effect of Biochar and *Bradyrhizobium japonicum* Inoculation on AMF Colonization, P uptake and BNF of Soybean [*Glycine max* (L.) Merrill] at Jimma, Southwest Ethiopia

ABSTRACT

Phosphorus deficiency is one of the most important soil factors, which affect soybean growth and its productivity. Biochar is one of the most important and easily available soil amendment resources that can improve soil properties and help plant roots access to mycorhyzal fungi. Nodulation and nitrogen fixation capacity of soybeans is affected by the extent to which the plant forms symbiosis with Bradyrhizobium japonicum. However, there are still many uncertainties about biochar, particularly whether it has positive effects with a particular soil and crop type. This study was, therefore, conducted to determine the effect of biochar application rate and Bradyrhizobium japonicum inoculation on Arbuscular Mycorhyzal Fungi (AMF) colonization, P uptake and Biological Nitrogen Fixation (BNF) of soybean (Glycine max) grown on Nitisol of Jimma, south-west Ethiopia. The experiment was conducted under greenhouse condition from February 2016 to May 2016 in randomized complete block design with three replications. The treatments consisted of four levels of biochar application rates (0, 6, 12 and 36 ton/ha with and without P supplement) with and without Bradyrhizobium japonicum strain (MAR1495) inoculation. Data on growth and yield parameters, AMF colonization, BNF and P up-take were collected and statistically analyzed using SAS 9.2 software. Analysis of Variance showed that application of biochar and B.japonicum inoculation had significant ($P<0.05$) effect on all parameters. Applications of 36 t ha^{-1} of biochar supplemented with 100kg/ha DAP showed a significant increase in all growth and yield parameters in both inoculated and non-inoculation treatments. Moreover, P uptake (4.81 and 4.27 g $plant^{-1}$), total N (4.19 and 2.66 g/plant), N_2 fixation (4.61 and 2.98 g $plant^{-1}$) and % Nitrogen derived from atmosphere (98.57 and 97.74) were also improved at 36 t ha^{-1} of biochar supplemented with 100kg/ha DAP in both inoculated and non-inoculation plots, respectively. Whereas inoculated treatments performed better as compared to the non-inoculated treatments for all the parameters. However AMF colorizations (79.79 and 78.65%) with and without inoculation, respectively, were significantly higher in a treatment containing of 36 t ha^{-1} biochar alone. The amount of N_2 fixed by soybean, and total N and P uptake were strongly and significantly correlated with number of nodules and total colonization of soybean. The results confirmed that biochar application with B.japonicum inoculation was beneficial for soybean growth, N and P up-take and BNF. Hence, application of 36 t ha^{-1} biochar with inoculation and P supplementation was considered as effective for soybean growth, BNF and P up-take. However, AMF root colonization percentage was high at 36 t ha^{-1} biochar without P supplement.

Key Words: *Glycine max*, Biochar, *B.japonicum*, BNF, uptake

1. INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is a legume crop native to East Asia perhaps in North and Central China (Laswai *et al.,* 2005) and it is grown for its edible beans and oil and protein around the world. It is eaten in fresh green state and as dry beans. Soybean belongs to the Family *Fabaceae* and species *Glycine max* (Shurtleff *et al.,* 2007). It is a short day self-pollinated C_3 plant that grows well in tropical, sub-tropical and temperate climates. Its germination is epigeal and the crop has a tap root system (Hymowitz, 1987). Soybean has been cultivated since the 11^{th} century with Brazil (36.1%) being the largest producer in the world, followed by United States (35.9%), Argentina (21.1%) and China (6.0) (Agboola and Moses, 2015). Soybeans have been introduced to Ethiopia in the 1950s because of its nutritional value, multipurpose use and wider adaptability in different cropping systems. Among food legumes grown in Ethiopia, soybean is gaining more importance in recent years (Zinaw *et al*., 2013).

Soybean is a multipurpose crop, which can be used for a variety of purposes, including preparation of different kinds of soybean foods, animal feed, soy milk, and raw material for the processing industry and recently for bio-energy. It is a good source of protein, unsaturated fatty acids, minerals like Ca and P including vitamins A, B and D that meet different nutritional needs (Rahman, 1982).The seed contains about 40-45% protein, 18-20% edible oil and 20-26% carbohydrate (Gowda and Kaul, 1982). In addition, soybean improves soil fertility through biological nitrogen fixation and can be used in rotation with cereals like maize and sorghum (Sanginga, 2003). World soybean productivity reached approximately 2.6 t ha^{-1} with an area coverage and total production of 117.7 million hectares and 308.4 million tons, respectively (FAOSTAT, 2015). Productivity of soybean in Ethiopia is around 2.1 t $ha^{-1,}$ which is lower than the world average of 2.6 t ha^{-1} (FAOSTAT, 2015). The crop is rapidly expanding in southwest Ethiopia as a potential market commodity particularly in Jimma and Illubabor zones. According to Illubabor zone Agriculture office, soybean cultivation data report of 2016, in the last three years (2013, 2014 and 2015), area covered by soybean in the zone alone was 4134.3, 4315.5 and 4738.8 hectares, respectively. These data shows that there is an average 14% area increment by the crop. However, the yield reported for the last three years was 2.4, 2.2 and 1.8 t ha^{-1} respectively. Although the area coverage has increased, yield

is decreasing progressively annually. The low national average yield (2.1 t ha^{-1}) and the zonal low yield (as low as 1.8 t ha^{-1}) may be attributed to the combination of several production constraints, among others; soil fertility depletion and soil acidity are the top ones.

Soil acidity is one of the most important soil factors which affect plant growth and ultimately limit crop production (Fageria, 2009). The high content of Al and Fe oxides and hydroxides are the main factors accounting for the strong P fixation in acidic soils in the highlands of Ethiopia and, consequently, limits crop production (Asmare, 2014). Phosphorus deficiency has also been shown to be an important fertility problem limiting legume production in the tropics and reduces nodulation, N_2 fixation and growth. Acidic soils are toxic to plant roots and inhibit microorganism's activity which influences nutrient uptake and crop growth. However, as suggested by many scholars, acidic infertile soils may be corrected through use of organic amendments. For instance using biochar is one of the most important and easily available reclamation methods as it raises soil pH (Lehmann *et al*., 2003; Gaskin *et al*., 2010; Van Zwieten *et al*., 2010). This is besides its many other potential uses, such as carbon sequestration (Laird, 2008) bioenergy generation (Lehmann, 2007; Laird, 2008); adsorbing organic and inorganic pollutants (Hale *et al*., 2011; Jiang, 2012) as well as improving soil fertility (Jeffery *et al*., 2011; Spokas *et al*., 2010).

Biochar (also commonly known as charcoal or agrichar) is a carbon (C) rich product derived from the pyrolysis of organic material at relatively low temperatures (<700 °C) (Lehmann and Joseph, 2009). Biochar has been reported to boost soil fertility and improve soil quality, such as by raising soil pH, increasing moisture holding capacity, attracting more beneficial fungi and microbes, improving cation exchange capacity and retaining nutrients in soil (Lehmann *et al*., 2006). Biochar is considered much more effective than other organic matter sources in retaining and making nutrients available to plants (Zheng *et al*., 2010). Its surface area and the complex pore structure made by pyrolysis process are hospitable to rhizosphere microbial community, such as bacteria and fungi, needed by plants to absorb nutrients from the soil and fix nutrients from atmosphere. These characteristics make biochar an exceptional soil amendment for use in sustainable agriculture (Chintana and Preeda, 2014).

Biochar holds carbon for long time improves degraded soils and reduces soil acidity for better crop production (IBI, 2012). It improves crop yield when applied as a soil amendment (Major *et al*., 2010). Biochar application improves crop productivity through enhancing soil water holding capacity, cation exchange capacity (CEC), and adsorption of plant nutrients and, creates suitable condition for soil micro-organisms (Glaser *et al*., 2002; Sohi *et al*., 2009; Lehmann *et al*., 2011). Thus, it can help to achieve a sustainable balance among productivity, environmental quality and profitability. However, evidences on its proper application based no results of tropical conditions are still scarce under both greenhouse and open-field experiments. Deep understanding of the processes involved in the biochar or organic fertilizers-soil-plant interaction is needed for successful use of biochar under various agro-ecological zones. Therefore, this study investigates the effect of coffee husk biochar and *Bradyrhizobium japonicum* inoculation on AMF colonization, biological nitrogen fixation and P up take of soybeans.

General objective:

- To investigate the effect of biochar and *Bradyrhizobium japonicum* inoculation on AMF colonization, P uptake and BNF of soybean grown in tropical Nitisols of Jimma, Ethiopia

Specific objective:

- To determine the effect of coffee husk biochar on P uptake, BNF, AMF root colonization, on growth and yield of soybean
- To evaluate the effectiveness of commercially available *B.japonicum* inoculant with biochar application at different rates

2. LITERATURE REVIEW

2.1. Economic Importance of Soybean

Soybean has been cultivated all over the world since ancient times for its high protein and lipid content. It has been cultivated for varying purposes during different periods of history in different parts of the world. Its earlier uses have varied from a green manure crop to a forage crop and a N_2 fixing crop due to its ability to fix substantial quantities of atmospheric nitrogen in association with nodule-forming bacteria (*Bradyrhizobium*) (Singh and Shivakumar, 2010). Besides its stated purpose as oil seed crop, soybean has several significant beneficial features. Its role in improving soil properties through its deep and proliferated tap root system, residue incorporation by way of shedding leaves as well as green manure crop, soil and moisture conservation due to its thick and dense foliage, contribution to soil N enrichment through BNF and improvement in the soil biological health have been recognized (Singh and Shivakumar, 2010).

Because of its potential for large-scale production, soybean has excelled in the world agricultural economy as a major oilseed crop. At present, soybeans are grown primarily for oil extraction and for use as a high protein meal for animal feed because it constitutes approximately 40% of protein and 20% of oil. Soybean has a high commercial value and high concentration of protein, calcium, phosphorus, fiber, and in addition it is cholesterol free (Imas and Magen, 2007).

2.2. Soil acidity and its constraints for soybean production

Food insecurity and diseases caused by malnutrition are permanent challenges of humankind since the down of history. Soil acidity is a major constraint to food crop production mainly in highly weathered soils of tropical and subtropical regions. Currently, about 41% of potential arable land of Ethiopia is acidic among which south Western part of the country is highly affected (Abebe, 2007). Acidic soils cause poor plant growth resulting from aluminum (Al^{+3}) and manganese toxicity (Mn^{+2}) or deficiency of essential nutrients like phosphorus, calcium and magnesium. Several other essential plant nutrients, which are present in the soil solution

as cations, are deficient. In acid soils, soybean is affected directly and indirectly. These effects include injury on plant roots therefore reducing water and nutrient uptake, reduced availability of essential plant nutrients, toxicity of Al and Manganese (Mn); and survival of microorganisms in the soil (Crawford *et al*., 2008; Onwonga *et al*., 2008).

To enable crop production in acid soils, several means to correct nutrient deficiency can be adopted. These include liming, addition of organic matter, and fertilization with mineral fertilizers (Masarirambi *et al*., 2012). Soybean as leguminous crop relies on microbial nitrogen fixation as source of N. However, under acid soils, the population of *rhizobia* bacteria is reduced and consequently nodulation and N fixation is impaired. This affects negatively on crop nutrition and yields. Restoring, maintaining and improving fertility of this soil is major priorities as a demand of food and raw materials are increasing rapidly. Therefore, addition of organic matter on acid soils for soybean production improves soils condition for microorganism development.

2.3 Biochar

Biochar is a fine-grained, carbon-rich, porous product remaining after plant biomass has been subjected to thermo-chemical conversion process (pyrolysis) at low temperatures(~350–600°C) in an environment with little or no oxygen (Amonette and Joseph, 2009).Biochar is not a pure carbon, but rather mix of carbon (C), hydrogen (H), oxygen (O), nitrogen (N), sulphur (S) and ash in different proportions (Masek, 2009). The unique characteristics of the biochar is its effectiveness in retaining most nutrients and keeping them available to plants than other organic matter such as for example common leaf litter, compost or manures. Addition of biochar to soil has also been associated with increased nutrient use efficiency, either through nutrients contained in biochar or through physico-chemical processes that allow better utilization of soil-inherent or fertilizer-derived nutrients. Compared to other soil amendments, the high surface area and porosity of biochar enable it to adsorb or retain nutrients and also provide a habitat for beneficial microorganisms to flourish (Lehmann and Rondon, 2006; Warnock *et al*., 2007; Sohi *et al.*, 2009).

Biochar is created by heating organic material under conditions of limited or no oxygen (Lehmann, 2007). A sustainable model of biochar production primarily uses waste biomass,

such as green waste from municipal landscaping, forestry, or agriculture. The process by which a biochar is produced is an important factor influencing its quality. The type of organic matter (or feed stock) that is used and the conditions under which a biochar is produced greatly affect its relative quality as a soil amendment (McClellan *et al*., 2007; McLaughlin *et al*., 2009). High cation exchange capacity (CEC), carbon levels and higher soil surface areas are some of the properties of better quality biochar.

2.4 Role of Biochar in Agriculture

2.4.1 Biochar effect on crop productivity

Biochar can retain applied fertilizer and nutrients and release them to agronomic crops over time. Biochar ability to retain water and nutrients in the surface soil horizons for long periods benefits agriculture by reducing nutrients leaching from the crop root zone, potentially improving crop yields, and reducing fertilizer requirements. The application of biochar to soil has been shown to improve crop yields which could be due to direct or indirect effect. The direct effect is explained by the fact that biochar being concentrated during pyrolysis contains higher amount of nutrients than the biomass from which they are prepared. The indirect effect is due to improvement in soil physical, chemical and biological properties due to biochar application. Several workers have reported that biochar applications to soils have shown positive responses for net primary crop production, grain yield and dry matter (Chan and Xu, 2009; Major *et al*., 2009 and Spokas *et al*., 2009).

A number of field and pot trials have assessed the impact of biochar on crop yield. In a review of biochar trials Jeffrey *et al*. (2011) found that amending with biochar produced statistically significant average increase of 10% in crop productivity. Additionally, some investigations showed that crop yields can be enhanced more compared to control soils if charcoal amendments are applied together with inorganic or organic fertilizers (Glaser *et al*., 2002; Lehmann *et al*., 2003; Srinivasarao, 2013).It is widely recognized that charcoal can be used as soil amendment in agriculture (Ogawa, 1994). Biochar has resulted in very high yield improvements on very poor soils such as acidic tropical soils. The effect of charcoal application on yield increases in soybean (Uddin *et al*., 1995) has been reported. Biochar

management may provide a significant opportunity for sustainable improvements of soil fertility and hence increase the growth and yield of soybean. Charcoal applied to the soil can also stimulate the activity of soil microorganisms and promote the formation of root nodules in soybean roots, thereby increasing yield and improving soil fertility (Agboola and Moses, 2015).

2.4.2 Biochar for sustainable agricultural development

Sustainable agriculture is a way of raising food that is healthy for consumers and animals without causing damage to ecosystem health. Low nutrient content and accelerated mineralization of soil organic matter are the two major constraints currently encountered in sustainable agriculture (Renner, 2007). Nutrients are retained in soil and remain available to crops mainly by adsorption to minerals and soil organic matter. Usually, the addition of organic matter such as compost and manure into soil can help to retain nutrients. Biochar is considered much more effective than other organic matter in retaining and making nutrients available to plants. Its surface area and complex pore structure are hospitable to bacteria and fungi that plants need to absorb nutrients from the soil. Moreover, biochar is a more stable nutrient source than compost and manure (Chan *et al*., 2007).

2.5 Biochar Effect on Soil Properties

As a soil amendment, biochar can greatly influence various soil properties and processes (Lehmann and Joseph, 2009). The presence of biochar in the soil can improve soil chemical (e.g. pH, CEC) (Liang *et al.*, 2006), and physical properties (e.g. soil water retention, hydraulic conductivity) (Major *et al*., 2010). Many of the benefits of biochar derive from its highly porous structure and associated high surface area. Charges on the high surface area can increase cation exchange capacity thereby increasing a soil's ability to retain and supply nutrients. Increased porosity can increase soil water holding capacity and the small pore spaces with positively charged surfaces can improve soil water retention and in turn reduce nutrient loss through leaching (Lehmann and Joseph, 2009; Verheijen *et al*., 2010). Biochar in soils has also been linked to increased soil microbial populations which may increase

beneficial soil processes mediated by soil organisms including nutrient availability (Kolb *et al*., 2009; Lehmann *et al*., 2011).

2.5.1 Effect of biochar on soil physical properties

The incorporation of biochar in to soil can alter soil physical properties such as texture, structure, pore size distribution and density with implications for soil aeration, water holding capacity, plant growth and soil work ability. Soil having good structure, porosity, hydraulic conductivity, bulk density and strength provide good medium for growth to beneficial microorganisms, better nutrient and water movement into the soil profile, higher nutrient and water retention and more root growth ultimately provide higher yield as compared to degraded soil having poor physical properties. Organic matter improves soil structure by increasing soil aggregation, increases soil porosity due to its high porous nature, boast up nutrient and water retention due to its high adsorption capacity and high surface area all these results in better root growth and crop yield (Bowman *et al*., 1990). Several soil benefits arise from the physical properties of biochar. Wide range of pore sizes within the biochar results in a large surface area and a low bulk density (Downie *et al*., 2009). Biochar can alleviate soil compaction by decreasing bulk density, which increase porosity and accentuates favorable soil processes (Laird *et al*., 2010). Application of biochar as a soil amendment reduces tensile strength and penetration resistance. In addition to improve soil mechanical properties, it also increase water infiltration rate, reduces runoff and decreases erosion (Busscher *et al*., 2010).

Evidence suggests that biochar application into soil may increase the overall net soil surface area (Chan *et al*., 2007) and consequently, may improve soil water and nutrient retention (Downie *et al*., 2009) and soil aeration, particularly in fine-textured soils (Kolb, 2007 ; Kristin, 2011). Many authors showed biochar effect on parameters such as bulk density, porosity, water-holding capacity, and aggregate stability.

2.5.2 Biochar effect on soil chemical properties

Important effects of biochar on soil chemical properties have also been reported, most notably increases in pH (in acid soils), cation exchange capacity (CEC), available P ,base saturation

and exchangeable bases, and organic carbon content, as well as decreases in Al saturation in acid soils . Cation exchange capacity of biochar is highly variable depending upon the pyrolysis conditions under which it is produced. Cation exchange capacity is lower at low pyrolysis temperatures and significantly increases when produced at higher temperatures (Lehmann, 2007). Biochar has a greater ability to absorb and retain cations in an exchangeable form than other forms of soil organic matter due to its greater surface area, and negative surface charge (Liang *et al.*, 2006). Elevated CEC are due to increases in charge density per unit surface of organic matter which equates with a greater degree of oxidation, or increases in surface charge area for cation adsorption, or a combination of both (Atkinson *et al*., 2010).

Biochar typically increases pH of acidic soils (Lehmann *et al*., 2003; Gaskin *et al*., 2010; Van Zwieten *et al*., 2010) due to the liming capacity of associated carbonate salts retained in the ash component of biochar. This can improve the availability of some nutrients, which is commonly thought to be responsible for positive plant growth responses to biochar amendments (Chan and Xu, 2009). Biochar has the potential to increase nutrient availability for plants. Nutrient availability can be affected by increasing cation exchange capacity, altering soil pH, or direct nutrient contributions from biochar. One potential mechanism for enhanced nutrient retention and supply following biochar amendment is increasing (CEC) by up to 50% as compared to unamended soils (Lehmann *et al*., 2003). Biochar application elevates total C, organic C, total N, available P, and exchangeable cations like Ca, Mg,Na, and K increase, and Al decreases in soil (Chan *et al*., 2008; Major *et al*., 2010b; Van Zwieten *et al*., 2010), then the plant uptakes several of these nutrients after biochar application (Chan *et al*., 2007; Major *et al*., 2010b). Major *et al*. (2010b) have reported that nutrient uptake by plants was increased in biochar amended soil.

2.6 Biochar Effect on Soil Microbial diversity

Biochar amended soil has more suitable pH for the growth of microbes, especially for fungal hyphae, (Wuddivira *et al*., 2009).An increase of soil microbial biomass and a changed composition of soil microbial community were observed after biochar amendments (Birk *et al*., 2009).Biochar properties may enhance soil microbial communities and create micro

environments that encourage microbial colonization. Biochar pores and its high internal surface area, and increased ability to absorb OM provide a suitable habitat to support soil micro biota that catalyze processes that reduce N loss and increase nutrient availability for plants .The pores are suggested to serve as a refuge by protecting microbes from predation and desiccation while the organic matter adsorbed to biochar provides C energy and mineral nutrient requirements (Saito and Muramoto 2002; Warnock *et al*., 2007).

Recent studies have reported that biochar addition often has a positive effect on soil microorganisms, e.g. increased biological N_2 fixation by rhizobia in legumes and elevated levels of mycorrhizal colonization and plant-growth promoting organisms in the rhizosphere (Rondon *et al*., 2007;Warnock *et al*., 2007; Graber *et al*., 2010). The micro–pores of biochar affect the population of soil micro-organism significantly by providing protection from soil predators and a food source in the form of adsorbed organic compounds .The application of biochar as a soil amendment can be beneficial to plant hosts due to increased mycorrhizal fungi abundance and/or functionality in soil by enhancing plant–fungui symbiosis (Thies and Rillig, 2009).

2.6.1 Arbuscular Mycorrhizal Fungi

Mycorrhiza is a name for a fungi and plant root association. Mycorrhizal fungi are a major component of the soil micro flora in many ecosystems. Many legumes can develop a double symbiosis with both nitrogen-fixing bacteria and mycorrhizal fungi. They are soil organisms that develop a filamentous network in the soil and in the roots of almost all tropical crops, which leads to symbiotic associations with low host specificity (Cardoso and Kuyper, 2006; Talaat and Abdallah, 2008). The filamentous network serves as transport system for nutrients and water from the fungi to the plant, which makes this symbiosis essential for the host plants growth. Furthermore, mycorrhizal fungi contribute to soil structure: their external hyphae network holds soil particles together, which allows formation of micro- and macro aggregates (Miller and Jastrow, 2000; Cardoso and Kuyper, 2006). However, the most important advantage of this symbiosis for the plant is the increased P absorbance and translocation by the extensive mycorrhizal hyphae network (Richardson, 2001).

It is well known that AMF improve the uptake of immobile mineral nutrients such as phosphate, thereby benefiting plant growth. The extent of such benefit varies with the soil environment, particularly available P content and soil moisture.AM fungi are especially important for sustainable farming systems because AM fungi are efficient when nutrient availability is low and when nutrients are bound to organic matter and soil particles. Many important agricultural crops can benefit from AM fungi, including soybean, especially under conditions where nutrient availability is limiting plant growth. Moreover, AM fungi not only can promote via direct effects, but there are also a number of indirect effects such as a stimulation of soil quality and the suppression of organisms that reduce crop productivity (Van der Heijden *et al*., 2008).

2.6.2 Biochar effect on AMF activity

Arbuscular mycorrhizal fungi functionality can be improved by addition of soil amendment (Warnock *et al*., 2010). One of soil amendments is biochar that is known to be able to reduce the soil carbon released because biochar is resistant to weathering and difficult to decompose (Verheijen *et al*., 2009). Biochar can serve as refuge for AMF hyphae and protect them from fungal grazers (Warnock *et al*., 2007), thus enhancing plant host-fungus symbiosis. Ishii and Kadoya (1994) argued that additions of biochar altered soil physico-chemical characteristics, leading to increased soil nutrient availability and enhanced mycorrhizal root colonization.

Nutrient changes can limit or stimulate fungal growth, and can control the plants root colonization by the fungi. Biochar may influence signaling processes, leading to altered root colonization by mycorrhiza. Signaling between plant roots, microbes and mycorrhizal fungi occurs in the rhizosphere .Several compounds that are excreted by the plant roots improve colonization of the roots by Arbuscular Mycorrhizal Fungi (Xie *et al*., 1995).Through the addition of biochar to agricultural soils, nutrient availability for plants improves and leaching is reduced (Gundale and DeLuca 2007; Rondon *et al*., 2007). Studies indicate that AMF benefit native plant production in severely degraded areas (Matias *et al*., 2009) in combination with biochar amendments can increase AMF percent root colonization among plants growing in acidic soils (Ezawa *et al*., 2002; Yamato *et al*., 2006).

2.6.3 Role of AMF in P uptake of soybean

Phosphorus (P) is an extremely important mineral for plant nutrition and its deficiency can greatly limit plant growth. For many plants, arbuscular mycorrhizal (AM) associations are one way of acquiring adequate supplies of P from soils. Arbuscular mycorrhizal fungi are known to form mutualistic relationships with more than 80% of plants (Ulrich *et al*., 2002). This mutualistic relationship can provide nutrients to the host plant in exchange for carbohydrates provided by the host plant for the fungi (Javaid, 2009). Plants roots with AMF are generally more efficient in acquisition of nutrients which leads to an improved plant growth (Mau and Utami, 2014). Many workers have reported enhancement of phosphate uptake and growth of leguminous plants by Arbuscular Mycorhizal Fungi (AMF).

The most prominent effect of the fungus is improved phosphorus nutrition of the host plant in soils with low phosphorus levels due to the large surface area of their hyphae and their high affinity P uptake mechanisms. Most studies indicated that the improved plant nutrition is due to increased root surface through extraradical hyphae which can extend beyond root depletion zone (Diriba, 2007).The hyphae of AMF have the potential to greatly increase the absorbing surface area beyond the root into the surrounding soil to improve the uptake of poorly mobile ions such as P. Fine AMF hyphae extend outside of the plant roots, thereby increasing the effective rooting zone and accessing P that is unavailable to the larger plant roots. These peculiar characteristics make the AMF very crucial in the tropics where phosphorus deficiencies appear to be a major factor limiting the establishment of most crops.

2.6.4 Role of AMF on BNF of soybean

Biological nitrogen fixation (BNF) demands high amounts of ATP energy, so an adequate supply of phosphorous offered by AMF benefits nodule formation. Phosphorus (P), an element that is scarce and has low mobility in the soil, is an important nutrient supplied to plants through AMF. Rhizobia, when in symbiotic association with leguminous plants, convert atmospheric N_2 to NH_3, which is used by the plants in various ways. Under conditions of phosphorus deficiency, legumes have low nodulation and nitrogen fixation unless their roots are colonized by mycorrhizas or if the soil is fertilized with high phosphorus levels.

Moreover, the mycorrhizal condition influences the efficient competition among strains of rhizobia to occupy the nodules in the roots of the host (Garg and Manchanda, 2008).

The widespread presence of symbiotic arbuscular mycorrhiza (AM) fungi in nodulated legumes and the role of AM fungi in improving nodulation and rhizobial activity within the nodules are both universally recognized processes (Barea *et al*., 2005). Mycorrhizas benefit the host through the mobilization of phosphorus from non-labile sources, whereas Rhizobium fixes nitrogen. AM colonization has been shown to improve nodulation and nitrogen fixation. The main effect of AM fungi in enhancing rhizobia activity is through the generalized stimulation of host nutrition. However, it should be noted that some localized effects may also occur at the root or nodule level (Barea *et al*., 1992). Suitable combinations of AM fungi and rhizobia bacteria may increase plant growth and improve nodulation and nitrogen fixation (Barea *et al*., 2002). Chalk *et al*. (2006) report a number of studies in which both native and inoculated AMF treatments increased legume dry matter, grain yield, P uptake, and the percent of plant N that was fixed.

2.7 Biochar Effect on Rhizobium Activity

The porous structure of biochar, its high internal surface area and its ability to adsorb soluble organic matter gases and inorganic nutrients are likely to provide a highly suitable habitat for microbes to colonize, grow and reproduce, particularly for bacteria. Inoculation with Rhizobia may be more effective in the presence of biochar due to the habitat offered by the biochar. Several authors have suggested that the biochar pores may act as a refuge site or micro habitat for colonizing microbes, where they are protected from being grazed upon by their natural predators (Saito and Muramoto, 2002; Warnock *et al*., 2007) or where microbes that are less competitive in the soil environment can become established (Lehmann and Joseph, 2009).

In fact, several studies indicate that biochar is an excellent support material for Rhizobium inoculants (Lal and Mishra, 1998). Consequently, BNF determined by nitrogen difference was found to be 15% higher when biochar was added to soil at early stages of alfalfa development and 227% higher when nodule development was greatest (Nishio, 1996). Biochar additions are, therefore, able to increase the net input of nitrogen into agricultural landscapes (Lehmann

and Rondon, 2006). Several studies found an increased nodulation and positive effects on rhizobium inoculants on soils with biochar. However, detailed information on interactions of biochar-rich soils and BNF has not been published. The main hypotheses for a higher biological nitrogen fixation in biochar-rich soils are: A decreased N availability because of the higher C/N ratio of biochar (Glaser *et al*., 2002); An increase in the availability of B, Mo and P, and an increased pH (Lehmann *et al*., 2003); A greater mycorrhizal infection, enhancing N_2 fixation due to the improved P-uptake by the plant roots (Saito and Marumoto, 2002).

2.7.1 Role of rhizobium on soybean production

Soybean is a member of the legume family of plants, most of which have relatively high protein content in their seeds and so need to take up a lot of nitrogen. Many of these plants have the ability to host bacteria in special structures, called nodules, on their roots. The plant forms nodules as a reaction to the infection of the root by these bacteria. Bacteria live in the nodules and are fed by sugars moving down from the leaves. In turn, the bacteria convert the nitrogen from air into forms usable by the plant. Active nodules have a pink color inside (Tairo and Ndakidemi, 2013).

Microorganisms such as *Bradyrhizobium* inoculants may significantly have an effect on the chemistry of nutrients in soils by enhancing nutrients uptake by plants. Most *Bradyrhizobium* inoculants have been developed and are primarily used for supplying N_2 to plants. Inoculation of soybean with specific *Bradyrhizobium* strains improves the plant dry matter, nitrogen concentration, nitrogen accumulation, and grain yield (Javaid *et al.*, 2010). Seed protein content increased when specific *Bradyrhizobium* species was used to inoculate soybean (Egamberdiyeva *et al*., 2004). However, Ndakidemi *et al*. (2011) and Makoi *et al*. (2012) reported *Bradyrhizobium* inoculation enhancing the uptake of P, K, Ca, Mg, S, Mn, Fe, Cu, Zn, B and Mo in leguminous plants. Among various factors that can contribute to soybean success rhizobial inoculation had quite prominent effects on nodulation, growth and yield parameters (Tairo and Ndakidemi, 2013).

Shahid *et al*. (2009) reported that seed production in soybean can increase by 70-75% when the proper bacterial strains were used to inoculate soybean seeds. The higher nodulation due

to inoculation resulted in higher nitrogen fixation by *Rhizobium* and eventually the number of pods per plant which bring about higher grain yields as a whole. In other studies, Ibrahim *et al*. (2011) reported increased yield and yield component of soybean by inoculating the seeds with specific strain of rhizobia. Biological nitrogen fixation and grain yields of legumes are normally increased when inoculated with effective and efficient strain of *Rhizobium.* It has also been reported that nodule number, dry weight and soybean shoot yield increased when seeds inoculated with *Rhizobium* (Tairo and Ndakidemi, 2013).

2.8 Biological Nitrogen Fixation (BNF)

Nitrogen is the most abundant element in the atmosphere, and it is mainly present in the diatomic form (N_2). Nitrogen is an essential macro nutrient for plant species. Some bacteria have enzymes with the ability to reduce N_2 and turn it into ammonia.N-deficiency is a serious problem for plant growth in Nitisols, resulting in reduced plant growth and yellowing of older leaves (chlorosis). To meet their N requirements, plants fully depend on soil mineral N and N derived from Biological Nitrogen Fixation (BNF). BNF is a natural process in legume crops, where atmospheric dinitrogen (N_2) is fixed into ammonia (NH_3) in plant root nodules by a symbiotic form of Rhizobia .The plant assimilates this NH_3 into proteins, nucleic acids and other nitrogenous compounds (Strodtman and Emerich, 2009).BNF can be symbiotic when there are mutualistic associations between plant species and fixing microorganisms. Nitrogen-fixing bacteria, rhizobia form mutualistic symbiotic associations with legumes.

According to Smil (1999), BNF from legume crops contributes 36.4 million tons N to agriculture globally. BNF has a high potential for low-input systems, as in large regions of Africa, where more N is removed from the soil than is replenished, which results in depletion of soil nutrients and land degradation (Cocking, 2009).BNF is important in terms of: saving fertilizer costs and thereby reducing costs for crop production and avoiding ground water pollution; enhancing protein production and thus improving nutrition status of the people; fixing N for succeeding crops and contributing to improved soil fertility (Hardarson, 1993).

2.8.1 The role of rhizobium in BNF of soybean

Rhizobium inoculation is a significant technology for the manipulation of rhizobia for improving crop productivity and soil fertility. Rhizobium inoculation can lead to establishment of large rhizobia in the rhizosphere and improved nodulation and nitrogen fixation (Peoples *et al*., 1995).Soybean rhizobium inoculation is the process of applying rhizobium inoculants to the soybean seed before planting in order to increase the nitrogen fixation and nodulation of the soybean roots. Inoculating soybean provides adequate number of bacteria in the soybean root zone, so that effective nodulation will take place (Lamptey *et al.*, 2014).

Leguminous plants are able to fix atmospheric N_2 through the association with rhizobia. The legume plant supplies the carbohydrate for bacterial growth while the bacteria fix atmospheric N_2 into $NH_4^{+,}$ to be converted into amino acids that can be used by the plant to synthesize proteins for its growth and development (Russel, 2008). Symbiotic association is a highly specified relationship between the host plant and the bacteria. *Rhizobium*-legume symbiosis involves the interaction between the plant and the bacteria leading to initiation and development of the root nodules. Soil bacteria like *Rhizobium* live in nodules as nitrogen fixing bacteroids. Rhizobia require a plant host; therefore, they cannot independently fix nitrogen. These bacteria are located around root hair and fixes atmospheric nitrogen using particular enzyme called nitrogenase. When this mutualistic symbiosis established, rhizobia use plant resources for their own reproduction whereas fixed atmospheric nitrogen is used to meet nitrogen requirement of both itself and the host plants. Supply of nitrogen through biological nitrogen fixation has ecological and economic benefits (Tairo and Ndakidemi, 2013).

A research done by Bambara and Ndakidemi, (2010) reported that *Rhizobium* inoculation in legumes stimulated growth and is an alternative source to the expensive commercial nitrogen fertilizers. Nitrogen is highly needed for all enzymatic reactions in a plant, also is a major part of the chlorophyll molecules and plays a necessary role in photosynthesis and is a major component of several vitamins .In legumes and other leafy vegetables, nitrogen improves the quality and quantity of dry matter and protein (Tairo and Ndakidemi, 2013).

3 MATERIALS AND METHODS

3.1 Description of the Study Area

The study was conducted at Jimma University College of Agriculture and Veterinary Medicine (located 7°, 33 N and 36°, 57' E and at an altitude of 1710 meter above sea level), in the Southwest Ethiopia during the year 2016 under greenhouse condition. The mean maximum and minimum temperatures of the area are 26.8°C and 11.4°C respectively, and the relative humidity is between 91.4% and 39.92%. The mean annual rainfall of the area is 1500mm. The dominant soil type in the study area is Nitisol (World Reference Base, 2006).

3.2 Biochar Production

To produce the biochar, coffee husk was collected from coffee processing sites around Jimma. The pyrolysis temperature to produce the biochar was 500°C with 3hrs retention time as suggested by Lehmann (2007) and Bayu *et al*. (2015). The biochar was ground to small granules and passed through 2mm sieve in order to have the same particle size as that of the soil.

3.3 Soil and Plant Sampling and Analysis

Soil samples were collected from Jimma agriculture research center (JARC). This soil from JARC was chosen for the fact that its pH was strongly acidic (4.59) compared to soils samples collected from Ela-dale and JUCAVM campus whose pH values were > 5. Five soil samples were collected in diagonal pattern from a depth of 0-20 cm and bulked to obtain one sample. The sample was air-dried, ground using a pestle and a mortar and allowed to pass through a 2 mm sieve. Before commencement of the experiment, the soil sample and biochar was analyzed for selected physical and chemical properties using standard laboratory procedures. Soil pH was measured using the in the suspension of 1: 2.5 soil to water ratio using a pH meter. Electrical conductivity (EC) was measured using the same suspension (soil to water ratio of 1:2.5). The particle size distribution (texture), of the soil sample was determined by the Boycouos hydrometric method (Van Reeuwijk, 1992). Soil organic carbon was determined by using Walkley and Black method (Walkley and Black, 1934). Total N was analyzed using

the Kjeldahl method by oxidizing the OM in 0.1N H_2SO_4 as described by Black (1965). Available phosphorus was determined by the Bray 2 method (Bray and Kurtz, 1945). Cation exchange capacity was determined at soil pH 7 after displacement by using 1N ammonium acetate method and, then, estimated titrimetrically by distillation of ammonium that was displaced by sodium (Gaskin *et al*., 2008).The analysis result were indicated in table 1.

The plants were collected from each pot during mid-pod setting stage and the collected plant samples were washed by distilled water. The oven dry plant tissues were then ground into 0.25 mm size subjected to wet digestion and analyzed for total N and P content. The N content of the plant tissue was determined by Kjeldahl procedure. The Kjeldahl procedure is based on the principle that by treating plant material with concentrated sulfuric acid it is oxidized and nitrogen in the plant material is being converted into ammonium sulfate during the oxidation. The ammonia liberated in the distillation process with NaOH is trapped by the acid. The ammonia is adsorbed in the form of NH_4^+ ion in boric acid and back titrated with standard H_2SO_4.Whereas, the phosphorus in the solution is determined colorimeterically by using molybdate and metavanadate for color development. The reading is made at 460nm wavelength.

Table 1: Analytical results of the soil and biochar for selected physico chemical properties before planting

Parameters	Soil	Biochar
pH-H_2O (1:2.5)	4.59	10.13
EC (mS cm^{-1}) (1:2.5)	0.03	5.24
CEC (me/100 g)	12.37	78.05
Organic carbon (%)	1.73	24.8
Organic matter (%)	2.98	42.76
Nitrogen (%)	0.15	2.14
Available P (mg/kg)	0.9	12.87
Texture	Clay	-
Sand (%)	23%	-
Clay (%)	53%	-
Silt (%)	25%	-

3.4 Experimental Material

A commercial effective *B. japonicum* strain (Fitsum *et al*., 2016), MAR 1495, was obtained from Holeta Research Center. Clark 63-K, a well-adapted soybean cultivar in the area was obtained from Jimma University College of Agriculture and Veterinary Medicine and used as a test crop.

3.5 Experimental Design, Treatments and Procedures

A pot experiment was laid down in randomized complete block Design (RCBD) with three replications. Plastic pots of about 10 kg capacity with surface diameter of 13 cm were filled with 10 kg of dry soil and different levels of biochar with and without inorganic P fertilizer. The experiment comprised of treatments combination of eight levels of coffee husk biochar application rates (0, 6, 12 and 36 t ha^{-1}) and two levels of P fertilizer (with and without):

Table 2: Treatment Explanation

Treatment	Treatment Explanation
T_1	Control
T_2	6 ton/ha Biochar+100kg/ha DAP
T_3	12 ton/ha Biochar+100kg/ha DAP
T_4	36 ton/ha Biochar+100kg/ha DAP
T_5	No biochar+100kg/ha DAP
T_6	6 ton/ha biochar
T_7	12 ton/ha biochar
T_8	36 ton/ha biochar

The treatments were evaluated with and without *Bradyrhizobium japonicum* (strain MAR 1495) inoculation. Teff (kuncho variety) was used as reference crop to estimate BNF via the N difference method. Two pots were used per treatment per replication for both soybean and the reference crop (teff).

Soybean seeds of variety Clark 63-K were washed with distilled water and surface sterilized with 70% ethanol. Seeds were then rinsed 3 to 4 times with distilled water; moistened with sucrose solution and inoculated with *B.japonicum* by covering with the paste of inoculum, which was made at the rate of 10 g of peat-based powder inocula per 100 g of seed, just before planting (Deaker *et al*., 2004).

Coffee husk biochar and Di-ammonium Phosphate (DAP) was applied to the pots as per the rate for each treatment and mixed with the soil evenly before seeding operation. Finally, 3 seeds were sown per pot. Seedlings were thinned to two when they attained two pairs of true leaves.

3.6 Data Collected

Growth, yield and yield components of soybean were measured throughout the experiment period. The details are described below:

3.6.1 Growth parameters

Plant height (cm): It was measured as the height from the ground level to the top most point using ruler and the average for each plant was calculated at physiological maturity.

Total number of leaves per plant:
The number of leaves per plant was determined by counting and averaging for each plant at flowering stage.

Number of branches per plant:

Number of primary branches was determined by counting the branches on the main stem of each plant at physiological maturity.

Shoot and root dry weight:
Two plants were taken for determination of dry weight of shoot and roots at maturity. After taking the fresh weight of shoot and root parts, the samples were dried in an oven at

70^{o}C to a constant weight and then the dry weight was measured using sensitive balance and average for a plant.

3.6. 2 Nodulation Parameters

Nodule number per plant:

At flowering, plants were carefully uprooted, gently washed over a fine sieve to remove any soil particle and the nodules were then counted. The number of nodules on each root was then determined and the average number of nodules per plant calculated.

Nodule fresh and dry weight per plant:

The fresh weight of nodules was recorded first and the nodules were oven dried at 70^{o}C for 24 hours after which, the nodule dry weight was measured using sensitive balance.

3.6.3 Yield and Yield components

Number of pods per plant:

The total number of pods from each plant was counted at the time of harvest and expressed as the average number of pods per plant.

Number of seeds per pod:

The number of seeds per pod was determined for 5 randomly sampled pods from the two plants, and the average was reported as number of seeds per pod.

Seed yield (g/plant)

Total seeds were collected from each plant at the time of harvest weighed at 12% moisture level using sensitive balance and expressed as the average seed yield per plant.

3.6.4 Arbuscular Mycorrhizal Fungi (AMF) Colonization

AMF root colonization rate was quantified using the magnified intersections method (McGonigle *et al*., 1990). The analysis was done at the Department of Microbial, Cellular and Molecular Biology, Addis Ababa University. Forty eight root samples were collected by

uprooting and the fine roots were used for the AMF detection. Fine Roots were gently washed with tap water over a fine sieve to remove any soil and debris. Then, only fine roots were cut in to segments of about 1 cm long. Staining of mycorrhizal roots was made according to the method described by Brundrett *et al*. (1996). About 0.5 g of root segments were cleared in 10% (w/v) KOH at 90°C in a water bath for 2 to 3 h depending on the structure of the root and its pigmentation (Brundrett *et al*., 1996). Dark roots were further bleached with alkaline hydrogen peroxide (10% H_2O_2) for 3min at room temperature. The roots were treated with 10% HCl (v/v) for 15 to 20 min at room temperature and finally stained in 0.05% w/v trypan blue in lactoglycerol (1:1:1 lactic acid, glycerol and water) at 90°C for 30 min in a water bath. With the exception of the HCl treatment, samples were drained and washed thoroughly with distilled water at the end of every step. The root samples were then left overnight in the lacto glycerol destaining solution (1:1:1; lactic acid, glycerol and water) in a dark room to remove coloration from root cells. Finally, roots were mounted in PVLG mountant on microscopic slides and covered with 40×22 mm cover slips. Fungal colonization was quantified using the magnified intersection method of McGonigle *et al*. (1990) under a compound-light microscope (OLYMPUSBX51) at a magnification of 200 X. The presence of arbuscular mycorrhizal hyphae, vesicles and arbuscules were recorded.

The presence of Arbuscular colonization (AC) and vesicular colonization (VC) were calculated by dividing the count for the Arbuscular and vesicles categories, respectively, by the total number of intersections. Hyphal colonization (HC) was calculated as the proportion of non-negative intersections.

Total intersections (G): N+A+V+H

%HC= (G-N)/G*100%

%AC= A/G*100%

%VC= V/G*100%, Where

G: total count, N: negative, A: Arbuscular, V: vesicles, H: hyphae**,** %AC: Percentage of Arbuscular colonization, %VC: percentage of vesicular colonization, %HC: percent hyphal colonization or Total colonization

3.6.5 Phosphorus (P) uptake

Phosphorus uptake was assessed by determining the P concentration (%) and then multiplying by the total biomass yield of plants (Johnston and Syers, 2009).

3.6.6 Estimation of BNF

The Total Nitrogen Difference (TND) method was used to quantify BNF. This was done by comparing total nitrogen of the legume with that of the reference crop (Peoples *et al*., 1989; Hardarson and Danso, 1993; Ukovich *et al*., 2008) which consists of the difference between N content in the soybean crop and the reference crop plus the difference in mineral N in the soil where the crops (soybean and the reference crop) are grown. The difference value is assumed to be N derived by BNF (N_2 fixed).

Thus, **N_2 fixed**= (N yield N_2-fixing plant – N yield reference plant) +
(Soil N under N_2-fixing plant – soil N under reference plant)

$$\text{Where Total N in plants} = \frac{\text{(Dry matter weight (g/plant) X \% N in plants)}}{100}$$

$$\%\ \textbf{Ndfa} = \frac{\text{[Total N in legume -Total N in reference crop]}}{\text{Total N in legume}} \times 100$$

Where % Ndfa is the percentage of N_2 derived from the atmosphere

3.7 Statistical Analyses

All the collected data were first checked for fitting the analysis of variance (ANOVA) assumptions including normality test and then those data which were found to have normal distributions were subjected to analysis of variance using SAS 9.2 version (SAS, 2008). Whenever ANOVA shows significant differences between treatments, mean comparison was done using the Least Significant difference (LSD) Test. Correlation analysis was carried out using Pearson's correlation analysis.

4. RESULTS AND DISCUSSION

4.1 Effect of biochar and *B. japonicum* inoculation on growth Parameters of soybean.

4.1.1. Plant height

The analysis of variance indicated significant ($P<0.05$) differences due to both *Bradyrhizobium* inoculation and biochar application (Table 3).The result of this experiment indicated that plant height ranged between 60.00 cm to 80.33 cm with inoculation and 60.00 to 76.33 cm without inoculation. The tallest plant (80.33 and 76.33 cm) from inoculated and uninoculated treatments, respectively, was obtained at 36 ton /ha biochar supplemented with 100kg/ha DAP, the shortest plant (60cm) was obtained from the control.

Significant increase in plant height as a consequence of biochar addition could have resulted from improved soil pH, EC and soil fertility, leading to better nutrient absorption. The other reason for the observed result may be due to more phosphorus availability, enhanced root growth and increased P adsorption leading to enhanced cell division and cell formation which ultimately result in increases in the height. Plant height was significantly increased with increasing rates of biochar application. These results are in agreement with the findings of Verheijen *et al.* (2004) who reported that biochar application to soils enhances plant growth.

In all biochar applied plots, *Rhizobium* inoculated treatments showed higher plant height than did the corresponding rates of biochar without inoculation. The variation in plant height due to inoculation may probably be due to N_2 fixation which can play a vital role in the vegetative growth of soybean. Abbasi *et al*. (2010) concluded that *Rhizobium* inoculation increased soybean plant height up to 12%. Wafaa *et al*. (2002) have also reported that *Bradyrhizobium japonicum* inoculums enabled soybean to display a better growth. A similar result was obtained by Afzal *et al.* (2010) who indicated that inoculation with strain of *B. japonicum* induced an increase of the height of soybean plants.

Plant height increased with increasing rates of biochar and tallest plants observed from biochar with 100 kg/ha DAP compared to biochar application alone. The increase in plant vegetative growth as a result of application of inorganic fertilizers in combination with

biochar could be attributed to increased up-take of P by the plants. These results are also in line with Chan *et al*. (2007) who reported that combined use of biochar and fertilizers enhanced plant height. Much greater yields in plant growth are observed with fertilizer additions plus biochar, as opposed to fertilizer additions alone (Yamato *et al*. 2006; Gundale and DeLuca 2007; Asai *et al*. 2009; Blackwell *et al.* 2009).

4.1.2 Number of primary branches per plant

Pod bearing branches are considered to be the major contributor to seed yield of legumes. There was significant ($P<0.05$) effect on number of primary branches because of both *Bradyrhizobium* inoculation and biochar application (Table 3). The result of this experiment revealed that the number of primary branches per plant ranged between 2.66 to 8.00 with inoculation and 2.66 to 6.66 without inoculation. The highest number of primary branches (8.00 and 6.66) in inoculated and uninoculated treatments, respectively, was recorded for 36 ton/ha biochar supplemented with 100kg/ha DAP, which was statistically similar with 12 ton/ha biochar supplemented with 100kg/ha DAP and 36 ton/ha biochar alone, in inoculated treatments. The lowest number of primary branches (2.66) was recorded for the control.

Number of primary branches was significantly increased with increasing rates of biochar application. Biochar can enhance plant growth by improving soil chemical, physical and biological properties all contributing to an increased crop productivity (Yamato *et al*., 2006). Plants with higher number of branches were observed for biochar application with 100 kg/ha DAP compared to biochar application alone. In line with this, it has been reported that biochar has the greatest ability to enhance plant growth and nutrient content when combined with fertilizer application (Blackwell *et al*., 2009).

In all biochar applied plots, *Bradyrhizobium japonicum* inoculation showed higher number of primary branches than those treated with biochar but without inoculation. In agreement with this result, Tairo and Ndakidemi (2013) have reported that *Bradyrhizobium japonicum* inoculation and P supply in green house and field experiment showed significant increase in the number of branches.

4.1.3 Number of leaves

The result of the present study showed that *Bradyrhizobium* inoculation and biochar application significantly ($P<0.05$) affected number of leaves per plant (Table 3). The number of leaves per plant ranged between 73.33 and 30.00 with inoculation and 66.00 and 31.33 without inoculation. The highest number of leaves per plant (73.33 and 66.00) in inoculated and uninoculated treatments, respectively, was recorded for 36 ton/ha biochar supplemented with 100kg/ha DAP. While the lowest number of leaves per plant (31.33 and 30.00) was recorded for the control treatment with and without inoculation, respectively. Spokas *et al*. (2010) reported that biochar application can significantly increase crop growth and productivity

Number of leaves increased with increasing rates of biochar and plants with higher number of leaves were observed for biochar application supplemented with 100 kg/ha DAP compared to biochar application alone. Van Zwieten *et al*. (2010) have noted increased crop biomass from the addition of biochar combined with a synthetic fertilizer, an effect that was not seen when the synthetic fertilizer was applied on its own. A number of researchers (Lehmann *et al*., 2003; Kimetu *et al*., 2008; Deal *et al*., 2012) have reported positive effects of biochar on plant growth and biomass yield.

In the present study, higher numbers of leaves were observed in all biochar applied plots under *Rhizobium* inoculated treatments compared to those without *Rhizobium* inoculation. Meghvansi *et al*., (2005) have reported that inoculation with *Rhizobium* strains can improve the vegetative growth in soybean.

4.1.4 Shoot and root Dry weight

Dry weight of shoot per plant was significantly different for the biochar application rates and inoculation. It ranged from 17.66 to 38.33g/plant with inoculation and 16.66 to 34.00 g/plant without inoculation (Table 3).The largest value (38.33g/plant and 34.00 g/plant) from inoculated and uninoculated treatments was recorded for 36 ton/ha biochar supplemented with 100kg/ha DAP, which was statistically similar with 12 ton/ha biochar supplemented with

100kg /ha DAP, followed by 6 ton/ha biochar supplemented with 100 kg/ha DAP .While the smallest value (17.66g/plant and 16.66 g/plant) was recorded for the inoculation without biochar and the control treatment.

Root dry weight ranged from 2.17 to 5.33 g/plant with inoculation and 2.07 to 4.17 g/plant without inoculation. The largest values (5.33 g/plant and 4.17 g/plant) of inoculated and non-inoculated treatments were recorded for 36 ton/ha biochar supplemented with 100kg/ha of DAP, followed by 12 ton /ha biochar supplemented with 100kg/ha DAP, while the smallest values (2.17, 2.07, 2.50 and 2.33) were recorded for inoculation without biochar, control and 100kg/ha DAP without biochar, respectively.

In the present study shoot and root dry weight were significantly increased with increasing rates of biochar application. Iswaran *et al.* (1980) have reported a 51% increase in dry biomass in soybean crops with biochar additions. In agreement with the present results, the increase in dry biomass production after biochar application has also been observed for other legumes, including clover (Mia *et al*., 2014b; Oram *et al*., 2014), common bean (Rondon *et al*., 2007), alfalfa (Nishio and Okano, 1991).

The improvement in growth parameters of soybean may be attributed to the fact that incorporation of biochar into soils changed the soil physico-chemical properties, such as soil pH, EC and CEC, enabling the plants to accumulate higher dry weight. The other reason for the improvement in agronomic parameters could be the incorporation of biochar into crop-growing soils changed the pore-size distribution (Asai *et al*., 2009), thereby making the pores to serve as a shield by protecting biochar decomposing microbes from predation and desiccation, while the organic matter adsorbed to biochar provides energy and mineral nutrient requirements for crop growth (Saito and Muramoto, 2002; Warnock *et al.*, 2007).

For the biochar application rates, with *Rhizobium* inoculation, a higher biomass yield was obtained compared to biochar application with no inoculation. The variation in plant growth due to inoculation may probably be due to N_2 fixation, which can play a vital role in the vegetative growth of soybean.Contribution of N_2 fixation due to inoculation, which supplied extra N_2 for the crop, can play a vital role in the plant dry matter accumulation. Nitrogen is a

key factor in many biological compounds that play a major role in photosynthetic activities and chlorophyll synthesis, which eventually result in vigorous vegetative growth and more biomass accumulation. In line with the present result, Abbasi *et al.* (2010) has pointed out that biomass yield of soybean was increased ranging from 39 to 75% by inoculating different strains of rhizobia. It has also been reported that inoculation of soybean with specific *Bradyrhizobium* strains improved the plant dry matter yield; nitrogen concentration and grain yield (Javaid *et al*., 2010).

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Table 3: Growth attributes of soybean in response to rhizobium inoculation and biochar application

		Plant height(cm)		Number of branches per plant		Number of leaves per plant		shoot dry weight(g)		Root dry weight(g)	
N^{o}	Treatment	With	Without	with	without	with	Wit out	with	without	with	without
1	Control	66.00^{e}	60.00^{e}	2.66^{d}	2.66^{d}	30.00^{f}	31.33^{h}	17.66^{e}	16.66^{d}	2.17^{e}	2.07^{e}
2	6 ton/haBc+100kg/ha DAP	74.33^{bc}	71.00^{b}	5.33^{c}	4.00^{c}	65.66^{b}	55.66^{c}	32.33^{b}	23.33^{b}	4.33^{c}	3.00^{c}
3	12 ton/haBc+100kg/ha DAP	76.33^{b}	72.3^{ab}	7.00^{ab}	5.66^{b}	66.00^{b}	60.66^{b}	37.00^{a}	31.33^{a}	4.83^{b}	3.66^{b}
4	36 ton/haBc+100kg/ha DAP	80.33^{a}	76.33^{a}	8.00^{a}	6.66^{a}	73.33^{a}	66.00^{a}	38.33^{a}	34.00^{a}	5.33^{a}	4.17^{a}
5	No biochar+100kg/ha DAP	66.00^{e}	60.33^{e}	3.33^{d}	3.00^{d}	44.00^{e}	38.00^{g}	20.33^{d}	18.66^{cd}	2.50^{e}	2.33^{e}
6	6 ton/ha biochar	69.33^{d}	64.3^{cd}	5.00^{c}	3.3^{cd}	45.33^{e}	42.00^{f}	28.66^{c}	21.00^{bc}	3.17^{d}	2.50^{de}
7	12 ton/ha biochar	72.33^{c}	68.3^{bc}	6.66^{b}	4.00^{c}	51.33^{d}	46.00^{e}	30.00^{bc}	22.3^{b}	3.50^{d}	2.83^{cd}
8	36 ton/ha biochar	75.00^{b}	72.33^{b}	8.00^{a}	5.66^{b}	57.33^{c}	53.00^{d}	31.66^{b}	23.33^{b}	4.00^{c}	3.17^{c}
	Mean	72.46	68.08	5.75	4.33	54.12	49.08	29.5	23.83	3.73	2.96
	CV %	1.6	3.5	10.3	11.1	6.2	2.7	4.6	6.4	6.2	8.8
	$LSD_{0.05}$	2.106	4.218	1.038	0.844	5.958	2.371	2.4	2.6	0.4	0.4

Where, with: with inoculation, without; without inoculation, CV: Coefficient of variation, LSD: Least significant difference. Means followed by the same letter with in a column for a given inoculation level and variable are not significantly different at 5% level of P.

4.2 Effect of biochar application and *B.japonicum* inoculation on nodulation Parameters

4.2.1 Number of nodule per plant

Analysis of variance revealed that total numbers of nodules per plant was significantly affected by inoculation and biochar application (Table 4). Both biochar and inoculation have significantly ($P<0.05$) improved the total number of nodules. The number of nodules per plant ranged from 50.83 to 148.17 with inoculation and 29.67 to 110.17 without inoculation. The maximum values, 148.17 and 110.17 in inoculated and uninoculated treatments, respectively, were recorded with application of 36 ton/ha biochar supplemented with 100kg/ha DAP, followed by 12 ton/ha biochar supplemented with 100kg/ha DAP (Table 4). Increased availability of soil P has been suggested to be responsible for increased nodulation following biochar application (Rondon *et al*., 2007). Under conditions of phosphorus deficiency; legumes have low nodulation and nitrogen fixation capacity, probably because of the fact that phosphorus is essential for the development and function of the nodules.

The increase in nodule number with biochar application has also been reported for white clover (Rillig *et al*., 2010), red clover (Mia *et al*., 2014b), and soybean (Tagoe *et al*., 2008). Ogawa and Okimori (2010) have found a 57% increase in nodule number in soybean with biochar and inorganic fertilizer. Number of nodules per plant increased with increasing rates of biochar and plants with the higher number was for inoculated compared to the uninoculated treatments. This result is due to the symbiotic relationship between N_2 fixing bacteria and the host plant. Neveen (2008) and Yoseph and Worku (2014) have also reported that inoculation has significantly increased soybean nodule number over the uninoculated treatments. Moreover, it has been shown that nodule number and dry weight and soybean shoot yield increased when seeds were inoculated with *Rhizobium* (Egamberdiyeva *et al*., 2004). Guo *et al*. (2010) have reported that rhizobia inoculation of legumes usually stimulates plant growth through its effects on nodulation and biological N_2-fixation.

4.2.2 Nodule fresh weight and dry weight

Analysis of variance revealed that inoculation and biochar application significantly affected nodule fresh and dry weights per plant (Table 4). Nodule fresh weight ranged from 8.67 to 16.66 g/plant with inoculation and 8.67 to 14.00 g/plant without inoculation. The highest values in both cases were obtained from application of 36 ton/ha biochar supplemented with 100kg/ha DAP. While the lowest value (8.67g/plant) was recorded for inoculation without biochar and the control (Table 4).

Nodule dry weight per plant ranged from 2.00 to 5.17g/plant with inoculation and 1.67 to 3.67 g/plant for uninoculated treatments. In both cases the highest values were obtained due to application of 36 ton/ha biochar supplemented with 100kg/ha. While the lowest values of 2.00, 1.67 and 2.50 were obtained from the inoculation without biochar, control and 100kg/ha DAP without biochar plots, respectively. Rhizobial inoculation significantly increased both the number and dry weight of nodules. The present finding is in agreement with the results of Zhang *et al*. (2011) and Abbasi *et al*. (2010) who reported that inoculation of soybean significantly increased nodule number over the control. Similarly, Peoples *et al*. (1995); Ndakidemi *et al*. (2006); Teymur *et al*. (2012) and Gicharu *et al*. (2013) have also reported significant increases in number of nodules, and nodule fresh weight in legumes following inoculation with rhizobium.

Table 4: Effect of biochar application and *B.japonicum* inoculation on nodule number and weight of soybean

		Number of nodule Per plant		Nodule fresh weight (g/plant)		Nodule dry weight (g/plant)	
N^{o}	Treatment	with	without	with	without	with	without
1	Control	50.83^{f}	29.67^{g}	8.67^{f}	8.67^{d}	2.00^{f}	1.67^{f}
2	6 ton/ha Bc+100 kg/ha DAP	138.33^{c}	90.17^{b}	13.33^{cd}	12.00^{bc}	3.17^{d}	2.53^{cd}
3	12 ton/ha Bc+100 kg/ha DAP	142.33^{b}	92.83^{b}	15.33^{b}	13.00^{ab}	4.50^{b}	3.00^{b}
4	36 ton/ha Bc+100 kg/ha DAP	148.17^{a}	110.17^{a}	16.66^{a}	14.00^{a}	5.17^{a}	3.67^{a}
5	No biochar+100 kg/ha of DAP	90.83^{e}	39.67^{f}	9.83^{e}	9.50^{d}	2.50^{ef}	2.00^{e}
6	6 ton/ha biochar	112.83^{d}	69.50^{e}	12.33^{d}	11.33^{c}	3.00^{de}	2.33^{d}
7	12 ton/ha biochar	136.00^{c}	77.17^{d}	13.67^{c}	12.17^{bc}	3.50^{cd}	2.67^{c}
8	36 ton/ha biochar	136.00^{c}	83.33^{c}	14.17^{c}	12.50^{b}	3.83^{c}	8.00^{b}
	Mean	119.42	74.06	13.000	11.646	3.458	2.61
	CV%	1.14	3.66	4.831	5.056	10.52	6.66
	$LSD_{0.05}$	2.38	4.75	1.1	1.0	0.6	0.3

Where, with: with inoculation, without; without inoculation, CV: Coefficient of variation, LSD: Least significant difference. Means followed by the same letter with in a column for a given inoculation level and variable are not significantly different at 5% level of P.

4.3 Effect of biochar and *B. japonicum* inoculation on Arbuscular Mycorrhizal Fungi (AMF) Colonization

The results of analysis of variance (Table 5) revealed that AMF colonization was significantly ($P<0.05$) affected by *Bradyrhizobium* inoculation and biochar application. The highest Arbuscular colorizations (AC) 17.30 and 19.97 % in inoculated and uninoculated treatments, respectively, where, recorded for 36 ton/ha biochar alone, while lowest values (1.096 and 1.397%) were for inoculation and uninoculated ones both without biochar in the control plot.

The highest vesicular colorizations (VC) (23.13 and 20.52 %) in inoculated and uninoculated treatments were recorded for the treatment with 36 ton/ha biochar alone. The lowest values

(1.64 and 1.76%) were recorded for inoculation and without inoculation both without biochar in the control treatment.

The highest values of hyphal colonization (HC) 79.79 and 78.65% were recorded for inoculated and uninoculated treatments respectively, both received biochar at a rate of 36 ton/ha. The lowest values of 12.1and 8.9% were recorded for inoculation without biochar and for the uninoculated plot with only maintained 100kg/ha DAP (Table 5).

In this study it was observed that percent mycorrhizal root colonization was at lower rates when biochar was added along with DAP. However, percent mycorrhizal root colonization was higher when biochar was added alone. The decrease in AMF colonization in those plots may be resulted from an increase in soil P availability (Table 10). It was argued that biochar amendments could increase AMF % root colonization in plant roots (Elmer and Pignatello 2011) grown in acidic soils (Ezawa *et al*., 2002; Matsubara *et al*., 2002; Yamato *et al*., 2006), where the rate of P fertilization is very high ,or decrease AMF abundance in some cases (Warnock *et al*., 2010). AMF root colonization in plants depends on availability of P in the soil as low P soils showed good colonization (Warnock *et al*., 2007). According to Steiner *et al*. (2009), nutrient additions in the form of fertilizers could reduce the enhancing effect of biochar on microbial reproduction rates. Similarly, Blackwell *et al*. (2010) have found significant increases in the proportion of root colonization of wheat with AMF in biochar amended soils at no or low rate of fertilizer additions, but there was no significant increase when large amounts of nutrients were applied.

Table 5: Effect of biochar application and *B.japonicum* inoculation on AMF root colonization in soybean

		AMF colonization (%)					
		Arbuscular colonization		Vesicular Colonization		Hyphal Colonization	
N^{o}	Treatment	With	with out	With	with out	with	with out
1	control	1.09^{f}	1.40^{e}	1.64^{g}	1.76^{d}	12.10^{g}	10.04^{ef}
2	6 ton/ha Bc+100 kg/ha DAP	5.54^{c}	4.79^{d}	4.83^{d}	5.00^{c}	22.83^{d}	14.82^{d}
3	12 ton/ha Bc+100 kg/ha DAP	4.64^{d}	3.22^{de}	4.38^{de}	4.64^{c}	18.72e	13.83^{d}
4	36 ton/ha Bc+100 kg/ha DAP	2.55^{e}	2.44^{e}	3.39^{ef}	3.00^{c}	15.70^{f}	12.84^{d}
5	No biochar+100 kg/ha DAP	1.65^{ef}	1.60^{e}	2.42^{gf}	2.43^{d}	13.54^{g}	8.90^{f}
6	6 ton/ha biochar	9.95^{c}	14.79^{c}	20.07^{c}	15.43^{b}	62.88^{c}	69.81^{c}
7	12 ton/ha biochar	15.51^{b}	17.63^{b}	21.71^{b}	19.15^{a}	65.91^{b}	73.97^{b}
8	36 ton/ha biochar	17.3^{a}	19.97^{a}	23.13^{a}	20.52^{a}	79.79^{a}	78.65^{a}
	Mean	7.27	8.22	10.19	8.99	36.43	35.35
	CV %	7.36	15.50	5.77	9.78	3.375	5.38
	$LSD_{0.05}$	0.93	2.23	1.03	1.54	2.15	3.33

Where, with: with inoculation, without; without inoculation, CV: Coefficient of variation, LSD: Least significant difference, Bc: biochar. Means within the same factor and column followed by the same letter with in a column for a given inoculation level are not significantly different at 5% level of P.

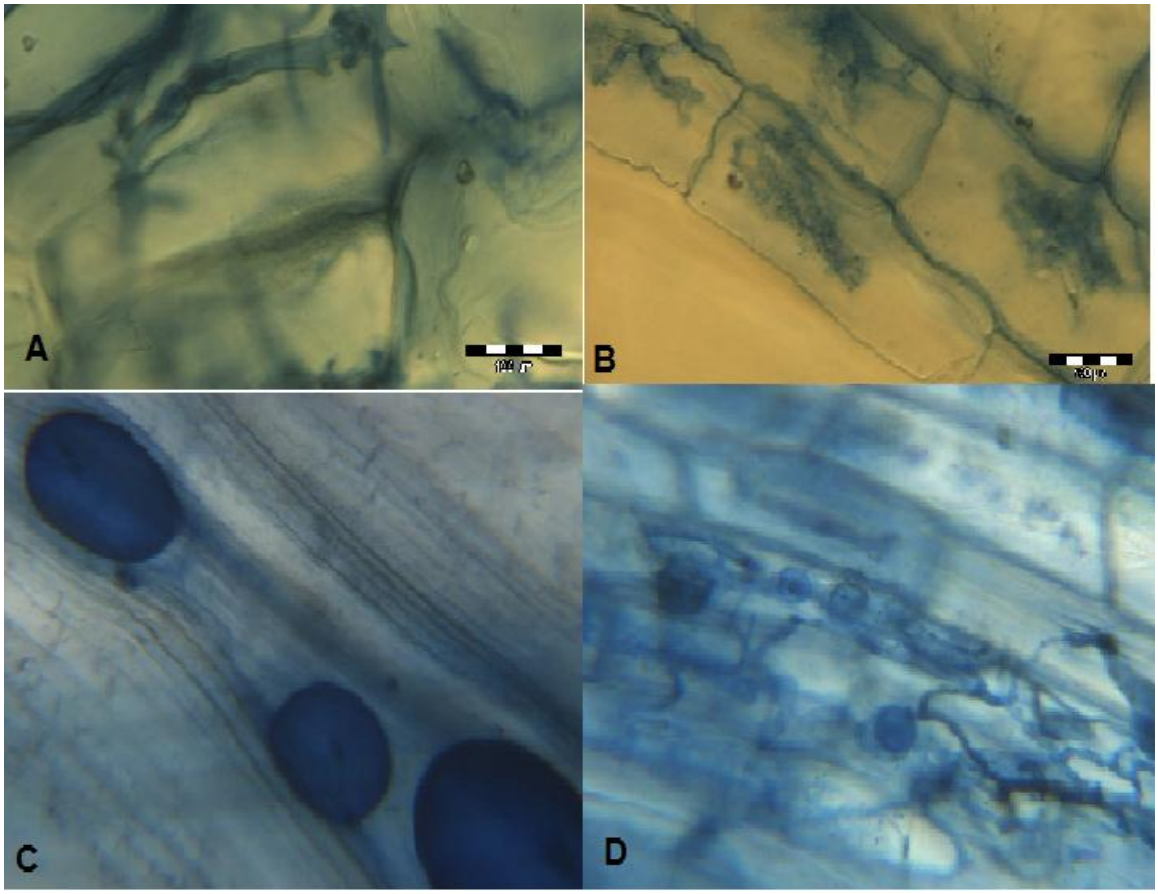

Figure 1: A), hyphae (taken from root samples treated with 36 ton/ha biochar); B) Arbuscule (taken from root samples treated with 6 ton/ha biochar); C) vesicle(taken from root samples treated with 12 ton/ha biochar); D) hyphae, vesicles and Arbuscular together (taken from root samples treated with 36 ton/ha biochar +100kg DAP).

4.4 Effect of biochar and *B.japonicum* inoculation on P uptake of Soybean

The results of analysis of variance (Table 6) revealed that P uptake of soybeans was significantly ($P<0.05$) affected by *Bradyrhizobium* inoculation and biochar application rate, where it was significantly increased by application of biochar. Both inoculated and uninoculated treatments resulted in the highest (4.81 and 4.27g/plant respectively) P up-take when amended with 36 t/ha biochar supplemented with 100kg/ha DAP, while the lowest respective values (0.88 and 0.83g/plant) were reported for those in the control plot (Table 6).

The results demonstrated that regardless of DAP application; there was an increase in phosphorus uptake with increasing rate of biochar application. However in most cases, the

effect of biochar was more pronounced with application of DAP at a rate of 100kg/ha. Increases in P uptake by plants as a result of biochar application could be attributed to high P content of the coffee husk biochar and thus, higher P concentration and availability in biochar-amended soils (Table 1and 10). Improved soil pH and CEC, leading to better nutrient absorption and reduced P fixation, could also be case. The increase in AMF activity due to application of biochar could also be the other reason for the highest nutrient uptake in biochar treated soils. Gundale and DeLuca, (2007) have demonstrated that biochar additions can change soil nutrient availability by affecting soil physico-chemical properties. Increases in soil nutrient availability may result in enhanced host plant performance and elevated tissue nutrient concentrations in addition to higher colonization rates of the host plant roots by AMF. Lehmann *et al*. (2003) have also observed an increase in P concentration in plants with increasing rates of biochar application.

Table 6: Effect of biochar application and *B.japonicum* inoculation on P up-take of soybean plants

		P uptake (g/plant)	
N^{o}	Treatment	With	with out
1	Control	0.88^{e}	0.83^{e}
2	6 ton/ha Bc+100 kg/ha DAP	3.17^{c}	2.88^{c}
3	12 ton/ha Bc+100 kg/ha DAP	3.91^{b}	3.35^{b}
4	36 ton/ha Bc+100 kg/ha DAP	4.81^{a}	4.27^{a}
5	No biochar+100 kg/ha DAP	0.92^{e}	0.84^{e}
6	6 ton/ha biochar	2.76^{d}	2.34^{d}
7	12 ton/ha biochar	2.91^{cd}	2.46^{d}
8	36 ton/ha biochar	3.12^{c}	2.79^{c}
	Mean	2.81	2.46
	CV %	7.02	5.23
	$LSD_{0.05}$	0.35	0.22

Where, with: with inoculation, without; without inoculation, CV: Coefficient of variation, LSD: Least significant difference, Bc: biochar. Means followed by the same letter with in a column are not significantly different at 5% level of P.

4.5 Effect of biochar and *B.japonicum* inoculation on BNF of soybeans

The results of analysis of variance (Table 7) revealed that Total N, N_2 fixed and N_2 derived from atmosphere (Ndfa) were significantly ($P<0.05$) affected by *Bradyrhizobium* inoculation and biochar application. Application of 36 ton/ha biochar supplemented with 100kg/ha DAP

resulted in the highest plant total N (4.19 and 2.66 g/plant for inoculated and uninoculated) treatments, respectively. The lowest values (0.42 and 0.38 g/plant) were recorded for the inoculation treatments without biochar and the control plot (Table 7). The highest total N concentration in inoculated plants implies a positive effect of nodulation on plant N accumulation. Hence, the ability of the rhizobia to establish an effective symbiosis had an impact on plant N. Increase in N contents due to *Rhizobium* inoculation was mainly due to significant increase in nodulation, which resulted in higher accumulation of N due to atmospheric N_2 fixation .Van Zwieten *et al*. (2010) have reported similar effect of biochar on N uptake, in which it was observed that application of biochar significantly increased uptake of N by plants.

Application of 36 ton/ha biochar supplemented with 100kg/ha DAP resulted in the highest amount of N_2 fixed (4.61 and 2.98 g/plant) and highest Percentage of Ndfa (98.57 and 97.74%) for inoculated and uninoculated treatments, respectively. The lowest N_2 fixed (0.54 and 0.51g/plant) was recorded for the inoculation treatments without biochar in the control plot. Rondon *et al*., (2007) have reported the positive effects of biochar on increased N_2 fixation which led to 30 to 40% increase in bean yield with biochar additions of up to 50 g kg^{-1} of soil. Increased N_2 fixation in the presence of biochar has been reported by a number of authors (Nishio, 1996; Rondon *et al*., 2007; Quilliam *et al*., 2013). In a study on common bean, an increase in N_2 fixation was observed with application until 60 g biochar kg^{-1} of soil (Rondon *et al*., 2007), while Tagoe *et al*. (2008) found an increased N_2 fixation in soybean even at 100 t ha^{-1}. Rondon *et al*. (2007) also reported that biochar significantly increased biological nitrogen fixation by *Rhizobium* and improved biomass production of plants.

In the present, study, higher amount of N_2 fixed (4.61 and 2.98g/plant) was due to biochar application with P supplementation in inoculated or uninoculated treatments compared to the treatments with biochar or P alone (Table 7). Increased availability of soil P has been suggested to be responsible for increased BNF following biochar addition (Rondon *et al*., 2007; Tagoe *et al*., 2008).The reasons for the improved BNF are most likely a combination of factors related to P availability in the soil (Lehmann *et al*., 2003), increased colonization of the host plant roots by AMF and, thus enhanced N_2 fixation due to the improved P-uptake by the plant roots (Saito and Marumoto, 2002). Lowering of soil acidity and increased pH by

biochar additions might have also contributed to the greater BNF. In addition to improved nutrient availabilities that are conducive to high BNF, inoculation with Rhizobia may be more effective in the presence of biochar due to the habitat it offer to microbes. The porous structure of biochar, its high internal surface area and its ability to adsorb soluble organic matter, gases and inorganic nutrients are likely to provide a highly suitable habitat for Rhizobia to grow and reproduce. In line with this Lehmann *et al*. (2006) have also indicated that biochar is an excellent support material for *Rhizobium* inoculants.

Table 7: Effect of biochar application and *B.japonicum* inoculation on Total nitrogen, N_2 fixation and %Ndfa of soybeans

		Total nitrogen (g/plant)		N_2 fixed(g/plant)		Ndfa (%)	
	Treatment	With	with out	with	with out	with	with out
1	Control	0.42^{e}	0.38^{f}	0.54^{e}	0.51^{e}	87.99^{c}	87.97^{e}
2	6 ton/ha Bc+100 kg/ha DAP	2.38^{c}	1.88^{cd}	2.76^{c}	2.14^{cd}	98.32^{a}	97.34^{abc}
3	12 ton/ha Bc+100 kg/ha DAP	3.19^{b}	2.11^{b}	3.58^{b}	2.39^{b}	98.43^{a}	97.63^{ab}
4	36 ton/ha Bc+100 kg/ha DAP	4.19^{a}	2.66^{a}	4.61^{a}	2.98^{a}	98.57^{a}	97.74^{a}
5	No biochar+100 kg/ha DAP	0.45^{e}	0.57^{e}	0.60^{e}	0.70^{d}	89.66^{b}	91.79^{d}
6	6 ton/ha biochar	2.14^{d}	1.74^{d}	2.48^{d}	2.03^{d}	98.13^{c}	96.55^{c}
7	12 ton/ha biochar	2.23^{d}	1.86^{cd}	2.57^{c}	2.14^{cd}	98.21^{a}	96.77^{bc}
8	36 ton/ha biochar	2.36^{c}	1.96^{cb}	2.71^{c}	2.30^{cb}	98.30^{a}	97.45^{abc}
	Mean	2.17	1.64	2.48	1.90	0.79	95.40
	CV %	2.91	4.48	2.49	3.85	95.97	0.55
	$LSD_{0.05}$	0.11	0.13	0.10	0.13	1.33	0.92

Where, with: with inoculation, without; without inoculation, CV: Coefficient of variation, LSD: Least significant difference, Ndfa is the percentage of N_2 derived from the atmosphere, Bc: biochar. Means followed by the same letters with in a column for a given variable and inoculation level are not significantly different at 5% level of P.

4.6 Effect of biochar and *B.japonicum* inoculation on Yield and yield Components of soybeans

4.6.1 Number of pods per plant

Analysis of variance results showed significant ($P<0.05$) variation in number of pods per plant

due to inoculation and biochar application (Table 8). Biochar in combination with *B. japonicum* inoculation resulted in significantly higher number of pods compared to biochar treatments without inoculation. The highest number of pods per plant (44.50 or 38.00) for inoculated or uninoculated treatments, respectively, was obtained from 36 ton/ha biochar supplemented with 100 kg/ha DAP. The lowest number of pods (20.50 or 13.50) was recorded for inoculated or uninoculated plots both without (control) (Table 8).

The productive potential of soybean is ultimately determined by number of pods per plant, which is a main yield component. The positive effect of inoculants might be due to sufficient nitrogen rendered through nitrogen fixation, which might have promoted vegetative growth and plant height and, thus, improving number of pods per plant. The higher nodulation due to inoculation resulted in higher nitrogen fixation by Rhizobium and eventually increased the number of pods per plant, which brings about higher grain yields as a whole (Singh *et al*., 2011).Tahir *et al*. (2009) have also observed a positive effect of seed inoculation on number of pods per plant. The authors have reported that *Rhizobium* inoculation boosted number of pods per plant by 85% over uninoculated treatment.

4.6.2 Number of seeds per pod

Number of seeds per pod was significantly ($P<0.05$) affected by biochar and *Bradyrhizobium* treatments (Table 8). The highest number of seeds per pod (4.00 and 3.83, respectively) for inoculated and uninoculated treatments respectively was recorded for 36 ton/ha biochar supplemented with 100kg/ha DAP,. Whereas the lowest value (2.00) was for the control plot. The number of seeds per pod is perceived as a significant constituent that directly plays a role in exploiting potential yield recovery in leguminous crops (Devi *et al*., 2012).

Number of seed per pod increased with increasing rates of biochar and, plants with higher number of pods were observed for inoculated compared to non-inoculated treatments. Ibrahim *et al*. (2011) have reported increased yield and yield components of soybean by inoculating the seeds with specific strain of rhizobia.

4.6.3 Seed yield

The results of analysis of variance of seed yield per plant indicated significant ($p<0.05$) response to *Bradyrhizobium* inoculation and biochar application (Table 8). Inoculated and uninoculated treatments showed the highest seed yields per plant (16.30 and 13.50 g/plant) for 36 ton/ha biochar supplemented with 100kg/ha DAP. The lowest values (8.33 and 7.37 g/plant) were recorded for both inoculated and uninoculated plot without biochar (control). Seed yield was significantly increased with increasing rates of biochar application. Lehmann and Rondon (2005) have reported that biochar application to soil helps to retain the nutrients which remain available to plants thus, increasing the plant growth and yield. Recent study by Yooyen *et al*. (2015) has indicated that the influence of biochar on dry weight and soybean seed yield is due to biochar properties in soil enabling the plant to accumulate its dry weight and seed yield better.

For all biochar applied plots, the *Rhizobium* inoculated treatment resulted in higher seed yield than those corresponding rates of biochar without inoculation. This could be due to significant contribution of N_2 fixation that supplied extra N_2 for the crop, as it is a major constituent of amino acids and many biological compounds which play major roles in photosynthesis, eventually increasing seed yields. This can be further linked to the positive and significant association observed between seed yield and N2 fixed ($r = 0.75^{**}$) (Table 11). Tairo and Ndakidemisi (2013) have reported that *Rhizobium* inoculation significantly increased seed yields of soybean by 91%. Furthermore, other workers (Bambara and Ndakidemi, 2010) have also reported significant increase in seed yield and all other yield components, such as number of pods and number of seeds per plant and seed weight, following rhizobia inoculation.

Seed yield of soybean increased when biochar application was supplemented with P compared to application of biochar or P supplementation alone. Highest seed yield was recorded for biochar application with P supplementation, while lower values were recorded for biochar alone or P supplementation alone (Table 8). In agreement with the present results, yield increases had been reported for biochar applied together with inorganic or organic fertilizers (Glaser *et al*., 2002; Van Zwieten *et al*., 2007). Positive plant growth and nutrient content responses to biochar are commonly observed in association with fertilizer application, while

lower plant growth responses have been observed for biochar amendments alone. Much greater yields and plant growth are observed with fertilizer additions plus biochar, as opposed to fertilizer additions alone (Yamato *et al*., 2006; Gundale and DeLuca, 2007; Asai *et al*., 2009; Blackwell *et al*., 2009).

Table 8: Effect of biochar application and *B.japonicum* inoculation on number of pods, number of seeds per pod, and seed yield per plant of soybean

N^{o}	Treatment	Number of pod per plant		Number of seed/pod		Seed yield (g/plant)	
		with	without	with	without	with	without
1	Control	20.50^{h}	13.50^{e}	2.00^{f}	2.00^{d}	8.33^{g}	7.37^{e}
2	6 ton/ha Bc+100 kg/ha DAP	41.83^{c}	28.67^{c}	3.50^{bcd}	2.83^{b}	12.60^{c}	10.83^{b}
3	12 ton/ha Bc+100 kg/ha DAP	43.00^{b}	33.50^{b}	3.83^{ab}	3.00^{b}	14.50^{b}	11.67^{b}
4	36 ton/ha Bc+100 kg/ha DAP	44.50^{a}	38.00^{a}	4.00^{a}	3.83^{a}	16.30^{a}	13.50^{a}
5	No biochar+100 kg/ha DAP	23.17^{g}	16.17^{d}	2.83^{e}	2.33^{c}	9.30^{f}	8.77^{d}
6	6 ton/ha biochar	33.83^{f}	28.00^{c}	3.17^{de}	2.50^{c}	10.00^{ef}	9.17^{cd}
7	12 ton/ha biochar	37.83^{e}	28.33^{c}	3.33^{cd}	2.83^{b}	10.53^{de}	10.33^{bc}
8	36 ton/ha biochar	39.17^{d}	32.17^{b}	3.66^{abc}	2.83^{b}	11.17^{d}	10.50^{bc}
	Mean	35.48	27.29	3.292	2.771	11.592	10.267
	CV %	1.652	5.309	6.732	5.392	4.1846	7.766
	$LSD_{0.05}$	1.027	2.538	0.388	0.262	0.8495	1.396

Where, with: with inoculation, without; without inoculation, CV: Coefficient of variation, Bc: biochar, LSD: Least significant difference. Means followed by the same letters within a column for a given inoculation level and variable are not significantly different at 5% level of P.

4.7 Effect of Biochar Application on Soil pH, EC and CEC

The effect of biochar application on pH and EC values of inoculated and uninoculated treatments is given in Table 9. The statistical analysis revealed a significant ($P<0.05$) increase in soil pH and EC due to addition of biochar. In both inoculated and uninoculated treatments, the highest mean values of soil pH and EC were observed for soils treated with 36 t ha^{-1} biochar supplemented with P followed by 12 t ha^{-1} biochar supplemented with P, while the lowest values were recorded for the control.

The increase in pH and EC values of the soil due to the application of biochar may generally be attributed to an increase in ash content, as ash residues are generally dominated by carbonates of alkali and alkaline earth metals, phosphates and small amounts of organic and inorganic N (Arocena and Opio, 2003). High pH level of the biochar and carbonate concentration which had a liming effect on the soil. Similar observations have been reported by Glaser *et al*. (2002) and Van Zwieten *et al*. (2007). Another reason for the increase in soil pH due to application of biochar could be the high surface area and porous nature of biochar that increases the cation exchange capacity (CEC) of the soil. Thus, there could be a chance for Al and Fe to bind with the exchange site of the soil. Agusalim *et al*. (2010) have also reported a decrease in exchangeable Al and soluble Fe in soils amended with biochar. Both pH and EC increased with increasing application rate of biochar. In line with this, Yuan and Xu (2011) showed that pH increased significantly with increasing application rates of biochar, reflecting the fact that its liming potential increased with increasing rates.

The effects of biochar addition on CEC in inoculated and uninoculated treatments are presented in Table 9. The analysis of variance showed that the cation exchange capacity significantly ($P<0.05$) increased with application of biochar. The highest values (27.65 and 27.33 me/100 g) of CEC for inoculated and uninoculated treatments, respectively, were obtained at 36 t ha^{-1} biochar amended with P application. The lowest CEC value (14.00me/100 g) was recorded for the control plot.

The increase in CEC due to application of biochar could be resulted from the inherent characteristics of biochar, since it has high surface area, highly porous and variable charge

organic material that has the potential to increase soil cation exchange capacity (CEC), surface sorption capacity and base saturation when added to soil (Glaser *et al*. 2002). Agusalim *et al*. (2010) and Chan *et al*. (2008) have also revealed the increase in soil cation exchange capacity after the application of biochar. Therefore, it is quite logical that soil treated with biochar had a higher CEC compared to untreated soil.

Table 9: Effect of biochar application and rhizobium inoculation of soybean on soil pH, EC and CEC

		pH-H_2O		EC (mS cm^{-1})		CEC(me/100 g)	
N^{o}	Treatment	with	without	With	without	with	Without
1	Control	4.71^{f}	4.58^{f}	0.046^{e}	0.047^{e}	14.70^{e}	14.00^{e}
2	6 ton/ha Bc+100 kg/ha DAP	5.38^{d}	5.30^{d}	0.09^{cd}	0.08^{cd}	19.40^{d}	18.66^{d}
3	12 ton/ha Bc+100 kg/ha DAP	5.66^{c}	5.62^{bc}	0.133^{b}	0.130^{b}	24.54^{b}	24.33^{b}
4	36 ton/ha Bc+100 kg/ha DAP	6.24^{a}	6.13^{a}	0.174^{a}	0.172^{a}	27.65^{a}	27.33^{a}
5	No biochar+100 kg/ha DAP	4.90^{e}	4.83^{e}	0.05^{e}	0.05^{e}	14.96^{e}	14.33^{d}
6	6 ton/ha biochar	5.41^{d}	5.36^{d}	0.08^{d}	0.07^{d}	18.37^{d}	18.00^{d}
7	12 ton/ha biochar	5.61^{c}	5.58^{c}	0.126^{bc}	0.122^{bc}	22.89^{c}	22.33^{c}
8	36 ton/ha biochar	5.85^{b}	5.81^{b}	0.138^{ab}	0.133^{ab}	25.08^{b}	24.33^{b}
	Mean	5.47	5.40	0.105	0.098	20.95	20.41
	CV%	1.816	2.13	19.9	19.4	3.0825	4.0169
	$LSD_{0.05}$	0.17	0.20	0.036	0.034	1.131	1.4362

Where, with: with inoculation, Without: Without inoculation, EC: Electrical conductivity, CEC; cation exchange capacity, Bc: biochar, LSD: Least significant difference, CV: Coefficient of variation. Means followed by the same letters within a column for a given inoculation level and variable are not significantly different at 5% level of P.

4.8 Effect of biochar application on OC, total N and available P content

Application of biochar on inoculated and uninoculated treatments significantly ($P<0.05$) increased mean OC, TN and available P content of the soil (Table 10). The highest OC, TN and available P levels were recorded for soil amended with 36 t /ha of biochar supplemented with P. The increase in OC and TN of the soil could be due to the high carbon and organic

matter content of coffee husk biochar. The other probable reason may be the decomposition of biochar added to the soil. High organic carbon in soils treated with biochar has been also reported by Lehmann, (2007). Solomon *et al.* (2007) and Liang *et al.* (2006) have also revealed a higher organic C and total N at the ancient *terra preta* compared with adjacent soils.

The highest value of soil available phosphorous (14.55 and 10.03 mg kg -1) for inoculated and uninoculated treatments, respectively, was obtained at 36 t ha^{-1} biochar amended with P fertilizer, while the lowest (3.83 mg kg^{-1}) was recorded for the control plot. The reason for improved P uptake was due to the beneficial effects of bio-char additions on P availability and the highest soil P concentration in biochar-amended soils. The other is due to high P content in the coffee husk biochar. The other reason is due to improved soil pH and CEC leading to better nutrient absorption and reduced P fixation due to release of P from complexes of Al and Fe under increasing soil pH. The increase in soil pH and CEC, that reduced the activity of Fe and Al, could also contribute to the highest values of available phosphorous in soils treated with biochar.

Several studies have demonstrated that the application of biochar to soil could increase the availability of major cations and phosphorus as well as total nitrogen concentrations (Glaser *et al.* 2002; Lehmann *et al.* 2003). Van Zwieten *et al*. (2010) and Chan *et al*. (2008) have also reported the increase in available phosphorous after the application of biochar.

Table 10: Effect of biochar application with and without inoculation on the status of soil OC, Total N and Available P in soybean plots

		Organic carbon (%)		Total Nitrogen (%)		Av.P(mg kg $^{-1}$)	
N^{o}	Treatment	with	without	with	without	With	without
1	Control	2.54^{f}	2.52^{d}	0.22^{f}	0.22^{d}	3.89^{e}	3.83^{e}
2	6 ton/ha Bc+100 kg/ha DAP	5.80^{c}	4.52^{c}	0.50^{c}	0.39^{c}	10.36^{c}	7.74^{c}
3	12 ton/ha Bc+100 kg/ha DAP	6.19^{b}	4.92^{b}	0.53^{b}	0.42^{b}	13.21^{b}	8.38^{b}
4	36 ton/ha Bc+100 kg/ha DAP	6.77^{a}	5.57^{a}	0.58^{a}	0.48^{a}	14.55^{a}	10.03^{a}
5	No biochar+100 kg/ha DAP	2.59 ^{f}	2.58^{d}	0.23^{f}	0.22^{d}	3.89^{e}	3.99^{e}
6	6 ton/ha biochar	4.77^{e}	4.52^{c}	0.42^{e}	0.39c	8.84^{d}	7.03^{d}
7	12 ton/ha biochar	5.13^{d}	4.91^{b}	0.45^{d}	0.42b	9.79^{cd}	7.44^{cd}
8	36 ton/ha biochar	5.58^{c}	5.55^{a}	0.48^{c}	0.48^{a}	10.78^{c}	7.92^{cb}
	Mean	4.92	4.3842	0.428	0.3783	9.4667	7.0461
	CV%	3.623	0.8609	2.816	1.0596	6.1124	5.0605
	$LSD_{0.05}$	0.3122	0.0661	0.0211	0.007	1.0133	0.6244

Where, with: with inoculation, Without: Without inoculation, Av.P: Available Phosphorus, LSD: Least significant difference, Bc: biochar, CV: Coefficient of variation. Means followed by the same letters within a column for a given inoculation level and variable are not significantly different at 5% level of P.

4.9 Correlation Analysis between selected Parameters

Correlation analysis showed that nodulation and yield parameters of soybean were significantly related to each other. Correlation among selected parameters was indicated in (Table 11). Correlation analysis showed that number of nodule had significantly positive correlation with seed yield per plant (r=0.825**), total N (r=0.895***), N fixed (r=0.892***), P up-take (r=0.908***) and total colonization (r=0.913***).Total seed yield shown significantly positive correlation with total N (r=0.764**), N fixed (r=0.757**), P up-take (r=0.769**) and total colonization(r=0.794**). Correlation analysis also showed that N fixation had significantly positive correlation with number of nodule (r=0.892***), P up-take (r=0.994***) and total colonization (r=0.983***). Generally strong positive correlation was

observed among selected parameters of soybean. These results showed that a rise in any of these variables would result in a corresponding increase in the other and vice versa. Rosario *et al*. (1997) have reported a similar observation, indicating that, the proportion of nitrogen in the plant contributed by fixation, was highly correlated with nodulation and BNF traits.

Table 11: Correlation matrix for the selected parameters of soybean

	NN	SY	TN	NF	P	TC
NN	1	0.825**	0.895***	0.892***	0.908***	0.913***
SY		1	0.764**	0.757**	0.769**	0.794**
TN			1	0.999***	0.994***	0.982***
NF				1	0.994***	0.983***
P					1	0.984***
TC						1

, * significant at P<0.01 and P<0.001, respectively; NN=Number of nodule; SY =seed yield; TN = total nitrogen; NF=Nitrogen fixation; P=P up take; TC=Total colonization

5. SUMMARY AND CONCLUSIONS

The present study was conducted to determine effects of biochar and *Bradyrhizobium japonicum* inoculation on P uptake, mycorrhizal colonization and BNF of soybean. The results showed that biochar was beneficial for P uptake, mycorrhizal colonization and BNF of soybeans. Biochar application at a rate of 36t ha^{-1} was found to be effective for soybean P uptake, mycorrhiza colonization and BNF. For all biochar application rates, *Bradyrhizobium* inoculated treatments performed better than those corresponding rates applied without inoculation. Treatments that were treated with biochar and *bradyrhizobium* showed greater P uptake, mycorrhiza colonization and N fixation than did plots treated with biochar alone. This showed that the biochar amendment enhanced the activities of *B. japonicum* strain and AM fungi that are indigenous to the soil. It is evident that biochar has a positive impact on soil microbe.

The results also reveal that addition of biochar increased soil pH, EC, organic carbon, total nitrogen, available phosphorous and CEC of the soil. The concentrations of nitrogen and phosphorous in soybean tissues were also increased after addition of biochar. The presence of plant nutrients and ash in the biochar, its high surface area, porous nature and capacity to act as a medium for microorganisms could be the main reasons for the increase in soil properties and higher nutrient uptake in biochar treated soils.

Increasing application rates of biochars also increased BNF of soybeans. Biochar application at a rate of 36 t ha^{-1} with 100 kg/ha DAP led to statistically significant increases in BNF compared to the control. For all biochar application rates, the *rhizobium* inoculated treatments fixed higher N than those corresponding rates without inoculation. Inoculation with Rhizobia is more effective in the presence of biochar due to the habitat offered by the biochar. The observed increases in BNF could be due to increased P availability in soil, improved P up-take by the plant roots and increased activity of rhizobia following biochar application.

Combination of biochar and Phosphorus fertilization was found to be the best for P uptake and BNF of soybeans compared to biochar application alone, while 36 t ha^{-1} biochar alone

was best for the mycorrhizal root colonization of soybean. AMF colonization decreased with biochar amendments with Phosphorus fertilization. The observed decrease in mycorrhiza colonization could be due to increased P availability in soil following biochar application. Positive plant growth and nutrient content responses were observed for biochar application with DAP fertilizer, while lower soybean growth and nutrient concentration have been observed for biochar amendments alone. Much greater yields were observed with fertilizer additions plus biochar, as opposed to fertilizer additions alone.

The amount of N_2 fixed by soybean, as well as total N and P uptake of the plants strongly and significantly correlated with number of nodules and AMF root colonization. Generally strong and positive correlation was observed among selected parameters of soybeans.

In conclusion, this short-term study shows a promising potential of using coffee husk biochar and inoculation to improve P availability, AMF colonization and BNF in acidic soils. However, these findings need to be further confirmed by long term field experiments for different soil types, which is critically important to further assess the potentials of biochar and scale up to other ecosystem functions for mitigation of the problem of climate change in Ethiopia.

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7. APPENDICES

Appendix Table 1 Mean squares for Soybean growth parameters

Source of Variation	Df	PH		NB		NL		SDW		RDW	
		With	Without	with	Without	With	Without	With	Without	With	Without
Rep	2	5.54	8.04	0.875	0.04	7.63	15.17	1.63	16.54	195.07	0.2
Treatment	7	77.23	106.93	12.263	6	609.9	418.55	159.43	106.48	3726.3	1.46
Error	14	1.446	5.804	0.351	0.232	11.577	1.833	1.911	2.351	0.054	0.069
P value		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Where, with: with inoculation, without; without inoculation, DF: Degree of freedom, PH: Plant height, NB: Number of Branch, NL: Number of leaf, SDW: shoot dry weight, RDW: Root dry weight.

Appendix Table 2.Mean squares for Soybean Nodulation

Source of Variation	Df	NN		NFW		NDW	
		With	with out	With	With out	With	With out
Rep	2	5.32	11.84	2.906	1.073	0.323	0.125
Treatment	7	3352.97	2222.4	21.452	9.463	3.208	1.186
Error	14	1.84673	7.35565	0.394	0.346	0.132	0.030
P value		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Where, with: with inoculation, without; without inoculation, DF: Degree of freedom, NN: Number of Nodule, NFW: Nodule Fresh Weight, NDW: Nodule Dry Weight.

Appendix Table 3.Mean squares for Soybean Yield Parameters

Source of Variation	Df	NP		NS/P		TSY	
		With	without	With	Without	With	Without
Rep	2	24.02	3.13	0.323	0.26	0.5329	2.6004
Treatment	7	246.915	211.89	1.23	0.88	22.0054	10.5705
Error	14	0.3437	2.0997	0.0491	0.022	0.2347	0.7648
P value		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Where, with: with inoculation, without; without inoculation, DF: Degree of freedom, NP: Number of Pod, NS/P: Number of Seed per Pod, TSY: Total Seed Yield

Appendix Table 4 Mean squares for Soybean P uptake, Total nitrogen, Ndfa and N_2 fixation

Source of Variation	Df	P uptake		Total N		N_2 fixation		Ndfa	
		with	without	With	without	with	without	with	without
Rep	2	0.00019	0.000953	0.0043	0.023	0.0026	0.023	0.6456	0.323
treatment	7	0.05455	0.041463	4.789	1.788	5.644	2.161	57.517	38.425
Error	14	0.0389	0.0167	0.0041	0.0054	0.0038	0.0054	0.582	0.274
P value		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Where, with: with inoculation, without; without inoculation, DF: Degree of freedom, Ndfa: Nitrogen derived from atmosphere, Total N: Total nitrogen.

Appendix Table 5 Mean squares for Soybean AMF colonization

Source of Variation	Df	Arbuscular Colonization		Vesicular Colonization		Hyphal Colonization	
		With	without	With	Without	With	Without
Rep	2	0.258	5.633	1.289	0.996	10.532	5.73312
Treatment	7	119.01	184.464	274.293	190.15	2354.33	3122.58
Error	14	0.2874	1.6277	0.34664	0.774	1.5127	3.626
P value		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Where, with: with inoculation, without; without inoculation, DF: Degree of freedom

Appendix Table 6. Mean squares for soil parameters after harvest

Source of Variation	Df	pH		EC		CEC	
		With	With out	with	Without	with	without
Rep	2	0.0074	0.0230	0.00089	0.00094	0.3529	0.2917
Treatment	7	0.7309	0.7674	0.00616	0.00323	69.6968	72.5476
Error	14	0.0099	0.0132	0.00044	0.00038	0.4171	0.6726
P value		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Where, with: with inoculation, without; without inoculation, DF: Degree of freedom, EC: Electrical conductivity, CEC; cation exchange capacity

Appendix Table 6.1 Mean squares for soil parameters after harvest

Source of Variation	Df	OC		TN		Av.P	
		With	With out	With	With out	With	With out
Rep	2	0.03788	0.00143	0.00052	0.00005	0.2993	0.8317
Treatment	7	7.46779	4.31465	0.05410	0.0322	43.0651	13.6471
Error	14	0.03177	0.00142	0.00015	0.000016	0.3348	0.1271
P value		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Where, with: with inoculation, without; without inoculation, DF: Degree of freedom, OC: organic carbon, TN: Total Nitrogen, Av.P: Available phosphorus