INFLUENCE OF BIOCHAR RATES FROM TWO TEMPERATURE REGIMES INTEGRATED WITH INORGANIC FERTILIZERS ON MAJOR NUTRIENT UPTAKE, AMF COLONIZATION, YIELD AND YIELD COMPONENT OF HOT PEPPER AT JIMMA ETHIOPIA

M.Sc THESIS

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Influence of Biochar Rates From two Temperature Regimes Integrated with Inorganic Fertilizers on Major Nutrient Uptake, AMF Colonization, Yield and Yield Component of Hot Pepper at Jimma Ethiopia

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A Thesis

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DEDICATION

I dedicate this Thesis manuscript to my father Melaku Habtemaryam who passed away without seeing my achievements and to my mother Abaynesh Weldeyohans, my brothers and sisters for nursing me with affections and love and their dedicated partnership for the success of my life.

STATEMENT OF THE AUTHOR

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BIOGRAPHICAL SKETCH

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ACRONYMS AND ABBREVATIONS

AC	Arbuscular Colonization
AMF	Arbuscular Mychrohizal Fungi
CEC	Cation Exchange Capacity
CHB	Coffee Husk Biochar
CSA	Central Statistical Agency
EARO	Ethiopian Agriculture Research Organization
EIAR	Ethiopia Institute of Agriculture Research
JARC	Jimma Agriculture Research Canter
Mo ARD	Ministry of Agriculture and Rural Development

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Influence of Biochar Rates From two Temperature Regimes Integrated with Inorganic Fertilizers on Major Nutrient Uptake, AMF Colonization, Yield and Yield Component of Hot Pepper at Jimma Ethiopia

ABSTRACT

Hot pepper production is an important activity in Southwestern Ethiopia as a means of income generation. Although it is one of the cash crops in the region, its production is constrained by many factors among which nutrient depletion and soil acidity can be mentioned. Biochar has been advocated to have a positive effect on crop yield improvements in acidic and highly weathered tropical soils via its effect on acidity amelioration. However, the dynamic use of biochar to a specific soil and crop type depends on how biochar is made, the amount applied and its interactions with soil-plant factors. Therefore, this experiment was conducted in a greenhouse to investigate the effect of biochar and inorganic fertilizers on N, P uptake, yield and root colonization by Arbuscular mychrohizal fungi (AMF). The experiment was laid out in 2×2×3 factorial arrangement with control consisting of two pyrolysis temperature (350^0 C and 500^0C), tow biochar application rate (6 and 18 t ha^{-1}) and three levels of the recommended inorganic fertilizer (N and P)as 0%, 60% and 100% in randomized complete block design with four replications. Analysis of variance (ANOVA) showed that interaction effect of pyrolysis temperature, biochar rate and inorganic fertilizer significantly ($P<0.001$*) affected growth, yield, N & P uptake, AMF root colonization and chemical properties of the soil. Besides, plant height, number of branches and soil total N content were significantly (*$P<0.001$*) affected by the interaction of biochar rate and inorganic fertilizer. The earliest days to flowering was obtained from the interaction of 6 t ha^{-1} biochar prepared at 350^0C along with 100% inorganic fertilizer. Similarly, the highest N and P uptake by fruit, haulm and total plant was obtained from the combination of biochar prepared at 500^0C, 18 t ha^{-1} biochar along with 100% inorganic fertilizer. The Arbuscul, Vesicular and Hyphal root colonization were also significantly (*$P<0.001$*) affected by the interaction of 18 t ha^{-1} biochar at 500^0C along with 60% inorganic fertilizer. The application of 18 t ha^{-1}biochar prepared at 500 ^{0}C pyrolysis temperature along with 100% or 60% inorganic fertilizer appeared to be a promising combination to optimize hot pepper yield, N and P uptake, AMF colonization and soil properties. However, to give strong conclusion and to determine the N and P use efficiency of hot pepper, this experiment need to be repeated on larger production field of hot pepper.*

Key words: *Biochar, Chemical fertilizers, Hot pepper, Mycorrhizal root colonization, N and P Uptake, Soil properties*

1. INTRODUCTION

Hot pepper (*Capsicum annuum* L.) is the most important vegetable crop belonging to the genus Capsicum family Solanaceae and grown as spice crop in different parts of the world (Rodriguez *et al*.,2008).The genus Capsicum to which pepper belongs is a member of the Solanaceae family that consists of about 22 wild species and five domesticated species. The five domesticated species include *C. annuum L., C. frutescens L., C.chinenses, C. baccatum L., and C. pubescens R.* (Bosland and Votava, 2000). Capsicum species can be divided into several groups depending on their fruit characteristics ranging in pungency, color, shape, intended use, flavor, and size.Despite their vast trait differences, most commercially cultivated peppers in the world belong to the species *C.annum* L. (Smith *et al*., 1987; Boslanad, 1992), and originated from the upland of central-eastern Mexico (Loaiza, 1989).

Capsicum fruit are consumed as fresh, dried or processed, as table vegetables and as spices or condiments (Geleta, 1998 and Delelegn, 2011). It is rich in Vitamins A, C and E and a good source of B2, potassium, phosphorus and calcium (Bosland and Votava, 2000); and (Delelegn 2011). Moreover, its vitamin C content is more than any other vegetable crops (Delelegn, 2011).Hot pepper pungency is a desirable attribute in many foods it produced by the capsaicinoids, alkaloid compounds (C18H27NO3) that are found only in the plant genus, *Capsicum* (Hoffman *et al*., 1983; Delelegn, 2011).Besides its ford benefits it also has medicinal values and eventually used for the treatment of diabetic neuropathy, osteoarthritis, post-herpetic neuralgia, and psoriasis (Jin *et al*. 2009), as well as there are many patents on insecticides, insect or animal repellents, and pesticides containing capsaicinoids (Eich, 2008).

After potatoes and tomatoes hot pepper ranks third in quantity of production among vegetables (Ochoa-Alej and Ramirez-Malagon, 2001; Ali , 2006). Hot pepper production was 31.44 million tons both dry and green fruit from 3.70 million hectares in the world FAO (2009). Africa's production of hot pepper was 7.70 million tons both dry and green fruit from 0.89 million hectares (FAO, 2009). This crop cultivated in many parts of the country and it is an important source of cash earning for smallholder producers both in green and dry forms. According to FAO (2009) report, the estimated productions of peppers were 220,791 ton from area 97, 712 ha in green form and 118,514t of dry pepper from an area of 300,000 ha. It is the leading vegetable crop produced in the country CSA (2011).

Despite its economic importance and allocation of relatively larger area allocated by Ethiopian farmers for pepper production, which is 339,305 ton is far below the world average, 98.02% and African average, 95.6% (FAO,2009).This is because of a lot of interrelated factors. Among others, P and N nutrient deficiency is the most yield limiting factor for vegetable production in smallholder farming system of Ethiopia (Agegnehu and Tsigie, 2004).Eventually these nutrients are lost, through leaching and P fixation in acidic soil (Elias *et al*, 1998; Amare *et al*., 2005; Aticho, 2011). Moreover, rapid organic matter oxidation and loss under tropical conditions would aggravate the situation (Tiessen *et al.*, 1994).

Biochar applications to soil are recently understood to influences various soil physicochemical properties. Due to the high specific surface area of biochar and because of direct nutrient addition via ash as organic fertilizer amendments, its application has been proved to reduce nutrient leaching (Downie *et al.*, 2009), After incorporation of biochar into soil, it was found to improve soil fertility (Lehmann *et al.*, 2003; Steiner *et al.*, 2007), Besides it caused nutrient retention ability, of many soils and brought improvement in fertilizer use efficiency and reduced leaching(Steiner *et al*., 2008;Roberts *et al.*,2010). N fertilizer use, efficiency noted to be maximized (Widowati *et al.*, 2012) and, it reduced the use of K fertilizers in Inseptisols (Widowati and Asnah, 2014). Biochar as a soil amendment has a potential for improving crop yields and quality of degraded soils. Particularly, biochar generated from black carbon biomass has been shown to increase yields (Lehmann *et al*., 2003). It also improves, the availability of P in soils; both directly through P addition from water-soluble P contained in biochar and/or indirectly through impact on soil chemical, physical and/or biological processes (DeLuca *et al*.,2009). Moreover, most benefits of biochar that have been used for soil fertility improvement were obtained in acidic and highly weathered tropical soils (Rondon *et al.*, 2004; Verheijen, 2009). It has been related that the effect of bio-char to mitigate some of the greatest soil–health constraints of crop productivity is different for different type of biochar, biochar application rate, soil properties, and environmental conditions, (Chan and Xu, 2009; Jeffery *et al*., 2011; Glaser, 2012).

Biochar can be generated from various sources of biomass and pyrolysis conditions. Pyrolysis is a thermo chemical process in which biomass is converted to biochar through heating with limited oxygen supply (<700^{0}C). Biochar is produced by incomplete combustion of organic matter which is relatively resistant to decomposition and degradation due to its condensed aromatic structure (Kuzyakov *et al*., 2009). Biochar is 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, it is a more stable nutrient source than compost and manure (Chan, *et al*., 2007). Therefore, biochar addition to soil cold is one of potential strategies for long-term C sequestration and offer ecosystem services and gradual soil improvements.

Biochar has different properties depending on raw materials and pyrolysis conditions used (Bonelli *et al*., 2001). According to Singh *et al*.,(2010), nutrients content of biochar is strongly influenced by the type of raw materials and pyrolysis conditions. The amount of biochar added to the soil will influence the effectiveness of biochar in reducing soil N loss, P fixation in the soil and improving plant growth. Plant response to biochar application has been reported to vary because of varying nature of biochar, depending on the biomass source and pyrolysis conditions (Major *et al*., 2009). Mineral contents of biochar generated from different raw materials also vary considerably (Yao *et al*., 2012). Therefore, wide application of biochar need further research focusing on the effect of biochar addition under site- specific conditions that onsiders available feedstock's, climate conditions, type of soil, soil management and crop type. There for efforts showed be made to study biochar using coffee husk feedstock in acidic soil conditions of Ethiopia.

General Objectives

To study the effect of coffee husk biochar produced at two different pyrolysis temperatures by integrating with the rates of inorganic fertilizer, and to optimize N and P uptake, Arbuscular mychrohizal fungi root colonization, yield of hot pepper, on dominated soil of Jimma areas.

Specific objectives

- To examine the effect of coffee husk biochar application rate changes on N and P uptake, AMF colonization, major soil chemical properties and yield of hot pepper.

- To determine the effect of coffee husk biochar produced by two different pyrolysis temperature (350^0C and 500^0C) on N and P uptake, AMF colonization, major soil chemical properties and yield of hot pepper.

- To investigate the interaction effect of coffee husk biochar application rate, pyrolysis temperature and inorganic fertilizer application on N and P uptake, AMF colonization, major soil chemical properties and yield of hot pepper.

2. LITERATURE REVIEW

2.1. Hot Pepper Production

Pepper (*Capsicum annum* L.) production is becoming commercially important in various regions of the world including Israel, Spain, southern Europe, and north Africa where the pepper is grown from fall to spring in greenhouses and net houses. Such production in protective structures commonly yields more than 100t $ha^{-1}$of high quality fruit seasonally (Yasuor *et al.,* 2013). It is one of the important spices that serve as the source of income particularly for smallholder producers in many parts of rural Ethiopia. According to the EEPA (2003) pepper generated an income of 122.80 million Birr for farmers in 2000/01. This value jumped to 509.44 million Birr for smallholder farmers in 2004/05. This indicates that hot pepper is the sources of income to smallholder farmers and as exchange earning commodity in the country (Beyene and David, 2007).

In Ethiopia, it is a seasonal plant of the family Solanaceae grown as an annual crop and produced for its fruits. Suitable altitude range for optimum production of pepper is between 1000 and 1800 m.a.s.l. Productions of peppers in Ethiopia were 339,305 tons of dry and green peppers from 397,712 ha of land (CSA, 2011/2012). which receives mean annual rainfall of 600 to 1200 mm, and has mean annual temperature of 25 to 28°C (EIAR, 2007).When the temperature falls below 15°C or exceeds 32°C for extended periods, growth and yield are usually reduced. High temperature associated with low relative humidity at the time of flowering increases the transpiration, resulting in abscission of buds, flowers and small fruit.

Hot pepper can grow best in a loam or sandy loam soil with good water-holding capacity, but can grow on many soil types, as long as the soil is well drained. It prefers a light porous and well-drained soil rich in organic matter. In poor drained soils, plants shed their leaves and turn sickly and fruit drop takes place due to water logging conditions. It can be grown successfully in sandy loam soil provided that adequate irrigation and manuring are carried out. So, heavy textured soils in locations where drainage facilities are inadequate should be avoided. The crop can be grown successfully with soil pH of 6-7.

2.2. Major Factor Which Affect Hot Pepper Production

The solanaceas vegetable crops of pepper and tomato generally take up large amounts of nutrients from the soil (Mengel and Kirkby, 1980).The application of N and P fertilizer are important for better nutrient management. Growers and farmers need to manage the fertilizer for better quality of fruit production. The amount of fertilizer to be applied depends on soil fertility, fertilizer recovery rate, and organic matter, soil mineralization of nitrogen(N), and soil leaching of N (Berke *et al*., 2005). Peppers require adequate amount of major and minor nutrients. However, they appear to be less responsive to fertilizer, compared with onion, lettuce and Cole crops (Cotter, 1986). Study by Hedge, (1997) showed that nutrient uptake and dry matter production (fruit yield) of hot pepper are closely related.

Sufficient N is essential for normal plant growth and development, being an integral part of protein and chloroplast structure, nucleic acid, hormone and vitamin synthesis and also helps in cell division and cell elongation function (Barker and Bryson, 2007). Nitrogen deficiency has been studied on the majority of horticultural crops, whereas the effects of oversupply of N are not as widely understood (Stefanelli *et al*., 2010).Several research works have reported increase in green pod yield of okra with application of N from 56 to 150kg/ha (Hooda *et al*., 1980; Mani & Raman than, 1980; Majanbu *et al*., 1985, Singh, 1995).

Similarly phosphorus for vegetable production is a key constituent of adenosine try phosphate (ATP) and plays significant role in energy transformation in plants and also in various physiological processes (Shivasankeb *et al*., 1982). It helps in nutrient uptake by promoting root growth and thereby ensuring a good pod yield through the increase in total dry matter (Roa, 1995). However, Phosphorus deficiency results in poor root development, poor pod setting and subsequently reduces yield (Jam *et al*., 1990).

Organic fertilizers are essential for the proper development of plants, vegetables, flowers and fruits, as they offer rapid growth with superior quality to all species. They have the nutrients necessary for better development. In addition, the organic matter serves as nutrients and energy sources for soil microorganisms (DaSilva *et al*., 2012). The suitability and usefulness of organic fertilizers has been attributed to high availability of NPK content (Waddington.1998), which capable to enhance soil fertility (Thomas, 1997).They also act as a substrate for soil microorganisms which lead to increase microbial activity, whereof increasing the rat of organic material decomposing and releasing nutrient for plant uptake. They improve the physical properties of the soil as well (Khalid and Shafej, 2005). Experiment conducted in Kenya also indicated that supplementing the inorganic fertilizers with well decomposed farmyard manure substantially increased both to improve soil fertility and potato tuber yield in a small holder farms (Muriithi and Irungu, 2004).This study also assessed that biochar considering as inorganic to reduce the acidity of the soil with negative effect on the environment with redaction of inorganic fertilizer use because it riches in alkaline compounds and it improve soil physicochemical property.

2.3. Fundamentals of Biochar

The deliberate mixing of burned biomass in soils around human settlements helped spark more recent interest in biochar. These deposits of enriched soils, known as *terra preta* in the Amazon region of South America, have a fascinating history of scientific study of their own (Lehmann *et al.*, 2003).Bio char is defined as charred organic matter that is applied to soil in a deliberate manner with the intent to improve soil properties (Lehmann, 2009). Bio char as a soil conditioner has also been defined as a porous carbonaceous solid produced by thermo chemical conversion of organic materials in an oxygen depleted atmosphere with pyrolysis-chemical properties suitable for the safe and long-term storage of carbon in the environment and for soil improvement (Shackley and Sohi, 2010). It is relatively recent development, emerging in conjunction with soil management and C sequestration issues (Lehmann *et al,* 2006). It is produced through an energy conversion process called *pyrolysis,* which is essentially the heating of biomass in the complete or near absence of oxygen. Pyrolysis of biomass produces char, oils and gases (NCAT, 2010). The longevity of Biochar in the soil environment and its effectiveness as a soil conditioner is directly influenced by the way it is produced, and the feed material used for its preparation (Downie, *et al*., 2009; Bayan *et al*., 2014).

2.3.1. Biochar preparation and properties

Bio-char is made by the pyrolysis of waste biomass such as coffee husk, corncobs, livestock wastes and plant residue. Preparation of biochar from waste material are important not only to obtain agricultural input but also produce energy for household activity and important for the management of domestic, agricultural and industrial wastes Cantrell *et al.*, 2007;Demirbas, 2002).Pyrolysis simply means partial combustion, or heating in the absence of oxygen. This is done in a controlled environment to minimize emissions. (Emissions reference points can be found- Supra footnote 5 pp. 132) Pyrolytic biochar can also potentially be used as a low cost sorbent (Ioannidou and Zabaniotou, 2007) or as a soil amendment to improve soil fertility and sequester carbon (Lehmann *et al*., 2006; Steiner, 2007)**.**

There are different ways to make bio char, but all of them involve heating biomass with little or no oxygen to drive off volatile gasses, leaving carbon behind (NICRA and Bulletin, 2013). This simple process is called thermal decomposition usually achieved from pyrolysis or gasification. Pyrolysis is the temperature driven chemical decomposition of biomass without combustion (Demirbas, 2004). In commercial biochar pyrolysis systems, the process occurs in three steps: first, moisture and some volatiles are lost; second, unreacted residues are converted to volatiles, gasses and biochar, and third, there is a slow chemical rearrangement of the biochar (Demirbas, 2004). Removal of crop residues for energy production can have deleterious effects on soil organic carbon (SOC) and consequently on soil fertility (Lal, 2004).

2.3.2. Preparation of biochar with deferent pyrolysis temperature

Biochar is deferring depending on the feedstock selected and pyrolysis process to store carbon and improve soil fertility. Because infertile soils in different regions around the world have specific quality issues and hence it follows that one biochar type will not solve all soil quality problems (Lehmann *et al.*, 2003). For instance, biochar with a highly aromatic composition may best suited for long term C sequestration because of their recalcitrant nature (Glaser *et al*., 2002; Novak *et al.,* 2009). Biochar with large amounts of C in poly-condensed aromatic structures is obtained by pyrolyzing organic feedstock at high temperatures (400-700°C) but also have fewer ion exchange functional groups due to dehydration and decarboxylation potentially limiting its usefulness in retaining soil nutrients (Glaser *et al*., 2002; Baldock and Smernik, 2002; Hammes *et al.*, 2006).

Other, found that maximum surface areas were obtained when oil palm stones were pyrolysed at 800°C with a retention time of three hours (Guo and Lua, 1998). On the other hand, biochar produced at lower temperatures (250-400°C) have higher yield recoveries and contains more C = O and C-H functional groups that can serve as nutrient exchange sites after oxidation (Glaser *et al.,* 2002). Moreover, biochar produced at these lower pyrolysis temperatures has more diversified organic character, including aliphatic and cellulose type structures. These may be good substrates for mineralization by bacteria and fungi (Alexander, 1977) which have an integral role in nutrient turnover processes and aggregate formation (Thompson and Troeh, 1978). Feedstock selection also has a significant influence on biochar surface properties (Downie *et al.,* 2009) and its elemental composition (Amonette and Joseph, 2009; Gaskin *et al.,* 2008). Because both feedstock and pyrolysis conditions affect physical (Downie *et al*., 2009) and chemical properties, biochar producers may wish to consider the goals for the biochar amendment and adjust their feedstock and pyrolysis protocol to create a designer biochar that is tailored to remedy a specific soil issue (Duma *et al.*, 2015).

On heating to higher temperature (300°C to 500°C), molecules are rapidly depolymerized to anhydroglucose units that further react to provide a tary pyro lysate. At even higher temperatures (>500°C), the an hydrosugar compounds undergo fission, dehydration, disproportionation and decarboxylation reactions to provide a mixture of low molecular weight gaseous and volatile products, as well as the residual biochar.

Table 1 Physical and chemical properties of contrasting biochars relevant to biological processes in soil

Feed stock	Temperature (^{0}C)	pH(KCL)	pH($_2$O)	CEC (mmolc kg^{-1})	CEC (molc m^{-2})	C (%)	C/N ratio	Total P Mg/kg^{-1}	Ash (%)	Volatilles	Fixed C^0(%)
Oak wood	60	3.16	3.73	182.1	ND	47.1	444	5	0.3	88.6	11.1
	350	5.18	4.80	294.2	0.65	74.9	455	12	1.1	60.8	38.1
	600	7.90	6.38	75.7	0.12	87.5	48.9	29	1.3	27.5	71.2
Corn Stover	60	6.33	6.70	269.4	ND	42.6	83	526	8.8	85.2	6.0
	350	9.39	9.39	419.3	1.43	60.4	51	1889	11.4	48.8	39.8
	600	9.42	9.42	252.1	0.48	70.6	66	2114	16.7	23.5	59.8
Poultry litter	60	7.53	7.53	363.0	ND	24.6	13	16.68	36.4	60.5	3.1
	350	9.65	9.65	121.3	2.58	29.3	15	21.25	51.2	47.2	1.6
	600	10.33	10.33	58.7	0.63	23.6	25	23.59	55.8	44.1	0.1

Source: Lehmann J, *et al*., 2011.

The charring temperature modifies the functional group, and thus aliphatic C groups decrease but aromatic C increases (Lee *et al*., 2010). Jindo K *et al*. (2014) worked in Chemical composition with spectra parameters show s that the pyrolysis process at 600^{0}C, which leads to a higher recalcitrant character by increasing the number of aromatic compounds, is a suitable method for carbon sequestration. However, when the charring temperature range is at 700–800^{0}C, the intensity of the bands such as that of the hydroxyl groups (3200–3400 cm1) and aromatic groups (1580–1600 and 3050–3000 cm 1) gradually diminishes. According to (Yuan *et al*., 2010) have shown that the number of bounds representing functional groups are present in biochar obtained at lower temperature (300 and 500^{0}C) and are absent in those derived at700^{0}C.

2.3.3. Importunes of biochar in agricultural systems

The quality and potential use of biochar are governed by its physical and chemical properties (Sohi *et al*., 2010).Therefore, characterization of biochar is the first step to understand the mechanism of action. Sustainable biochar is a powerfully simple tool overcome global warming challenges and produce a soil enhancer that holds carbon and makes soil more fertile. In some biochar systems objectives related to management of agricultural waste: and production of clean and renewable energy for small holder farmers can also be met (IBI, 2015). Bio char is rich in alkaline components (Ca, Mg, K), and this may contribute to the neutralization of soil acidity and to a decrease in the solubility of phytotoxic metals such as aluminum in soils. In addition, biochar can bind and release nutrients (N, P, K, Ca) and could therefore reduce nutrient leaching in a highly weathered, low-cation exchange capacity soils. NGI,(2014).

2.3.3.1. Effect of biochar on soil properties

The potential impacts of bio char as a soil amendment have been extensively reviewed in the literature, e.g. Sohi *et al,* (2010) and Jeffery *et al* (2011). Bio char may alter the physical properties of the soil, including increasing aeration and water holding capacity of certain soils (Sohi *et al*, 2010 – Haefele, 2011). Bio char can increase pH by 0.5–1.0 unit in most cases for application rates of 30 Mg ha^1 of bio char (Shackley, 2012 b), nutrients are directly available through the solubilizeation of ash in the solid bio char residue and other nutrients may become available through microbial utilization of a small labile carbon component of bio char (Philippines, 1997). Gasification chars typically contain more nutrients, than those produced in slow pyrolysis for example (Shackley, 2012). While “fresh RHC” does not have a very high cation exchange capacity (CEC), it is still higher than weathered sandy tropical soils, and the CEC increases over time in soil (Glaser,2002; Lehmann, 2007).

According to National Sustainable Agriculture Information Service (2010), scientists still do not have a specific understanding of how biochar provides fertility for crops. Biochar has little plant nutrient content itself but acts more as a soil conditioner by making nutrients more available to plants and improving soil structure. The high surface area and pore structure of biochar likely provides a habitat for soil microorganisms, which in turn may aid in making some nutrients available to crops. Biochar may provide an indirect nutrient effect by reducing leaching of nutrients that otherwise would not be made available to crops.

Bio char is a high carbon containing material (more than 50%) produced by heating of biomass in absence of oxygen. Bio char application to soil leads to several interactions mainly with soil matrix, soil microbes, and plant roots (Lehmann and Joseph, 2009). The types and rates of interactions depend on different factors like composition of biomass as well as bio char, methods of biochar preparation, physical aspect of bio char and soil environmental condition mainly soil temperature and moisture. Bio char can act as a soil conditioner by improving the physical and biological properties of soils such as water holding capacity and soil nutrients retention, and also enhancing plant growth (Sohi *et al*., 2010).

The application of biochar in soils is based on its properties such as: (i) agricultural value from enhanced soils nutrient retention and water holding capacity, (ii) permanent carbon sequestration, and (iii) reduced GHG emissions, particularly nitrous oxide (N_2O) and methane (CH_4) release (Bracmort, 2010; Brown, 2009; Glaser *et al*., 2002; Kammen and Lew, 2005; Lehmann *et al*., 2006; Steiner, 2010; Steiner *et al*., 2008). Farmers will be motivated to apply biochar on their farms if these benefits can be demonstrated explicitly. At the local scale, soil organic carbon levels shape agro-ecosystem function and influence soil fertility and physical properties, such as aggregate stability, water holding capacity and cation exchange capacity (CEC) (Milne *et al*., 2007). The ability of soils to retain nutrients in cation form that are 0available to plants can be increased using biochar. The addition of biochar to agricultural soils is receiving considerable interest due to the agronomic benefits it may provide (Quayle, 2010) Several authors have reported that biochar has the potential to: (i) increase soil pH, (ii) decrease aluminum toxicity, (iii) decrease soil tensile strength, (iv)Improve soil conditions for earthworm populations, and (v) improve fertilizer use efficiency (Table 2).

Table 2 Effect of bio char on different soil properties

Factor	Impact	Source
Cation exchange capacity	50% increase	(Glaser *et al.,* 2002)
Fertilizer use efficiency	10-30 % increase	(Gaunt and Cowie, 2009)
Liming agent	1 point pH increase	(Lehman and Rondon, 2006)
Soil moisture retention	Up to 18 % increase	(Tryon, 1948)
Crop productivity	20-120% increase	(Lehman and Rondon, 2006)
Methane emission	100% decrease	Rondon *et al.,* 2005)
Nitrous oxide emissions	50 % decrease	Yanai *et al.,* 2007)
Bulk density	Soil dependent	(Laird, 2008)
Mycorrhizal fungi	40 % increase	(Warnock *et al.,* 2007)
Biological nitrogen fixation	50-72% increase	(Lehman and Rondon, 2006)

National initiative on climate resilient agriculture central research institute for dry land agriculture Hyderabad (2012)

2.3.4. Effect of biochar on P uptake

Phosphorus (P) availability is low in many soils, particularly in acid soils with high P sorption capacity. Biochar may improve available P in soils; both directly through P addition from water-soluble P contained in biochar and/or indirectly through impact on soil chemical, physical and/or biological processes (DeLuca *et al*., 2009). Many biochar have a liming effect, so increased soil pH may increase negative charge which in turn reduces P sorption (DeLuca *et al*., 2009). The extent of this effect depends on the Acid Neutralizing Capacity (ANC) of biochar (Van Zwieten.,2010)Biochar may also increase microorganism activities through application of C, especially aliphatic C compounds (Zimmermann,2010). The increase in microbial activities may affect microbial biomass phosphorus (MBP) (Liptzin and Silver,2009) and phosphatase activity (Trasar- Cepeda *et al*.,1990; Saa *et al.*, 1993) resulting in increased plant available P.

2.3.5. Effect of biochar on N uptake

A lot of works have shown that biochar is able to improve soil properties, included soil pH, and CEC (Chan *et al.,* 2008; Masulili*et al*., 2010), soil aggregation, soil water holding capacity and soil strength (Chan *et al*., 2008), and to increase soil biology population and activity (Rondon *etal.,* 2007) . Observation of Steiner *et al.* (2007) indicates that in the long term, application of biochar increases plant nutrient availability and soil productivity. Increasing of crop yield with biochar application has been shown by Islami *et al*., (2011a), Sukartono *et al*., (2011) and Yamato *et al*.,(2004) for maize (Zea maysL.); Tagoe *et al*., (2008) for soybean (Glicine max(L) Merr.); and Islami *et al*.,(2011b) for cassava (Manihot esculenta Cranz). One of the reasons for increasing crop yield with biochar application is the increasing of nitrogen uptake from the applied fertilizer (Steiner *et al*., 2007; Widowati *et al*., 2011). This is as the result from the decrease of nitrogen lost due to the increase of soil CEC with biochar application (Chan *et al*., 2008; Masulili *et al*., 2010) or because of the biochar ability to inhibit N-NO3 transformation from N-NH4 released by fertilizer (Widowati *et al*., 2011).Application of a nutrient rich wheat-straw biochar (20 and 40 t ha $^{-1}$) to a calcareous loamy soil resulted in no changes in soil mineral N concentrations but nevertheless there was a significant maize yield increase, accompanied by increased total soil N content and agronomic N-use efficiency during a 4-month field trial (Zhang, 2012). Uzoma*et al*., (2011) conducted a glasshouse experiment where a biochar manufactured from cow manure (500^{0}C) was applied at increasing rates to a sandy soil, subsequently planted with maize. Both maize yield and N uptake increased with increasing biochar rate, indicating N release from the biochar.

N-enriched biochar, Day *et al*. (2004) suggested that biochar produced at a lower temperature of 400°C to 500°C is more effective in adsorbing ammonia than that produced at higher temperatures (700°C to 1000°C). Similarly, Asada *et al.*(2002) compared adsorption properties of bamboo biochar prepared at 500°C, 700°C and 1000°C and found that only the biochar prepared at 500°C was effective in adsorbing ammonia. They attributed this to the presence of acidic functional groups, such as carboxyl, formed as a result of the hemolysis of cellulose and lignin at temperatures of 400°C to 500°C. Acidic functional groups are effective in chemical adsorption of basic ammonia. Day *et al*. (2004) also proposed using biochar to scrub fossil fuel exhausts from coal-fired power plants in combination with hydrated ammonia. In the process, CO_2, NO x and SO x emissions are directly captured at the smokestacks, which reduce air pollution and greenhouse gas emission. The biochar is converted in the process to valuable N and S fertilizers with C sequestration value. However, as pointed out by Asada *et al*. (2002), the effectiveness of gas capture by biochar depends upon the pyrolysis temperature, which is different for varying nutrient elements.

2.3.6. Influence of biochar addition on soil microbial diversity

Bio chars, when incorporated into soils, can improve soil quality and may also serve as a means to increase sequestration rates of atmospheric carbon (Lehmann *et al.,* 2006; Lehmann, 2007a). Biochar is produced by thermally degrading (charring or pyrolyzing) biomass-derived feed stocks under oxygen limited conditions. Despite the potential usefulness of biochar for soil management applications, our knowledge of how these materials influence soil physical, chemical and biotic properties is limited compared to other soil amendments (Lehmann, 2007b).

During biomass pyrolysis, the molecular structure of the biochar feedstock changes, yielding highly aromatic, and graphitic C containing bio chars (Glaser *et al*., 1998), which are often highly resistant to microbial decomposition (Preston and Schmidt, 2006). Due to its complex chemical structure, biochar exhibits a long mean residence time in soil, estimated between 1,000 to 10,000 years (Skjemstad *et al*., 1998; Swift, 2001; Cheng *et al.*, 2008; Lehmann *et al*., 2008; Liang *et al.*, 2008; Kuzyakov *et al*.,2009; Major *et al*., 2010). Given this recalcitrance, biochar is beginning to receive attention as a potential means for delivering and storing C in soils on a stable and long-term basis (Lehmann, 2007a,b).The implications of biochar additions on the soil microbial community are less clear. Investigation of this interaction has predominantly shown that the soil microbial biomass and/or microbial activity have increased with biochar additions (e.g. Steiner *et al*., 2008; Kolb *et al*., 2009). It has been hypothesized that biochar can provide a microbial refuge due to its porous nature (Peitikainen *et al*., 2000).

The size of the microbial community can be linked to nitrogen mineralization within the soil (Zaman *et al*., 1999). As a large portion of crop nitrogen is derived from biological processes, changes in microbial processes derived from biochar addition to soil must be both investigated and documented. Biochar additions can enhance net nitrification in pine forest ecosystems (DeLuca *et al*., 2006); such changes have yet to be seen in agricultural soils.

Arbuscular mycorrhizal fungi (AMF) are thought to be one of the most important soil microbial groups in the context of modern organic agricultural practices (Piotrowski and Rillig, 2008) and land reclamation (Renker *et al.,* 2004). AMF fungi are obligate symbionts that colonize the roots of more than 80% of terrestrial plants. They provide a variety of benefits to their hosts, including increased nutrient uptake under low-input conditions (Smith, 2008), resistance to plant pests, improved water relations and drought resistance, and increased growth and yield. Although the benefits of AM fungi are more common for nutrients that are immobile in the soil solution, such as phosphorus (P) and zinc (Abdel and Piao., 2011), AM fungi can also enhance the nitrogen (N) nutrition of their hosts (Cheng,2004 and McFarland, 2010). The extra radical AM fungal hyphae effectively acquire nitrate (NO_3) (Tobar, 1994 and Bago,1996), ammonium (NH_4^+) (Johansen, 1996 and Breuninger, 2004), and amino acids (AAs) from the external medium (Azcón, 2001 and Hodge,2001).

The passage of AM into the plant roots is through their hypha, which would eventually form the arbuscules and the vesicles. Arbuscules are branched structural hypha, which are the place of nutrient exchange with the plant roots. Vesicles are the specialized storage organelles with numerous and large vacuoles, which can greatly help the host plant especially under different stresses such as salinity and heavy metals. The onset of the symbiosis and the beneficial effects of the two symbionts become likely through the communication of some signal molecules. Harrison, (1999), Matusova *et al*,(2005).

AM are able to enhance plant tolerance to different stresses such as soil salinity and drought, soil compaction, heavy metals and pathogens Davies,(1993) Miransari *et al.*, (2008). Plant responses to different species of AM are different, and since AM greatly affect the variousness, biomass and nutrients uptake of plants, AM species determine very much the structure of plant communities van der Heijden, *et al.,* (1998), Scheublin *et al.,* (2004). Different efficiency of plants species in symbiosis with AM species affects their ecological functioning. Miransari *et al*, (2007).

AMF form symbioses by colonizing the root tissues of approximately 2/3 of known plant species, including many important crops (Trappe, 1987). AMF are obligate biotrophs, which cannot complete their life cycle without receiving fixed carbon (simple sugars) from their host plant (Smith and Read, 2008). In exchange for these sugars, AMF provide their hosts with benefits including increased access to immobile nutrients, especially phosphorus, improved water relations, and greater pathogen resistance (Newsham *et al*., 1995; Smith and Read, 2008). Therefore, soil amendments which increase AMF abundance and/or functionality could be beneficial to plant hosts and result in improved soil quality via influences on soil structure (Rillig and Mummey , 2006).

Recent studies indicate that soil biochar amendments can increase AMF percent root colonization among plants growing in acidic soils (Ezawa *et al*., 2002; Matsubara *et al*., 2002; Yamato *et al*.,2006). Although the mechanisms responsible are poorly understood, modulation of soil pH likely plays a role (Warnock *et al*., 207). In soils having near neutral pH, where modulation of soil pH would be less pronounced, bio char influences on AMF abundances, e.g. root colonization, are not known.

The physical and chemical properties of biochar are influenced by both the feedstock (Keech *et al*., 2005; Gundale and DeLuca, 2006) and the maximum temperature attained during pyrolysis (Gundale and DeLuca, 2006; Lehmann, 2007 b). In terms of feed stocks, the studies reporting positive interactions between biochar and AMF also reported using biochars derived from herbaceous plant materials, most commonly rice husks (Warnock *et al.*, 2007).

2.3.7. Influence of bio char on AMF colonization

Pioneering studies, conducted primarily in Japan, where biochar application to soil has a long tradition (Ishii and Kadoya 1994), provided evidence that biochar can have positive effects on the abundance of mycorrhizal. Soil micro-organisms, especially arbuscular mycorrhizal fungi (AMF), in addition to ectomycorrhizal fungi (ECM) and ericoid mycorrhizal fungi (ERM), have well-recognized roles in terrestrial ecosystems (Zhu and Miller 2003; Rillig; Read *et al.*, 2004;

Rillig and Mummey, 2006). Mycorrhizal fungi are frequently included in management, since they are widely used as soil inoculum additives (Schwartz *et al.*, 2006). With both biochar additions and mycorrhizal abundance subject to management practices, there clearly are opportunities for exploiting a potential synergism that could positively affect soil quality (Daniel, 2007).

The high root colonization and arbuscule abundance, frequently observed when +M plants were fertilized with the highest P rate, does not seem to be linked with the improvement of plant P uptake. These results in agreement with Feddermann *et al*. (2010) who underlined that the effective plant P uptake from the fungus is not directly correlated with the potential P supply capacity, i.e., a high colonization degree or a high proportion of intraradical structures capable of nutrient exchange. Arbuscular mychrohizal fungi (AMF) are symbiotic soil fungi that colonize roots of about 80% of vascular plants (Vierheirlig,2004) and is one of the soil microorganism that form essential components of sustainable soil-plant system (Hause and Fester,2005; Budi *et al*. 2012). They improve plant fitness and soil quality (Barea *et al*., 2002; Duponnois *et al*., 2005; Wu *et al.,* 2010), increased plant uptake of P and N mainly in acidic soil (Goussous and Mohammad,2009; Guissou,2009; He *et al*.,2009; Rotor and Delima, 2010; Budi and Christina 2013). They also produced plant growth hormones (Herrera-Medina *et al.*,2007), and defending root against some plant pathogens (Bachtiar *et al.*,2010).Colonization of roots by AMF enhanced crop productivity by enhancing tolerance to various biotic and abiotic stress factors (Al-Garni, 2006; Khaosaad *et al*., 2007; Javaid and Riaz, 2008).

2.3.7.1. Role of AMF on P uptake by pepper

Colonization of roots with Arbuscular mychrohizal fungi (AMF) often improves the phosphorus nutrition of host plants growing on soils with sparingly soluble P forms (Shenoy and Kalagudi, 2005). Increased absorption surface and lower threshold concentration for P uptake in mychrohizal root systems are contributing factors (Peterson and Massicotte, 2004). Recent evidences suggest that AMF can provide the dominant route for plant P supply, even when overall growth or P uptake remains unaffected (Smith *et al.,* 2003). Improved uptake of other mineral elements by mychrohizal roots has also been demonstrated for nitrogen, potassium(George *et al*., 1995).

According to (Giulia, 2012) the AMF and P uptake which is the fruit showed the highest P concentration in the plant. Soil inoculation is effective in improving fruit P concentration both in not and low P fertilized plants up to the maximum value registered in the most fertilized ones. Therefore, the association with introduced G. intraradices seems to be more effective in acquisition and uptake of P when P soil availability is not excessive. Probably, the introduced G. intra radices is involved in P acquisition (abundance or functionality of extra radical mycelium), P delivery at the root–arbuscule interface, and P uptake (i.e., P transporters activity; Smith *et al*., 2010; Feddermann *et al*., 2010).

AMF colonization had a significant effect on uptake of P and Zn. Root colonization by AMF improves P (Smith *et al.*, 2003) and Zn (Gao *et al*., 2007) uptake per unit of root length due to the enhancement of root surface area by hypha growth and providing an extra route for P uptake as mychrohizal pathway.

2.3.7.2. Role of AMF on N uptake by pepper

The role of AMF In terms of the mechanism of N uptake, assimilation of $NH4^+$ is the principal means of N absorption in AM fungal systems. The uptake of $NH_4{}^+$ by the fungi is probably mediated by a specific carrier (Botton, 1999). This mechanism is supported by characterization of GintAMT1, which encodes the high-affinity NH_4^+ transporter in the AM fungus G. intraradices (López, 2006). Whereas Gint AMT1 plays a role in mycelia uptake of NH_4^+ at low concentrations in the medium, GintAMT2, which is constitutively expressed in N-limiting conditions and transiently induced after exposure to different N sources, plays a role in retaining the NH_4^+ derived from the metabolism of different N sources (Pérez , 2011).

The importance of other soil organisms for plant growth is well demonstrated by the symbiotic Relationship between mychrohizal fungi and plants. Mycorrhizae are able to form a symbiosis with about 90% of all terrestrial plants (He and Nara, 2007). Arbuscular mycorrhizal (AM) fungi are by far the most abundant of all mychrohizal. They are characterized by the formation of an extra radical mycelium and branched haustorial structures within the cortical cells, termed arbuscules (Hock and Varma, 1995).

According to Kyounga (2009) an increased N content both in the roots and shoots was found for the plantlets raised with Methylobacterium and AMF treatments, singly as well as in combination. Inoculation of red pepper plants with the either of the two bacterial strains had a profound effect on various growth parameters, including length and biomass of the plants. Inoculation of AM fungi alone increased the total dry weight in comparison to the un inoculated plants, but better results were observed when AM fungi was co-inoculated with the two bacterial strains. Plants co-inoculated with M. or yzae CBMB110 and AM fungi had the highest weight (dry and fresh) compared to all other treatments. It has been previously reported that the dual inoculation of AM fungi with other beneficial microorganisms increased dry matter yields (Antunes and Cardoso, 1991; Boby *et al*., 2008; Medina *et al*., 2003; Ortas *et al*.,2002).

2.8. Effect of Biochar as Affected by its Application Rate

It depends on many factors including the type of biomass used, the degree of metal contamination in the biomass, the types and proportions of various nutrients (N, P, etc.) and also on edaphic, climatic and topographic factors of the land where the biochar is to be applied. Given the variability in biochar materials, nature of crop and soils, users of biochar should consider testing several rates of biochar application on a small scale before setting out to apply it on large areas. Experiments have found that rates between 5-50 t / ha (0.5-5 kg / m2) have often been used successfully. While no recommended application rates for biochar can be given, biochar should be applied in moderate amounts to soil. Rates around 1% by weight or less have been used successfully so far in field crops (Major, 2013).

Research suggests that even low rates of biochar application can significantly increase crop productivity assuming that the biochar is rich in nutrients which that soil lacks (Winsley, 2007). In the case of piggery and poultry manure biochar, the biochar works both as an organic fertilizer and soil conditioner with agronomic benefits observed at low application rates (10 t/ha) (Chan *et al*., 2007). Application to soils of higher amounts of biochar may increase the carbon credit benefit; but, in nitrogen-limiting soils it could fail to assist crop productivity as a high C/N ratio leads to low N availability (Lehmann and Rondon, 2006). Crop productivity benefits of higher biochar application rates can be maximized only if the soil is rich in nitrogen, or if the crops are nitrogen-fixing legumes. Therefore, application of biochar to soils in a legume-based (e.g. peanut and maize) rotational cropping system, clovers and lucerne is more beneficial. Biochar application rates also depend on the amount of dangerous metals present in the original biomass. The chance of bio-magnification also depends on the amount of a given metal in the soil.

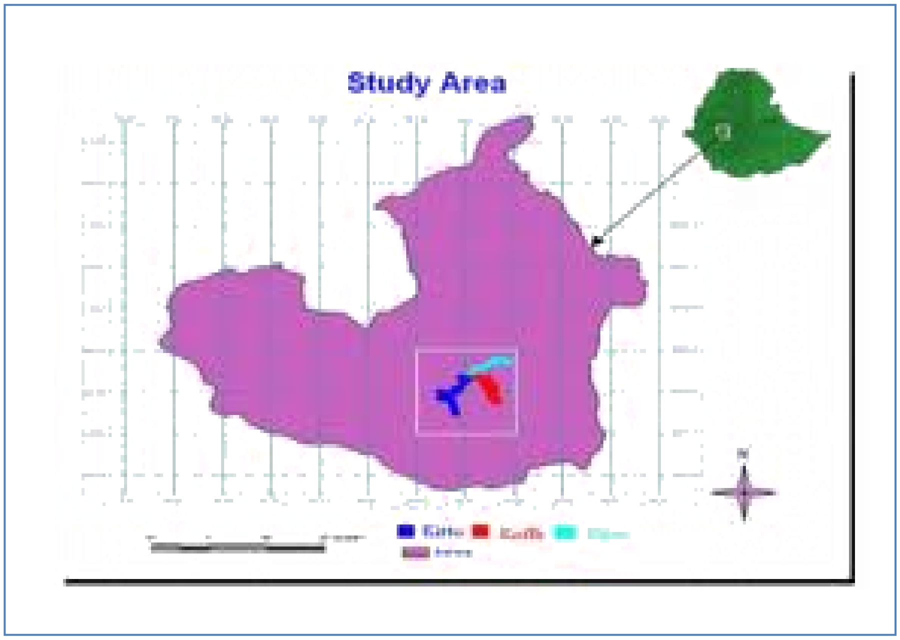
Biochar materials can differ widely in their characteristics, thus the nature of a specific biochar material (e.g. pH, ash content) also influences application rate. Several studies have reported positive effects of biochar application on crop yields with rates of 5-50 tons of biochar per hectare, with appropriate nutrient management. This is a large range, but often when several rates are used, the plots with the higher biochar application rate show better results (Chan *et al.*, 2007, 2008; Major *et al*., 2010b). Since the C content of biochar materials varies, it may be appropriate to report application rates in tones of biochar-C per hectare, as opposed to tons of bulk biochar material. A 10 t/ha application of poultry manure biochar contains much less C (and more ash) than an equivalent application of wood waste biochar. However, “high-ash” biochar can constitute managing soil fertility at the field level. Most biochar materials are not substitutes for fertilizer, so adding biochar without necessary amounts of nitrogen (N) and other nutrients cannot be expected to provide improvements to crop yield. Instances of decreasing yield due to a high biochar application rate were reported when the equivalent of 165 t of biochar / ha was added to a poor soil in a pot experiment (Rondon *et al*., 2007).

3. MATERIALS AND METHODS

Description of the Study Area

The study was conducted under green house at Jimma University College of Agriculture and Veterinary Medicine (JUCAVM), Southwest Ethiopia in the year of 2015-2016. The area is situated at about 356 km to South west of Addis Ababa which is located at 7°, 33 N and 36°, 57' E at an altitude of 1710 meter above sea level. The mean annual maximum and minimum temperatures are 28.9°C and 11.4°C and the relative humidity is between 91.4% and 39.92%;the mean annual rainfall of the study area is 1500 mm (BPEDORS, 2000).The soil of the area is dominated by Nitisol (Michéli,2006).

During the study period, the average mean greenhouse temperatures was28.63^0 C and relative humidity was 49.88%from December to April, 2015/16.The initial soil samples on analysis gives soil pH 4.59, EC 0.06, CEC 7.42 and organic C 2.16 %, total N 0.15% and available P 1. 96 mg / kg.



Source: Jimma Agricultural Research Center

Figure 1. Illustrates soil sample collection site for the study from Jimma Agricultural Research Center at Melko field crops research site 1.

3.1. Experimental Materials

3.2.1. Biochar material production

Biochar was prepared from coffee husk which is selected based on its wide spread availability in the region. Coffee husk biomass was obtained from Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) using a pyrolysis unit at two different pyrolysis temperatures 350^0C and 500^0 C for three hours of residence time (Duma*et al.*, 2015). According to (Lehmann, 2007; Abebe, 2012), the materials were placed in the tight containers for pyrolysis in a deferent temperature ^{0}C for some hour residence time. A portion of biochar was grind and sieved with 0.25 mm square-mesh for the physicochemical analysis of the biochar.

3.2.2. Soil and biochar sampling and analysis

Prior to planting, soil used in this study was collected from the field located at Jimma agricultural research center at Melko field crop research site 1 the sample profile depth of 0 to 20 cm and brought to JUCAVM research site in October 2015. The soil was selected due to highest acidic nature (pH 4.5) the area which is Nitisol type. Then the soil was air-dried, mixed thoroughly and a portion of it was sieved to < 2 mm sieve and used for the analysis of soil physicochemical parameters. After harvest of hot pepper soil samples were collected from each treatment pot and analyzed. A composite coffee husk Biochar samples was collected after pyrolysis (350^0C and 500^0C) for physicochemical characterization of the biochar before application to the pot. The entire soil and Coffee husk biochar samples were analyzed at JUCAVM soil testing laboratory following standard laboratory procedures.

The soil samples were analyzed for soil texture, soil pH, EC, organic C, total N, available P and cation exchange capacity (CEC). Soil pH was determined in water suspension, at the soil: water ration of 1:2.5 using glass electrode attached to digital pH mater as described by (Van Reeuwijk, 1992).The EC of the soil soluble salt was analyzed at the soil: water ration of 1:5 using conductivity mater. Soil texture was determined by hydrometer method (differential settling within a water column) using particles less than 2mm diameter (FAO, 2008). This procedure measures percentage of sand (0.05 - 2.0 mm), silt (0.002 - 0.05 mm) and clay (<0.002 mm) fractions in soils.

3.2.2.1. Organic carbon content %, total nitrogen% and available phosphorus (ppm)

Organic carbon content of soil and biochar was determined by the dichromate oxidation method and organic matter content was estimated from it multiplying by 1.724 (Walkely and Black, 1934).Percent total nitrogen in the soil and biochar was also determined by the modified Kjeldal methods (Bremner and Mulvancy, 1982).where by the ammonia evolved was collected in a boric acid solution in the presence of indicators (methyl red and bromocresol green) and titrated with 0.1N H_2SO_4 to pink end color (Sahlemedhin and Taye ,2000). Available phosphorus in the study soil was determined using the Bray II method in extraction method as described by (Bray and Kurtz, 1945).0.2g of soil was mixed with 14 mL extracting solution bray, containing 0.03M NH_4F and 0.025 M HCL. The solution was shaken for one minute and filtered through what man filter paper. The 2 mL of the sample was pipetted to a test tube and 8mL boric acid as well as 2 mL mixed reagent were added. Solutions were left for about one hour to develop the blue color. Absorbance was measured at 882nm with a UV/VIS spectrophotometer and a plant available P concentration in the soil samples was derived from the calibration curve.

Biochar was characterized for pH, EC, CEC organic C, total N, Available P and ash content. Biochar pH and Electrical conductivity (EC) were measured in distilled water at 1:10 biochar to water mass ratio after shaking for 30 min (ASTM Standard, 2009). Available phosphorous (P) was determined by using the Olsen extraction method (Shaheen *et al*., 2009). Soil and biochar 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 ash content of the biochar was also done in dry ash procedures using a high temperature muffle furnace capable of maintaining temperatures between 500 and 600 ^{0}C. The percentage of ash was calculated on wet weight basis as follows:

$$\%ASH(wet) = \left[\frac{(wt.crucible + ash) - wt.crucible}{wt.crucible + sample - wt.crucible} \right] x100$$

3.2.3. Plant material

Hot pepper (*Capsicum annuum* L.) variety Mareko fana was obtained from Jimma Agricultural Research Center (JARC).This Varity released in 1976 from Melkassa Agricultural Research Center (MARC) is adapted to 1400-2200 m.a.s.1 with the temperature of 20/29^{0}C and rain fall of 600-1337 mm (EARO, 2004). This variety was selected due to its high yielding capacity and marketable qualities (Delelegn, 2011).

3.2.4. Fertilizer materials

In this study UREA (46% N) and DAP (18% N and 46% P_2O_5) were used as a sources of Nitrogen and phosphorus fertilizers. In Ethiopia, 100 kg N ha $^{-1}$ and 100 kg P ha $^{-1}$ are recommended to optimize hot pepper productivity according to (EIAR, 2007). For this recommendation the amount of application which is 132 kg ha $^{-1}$ of urea and 217kg DAP ha $^{-1}$ were used.

3.2.5. Treatments combination and experimental design

The experiment was conducted with treatment combination of two biochar pyrolysis temperature (350^0 C and 500^0 C), three different biochar application rates 0, 6 and 18t ha^{-1}(0, 15.24 and 45.7 gm.), and three inorganic fertilizer application rates 0 %, 100% and 60% Urea and DAP (For 100 % urea and DAP at 0.55gm of DAP and 0.33gm of urea/pot, for 60% of urea and DAP 0.33gm of DAP and 0.20gm of urea. The treatments were arranged in Randomized Complete Block Design (RCBD) in factorial arrangements (2x2x3=12) treatment plus a negative control (with no application) and positive control (with 100% and 60% inorganic fertilizer) with four replication and 60 observations and two pots per treatment was used. The details of the treatment combinations are indicated in table 3 below. The design (RCBD) was found appropriate due to greenhouse situation that manifest semi field condition.

Table 3 Details of the treatment combinations of the experiment

No	Symbol	Description
1	Control	Negative control
2	Control	Positive control 100% inorganic fertilizer
3	Control	Positive control 60% inorganic fertilizer
4	T1 R1 IF0	Temperature 350^0C+6t ha^{-1} biochar rate + 0% inorganic fertilizer
5	T1 R1 IF1	Temperature 350^0C+6t ha^{-1} biochar rate + 100% inorganic fertilizer
6	T1 R1 IF2	Temperature 350^0C+6t ha^{-1} biochar rate + 60% inorganic fertilizer
7	T1 R2 IF0	Temperature 350^0C+18t ha^{-1} biochar rate + 0 %inorganic fertilizer
8	T1 R2 IF1	Temperature 350^0C+18t ha^{-1} biochar rate + 100% inorganic fertilizer
9	T1 R2 IF2	Temperature 350^0C+18t ha^{-1} biochar rate + 60% inorganic fertilizer
10	T2 R1 IF0	Temperature 500^0C+6t ha^{-1} biochar rate + 0% inorganic fertilizer
11	T2 R1 IF1	Temperature 500^0C+6t ha^{-1} biochar rate + 100% inorganic fertilizer
12	T2 R1 IF2	Temperature 500^0C+6t ha^{-1} biochar rate + 60% inorganic fertilizer
13	T2 R2 IF0	Temperature 500^0C+18t ha^{-1} biochar rate + 0% inorganic fertilizer
14	T2 R2 IF1	Temperature 500^0C+ 18t ha^{-1} biochar rate + 100% inorganic fertilizer
15	T2 R2 IF2	Temperature 500^0C+18t ha^{-1} biochar rate + 60% inorganic fertilizer

3.3. Experimental Procedures

3.3.1. Pot filling and preparation

One hundred forty-four pots were prepared and given tag for identification purpose. The diameter of each pot was 18 cm with the volume of ($V = 0.00458$ m^3).These plastic pots were holed up uniformly at 4 places at the bottom to improve soil water drainage. Of these soils, 5.00 kg was filed in the each pot. Coffee husk Biochar was prepared in the treatment of 0, 6 and 18t ha^{-1} (0, 15.24 and 47.7gm) was mixed with the acidic soil in the middle and watering before transplanting of the seedling. Urea and DAP were applied at three application rate. 100% (0.55gm of DAP/pot and 0.33gm of urea /pot), 60% (0.33gm DAP/pot and 0.20gm of urea/ pot) and 0% without application of urea and DAP. DAP was applied at transplanting time and urea was applied 3rd week after transplanting.

Crop management practice done based the following stapes, irrigation water was applied to the transplants on surface to facilitate plants establishments, and then up to the time of full plant establishments, water was applied using watering can once a day equally for all pot. Then based on the environmental conditions watering was done every two days interval that was three times a week afterwards. Hand weeding was done frequently as per the emergence of the weeds. Randomization of the pot with in the blocks was done in every three days.

3.3.2. Preparation of seedling

Hot pepper seedlings were raised in the nursery plastic well prepared seedbed. The seeds were sown in hall of the plastic seed bed two seed per hall was planted and the beds were covered with dry grass mulch until emergence. Complete germination of the seeds took place within 5 to seven days after sowing. Watering of the seedbed was done always in the morning and afternoon using watering can. The seed beds were watered before uprooting the seedlings in order to minimize the damage of the roots. Healthy, uniform and four weeks old seedlings were transplanted to well-prepared pots.

3.4. Data Collection, Sampling, and Analysis

Data were collected from two pots per treatment one pot was used for AMF analysis and the another for growth and yield ,tissue analysis and soil character was used at the time of recording and sampling was recorded per pot at each block, as indicated below.

3.4.1. Growth parameter

1. **Days to flowering**: It was determined by taking of flowered plant at different days after transplanting.
2. **Plant height (cm):** Plant height was measured at physiological maturity from the ground level to the tip most growing points.
3. **Number of branches per plant:** Number of branches per plant was measured by counting numbers of primary, secondary and tertiary branches per stem at physiological maturity.

4. **Number of leaves per plant**: Number of leaves was measured by counting the total numbers of leaves after flowering.

5. **Days to Maturity**: The total days from transplanting to the date of first harvest was recorded in each pot in days of maturity.

3.4.2. Yield and yield related component

1. Number of fruits per plant: was measured by counting number of green fruits from individual plants during first harvest up to the last harvest.

2. Total fresh fruit weight (gm /plant): Weight of total (marketable and unmarketable) fruits was measured by weighing its fresh weight at each successive harvesting by using balance. From the plants was recorded and summed up to estimate yield per plant.

3. Fruit and haulm dry weight (gm/ plant): a matured fruit pod from each pot was allowed for each pinking and lastly uprooted the whole plant to dry in an oven at 70^0C until constant weight was reached. Then the weight obtained was recorded as fruit and haulm dry weight.

3.4.3. Assessment of AMF root colonization

For AMF Root Colonization sample was determined from fifty for (18x3) pots. The fine root with 10-15 cm length from each sample pot was collected, washed with water, cut into 4-5 cm pieces, placed inside sample collecting test tubes and fixed in 50% Formalin-Acetic acid-Alcohol (FAA).They were then taken, to the Microbiology laboratory in Addis Ababa University and stored at 4^0C until analysis begun (Zhoa *et al*., 2001).

The stored roots were taken from 50 % FAA-fixed sample, washed several times in tap water to remove the soil from the root and cut into segments about 1 cm long. About 0.5 g of root segments were cleared in 10 % (w/v) KOH at 90^0 C in a water bath for 2-3h 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 3 minutes at room temperature. Thereafter, the roots were treated with 10% HCl (v/v) for 15-20 minutes at room temperature and finally stained in 0.05% w/v try pan blue in lacto glycerol (1:1:1 lactic acid, glycerol and water) at 90 ^{0}C for 30 minutes in a water bath (Brundrett *et al*., 1996). Samples were drained and washed thoroughly with distilled water at the end of every action except HCl treatment. The samples were left in distaining solution (14:1:1 lactic acid: glycerol: water) for more than two days in a dark room. The roots were then mounted on microscopic slides beneath a 50x24 mm cover slip. Fungal colonization was quantified using the magnified intersection method of Mc Gonigle *et al*. (1990) under a compound-light microscope (OLYMPUS-BX51) at a magnification of x200. Accordingly, 150 intersections were observed for each sample. The presence of Arbuscular mycorrhizal hyphae, vesicles, and Arbuscules were recorded.

Staining of root (Brundrett *et al.,* 1996)

Quantified using the magnified intersections method (McGonigle.,1990)

- Total intersection (G) N+A+V+H
- %N= (N)
- %HC= (G-N)/G*100%
- %AC= A/G*100%
- %VC= V/G*100%

G: total count N+A+V+H

A: arbuscules% AC: Percentage of Arbuscular colonization

V: vesicles% VC: percentage of vesicular colonization

H: hyphae% HC: hyphal coloinzation or Total colonization

N: negative %N: negative colonization

3.4.4. Plant tissue sampling and analysis

All plant samples were prepared and analyzed at Jimma University College of Agriculture and Veterinary Medicine animal nutrition laboratory following standard laboratory procedures. Three hot pepper plants per treatment were taken from each potted plant just at maturity of vegetative stag (at tender and dipper green color fruit) of hot pepper. Each collected fruit and uprooted sample tissues per plant were separately washed with clean water, chopped and then oven dried at $65-70^0$ C for 24 hours, grind and sieved to1mm (Fahmy,1977) put in a paper bag and labeled accordingly for nutrient content determination (tissue analysis). The finely dried fruit and tissues were wet digested as described by Wolf (1982).

Nitrogen in fruit and haulm sub-samples were quantitatively determined by a Kjeldahl procedure, (Bremmer and Mulvancy, 1982). Whereby the ammonia evolved was collected in a boric acid solution in the presence of indicators (methyl red and bromocresol green) and titrated with 0.1N H_2 SO_4 to pink end color (Sahlemedhin and Taye, 2000). The total N uptake biomass was calculated by summing up the N uptake by fruit and haulm.

Phosphorus in fruit and haulm sub-samples were carried out on the digest aliquot obtained through wet digestion by (Chapman and Pratt, 1996; Ryan *et al.,* 2001). Plant samples of 0.5 g were ashed in porcelain crucibles for 5 hours at 550^0C. The phosphorus in the solution is determined calorimetrically by using molybdate and metavanadate for color development. Plant phosphorus is converted to orthophosphates during digestion. These orthophosphates react with 10ml molyb date and give yellow colored unreduced vanado-molybdo-phosphoric hetropoly complex in acid medium. The yellow color is attributed to a substitution of oxyvanadium and oxymolybdenum radicals for the oxygen of phosphate. The reading is made at 460nm wavelength. The P concentration (PC) was expressed in gm P/plant dry weight (Khair *et al*., 2002).Total P uptake by hot pepper was calculated by summing up the P concentration by fruit and haulm then multiplying by the total biomass.

3.7. Data Analysis

All the data were examined for homogeneity of variance and normality. Then, those data which were found to have normal distributions were subjected to analysis of variance using SAS statistical software package 9.3 (SAS, 2008). The differences between treatment means were compared using least significance difference (LSD) test at 5% level of significance. Pearson’s correlation analysis was done to observe the relationship between crop yield parameters and soil properties.

4. RESULTS AND DISCUSSION

4.1. Variation in Major chemical Characteristics of Biochar with Temperature

The selected chemical properties of biochar produced from coffee husk at two different pyrolysis temperatures were shown in (Table 4). The mean pH, EC, CEC, OC, Av.P and ash of the biochar were significantly increase at ($P<0.001$) at elevated pyrolysis temperature of 500^0C. However, total N percentage of the biochar showed non-significant difference at ($P<0.05$) in the pyrolysis. The highest mean values were at pyrolysis temperature of 500^0Cand resulted men value pH (10.55), EC (6.07) and CEC (48.30), OC (19.67), Av. P(15.92) and Ash(29.80) which is significantly higher than pyrolysis temperature of 350^0C.This finding is in agreement with the result reported by Bagreev *et al*. (2001) and Bayu *et al*. (2015). For instance, an increasing pyrolysis temperature from 350 ^{0}C to 500 ^{0}C during production of biochar from coffee husk and corn-cop accompanied by an increase in the pH, EC, CEC, OC and available P (Bayu *et al.*, 2015). In general this increase in the nutrient may be due to the complex and varying changes of biomass during pyrolysis affect both the composition and chemical structure of the resulting biochar, with significant implications for nutrient contents.

Table 4 Selected chemical properties of the biochar produced from coffee husk at two pyrolysis temperature (350 and 500°C).

Biochar pyrolysis temperature	pH-(H_2O)	EC (mS cm $^{-1}$)	CEC (me/100g)	OC (%)	OM (%)	TN (%)	Av.P (ppm)	Ash
CHB(350^0C)	9.68^b	4.70^b	36.30^b	12.09^b	20.84^b	1.68^a	11.01^b	24.05^b
CHB(500^0C)	10.55^a	6.07^a	48.30^a	19.67^a	33.91^a	1.91^a	15.92^a	29.80^a
CV	0.69	1.73	0.30	0.99	0.99	7.90	0.9	0.13
LSD	0.16	0.21	0.32	0.35	0.61	0.32	0.27	0.08

Where: EC: Electrical conductivity, CEC: Cation exchange capacity, OC: Organic carbon, OM: Organic matter, TN: Total nitrogen, Av. P: Available phosphorus, CHB 350^0*C: Coffee husk biochar at 350°C, CHB*500^0*C: Coffee husk biochar at500°C.*

4.2. Effect of Biochar Rate from two Pyrolysis Temperature regimes and integration of Inorganic Fertilizer on Growth and Yield of Hot Pepper

The ANOVA revealed that all of the response variables except for plant height and number of branches were significantly ($P<0.05$) affected by the interaction effect of pyrolysis temperature, biochar rate and inorganic fertilizers. The results are therefore presented and discussed below.

4.2.1. Days to flowering

ANOVA showed that there was a highly significant ($P<0.001$) interaction effect of biochar application rate, pyrolysis temperature, and inorganic fertilizer on days to flowering of hot pepper (Appendix Table1).The treatment with 6t ha^{-1} biochar obtained from 350^{0}C pyrolysis temperature with 100% inorganic fertilizer that gave significantly earlier flowering days of 38.25 and followed by 41.50 days from 6t ha^{-1}biochar obtained from 500^{0}Cwith 100% inorganic fertilizer compared to other treatment (Table 5). Flowering was delayed (69.25days) and (65.25days) at the application of 18 t ha^{-1}and 6t ha^{-1} of biochar prepared at pyrolysis temperature of 350^{0}C and with negative control respectively. The result indicated that soil amendment with biochar and inorganic fertilizers improve the level of available nutrients to enhance plant growth and early flowering. Medina-Lara *et al*.(2008) reported that flower formation increased as soil N and P concentration increased as a result of N fertilizer amendment which might result in higher fruit set and development.

This finding was in agreement with the observations of Giulia(2015) who worked on Pelargonium plants grown in pot biochar 70:30 (v: v) in combination with fertilizer was more effective in enhancing nitrogen and chlorophyll leaf concentrations, and leaf and flower numbers. The same author further discussed that a better plant nutritional status that could be considered the reason for the promotion of leaf formation and flowering. Similarly Graber *et al* (2010) found that well fertilized pepper crop by the addition of biochar prepared from coconut fiber showed an increase in flower number, growth and productivity than the control

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4.2.2. Days to first harvest

ANOVA showed that there was a highly significant ($P<0.001$) effect of biochar rate, pyrolysis temperature and inorganic fertilizer (Appendix Table 1) on days to first harvest (Table 5). Accordingly, the earliest number of days to first harvest maturity were 82, 82and 83 days, were respectively from the treatment of 18t ha^{-1} biochar obtained from 500^{0}C with 100% of the recommended inorganic fertilizer, 6 t ha^{-1} biochar from 350^{0}C with 100% of the recommended fertilizer and 18t ha^{-1} biochar obtained from 350^{0}C with 100% of inorganic fertilizer respectively. The longest days to attain first harvest, 122 days was recorded from the treatment of 18t ha^{-1} from 350^{0}C with 0% inorganic fertilizer,6t ha^{-1} from 350^{0}C with 60% inorganic fertilizer and 6t ha^{-1} from 350^{0}C with 0 % inorganic fertilizer statistically on par 118.25 days recorded from the negative control(Table 5).

Thus the observed variation in the days to harvest might be due to the direct or indirect effects of biochar in the soil on plant nutrition for early maturity in hot pepper. Biochar can act as a soil conditioner by improving the physical and biological properties of soils such as water holding capacity and soil nutrients retention, and also enhancing plant growth (Sohi *et al*., 2010). On the other hand application of biochar in acidic soils in the absence of inorganic fertilizers may increase the carbon credit benefit; however in nitrogen-limiting soils it could fail to improve crop productivity because of a high C/N ratio which leads to low N availability (Lehmann and Rondon, 2006). Hence it might reduce crop productivity temporarily (Lehmann *et al.* 2003). Crop productivity benefits of higher biochar application rates could be maximized only if the soil is rich in nitrogen, or if the crops are nitrogen-fixing legumes.

4.2.3. Plant height

ANOVA revealed that there was a significance interaction effect ($P<0.05$) (Appendixes table 2) of biochar rate and inorganic fertilizer on the plant height of hot pepper (Table 6). The highest plant height of 59.25 cm was measured from the treatment of 6 t ha^{-1} biochar with 100% inorganic fertilizer. Similarly biochar integrated with fertilizer and sole fertilizer application gate similar plant height. The lowest plant height 41.25 cm was measured from 6t ha^{-1} biochar with no inorganic fertilizer and control (42.00 cm).This finding is in agreement with the result reported by Ellen *et al.* (2010) tomato plant heights were significantly greater in the 1 and 3% biochar treatments at all measurement times as compared with the control. George (2013) has also observed a significant increase in plant height of hot pepper among the various days after transplanting in the major and minor planting season with treatment of 100% fertilizer + 3.5 t biochar.

This improvement in growth was due to application of biochar and some amount of inorganic fertilizer that raised the availability of plant nutrient. Specially nitrogen and phosphorus which have enhanced the vegetative growth of plants by increasing cell division and elongation. This result is in agreement with the finding of Lehmann *et al*, (2003) who reported that using wood biochar at rates of 68 t ha $^{-1}$ to 135 t ha $^{-1}$ increased rice biomass by 17 per cent and cowpea by 43 per cent in a pot experiment.

4.2.4. Number of branch per plant

ANOVA revealed that there was a significance interaction effect ($P<0.05$) (Appendixes Table 2) of biochar rate and inorganic fertilizer on branch number of hot pepper (Table 5). The highest number of branches of 5.25 was recorded from the treatment combination of 6 t ha^{-1} biochar with 100% inorganic fertilizer. This value was not significantly different from the 18 t ha^{-1} biochar with 100% inorganic fertilizer and 18 t ha^{-1} biochar with 60% inorganic fertilizer treatment and all applied biochar with inorganic fertilizer treatment (table 5). The lowest number of branches which is 1.75 recorded from the control. Thus the observed variation among treatments might be due to the ability of biochar to alter physicochemical properties of the acidic soil so that the applied inorganic fertilizers are readily available. Similar study suggested that biochar amended pepper plants develop rapidly and grater difference in canopy width was observed as a result of enhanced branch number (Graber R.*et al.*, 2010). Similarly, Carter *et al.* (2013) reported that rice-husk biochar produced by gasification process increased final biomass, root biomass, plant height and number of leaves compared to no biochar application.

4.2.5. Number of leaves per plant

The number of leaves per plant was significantly affected by the interaction effect of biochar rate, pyrolysis temperature and inorganic fertilizer (Appendix 1). The highest mean number of 34.5 leaves was counted from the treatment of 18 t ha^{-1} biochar obtained from 500 ^{0}C with 100% inorganic fertilizer and the lowest mean number of 13.5 leaves per plant was counted from control. This result is in agreement with the finding of Appah (2013) who reported that application of 7 t biochar along with 50% inorganic fertilizer resulted in the highest number of leaves.

The higher number of leaves per plant might be due to the amended acidic soil by biochar and sole recommended inorganic fertilizer application which could be attributed to the significant role for the increment of available nutrients in the plant systems for efficient photosynthesis, metabolic processes and brought improve pepper growth. Giulia, *et al.* (2015) have also reported that application different rates of biochar along with 30% inorganic fertilizer resulted in the highest leaf number, flower number and floral clusters in pelargonium plants.

4.2.6. Number of fruit per plant

The number of fruits per plant was significantly ($p< 0.01$) affected by the interaction effect of biochar rate, pyrolysis temperature and inorganic fertilizer (Table 5) (Appendix Table 1). The highest mean number of 36.5 fruits per plant was counted from the treatment of 18 t ha^{-1} biochar from 500^{0}C with 100% inorganic fertilizer application and the lowest fruit number 3.5 was counted from the control. The increase in fruit number could be attributed to the increased leaf number which might have worked as an efficient photosynthesis organ and produced high amount of carbohydrates in the plant system. Because the treatment that resulted in high number of leaves also coincided with high fruit number. It could also be associated with other yield attributes like the number of branches per plant which can directly influence the fruit yield per plant. Nanthakumar and Veeraraghathatham (2001) have also reported similar findings in brinjal. Similarly, Chan *et al*. (2008) showed that biochar can significantly improve yield of radish when applied at the rate of 10 t/ha.

4.2.7. Fresh fruit yield

Fruit yield was highly significantly ($P<0.001$) affected by the interaction effect of the three factors in the present study (Appendix Table 1). The mean maximum fresh fruit yield of 0.121 kg/plant was recorded from the treatment of 18 t ha^{-1} biochar from 500^{0}C with 100% inorganic fertilizer and the lowest fresh fruit yield per plant of 0.014 kg/plant was measured from the control treatment. Integrated use of biochar with inorganic fertilizer obviously brought yield increment by 85 % over the control treatment. The significant differences in fruit yield may be attributed to the direct effect of the yield components like fruit number, leaf number and branch number which, by themselves, are influenced by the bio-availability of soil nutrients.

Yield components are one of the most vital attributes to yield and are positively affected by the optimal supply of nutrients. The biochar with increasing of application rate incorporated in the soil is assumed to balance the C/N ratio of the soil, higher organic matter build up and ameliorates acidic soils. This can result in more supply of availability and better translocation of nutrients, to the areal parts of the plant and other compounds led to an improvement in yield and yield related attributes (Malik *et al.*, 2011). The results are also in agreement with the findings of Shashidhara (2000) in chilli.

Table 5 Effect of biochar rate integrated with inorganic fertilizer on hot peppers days to flowering, days to first harvest, plant height, number of leaf, number of fruit, fresh fruit yield kg per plant

Biochar pyrolysis temperature(^{0} C)	Biochar application rate (t ha^{-1})	NP(% RR)	Days to Flowering	Days to first harvest	Number of leaf	Number of Fruit	Fresh Fruit Yield kg /plant
Control	0	0	57.25^{c}	118.25^{a}	13.50^{g}	3.50^{g}	0.014^{m}
		100	50.75^{d}	103.0^{c}	25.00^{b}	20.50^{c}	0.081^{e}
		60	50.75^{c}	103.0^{d}	25.00^{b}	20.50^{d}	0.081^{e}
350	6	0	65.75^{a}	122.0^{a}	13.50^{g}	7.75^{f}	0.050^{i}
		100	38.25^{h}	82.00^{e}	22.75^{bc}	20.50^{c}	0.061^{h}
		60	61.50^{b}	122.0^{a}	21.00^{cd}	17.00^{d}	0.049^{i}
	18	0	69.25^{a}	122.0^{a}	16.00^{fg}	12.25^{e}	0.040^{j}
		100	51.00^{d}	83.00^{e}	24.25^{b}	25.50^{b}	0.097^{c}
		60	44.25^{fg}	103.0^{bc}	17.75^{ef}	19.50^{c}	0.084^{e}
500	6	0	50.50^{d}	110.75^{b}	18.25^{ef}	13.25^{e}	0.036^{k}
		100	41.50^{gh}	$103.0b^{c}$	23.25^{bc}	20.00^{c}	0.080^{f}
		60	50.75^{d}	108.25^{b}	21.00^{de}	18.50^{cd}	0.070^{g}
	18	0	49.50^{de}	98.00^{d}	19.75^{de}	18.75^{e}	0.086^{de}
		100	45.75^{ef}	82.00^{e}	34.50^{a}	36.50^{a}	0.121^{a}
		60	57.50^{c}	103.0^{c}	24.00^{b}	23.75^{b}	0.110^{b}
Mean			**52.38**	**104.86**	**21.27**	**17.90**	**0.069**
LSD(0.001)			**3.76**	**4.32**	**2.67**	**2.35**	**0.0022**
CV (%)			**5.01**	**2.86**	**8.79**	**9.35**	**2.31**

Where: *Where: NP% RR nitrogen and phosphorus with recommended rate. LSD = least significant difference; CV = coefficient of variation. Means sharing the same letter(s) in each column do not differ significantly at 5% P level according to the LSD test.*

Table 6 Effect of biochar rate integrated with inorganic fertilizer on hot peppers plant height and number of branch/plant

Biochar pyrolysis temperature (^{0} C)	Biochar Application rate (t ha^{-1})	NP(% RR)	Plant height cm	Number of branch/plant
Control	0	0	42.00^{d}	1.75^{c}
		100	55.25^{ab}	5.00^{a}
		60	55.25^{ab}	5.00^{a}
300	6	0	41.25^{d}	2.00^{c}
		100	59.25^{a}	5.25^{a}
		60	55.50^{ab}	5.00^{a}
	18	0	47.25^{c}	4.75^{ab}
		100	55.25^{ab}	4.75^{ab}
		60	54.50^{b}	4.50^{ab}
500	6	0	46.75^{c}	2.50^{c}
		100	56.00^{ab}	4.25^{ab}
		60	55.75^{ab}	3.75^{b}
	18	0	48.00^{c}	4.75^{ab}
		100	57.00^{ab}	5.25^{a}
		60	55.50^{ab}	5.25^{a}
	Mean		**52.05**	**4.19**
	LSD(0.05)		**4.34**	**1.04**
	CV(%)		**5.90**	**18.38**

Where: NP% RR nitrogen and phosphorus with recommended rate. LSD = least significant difference; CV = coefficient of variation. Means sharing the same letter(s) in each column do not differ significantly at 5% P level according to the LSD test.

4.4. Effect of Biochar Rate, Pyrolysis Temperature and Inorganic Fertilizer on AMF Root Colonization of Hot Pepper.

AMF root colonization was assessed in terms of the typical fungal structures (Arbuscules, vesicles and hyphal colonization). Hence, each of the fungal structures were highly significantly ($P<0.001$) influenced by the interaction effect of the factors in the present study (Appendix Table 3). The maximum Arbuscular colonization (AC) of 10.87%, vesicular colonization (VC) of 7.75% and hyphal colonization (HC) of 66.49 was attained at the treatment of 18 t ha^{-1} biochar from 500^0C with 60% inorganic fertilizer (Table 7). The lowest root colonization percentage was attained from the control treatment with values of 0.37%, 0.04%, and 1.06% for AC, VC and HC respectively.

In general, the AMF colonization pattern showed heterogeneity among the roots of hot pepper in the present study. It was also observed that percent mycorrhizal root colonization was found at a lower rate when biochar was added along with 100% of the recommended inorganic fertilizer compared to colonization rate when biochar was added alone under both pyrolysis temperatures. The decrease in root colonization by AMF in 100% fertilizer amended treatments may have resulted from an increase in plant available soil P that was in the range of 4.5 – 10.8 ppm (Table 9) as compared to the initial plant available soil P which was 1.96 ppm. It is reported that phosphorus is central to the interaction between plant roots and AMF colonization (Smith and Read, 2008). Various studies suggest that either low or high soil P availabilities could adversely affect AMF abundances in plant roots and soil (Corbin *et al*., 2003; Drow *et al.*,2006). The study was in line with the positive interaction of rice husks biochar with AMF (Warnock *et al.*, 2007).

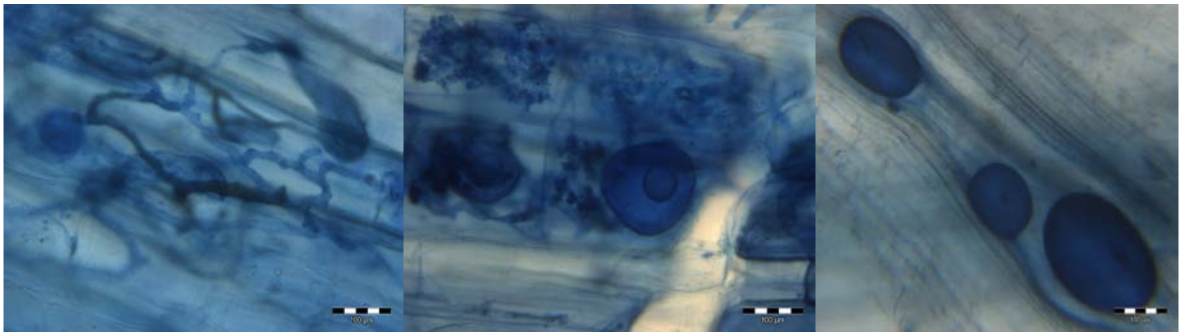

Figure 2. Arbuscular, Vesicle and Hiphale colonization of hot pepper at the study time.

Table 7 Effect of variation in biochar rate, pyrolysis temperature and inorganic fertilizer on hot pepper Arbuscular colonization (AC), Vesicular colonization (VC) and Hyphal colonization (HC).

Biochar pyrolysis temperature (^{0}C)	Biochar application rate (t ha^{-1})	NP (%RR)	AC%	VC%	HC%
Control	0	0	0.37i	0.04^{g}	1.06^{j}
		100	2.42^{g}	0.13^{fg}	2.49^{j}
		60	2.42^{g}	0.13^{fg}	2.49^{j}
350	6	0	8.42^{cd}	6.34^{d}	44.39^{e}
		100	2.68^{gh}	1.05^{f}	12.40^{h}
		60	5.50^{e}	0.45^{g}	33.90^{f}
	18	0	7.67^{d}	6.38^{b}	57.90^{d}
		100	4.00^{f}	0.32^{g}	17.49^{g}
		60	9.88^{b}	6.80^{bc}	64.39^{b}
500	6	0	8.91^{c}	6.81^{b}	63.64^{b}
		100	8.35^{cd}	0.09^{g}	11.49^{hi}
		60	5.91^{e}	1.13^{f}	10.20^{i}
	18	0	9.68^{b}	1.71^{e}	60.14^{c}
		100	8.31^{cd}	0.95^{f}	15.94^{g}
		60	10.87^{a}	7.75^{a}	66.49^{a}
Mean			5.59	2.24	26.13
LSD(0.001)			0.76	0.40	1.70
CV (%)			8.20	10.83	3.93

LSD = least significant difference; CV = coefficient of variation. Means sharing the same letter(s) in each column do not differ significantly at 5% P level according to the LSD test. Where: NP% RR: nitrogen and phosphorus with recommended rate.

4.5. Nitrogen uptake by Hot pepper as Affected by biochar and inorganic fertilizer

Nitrogen uptake by hot pepper fruit, haulm and total plant N was highly significantly affected ($P<0.001$) by the interaction of factors in the present study (Appendix table 4). The highest N uptake by fruits was 5.226g/plant, haulm (2.133g/plant) and total plant was 7.360g/plant which recorded from the treatment of 18 t ha^{-1} biochar from 500^{0}C with 100% inorganic fertilizer respectively. The lowest mean N uptake by fruits was 0.247g/plant, haulm was (0.074 g/plant) and total N was 0.321g/plant recorded from the control treatment (Table 8). The current finding is in agreement with the privies report of Blackwell *et al*. (2010) who observed significantly greater N uptake in wheat where biochar was applied along with N fertilizer rates of 100 kg/ha.

The improvement in the availability of N nutrient was because of the fact that biochar has a macromolecular structure dominated by aromatic C and at the same time biochar is more recalcitrant to microbial decomposition than uncharred organic matter (Baldock and Smernik. 2002). Thus the application of biochar along with inorganic N fertilizer could result in a significantly higher N uptake with an increasing rate of biochar application (Chan *et al.*, 2008).According to Day *et al*.(2004), biochar produced at a temperature of 500°C is more effective in adsorbing ammonia than that produced at higher temperatures (700°C to 1000°C). Similarly, Asada *et al*.(2002) compared adsorption properties of bamboo biochar prepared at 500°C, 700°C and 1000°C and found that only the biochar prepared at 500°C was effective in adsorbing ammonia. They attributed this to the presence of acidic functional groups, such as carboxyl, formed as a result of the pyrolysis of cellulose and lignin at temperatures of 400°C to 500°C. Acidic functional groups are effective in chemical adsorption of basic ammonia.

4.6. Phosphorus Uptake by Hot Pepper as Affected By the Treatments

The interaction of biochar preparation pyrolysis temperature, application rate and inorganic fertilizer had a highly significant effect ($P<0.001$) on P uptake by fruit, haulm and total plant P uptake by hot peppers (Appendix Table 4). The highest P uptake by fruits was 3.313g/plant, haulm was 1.706 g/plant and total plant was 4.847 g/plant was recorded from the treatment of 18 t ha^{-1} biochar at 500^0C with 100% inorganic fertilizer. The lowest mean P uptake by fruits was 0.123g/plant, haulm was 0.072 g/plant and total plant was 0.195 g/plant recorded from the control treatment (Table 8).

All the treatments with no biochar addition showed lower P uptake as compared to biochar treatments along with either 60% or 100% inorganic fertilizers. The increased P uptake due to biochar application could be attributed to the high P content in the biochar and the raised soil pH from 4.59 to 5.66 which cause improved P uptake at the modified soil pH was highly significantly correlated with available P ($r=0.975^{***}$). The modified pH may reduce the activity of Fe and Al. Lehmann and Rondon. (2006) and Uzoma *et al*. (2011) also reported increased P uptake due to addition of biochar in the tropical soils. Moreover, the observed improvements in P uptake were due to biochar application. It was also related to better microbial activity that biochar served as habitat for AMF. The positive association between AMF hyphal colonization and total plant P uptake (r=0. 418***) was positive and significant in the present study which may justify that AMF colonization helped in P uptake by hot pepper. The result of the present study agrees with the earlier finding of Nigussie (2012) who observed that an increase in available phosphorus due to application of biochar was related to the presence of high P content in the biochar feedstock.

Table 8 Effect of variation in biochar rate, pyrolysis temperature and inorganic fertilizer on hot peppers Nitrogen and Phosphorus Fruit, straw and total uptake

Biochar pyrolysis temperature(^{0}C)	Biochar application rate(t ha^{-1})	NP (%RR)	Fruit N(g/plant)	Haulm N(g/plant)	Total N(g/plant)	Fruit P(g/plant)	Haulm P(g/plant)	Total P(g/plant)
Control	0	0	0.247^{g}	0.074^{g}	0.321^{i}	0.123^{f}	0.072^{g}	0.195^{i}
		100	2.283^{d}	1.082^{c}	3.366^{d}	1.883^{c}	1.032^{b}	2.915^{c}
		60	2.283^{d}	1.082^{c}	3.366^{d}	1.883^{c}	1.032^{b}	2.915^{c}
350	6	0	1.032^{e}	0.836^{e}	1.868^{f}	0.599^{e}	0.263^{f}	0.863^{g}
		100	0.910^{ef}	0.176^{fg}	1.086^{h}	1.146^{d}	0.201^{fg}	1.348^{f}
		60	0.687^{f}	0.094^{f}	0.782^{h}	0.656^{e}	0.170^{fg}	0.602^{gh}
	18	0	0.810^{ef}	0.330^{f}	1.140^{h}	0.593^{e}	0.193^{fg}	0.787^{g}
		100	4.126^{c}	1.670^{b}	5.796^{c}	2.075^{c}	1.056^{b}	2.966^{c}
		60	2.160^{d}	1.076^{cd}	3.236^{de}	1.165^{d}	0.790^{cd}	1.955^{de}
500	6	0	0.586^{f}	0.220^{fg}	0.086^{f}	0.210^{f}	0.080^{dg}	0.290^{hi}
		100	1.063^{e}	0.742^{e}	2.786^{f}	1.225^{d}	0.758^{de}	1.983^{de}
		60	1.063^{e}	0.846^{de}	2.786^{f}	1.037^{d}	0.654^{e}	1.692^{e}
	18	0	1.986^{d}	0.919^{cde}	2.906^{f}	1.201^{d}	0.900^{c}	2.024^{d}
		100	5.226^{a}	2.133^{a}	7.360^{a}	3.313^{a}	1.706^{a}	4.847^{a}
		60	4.830^{b}	1.952^{a}	6.782^{b}	2.665^{b}	1.061^{b}	3.657^{b}
Mean			1.949	0.859	2.809	1.314	0.672	1.948
LSD(0.001)			0.33	0.28	0.38	0.23	0.12	0.32
CV(%)			10.25	16.45	8.31	10.59	11.62	10.19

LSD = least significant difference Mean; CV = coefficient of variation. Means sharing the same letter(s) in each column do not differ significantly at 5% P level according to the LSD Where: NP test. % RR nitrogen and phosphorus with recommended rate.

4.7. Soil Chemical Properties after Crop Harvest

Analysis of soil chemical properties after crop harvest indicate highly significant ($P<0.001$) differenc0e on soil pH, EC, CEC, OC and available P due to the interaction effect of the factors (Appendix Table 5). The interaction effect is shown in (Table 9). Total N was however significantly affected only by the interaction effect of biochar rate and inorganic fertilizer (Table 10). The highest mean values of pH 5.66, EC 0.163 and CEC 19.40, OC 5.22 and available P 10.84 ppm were recorded in soils treated with 18 t ha^{-1} biochar (at 500 ^{0}C) along with100% inorganic fertilizer. The lowest value for these soil parameters were recorded from the control treatment.

The improvement in pH and EC might have resulted from the alkaline nature of the added coffee husk biochar its pH value was 10.55 at 500°C it said to see dominated by carbonates of alkali and alkaline earth metals (Gaskin *et al.*, 2010). Hence, the application of coffee husk biochar had shown alkaline condition that might have a liming effect at increasing application rate. According to earlier finding of Bayu *et al.* (2015), application of coffee husk biochar produced at 500^{0}C and applied at the rate of 15 t ha^{-1} significantly improved physicochemical properties of soil. Similarly, Kloss *et al.* (2014) and Prasad (2015) have shown that application of biochar affects pH, EC and CEC of the soil at different application rates compared to a control. Other studies suggested that application of biochar has decreased soil acidity (Asai *et al*., 2009), increased CEC (Liang *et al*., 2006) of the soil. On the other hand, the increase in CEC of the soil as a result of biochar addition at the higher rate could arise from the inherent characteristics of biochar itself. Biochar is known to have a high surface area, is highly porous, and is variable charged organic material that has the potential to increase soil's CEC, surface sorption capacity and base saturation (Glaser *et al*., 2002). Moreover, the inherent CEC of biochar is consistently higher than that of soil, clays or soil organic matter (Peitikainen *et al*., 2000).The observed increase in OC may be related to the high carbon content of the coffee husk biochar that was incorporated into the soil. This was in agreement with Lehmann (2007) who suggested that biochar rich in recalcitrant carbon can increase the soil OC pool.

The observed increase in available phosphorus in the present study could be due to the presence of high phosphorus in the coffee husk biochar. Biochar may improve available P in soils; both directly through P addition from water-soluble P contained in biochar and/or indirectly through impact on soil chemical, physical and/or biological processes (DeLuca *et al*., 2009). Many biochars have a liming effect, so increased soil pH may increase negative charge which in turn reduces P sorption (DeLuca *et al*., 2009). Hence, the improvement in the soil pH and CEC in the present study might have also contributed to reduce the activity of Fe and Al. The increased available P of the soil due to the application of biochar along with inorganic fertilizers may also be resulted from the release of P from complexes of Al and Fe under high soil pH conditions. Kleineidam *et al*. (2002) also suggested that the higher sorption affinity of biochar for organic and inorganic compounds and higher nutrient retention ability of biochar can modify the nutrient status in soil. The increase of soil available P due to biochar amendment indicated that biochar provided benefits of adsorption complex for cations and anions needed for plant growth (Major *et al*., 2010).

The highest mean N content of 0.336% was recorded from the treatment of18 t ha^{-1} with 100% inorganic fertilizer and the lowest N content 0.123% was from the control in (Table 10). The positive effect of biochar on soil N percentage has been reported previously by Kaleem Abbas (2015) that total N content in the soil amended with biochar was significantly higher than the control. Biochar effects on N cycling in soils and that it offers potential options for tightening the N cycle in agricultural ecosystems. The beneficial agricultural management tool of biochar in case of reduction of NH_3volatilisation via adsorption processes and the development of slow release N fertilizers using fresh biochar additions to soils (Clough *et al.,*2013).

Table 9. Effect of biochar rate, pyrolysis temperature integrated with inorganic fertilizer on soil major chemical properties

Biochar Pyrolysis Temperature(^{0}C)	Biochar application rate(t ha^{-1})	NP(% RR)	pH- H_2O	(EC mSm^{-1})	CEC (me/100g)	OC%	OM%	Av. P(ppm)
Control	0	0	4.58^{h}	0.056^{h}	7.40^{k}	1.32^{j}	2.28^{k}	1.39^{k}
		100	4.94^{de}	0.083^{fg}	9.42^{i}	2.26^{fg}	3.89^{gh}	5.20^{f}
		60	4.94^{de}	0.083^{fg}	9.42^{i}	2.26^{fg}	3.89^{gh}	5.20^{f}
350	6	0	4.68^{g}	0.093^{efg}	11.40^{f}	2.51^{e}	4.33^{ef}	3.04^{j}
		100	4.93^{e}	0.080^{g}	12.40^{f}	1.73^{i}	2.99^{j}	4.54^{h}
		60	4.90^{ef}	0.080^{g}	10.40^{h}	1.95^{h}	3.36^{i}	3.18^{i}
	18	0	5.14^{c}	0.096^{ef}	13.40^{e}	2.59^{e}	4.47^{e}	6.45^{e}
		100	5.41^{b}	0.133^{b}	11.40^{g}	2.88^{d}	4.97^{d}	8.31^{d}
		60	5.40^{b}	0.113^{cd}	10.40^{h}	3.10^{c}	5.34^{c}	9.91^{b}
500	6	0	4.83^{f}	0.103^{de}	9.40^{i}	2.81^{d}	4.41^{gh}	4.07^{i}
		100	5.01^{d}	0.123^{c}	14.60^{d}	2.20^{g}	3.79^{h}	4.94^{g}
		60	5.17^{c}	0.126^{bc}	12.40^{f}	2.40^{ef}	4.1^{fg}	6.40^{e}
	18	0	5.21^{c}	0.120^{cd}	17.40^{b}	2.47^{e}	4.27^{ef}	8.33^{d}
		100	5.66^{a}	0.163^{a}	19.40^{a}	5.22^{a}	9.00^{a}	10.84^{a}
		60	5.41^{b}	0.133^{b}	15.40^{c}	3.88^{b}	6.69^{b}	9.11^{c}
Mean			5.04	0.100	11.69	2.52	4.35	5.29
LSD(0.001)			0.07	0.01	0.05	0.18	0.32	0.23
CV (%)			0.90	8.10	0.29	4.48	4.49	2.29

Where: EC: electrical conductivity, CEC: Cation exchange capacity, OC: Organic carbon, OM: Organic matter, Av. P: Available phosphorus, NP% RR nitrogen and phosphorus with recommended rate. Where: NP% RR nitrogen and phosphorus with recommended rate. Means sharing the same letter(s) in each column do not differ significantly at 5% P level according to the LSD test.

Table 10 Effect of variation in biochar rate and inorganic fertilizer on soil character of total nitrogen

Pyrolysis Temperature ^{0}C	Biochar application rate(t ha^{-1})	NP(% RR)	TN%
Control	0	0	0.123^{h}
		100	0.226^{de}
		60	0.226^{de}
350	6	0	0.200^{fg}
		100	0.183^{g}
		60	0.203^{efg}
	18	0	0.226^{de}
		100	0.270^{bc}
		60	0.243^{d}
500	6	0	0.226^{de}
		100	0.210^{ef}
		60	0.25^{cd}
	18	0	0.25^{cd}
		100	0.336^{a}
		60	0.286^{b}
Mean			0.21
LSD(0.001)			0.026
CV (%)			7.2

LSD = least significant difference; CV = coefficient of variation. Means sharing the same letter(s) in each column do not differ significantly at 5% significant level according to the LSD test. Where: NP% RR nitrogen and phosphorus with recommended rate, TN: Total nitrogen.

4.8. Relationship between Hot Pepper Yield, N and P Uptake, AMF Colonization and Soil Chemical Parameter.

Correlation coefficient values computed to display the relationships between and within yield, N and P uptake and AMF root colonization of the selected from parameter of hot pepper and soil physiochemical parameter are shown in (Table 11). The correlation values showed apparent association of the parameters of the crop with each other and indicate the magnitude and direction of the association and relationships.

4.8.1. Growth and yield component with AMF colonization, nutrient uptake and with soil

Among growth and yield components, days to flowering was negatively and significantly correlated with number of leaves per plant (r= -0.471$^{**)}$, number of fresh fruits per plant (r= -0.507***), fresh fruit yield per plant (r= -0.451***) and N uptake (r=-0.430**). Days to flowering was also negatively correlated with soil properties like pH (r = -332*), EC (r = -377**), CEC (r = -377*), OM (r = -315*) and AvP (r = -0.358**) (Table 11). Days to first harvest was however positively and significantly (r = 0.735***) correlated with days to flowering and negatively and significantly correlated with number of fruits per plant (r = -0.711***) and fresh fruit yield(r = -0.646***). Number of leaves was positively and highly correlated with all parameters except with hyphal colonization. Similarly, fresh fruit yield was positively and significantly correlated with total phosphorus (r=0.800***) and nitrogen (r=0.772***) uptake indicating that yield is dependent on nutrients available in the soil.

4.8.2. AMF colonization with N and P uptake and soil character

AMF Hyphal colonization percentage was positively and significantly correlated with P uptake (r= 0.418**), soil pH (r = 0.422**), EC (r = 0.404**), CEC(r = 0.418**),OC (r =0.491**), total nitrogen (r=0.454**) and Av.P (r=0.497**) (Table 11).The biochar which influenced soil physicochemical characteristics to improve colonization correlated with values explain the apparent association of HC and soil parameters with each other and with soil. Biochar act as a habitat for soil microorganisms involved in N, P or S transformations (Pietikäinen *et al.,* 2006) and also to support bacteria from the organisms may influence soil processes.

4.8.3. Nitrogen and Phosphorus uptake with soil characters

Total N and P uptake of hot pepper were positively and significantly correlated with selected soil chemical characteristics (Table 11). P uptake showed positive and highly significant correlation ($r=0.787^{***}$) with N uptake and with other soil parameters. N uptake was correlated with pH ($r=0.761^{***}$), EC($r=0.727^{***}$), CEC ($r=0.585^{***}$), OC ($r=0.682^{***}$), total nitrogen ($r=0.658^{***}$), available phosphorus ($r=0.730^{***}$). Like that of N, P uptake also positively and highly correlated with soil parameters like pH ($r=0.863^{***}$), EC ($r=0.743^{***}$), CEC ($r=0.762^{***}$), OC ($r=0.764^{***}$), total nitrogen ($r=0.774^{***}$) and Av.P ($r=0.868^{***}$). The strong correlation matrix of uptake with soil parameters may indicate that the biochar treated soils have improved pH may favor availability of nutrients. This study is in agreement with Abebe *et al*. (2012), who reported that correlation matrix showed a significant association of soil available phosphorus with biochar applied and uptake by lettuce and growth.

Table 11 Correlation coefficients (r) among hot pepper yield, N and P uptake, AMF colonization and soil physic-chemical parameters.

	DF	DFH	NL	NFF	FF kg	HC	Pup	Nup	pH	EC	CEC	OC	OM	TN	Av.P
DF	1														
DFH	0.735***	1													
NL	-0.471**	-0.659***	1												
NFF	-0.507***	-0.711***	0.895***	1											
FFkg	-0.451**	-0.646***	0.805***	0.918***	1										
HC	0.131ns	0.131ns	-0.093ns	0.13ns	0.222ns	1									
Pup	0.24ns	-0.566***	0.634***	0.779***	0.800***	0.418**	1								
Nup	-0.430**	-0.631***	0.633***	0.758***	0.772***	0.166ns	0.787***	1							
pH	-0.332*	-0.572***	0.687***	0.869***	0.862***	0.422**	0.863***	0.761***	1						
EC	-0.377**	-0.554***	0.653***	0.828***	0.826***	0.404**	0.743***	0.727***	0.893***	1					
CEC	-0.271*	-0.465**	0.611***	0.738***	0.726***	0.418**	0.762***	0.585***	0.776***	0.855***	1				
OC	-0.194ns	-0.427**	0.596***	0.790***	0.760***	0.491**	0.764***	0.682***	0.851***	0.840***	0.714***	1			
OM	-0.315*	-0.527***	0.598***	0.795***	0.779***	0.448**	0.836***	0.829***	0.897***	0.849***	0.725***	0.776***	1		
To N	-0.245ns	-0.465**	0.713***	0.873***	0.873***	0.454**	0.774***	0.658***	0.903***	0.885***	0.777***	0.901***	0.811***	1	
Av.P	-0.358**	-0.559***	0.636***	0.832***	0.874***	0.497**	0.868***	0.730***	0.975***	0.879***	0.773***	0.861***	0.863***	0.903***	1

*Where : DF: date of flowering, DFH: date of first harvest , NL: number of leaf ,NFF: number of fresh fruit, FFkg: fresh fruit kilogram/plant , HC: hifal colonization , Pup: phosphorus uptake , Nup: nitrogen uptake , EC: electrical conductivity ,CEC: cations exchange capacity ,OC: organic carbon ,OM: organic matter, TN: total nitrogen Ns = Level of significance ns, *, **, *** denoting ($P>0.05$ = ns), significant at ($P<0.05$), ($P<0.01$), and ($P<0.001$), respectively*

5. SUMMARY AND CONCLUSION

Crop growth and development with their subsequent yield are governed by the availability of optimum levels of water, nutrients and favorable environmental conditions. Although hot pepper is one of the cash crops in the Southwestern Ethiopia, its production and productivity are limited by many factors including nutrient depletion and soil acidity. Biochar has been shown to have a positive effect on crop yield improvements in acidic soils due to its liming effect. In view of this, eighteen treatment combinations were evaluated to assess the effect of variation in biochar prepared from coffee husk produced at two pyrolysis temperatures along with application of inorganic fertilizers on N and P uptake and mychrohizal root colonization and yield and yield components of hot pepper.

The findings of the present study revealed that variation in biochar production under two pyrolysis temperatures, and its application rate, with integration of inorganic fertilizer had significant differences among values of most growth parameters and yield. Combination of biochar rate 18 t ha^{-1}, at pyrolysis temperature of 500 ^{0}C along with 100% inorganic fertilizer led to significantly higher hot pepper yield and nutrient uptake. Besides, highest AMF root colonization was observed at the interaction of 18 t ha^{-1} biochar, at pyrolysis temperature of 500 ^{0}C along with 60% inorganic fertilizer. Soil chemical properties and N and P uptake by hot pepper were significantly higher from 18 t ha^{-1} biochar at 500 ^{0}C along with 100% inorganic fertilizer.

Moreover, the correlation analysis showed that significant and positive relationship between and among most yield components with total N and P uptake and all soil characteristics. AMF root colonization showed significant and positive correlation with P uptake and with soil chemical properties such as pH, EC, CEC, OC, total N and Av. P. In addition, total nitrogen and phosphorus uptake showed significant and positive relation with soil chemical characteristics. In the present study, coffee husk biochar prepared at 500^{0}C pyrolysis temperature, with the application rate of 18 t ha^{-1} in combined with 100% and 60% of the recommended rate of inorganic fertilizers could optimize hot pepper yield, nutrient uptake, AMF root colonization. The same treatment had shown better improvement in soil chemical properties.

Therefore, it is suggested that further studies are required to determine the N and P use efficiency of hot pepper in the presence of biochar and it is also important to extend the experiment to a larger field-scale in order to test whether the pot-trial results will be acceptable.

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7. APPENDICES

Appendix Table 7. analysis of variance for days to flowering, days to first harvest, number of leaf , number of fruit and fresh fruit yield where Effects of variation in biochar pyrolysis temperature, application rate and inorganic fertilizer application in greenhouse condition at Jimma, Ethiopia 2015/16.

		Mean square values				
Sores	Degrees of Freedom	**DF**	**DFH**	**NL**	**NFR**	**FFY kg.**
Biochar Temperatures(T)	1	264.50***	186.88**	144.50***	177.34***	0.0032***
Biochar Rate (R)	2	18.510ns	728.38***	45.59***	426.34***	0.0078***
Inorganic Fertilizers (IF)	2	852.72***	3044.84***	623.51***	1266.09***	0.0151***
T*R	2	86.29***	120.22***	73.62***	83.09***	0.0016***
T*IF	2	279.50***	517.09***	3.3700ns	7.0900ns	0.00004***
R*IF	4	163.73***	236.97***	60.93***	98.63***	0.0014***
T*R*IF	4	226.91***	301.43***	15.870*	17.59**	0.0005***
Error	54	6.89	9.02	3.50	2.80	0.00

*Ns = Level of significance ns, *, **, *** denoting (P>0.05 = ns), significant at (P<0.05), (P<0.01), and (P<0.001), respectively. Where: DF: days to flowering, DFH: days to first harvest, NL: number of leaf, NFR: number of fruit and FFY km: fresh fruit yield kilogram per plant.*

Appendix Table8. Analysis of variance for Plant height, Number of branch per plant as Effects of variation in biochar application rate and inorganic fertilizer application in green haws condition at Jimma, 2015/16 South Ethiopia.

		Mean square values	
Sores	Degrees of Freedom	**PHT**	**NB**
Biochar Temperatures(T)	1	8.000ns	0.05ns
Biochar Rate (R)	2	28.38*	8.43***
Inorganic Fertilizers (IF)	2	1022.76***	29.55***
T*R	2	2.160ns	1.51ns
T*IF	2	10.29ns	0.22ns
R*IF	4	24.49*	6.63***
Error	54	9.43	0.59

*Where: PHT: plant height, NB: number of branch. Ns = Level of significance ns, *, **, *** denoting (P>0.05 = ns), significant at (P<0.05), (P<0.01), and (P<0.001), respectively.*

Appendix Table 9. Analysis of variance for Arbuscular colonization, vesicular colonization and hyphal colonization where subjected to various biochar pyrolysis temperature, application rate and inorganic fertilizer in green haws condition on (Capsicum annem L.) at Jimma, Ethiopia.

Source	Degrees of Freedom	Mean square values		
		AC	**VC**	**HC**
Biochar Temperatures(T)	1	32.18***	1.410***	1.08000ns
Biochar Rate (R)	2	214.26***	69.99***	9270.16***
Inorganic Fertilizers (IF)	2	11.04***	46.70***	3641.02***
T*R	2	8.11***	1.700***	8.56000*
T*IF	2	10.93***	4.410***	232.90***
R*IF	4	21.56***	44.51***	1730.86***
T*R*IF	4	3.54***	5.860***	233.150***
Error	36	0.21	0.059	1.05

*Ns = Level of significance ns, *, **, *** denoting (P>0.05 = ns), significant at (P<0.05), (P<0.01), and (P<0.001), respectively. Where: AC: Arbuscular colonization, VC: vesicular colonization and HC-:hyphal colonization.*

Appendix Table 10 Analysis of variance for Nitrogen and phosphorus uptake of fruit, straw and total hot pepper was sowed in the variation of biochar pyrolysis temperature, application rate and fertilizer at Jimma, Ethiopia, 2015/16.

Source	Degrees of Freedom	Mean square values					
		FN	**HN**	**TN**	**FP**	**HP**	**TP**
Biochar Temperatures(T)	1	6.0233***	1.1528***	12.446***	1.9440***	1.0292***	5.9461***
Biochar Rate (R)	2	22.136***	3.5092***	43.251***	4.7149***	1.6227***	11.233***
Inorganic Fertilizers (IF)	2	18.865***	2.8133 ***	36.114***	10.149***	2.3980***	21.488***
T*R	2	3.3892***	0.4764***	6.3743***	1.8275***	0.3313***	3.1680***
T*IF	2	0.6898***	0.3508***	2.0244***	0.3575***	0.0604**	0.6694**
R*IF	4	3.3890***	0.9000 ***	7.6286***	0.9215***	0.3261***	2.2629***
T*R*IF	4	0.7111***	0.2710***	1.4741***	0.0920**	0.1366***	0.3023***
Error	36	0.0399	0.0200	0.054	0.0194	0.0061	0.0394

*Ns = Level of significance ns, *, **, *** denoting (P>0.05 = ns), significant at (P<0.05), (P<0.01), and (P<0.001), respectively. Where: FP: fruit phosphorus uptake, SP: Hulen phosphorus uptake, TP: total phosphorus uptake, FN:fruit nitrogen uptake, SN:straw nitrogen uptake and TN:total nitrogen uptake.*

Appendix Table 11: Analysis of variance for pH, EC,CEC ,OC, OM and Available P effects of variation in biochar pyrolysis temperature, application rate and inorganic fertilizer .

		Mean square values					
Source	Degrees of Freedom	**pH**	**EC**	**CEC**	**OC**	**OM**	**Av,P**
Biochar Temperatures(T)	1	0.1157***	0.00500***	61.44***	2.986***	8.873***	11.371***
Biochar Rate (R)	2	1.5587***	0.0122***	152.49***	9.847***	29.289***	132.206***
Inorganic Fertilizers (IF)	2	0.5467***	0.0025***	15.88***	1.727***	5.132***	34.368***
T*R	2	0.03240***	0.0013***	42.74***	1.142***	3.401***	2.981***
T*IF	2	0.00142ns	0.00022*	8.540***	0.873***	2.597***	0.041ns
R*IF	4	0.02130***	0.00068***	9.899***	2.156***	6.410***	3.471***
T*R*IF	4	0.01844***	0.00021**	3.190***	0.728***	2.171***	3.979***
Error	36	0.0020	0.0000	0.0011	0.012	0.038	0.014

*NB: Level of significance ns, *, **, *** denoting ($P>0.05$ = ns), significant at ($P<0.05$), ($P<0.01$), and ($P<0.001$), respectively. Where: EC: electrical conductivity, CEC: Cation exchange capacity, OC: Organic carbon, OM: Organic matter, Av.P: Available phosphorus.*

Appendix Table12: Analysis of variance for TN, in biochar application rate and inorganic fertilizer application.

		Mean square values
Source	Degrees of Freedom	**TN**
Biochar Temperatures(T)	1	0.00907***
Biochar Rate (R)	2	0.0284***
Inorganic Fertilizers (IF)	2	0.0145***
T*R	2	0.00240**
T*IF	2	0.00029ns
R*IF	4	0.0072***
Error	54	0.00025

*NB: Level of significance ns, *, **, *** denoting (P>0.05 = ns), significant at (P<0.05), (P<0.01), and (P<0.001), respectively. Where: TN: Total nitrogen*

Appendix Figure6: preparation of coffee husk biochar pot.

Appendix Figure 7: hot pepper seedling preparation and transplanting

Appendix Figure 8: fruit of hot pepper at the physiological maturity stage.

Appendix Figure 9: sample preparation and laboratory work of AMF root colonization.

Appendix Figure 10: Jimma University college of Agriculture soil laboratory work