EFFECT OF BIOCHAR AND INORGANIC FERTILIZERS ON NITROGEN AND PHOSPORUS UPTAKE, MYCORRHIZAL FUNGI COLONIZATION, GROWTH AND YIELD OF TOMATO (*Lycopersicon lycopersicon*) AT JIMMA, SOUTH WEST ETHIOPIA

M.Sc. THESIS

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Effect of Biochar and Inorganic Fertilizers on Nitrogen and Phosphorus Uptake, Mycorrhizal Fungi Colonization, Growth and Yield of Tomato (*Lycopersicon lycopersicon*) at Jimma, South West Ethiopia

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As Thesis Research advisors, we here by certify that we have read and evaluated this thesis prepared, under our guidance, by Girma Abebe, entitled "Effect of Biochar and Inorganic Fertilizers on Nitrogen and Phosphorus Uptake, Mycorrhizal Fungi Colonization, Growth and Yield of Tomato (*Lycopersicon lycopersicon*) At Jimma, South West Ethiopia

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DEDICATION

I dedicate this thesis manuscript to my wife Betelihem sime, my mother Abebech w/mikael, and my father Abebe Adelo for their cooperation and kind support.

STATEMENT OF AUTHOR

First, I declare that this thesis is my bona fide work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements of M.Sc. degree at Jimma University, College of Agriculture and Veterinary Medicine and is deposited at the University Library to be made available to users under rules of the Library. I seriously declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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BIOGRAPHICAL SKETCH

The author, Girma Abebe was born on August 19, 1979 G.C in Kaffa Zone, South Nation, and Nationalities Regional State. He pursued his Elementary School at Saylem Elementary School, and attended his Secondary School at Gore Senior Secondary School. After completion of his high school education, he joined Ardayta co-operative institute and completed one year's certificate programs in General agriculture in 2000 G.C. After graduation he was employed at Ministry of Agriculture and served the Organization for more than 2 years. He got an opportunity to joined Wolayta Soddo Agricultural Technique and Vocational Education and Training College and obtained diploma in Plant Sciences in 2005 G.C. After graduation he was employed at Ministry of Agriculture and served the Organization for more than 4 years. He got an opportunity to join Jimma University College of Agriculture and Veterinary Medicine, for his B.Sc degree in Plant science in continuing Education in-service Program in 2010 and obtained B.Sc. degree in Plant Sciences in 2012. After graduation he was employed at Saylem woreda Ministry of Agriculture and served Organization until he joined Jimma University, School of Postgraduate in 2014 to pursue his M.Sc. study in Agronomy.

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LIST OF ABBREVIATION

AC	Arbuscular Colonization
AMF	Arbuscular Mycorrhizal Fungi
ASTM	American Society for Testing and Materials
BC	Black Carbon
BPEDORS	Bureau of Planning and Economic Development of Oromia Regional state
CIMMYT	International Center for Wheat and Maize Improvement
CSA	Central Statistical Agency
EARO	Ethiopia Agriculture and Research origanization
ECM	Ectomycorrhizal Fungi
ERM	Ericoid Mycorrhizal Fungi
FAOSTAT	Food and Agriculture Organization Statistical Database
HC	Hyphal Colonization
MoA	Ministry Of Agriculture
MoARD	Ministry of Agriculture and Rural Development
NSL	National Soil Laboratory
PR	Pathogenesis Related
SOC	Soil origanic carbon
TSS	Total soluble solids
USDA	United State Department of Agriculture
VAM	Vesicular Arbuscular Mycorrhizae
VC	Vesicular Colonization

Effects of Biochar and Inorganic Fertilizers on Nitrogen and Phosphorus Uptake, Mycorrhizal Fungi Colonization, Growth and Yield of Tomato (*Lycopersicon lycopersicon*) at Jimma, South West Ethiopia

ABSTRACT

Soil fertility management practices are the most important factors that affect tomato growth and its productivity on wide range of soil types. Tomato production in South west Ethiopia concentrates mainly on acidic soils where Nitrogen and Phosphorus nutrients are limiting. Biochar is one of the most important and easily available soil amendment resources that can improve soil conditions and help plant root access to mycorrhizal fungi thereby improve nitrogen and phosphorus nutrition of the plant. However, there are still many uncertainties about biochar, particularly in terms of making sure that it has positive effects with a particular soil and crop type. An experiment was therefore, conducted to determine the effect of biochar integrated with inorganic fertilizer application on Nitrogen and Phosphorus uptake and arbuscular mycorrhizal fungi (AMF) root colonization by tomato. The experiment was conducted under greenhouse conditions from January 2016 to may 2016.The experiment was laid out as randomized complete block design and replicted four times. The experiment consisted of five levels of biochar application (0, 6, 12, 36, and 72t/ ha) that were integrated with chemical fertilizers. Data on growth and yield parameters, AMF colonization, and Nitrogen and Phosphorus uptake were collected and statistically analyzed using SAS version9.2 software. Analysis of variance showed that application of biochar significantly ($P<0.05$) affected all the studied parameters. Application of 36 t/ha biochar supplemented with 96 N and 92 P_2O_5 kg/ha showed a significant increase in all growth and yield parameters. Moreover, Nitrogen uptake showed improvment by haulm (1.4gm/plant), fruit (7.47gm/plant) and total plant uptakes (8.87gm/plant) and P uptake by haulm (1.4gm/plant), fruit (4.9gm/plant) and total plant (6.2gm/plant) were also improved at 36 t/ha biochar supplemented with 96 N and 92 P_2O_5 kg/ha. On the other hand, tomato roots showed significant ($P<0.05$) mycorrhizal association with typical fungi structures (arbuscules, hyphae and vesicles). The height hyphae (HC) colonization (76.9%) was found from 72 ton biochar alone. However, significantly lower HC colonization (13%) was recorded from the control. Moreover, significant and positive correlation was also observed between total N and P uptake with number of fruit and mycorrhizae hyphal colonization (AMF). In conclusion, application of 36t/ha biochar supplemented with 96 N and 92 P_2O_5 kg/ha was found to give highest N and p uptake and better fruit tomato yield. Therfore, extending the experiment to a field-scale is suggested in order to test whether the pot-trial results can be reproduced or not.

Key words: Biochar, Inoriganic ferilizer, Mycorrhizal colonization, N, P uptake, Tomato

1. INTRODUCTION

Tomato (*Lycopersicon lycopersicon*) belongs to Solanaceous family and it is originated in the South American Andes however, cultivated tomato originated in Mexico (Maria and Fernando, 2008).This family also includes other well-known species, such as potato, tobacco, hot peppers and eggplant. The cultivated tomato was brought to Europe by the Spanish conquistadors in the sixteenth century and later introduced from Europe to southern and eastern Asia, Africa and Middle East (Shankara *et al*., 2005).There is no definite time recorded regarding the introduction of cultivated tomato to Ethiopia. However, cherry type has been grown for long time around big cities and in small gardens (Lemma, 2002).

Currently tomato is the world's third largest vegetable crop after potato and sweet potato (FAO, 2006). It is one of the vegetable with the highest production both in the world and at country level. Asia is by far the content with the greatest production, China is the main producer of tomato with the area coverage 920,803 ha and production of 45,365,543 tons with productivity of 49.27 t /ha, followed by the US, Turkey, India and Italy. From Africa Egypt and Nigeria are the main tomato producers. The total tomato production and productivity in Ethiopia is far below the average of major producers in Africa. In 2010 cropping season the area coverage by this crop in Ethiopia was 4,593 ha and production was 40,426 tons with the productivity of 8.8 t/ha, which is very low compared to other countries (FAOSTAT, 2011).

In Ethiopia, farmers get lower yield mainly due to diseases and pests as well as due to sub-optimal fertilization. Mehla *et al*. (2000) reported that fruit yield in tomato is highly influenced by Nitrogen and Phosphorus. Although soils contain higher quantity of both, most of them are not readily available for the plant use. Most of Nitrogen is tied into soil organic matter. Even after fertilization, plants have to compete with soil microbes for easily available soluble Nitrogen but problem with Phosphorus are different. In acidic soils, even when phosphorus fertilizers are added in substantial quantities, it becomes unavailable as Phosphoures fertilizer precipitates with iron or aluminum (Glaser *et al*., 2001). Accordingly, P limitation may be a difficult problem to overcome through the addition of P-containing fertilizers.

Biochar is a charcoal substance produced from controlled, incomplete combustion of biomass in an oxygen- free environment. It creates virtually a permanent carbon sink in prime soils that have multiple environmental benefits when used as soil amendments. Thus, it can remove CO_2 in large quantities to combat climate change. Biochar gives the soil its black colour and improves soil structure, aggregation, water infiltration and retention and nutrient storage capacities (Lehmann *et al*., 2003). One significant feature of biochar is that it may increase stabilization of organic matter nutrient sources in the soil (Glaser *et al*., 2001) and reduce nutrient leaching losses (Lehmann *et al.,* 2003) and hence improve nutrient retention. Biochar is important as a soil conditioner and also helps to spread and transform nutrients (Glaser *et al*., 2002; Lehmann *et al*., 2003). This makes it possible to modify N, P and S transformation in mineral soils. Besides, it has high surface areas that are highly porous and so has the ability to increase soil water holding capacity, Cation Exchange Capacity (CEC) and surface sorption capacity when added to the soil (Glaser *et al*., 2002; 2004; Keech *et al*., 2005; Liang *et al*., 2006). It has been proven to have the ability to influence the population of soil microbes (Pietikainen *et al.,* 2000) and also lower soil bulk density (Gundale and Deluca, 2006).

Arbuscular mycorrhizal fungi (AMF) are obligatory symbiotic soil fungi which colonise roots of most plants (Douds and Millner, 1999). These fungi form mutualistic relationships with more than 80% of terrestrial plants (Ulrich *et al*., 2002) and provide the host with mineral nutrients in exchange for carbohydrates (Tahat *et al*., 2008). In exchange for 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). In soilless tomato cultivation, AMF improves plant growth and vegetative development, as well as increases the fruit yields (Demir 2004, Utkhede 2006, Dasgan *et al.* 2008). It was justified that better utilization of nutrients, in particular nitrogen, phosphorus, and microelements, from rhizosphere is the main benefit due to root settlement by AMF (Dasgan 2008; and Salvioli 2012). In opinion of Mwangi *et al*. (2011), a positive influence of AMF on plant growth and development is enhanced along with the decrease of rhizosphere abundance. Salvioli *et al*. (2012) and Candido (2013) proved that utilization of artificial fertilizers can be reduced by means of AMF inoculation, which is crucial for sustainable plant production. In tomato, AMF (Arbuscular mycorrhizal fungi) are widely used

to improve plant growth and health (Oseni *et al*., 2010). However, even with nursery inoculation with AMF or field application, tomato plants exhibit low root mycorrhizal colonisation. Low AMF colonisation in field grown plants has been probably attributed to use of unsuitable strains, relatively high available soil P, cultural practices and some other microbial competition in the rhizosphere (Tahat *et al*., 2008).

In Ethiopia, tomato production is subsistence in nature and highly constrained by low soil fertility and nutrient depletion. According to CSA, (2012) the national average of tomato fruit yield under farmers 'conditions is 8.8 t/ ha which are very low as compared to 25 and 40 t/ ha at demonstration and experimental research plots, respectively. In the soils appear to be highly depleted because farmers remove the soil nutrients without putting back anything to replenish the soil and constantly under cultivation all year round. Biochar has the potential characteristic of increasing water retention capacity and nutrient availability especially in depleted tropical soils (Bakewell-Stone, 2011). Biochar addition can result in elevated quantities of bio-available nutrients such as N and P, in the depleted soils (Lehmann *et al.*, 2003).

The addition of biochar to soil can increase soil fertility, improve crop yields, and improve plant response to fertilizer. Early adoption by farmers increased yields by approximately 23% in the first season of application, and 30% a year later (USAID, 2013). The effect of biochar amendment on crops such as maize (*Zea mays*), soybean (*Glycine max*), radish (*Raphanussativus*), sorghum (*Sorghum bicolor*), potato (*Solanumtuberosum*), wheat (*Triticumaestivum*), pea (*Pisumsativum*), oats (*Avena*sp), rice (*Oryza sativa*), and cowpea (*Vignaunguiculata*) (Lehmann *et al*., 2003; Chan *et al.,* 2007) and on sweet potato (*Ipomoea batatas*) yield and quality (Dou *et al*., 2012) has been studied in different parts of the world but little literatures are sofare available on studies done on the effect of biochar amendment for tomato production else where.

Tomato is a widely cultivated vegetable crop by farmers in Ethiopia, such as Jimma area, for economic as well as consumption purposes but its productivity is low due to poor soil fertility and lack of innovative technology that address soil fertility problems in the region. On the other hand, the potential of biochar as a soil amendment in agricultural fields is recently

recognized as a viable option to remediate many of soil fertility problems in the tropics, yet it is underutilized technology and specific mechanisms underlying the contribution of biochar to plant response to nutrient from soil are poorly understood. Moreover, no works have been conducted on determination of optimum rate of biochar for improved tomato production under major soil type of the area. Therefore, the aim of this research was to investigate the effects of biochar and inorganic fertilizers on Nitrogen and Phosphorus uptake, mycorrhizal fungi colonization, growth and yield of tomato in the soil type of Jimma zone. Therefore, the present study was undertaken with the following objectives:

- To produce biochar from coffee husk and process it for further used as soil organic amendment
- To study the effect of biochar coffee husk on soil physical, biological and chemical properties
- To determinate the rate of biochar application by integrating at with inorganic fertilizer and Optimize N and P uptake, AMF root colonization and tomato yield and yield components

2. LITERATURE REVIEW

2.1. Origin, Botany and Ecology of Tomato Crop

Tomato (*Lycopersicon lycopersicon*) is a member of the *Solanaceae* family and was first domesticated in the Central America as early as 700 B.C. Tomato plants are decocts, and grows as a series of branching stems, with a terminal bud at the tip that does the actual growing. When that tip eventually stops growing, whether because of pruning or flowering, lateral buds take over and grow into other, fully functional, vines. Tomato plant vines are typically pubescent (covered with fine short hairs). These hairs facilitate the vining process, turning into roots wherever the plant is in contact with the ground and moisture, especially if there is some issue with the vine's contact to its original root.

The leaves are 10–25 centimeters long, odd pinnate, with 5–9 leaflets on petioles, each leaflet up to 8 centimeters long, with a serrated margin; both the stem and leaves are densely glandular-hairy (David, 2010). Tomatoes can be grown both in temperate and tropical zones. Its fruit is fleshy berry, globular to oblate in shape and 2-15 cm in diameter. The immature fruit is green and hairy. Ripe fruits range from yellow, orange to red. It is usually round, smooth or furrowed. Tomato fruits mature in about 25-30 days after fertilization. Maturity is correlated with increased fruit size, weight, specific gravity, total acidity, and hydrogen concentration. Time from transplant to first harvest is 70-75 days for cherry types, 75-80 days for the plum types and 80-90 for the large fruited type tomatoes. The ripening phase of tomato fruit is also characterized by fruit softening, coloring, and sweetening.

MOARD (2009) reported that in Ethiopia, tomato is produced in altitudes between 700 and 2000, which is characterized as warm and dry day and cooler night, are favorable for optimum growth and development of tomatoes. A temperature range between 21 to 27^0_C day and 10 to 20^0_C night is favorable for plant development, and fruit set in the country. It grows better at a constant day and night temperature. A difference of 6^0_C between day and night temperatures was found sufficient for good plant growth and development. Fruit setting is poor when the temperature is either high or low. Extreme temperatures cause flower drops and poor fruit set.

2.2. Importance of Tomato Fruit

Tomato (*Lycopersicon lycopersicon*), is cultivated both in backyard for home consumption and commercially for domestic and export market. It is one of the world's most popular vegetables. Cultivation of tomatoes improves diet of the people, as they area part of every salad in combination with leaf vegetables, green onions, cucumbers, peppers, and other vegetables (AVRDC, 2005). As a processing crop, it ranks first among all vegetables grown throughout the world. It also possesses valuable medicinal properties, an excellent purifier of blood and a rich source of vitamins like vitamin A and C than any other vegetables.

It is an important cash-generating vegetable crop to small-scale growers and provides opportunities for employment in the production and processing plants (Lemma, 2003). Its production is more attractive than any other vegetable crops for its multiple harvests, which results in high profit per unit area of land. Tomato is the most profitable vegetable with net income of about 11,000 to 14,000 Birr per hectare. Both fresh and processing tomato varieties are popular and economically important vegetable crops produced in the country (Geleta *et al*., 1995).

Besides its importance for consumption, fruit acidity affects the industrialization processes by reducing of pH of the pulp and preventing the growth of microorganisms that are harmful to the conservation of the product (Frusciante *et al.*, 2000). Moreover, low pH decreases the period of heating needed for sterilization during processing. Total soluble solids contents (TSS) are important for the industrialization process as product yield is directly related to ^{o}Brix, especially when the objective is dehydration, concentration of the pulp, or both. Lycopene, ascorbic acid (vitamin C), and potassium contents are important for the nutritional value of tomato; they have beneficial effects on human health.

Geleta *et al*. (1995) indicated that tomatoes contribute to a healthy, well-balanced diet. They are rich in minerals, vitamins, essential amino acids, sugars and dietary fibers. It contains much vitamin B and C, iron and phosphorus. Tomato fruits are consumed fresh in salads or cooked in sauces, soup and meat or fish dishes. They can be processed into purées, juices and ketchup. Canned and dried tomatoes are economically important processed products.

Lycopene is a very powerful antioxidant which can help prevent the development of many forms of cancer. Cooked tomatoes and tomato products are the best source of lycopene since the lycopene is released from the tomato when cooked. A raw tomato has about 20% of the lycopene content found in cooked tomatoes. However, raw or cooked tomatoes are considered the best source for this antioxidan

2.3. Biochar Production and Its Property

Biochar is a term reserved for the plant biomass-derived materials contained within the black carbon (BC) continuum. This definition includes chars and charcoal, and excludes fossil fuel products or geogenic carbon (Lehmann *et al.*, 2006). Materials forming the BC continuum are produced by partially combusting (charring) carbonaceous source materials, e.g. plant tissues (Schmidt and Noack 2000; Preston and Schmidt 2006; Knicker 2007), and have both natural as well as anthropogenic sources. Restricting the oxygen supply during combustion can prevent complete combustion (e.g., carbon volatilization and ash production) of the source materials. When plant tissues are used as raw materials for biochar production, heat produced during combustion volatilizes a significant portion of the hydrogen and oxygen, along with some of the carbon contained within the plant's tissues (Antal and Gronli 2003; Preston and Schmidt 2006). The remaining carbonaceous materials contain many poly-aromatic (cyclic) hydro carbons, some of which may contain functional groups with oxygen or hydrogen (Schmidt and Noack 2000; Preston and Schmidt 2006).

Biochar has a wide range of pH values ranging from between 4 and 12 depending on the feedstock and operating conditions (Lehmann, 2007). Biochar produced at low pyrolysis temperatures ($< 400^{o}_{C}$) gives acidic biochar. Conversely biochar produced at higher temperatures becomes alkaline due to reaction of water, O_2 and various soil agents, surfaces oxidation occurs when incorporated to the soil (Lehmann, 2007). The cation exchange capacity (CEC) of fresh biochar is very low but increases with time as it ages in the presence of water (Lehmann, 2006; Liang, 2006: Cheng *et al.*, 2008). Depending on the temperatures reached during combustion and the species identity of the source material, a biochar's chemical and physical properties may vary (Keech *et al.*, 2005; Gundale and DeLuca, 2006). Coniferous biochars generated at lower temperatures, e.g. 350^{o}C, can contain larger amounts

of available nutrients, while having a smaller sorptive capacity for cations than biochars generated at higher temperatures, e.g. 800^{o}C (Gundale and DeLuca, 2006).

Furthermore, plant species with many large diameter cells in their stem tissues can lead to greater quantities of macro pores in biochar particles. Larger numbers of macro pores can for example enhance the ability of biochar to adsorb larger molecules such as phenolic compounds (Keech *et al*., 2005). Because of its macromolecular structure dominated by aromatic C, biochar is more recalcitrant to microbial decomposition than un charred organic matter (Baldock and Smernik, 2002). In contrast to the organic C-rich biochar, burning biomass in a fire creates ash, which mainly contains minerals such as calcium (Ca) or magnesium (Mg) and inorganic carbonates. Also, in most fires, a small portion of the vegetation is only partially burned in areas of limited O_2 supply, with a portion remaining as char (Kuhlbusch andCrutzen, 1995). Biochar is believed to have long mean residence times in soil, ranging from 1,000 to 10,000 years, with 5,000 years being a common estimate (Skjemstad, 1998; Swift, 2001; Krull *et al*., 2003). However, its recalcitrance and physical nature represent significant obstacles to the quantification of long-term stability (Lehmann, 2007).

Biochar can be produced from different plant materials including wood chip and wood pellets, tree bark, crop residues, grasses, organic wastes (distillers fruit, bagasse, olive waste) (Yaman, 2004). Except olive waste, all sources are found in Ethiopia. Various publications report a generally positive effect of biochar soil amendment on field crops and trees grown under greenhouse and commercial conditions. Charcoal added to soil increased the yield of moong, soybean and pea (Iswaran *et al.*, 1980) and of soybean (Kishimoto and Sugiura, 1985). Shoot and root biomass of birch and pine were greater in charcoal-amended soil (Wardle *et al.*, 1998).

2.4. Nutrient Contents of Biochars

Since biochars are manufactured from biomass, it is expected that they are high in C and contain a range of plant macro- and micro-nutrients. The composition of biochars depends upon the nature of the feedstocks and the operating conditions of pyrolysis. Most of the

research on pyrolysis of biomass has focused on energy and fuel quality (Horne and Williams, 1996; Tsai *et al,* 2006) rather than on biochar as a soil amendment. Often, biochar is looked upon as a fuel for further energy production or as a by-product to be upgraded to activated carbon and used in purification processes (Horne and Williams, 1996).

In the case of pH, biochars used as a soil amendment in prior research are usually alkaline in nature (pH>7.0). However, biochars can be produced at almost any pH between 4 and 12 (Lehmann, 2007) and can decrease to a pH value of 2.5 after short-term incubation of four months at 70°C (Cheng *et al*., 2006). It is important to note that the same type of feedstock can produce very different biochars. For example, Chan *et al.* (2007) reported total N contents of 20g kg^{-1} for biochar produced from poultry litter compared to 7.5g kg^{-1} and 6.0g kg^{-1} for two biochars made from different poultry litter.

For 16 biochars made from different plant biomass as well as poultry litter, bicarbonate extractable available P (Colwell, 1963) was found to range between 15 mg $kg^{-1}$and 11,600mg kg–1 .Significantly higher levels of available P were found in biochars produced from poultry litter than those from plant biomass. However, high contents of heavy metals have been reported in biochars produced from a range of feedstocks (e.g. sewage sludge and tannery wastes). Bridle and Pritchard (2004), reported high concentrations of copper (Cu), zinc (Zn), chromium (Cr) and nickel (Ni) in a biochar produced from sewage sludge. Biochar produced from tannery wastes can be very high in Cr (Muralidhara, 1982) as this metal can make up 2 per cent of total dry weight of the wastes.

2.5. Biochar as a Soil Amendment

Soil improvement is not a luxury but a necessity in many regions of the world. Lack of food security is especially common in sub Saharan Africa and South Asia, with malnutrition in 32 and 22 per cent of the total population, respectively .While malnutrition decreased in many countries worldwide from 1990–1992 to 2001–2003,many nations in Asia, Africa or Latin America have seen increases (FAO, 2006). The ‘Green Revolution’ initiated by Nobel Laureate Norman Borlaug at the International Centre for Maize and Wheat Improvement (CIMMYT) in Mexico during the 1940s had great success in increasing agricultural

productivity in Latin America and Asia. These successes were mainly based on better agricultural technology, such as improved crop varieties, irrigation, and input of fertilizers and pesticides. Sustainable soil management has only recently been demanded to create a 'Doubly Green Revolution' that includes conservation technologies (Tilman, 1998; Conway, 1999).

Biochar provides great opportunities to turn the Green Revolution into sustainable agro ecosystem practice. Good returns on ever more expensive inputs such as fertilizers rely on appropriate levels of soil organic matter, which can be secured by biochar soil management for the long term (Kimetu, 2008; Steiner *et al*., 2007). Specifically in Africa; the Green Revolution has not had sufficient success (Evenson and Gollin, 2003), to a significant extent due to high costs of agrochemicals (Sanchez, 2002), among other reasons (Evenson and Gollin, 2003).

Use of biochar could provide a unique opportunity to improve soil fertility and nutrient-use efficiency using locally available and renewable materials in a sustainable way. Adoption of biochar management does not require new resources, but makes more efficient and more environment frindly, in both industrialized and developing countries.Currently, soil loss and degradation is occurring at unprecedented rates (Stocking, 2003) that could cause a profound consequence for disturbed soil ecosystem (Matson *et al*., 1997). In many regions, loss in soil productivity occurs despite intensive use of agrochemicals, concurrent with adverse environmental impact on soil and water resources (Foley *et al*., 2005). Biochar is able to play a major role in expanding options for sustainable soil management by improving upon existing best management practices, not only to improve soil productivity conscious use of existing resources.

2.6. Effect of Biochar Application on Soil Physical and Chemical Properties

Mineral fertilized fields show yield decreases, reduced nutrient cycling and reduced nutrient-use efficiency of applied fertilizer associated with a loss of SOC (Zech *et al*., 1990). Biochar addition to soils has a multitude of potential agricultural benefits. These include liming of acid soils, addition of basic cations and micronutrients, improving water holding capacity, and a gradual release of nutrients to the growing plant (Glaser *et al*., 2002). Leached sandy soils

typically have low soil pH values, poor buffering capacities, low cation exchange capacity (CEC), with values ranging from 2-8 cmolc kg-^{1}, and can have Al toxicity (Novak *et al*., 2009).

The addition of biochar to these highly leached, infertile soils gives an almost immediate increase in the availability of some basic cations (Glaser, 2002; Liang *et al*,. 2006), as well as a significant improvement in crop yields, particularly where nutrient resources are in short supply (Lehmann and Rondon 2006). Over time, these additions continue to promote soil nutrient availability by giving rise to greater stabilization of organic matter and a subsequent reduction in the release of nutrients from organic matter (Glaser *et al*., 2001; Lehmann and Rondon 2006).

Biochar is becoming a popular alternative to organic amendments that are being applied to soils to increase and sustain soil productivity (Lehmann and Joseph, 2009). This is attributed to the large amounts of highly porous black carbon found in biochar. The carboxylate groups found in black carbon provide CEC, increase the O/C ratio, and are the primary source of biochar‘s high nutrient retention ability (Glaser *et al*., 2001). In addition, biochar may aid in maintaining or increasing nutrient cycling and the stable pools of soil organic carbon (Gaskin *et al*., 2008). Despite biochar being able to improve and sustain soil fertility, fresh biochar shows moderately low cation retention properties relative to aged biochar (Lehmann, 2007). Biochar has the potential to increase nutrient availability for plants (Lehmann *et al*., 2003). Nutrient availability can be affected by increasing cation exchange capacity, altering soil pH, or direct nutrient contributions from biochar. One potential mechanism for enhanced nutrient retention and supply following biochar amendment is increasing (CEC) by up to 50% as compared to unamended soils (Lehmann 2003 and Liang, 2006). Biochar can act as a soil conditioner enhancing plant growth by supplying and, more importantly, retaining nutrients and by providing other services such as improving soil physical and biological properties (Glaser, 2002; Lehmann *et al*., 2003; Lehmann and Rondon 2005).

2.7. Effect of Biochar on Soil Biological Properties

Soil microbial biomass and activities increase with biochar additions (Steiner *et al*., 2008). Biochar provides 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 (McElligott, 2011). As large portion of crop nitrogen is derived from biological processes, changes in microbial processes derived from biochar addition to soils are enhanced. The addition of biochar to soil via microbial habitat provision (Peitikainen *et al*., 2000) induce an increased microbial biomass, nitrogen mineralization also increases, due to the increased microbial biomass and its intimate link to enzyme production (McElligott, 2011).

A major family of soil micro-organisms that is well known for its positive impact on plant productivity is Arbuscular-mycorrhizal (AM) fungi. AM fungi are obligate symbiotic soil fungi which colonize the roots of vascular plants (Smith and Read, 1997). A conservative estimate suggests that 80% of terrestrial land plants are potential hosts to these fungi (Bonfante-Fasolo, 1987). This symbiosis typically results in enhanced host vigor, most frequently demonstrated in increased uptake of immobile nutrients, principally phosphorus, from the soil (Harrison, 1999). Modulation of pathogenesis related (PR) proteins and phytohormones (especially gibberellins) in the host plant are known to play a role in AM fungal–host symbiosis (McElligott, 2011). Addition of biochar to soil often results in significant augmentation of mycorrhizal fungi-plant symbiotic interactions (Warnock *et al.*, 2007).

Beyond the well-known role of arbuscular mycorrhizal (AM) fungi in promoting plant growth, it is known that rhizosphere microorganisms in general, and selected strains belonging to the genera *Pseudomonas*, *Bacillus*, and *Trichoderma* in particular, can improve plant growth in many cropping systems. For instance, increased growth was triggered by species of *Trichoderma* in tomato (Windham *et al.*, 1986), and by species of *Bacillus* (Kloepper *et al.*, 2004) and *Pseudomonas* (Mercado-Blanco and Bakker, 2007) in several crops.

Mycorrhizae are common root-fungal mutualisms with key roles in terrestrial ecosystems (Rillig, 2004). There are several types of mycorrhizas, the most common of which are arbuscular mycorrhizae (AM) and ectomycorrhizae (EM) (Smith and Read, 1997).These two groups are distinct morphologically, physiologically and ecologically with respect to the plant hosts, and also in regard to phylogeny of the fungal partner. Thus, it is highly likely that they

also respond differently to biochar additions. The interest in mycorrhizae and biochar is probably due to three reasons. First, mycorrhizal fungi are ubiquitous key components in virtually all biomes (Treseder and Cross, 2006).Therefore, it is important to understand how any soil additive, including biochar, may affect their performance.

AMF colonize most of the important crop species (maize, rice, wheat, etc.) so that they are also of interest from a perspective of agro-ecosystem productivity and sustainability. Second, mycorrhizae are sensitive to management interventions (Schwartz *et al.*, 2006), such as adding biochar, and it is tempting to speculate on the possible synergistic effects of mycorrhizal inoculation and biochar application in enhancing soil quality and plant growth. Applying biochar to soil stimulated the colonization of crops by AM fungi. Nishio and Okano (1991) reported that root infection by AM fungi significantly increased alfalfa yield by 40 to 80 percent when 1kg m_{-2} of biochar was added to an alfalfa field in a volcanic ash soil and strongly positive effect of biochar on mycorrhiza abundance (Warnock *et al*., 2007).

2.7.1. Role of AMF in tomato production

AM fungi have the potential to increase the sustainability of tomato production by providing an alternate path way of plant Nutrient uptake (Smith *et al*., 2004) that can access greater soil volumes and possibility of nutrients sources other than those directly accessible to planting roots (Marschner and Dell,1994;Smith and Ready,1997). It also reduces the need to apply chemical fertilizers, particularly phosphorus. AM fungi are members of the Glomeromycota (Schussler *et al*.,2001) and, with few exception, they are ubiquitous in all terrestrial soil ecosystems and colonise the root of the majority of plants, including agricultural crops,by forming symbiosis (Gerdemann,1988).They are obligate symbionts, the rely on sugars supplied by host plants for their energy requirements (Bago *et al*,.2002) and supply their host plants with mineral nutrients.

AMF colonise the roots of most plants (Douds and Millner, 1999) and form mutualistic relationships with more than 80% of terrestrial plants (Ulrich *et al*., 2002) and provide the host with mineral nutrients in exchange for carbohydrates (Tahat *et al*., 2008; Javaid, 2009). Generally, plants inoculated with AMF are more efficient in nutrient and water acquisition,

thus resulting in an improved plant growth (Oseni *et al*., 2010). Colonisation of roots by AMF has also been shown to enhance tomato productivity by enhancing tolerance to various biotic and abiotic stress factors (Al-Garni, 2006; Khaosaad *et al*., 2007; Javaid and Riaz, 2008). In tomato, AMF are widely used to improve plant growth and health (Oseni *et al*., 2010). Copetta *et al.* (2011) reported tomato mycorrhization has positive effects on fruits quality. (Salvioli *et al*, 2012 and Colella *et al.*2014), proved that application of mycorrhization remarkably improved the plant nutrition, mainly with nitrogen and phosphorus mycorrhizal mycelium actively uptakes the nutrients and transfers them directly to the roots of a host plant.

2.7.2. Role of AMF on Phosphorus and Nitrogen uptake by tomato

Phosphorus is a major plant nutrient required in relatively large amounts and plays a vital role in all biological functions in energy transfer through the formation of energy-rich phosphate esters and is also an essential component of macromolecules such as nucleotides, phospholipids and sugar phosphates (Marschner, 1995). The most important benefits of mycorrhizae are the increase in the phosphorus uptake by the plant. The general process of phosphorus uptake consists of three sub-processes; (i) absorption from soil by AMF hyphae, (ii) translocation along the hyphae from external to internal (root cortex) mycelia, (iii) the transfer of phosphate to cortical root cells (Barea, 1991). The various mechanisms proposed to account for enhanced nutrient uptake include (i) increased exploration of soil; (ii) increased translocation of phosphorus into plants through arbuscules; (iii) modification of root environment; (iv) efficient utilization of P within plants; (v) efficient transfer of P to plant roots; and (vi) increased storage of absorbed P. Uptake of phosphate by roots is much faster than diffusion of ions to the absorption surfaces of the root (Bhat and Kaveriappa, 2007).

The extensive extrametrical hyphae of AMF extend out into the soil for several centimeters so that it bridges the zone of nutrient depletion. Thus, the plant is able to exploit microhabitats beyond the nutrient depleted area where rootlets and root hair cannot thrive (O'keefe and Sylvia, 1992). Arbuscular mycorrhizal fungi phosphatase are able to mineralize organic P sources. Alkaline phosphatase activity is related to phosphate metabolism of fungus as it is present within the fungal vacuoles where polyphosphate granules were observed. The

polyphosphate granules in fine branches of arbuscules are broken down by enzymatic activities releasing inorganic phosphorus in the cytoplasm.

Nitrogen is needed for the formation of amino acids, purines, pyrimidines and, is thus, indirectly involved in protein and nucleic acid synthesis. AMF associated plants have increased nitrogen content in shoots. A number of mechanisms are suggested for this effects, namely (i) improvement of symbiotic nitrogen fixation; (ii) direct uptake of combined nitrogen by mycorrhizal fungi; (iii) facilitated nitrogen transfer, a process by which a part of nitrogen fixed by nodulated plants benefits the non-nodulated plants; (iv) increased enzymatic activities involved in nitrogen metabolism like pectinase, xyloglucanase and cellulose which are able to decompose soil organic matter (Barea, 1991). The hyphae of AMF have the tendency to extract nitrogen and transport it from the soil to plants (Smith *etal*,.2004). They contain enzymes that breakdown organic nitrogen and contain nitrogen reductase which alters the forms of nitrogen in the soil (Guether *et al*., 2009).

Arbuscular mycorrhizal fungi improves growth, nodulation and nitrogen fixation in legume-*Rhizobium* symbiosis. They also uptake NH_4^+readily from soil which forms the larger fraction of available nitrogen in many natural ecosystems (Peitikainen *et al*., 2000). In soils where nitrate is the dominant nitrogen source, AMF have only a minor influence in acquisition of nitrogen by plants (Johnson *et al*., 1992). AMF hyphae improve nitrogen transfer in communities, since the network of AM mycelia links different plant species growing nearby and helps overlap the pool of available nutrients for these plants. According to McFarland *et al*. (2010) more than 50% of plant N requirement is supplied by mycorrhizal association. Mycorrhizal inoculation enhanced activities of nitrate reductase, glutamine synthetase and glutamine synthase in the roots and shoots of mycorrhizal corn (*Zea mays* L.) (Subramanian and Charest 1999). Recently, a plant ammonium transporter, which is activated in the presence of AMF has been identified and indicated that the way by which N is transferred in plant may be similar to P transfer (Guether *et al*., 2009).

2.7.3. The Role of AM fungi on tomato growth and yield quality

Arbuscular mycorrhizal fungi have the potential to increase the sustainability of tomato production by providing an alternate path way of plant nutrient uptake (Smith *etal*.,2004). It helps to access greater soil volumes and reach possible nutrients sources other than those directly accessible to plant roots and could reduce the rate to apply chemical fertilizers particularly P.The contribution of arbuscular-mycorrhizal (AM) fungi to yield and fruit quality of field-grown processing tomatoes, and the potential to increase the sustainability of commercial tomato production through more efficiency fertilizer use by inoculating tomato seedlings with beneficial AMfungi (Barea, 1991).

Arbuscular mycorrhizal fungi may have the potential to improve the sustainability of commercial tomato production by improving yield quality. Such improvements of crops at given level of inputs increases production efficiency and consequently reduce input level to achieve the same yield (Johnson *et al*., 1992). Arbuscular mycorrhizal fungi must demonstrate a practically benefit in production to important economic crops. Tomato (*Solanum lycopersicum* L) previously known as (*Lycopersicum esculentumMill*) (Barea, 1991) is readily colonized by AM fungi and one of the most widely any contributions of AMfungi to improving the sustainability of tomato production yield and quality will be far-reaching.

Increase tomato yield and quality could be achieved by the well-known capacity of AM fungi to enhance plant uptake of nutrients such as phosphorus (P) and Zink (Zn), which in turn can increase plant growth and fruit nutrient density. It is widely recognized that AM fungi enhance plant uptake of a number of macro-nutrients (Marschner, 1995), which may translate into decreased need for applied fertilizers in commercial production and an increase in the plant nutrient density for a given level of nutrient availability (Barea, 1991),AM fungi have also been shown to enhance the uptake of a number of other nutrients essentially to plant and humans. These include Zn and Calcium (Ca) (Marschner, 1995) and increase in their density in tomato fruit has been demonstrated by (Cavagnaro *et al,. (2006).* Increased fruit nutrient AM response for tomato processers and growers because it determines the quality of paste produced.

2.8. Effect of Biochar on Plant Growth

Biochar can be used as a soil amendment to improve soil quality and crop productivity in a variety of soils (Blackwell *et al*., 2009). In a pot experiment, (Lehmann *et al*., 2003) found biochar to increase rice biomass by 17% and cowpea by 43% when applied at rates of 68t C ha^{-1} to 135t C ha^{-1}. This growth was attributed to direct nutrient additions from biochar of P, K and Copper (Cu). Iswaran *et al*. (1980) reported a 51% increase in biomass in soybean crops with biochar additions of 0.5t ha^{-1} and (Hoshi 2001) found a 20% increase in volume and 40% increase in height of tea trees with biochar additions. Chidumayo (1994) reported better seed germination (30% enhancement), shoot heights (24%) and biomass production (13%) among seven native woody plants on soils under charcoal kilns compared to the undisturbed Zambian Alfisols and Ultisols.

Positive plant growth and nutrient content responses to biochar are commonly observed in association with fertilizer application, while neutral or even negative plant growth responses have been observed succeeding biochar only amendments. Much greater yields in plant growth are observed with fertilizer additions plus biochar, as opposed to fertilizer additions alone (Gundale and DeLuca, 2007).

3. MATERIAL AND METHODS

3.1. Description of the Study Area

The experiment was conducted in a green house at Eladale Research site of the College of Agriculture and Veterinary Medicine, Jimma university, Ethiopia from January to May 2016. Eladale is geographically located 360 km south west of Addis Ababa at about 7^{o}, 41^{o} N latitude and 36^{o}, 50^{o} E longitude at an altitude of 1813 m.a.s.l. The mean annual rainfall of the study area is 1500 mm. The soils of the study area are dominated by Nitisol with pH of 5.5 (BPEDORS, 2000).The greenhouse mean maximum and minimum temperature are 34.1^{0}C and 19^{0}C and relative humidity was 38.92%, and 89% respectively.

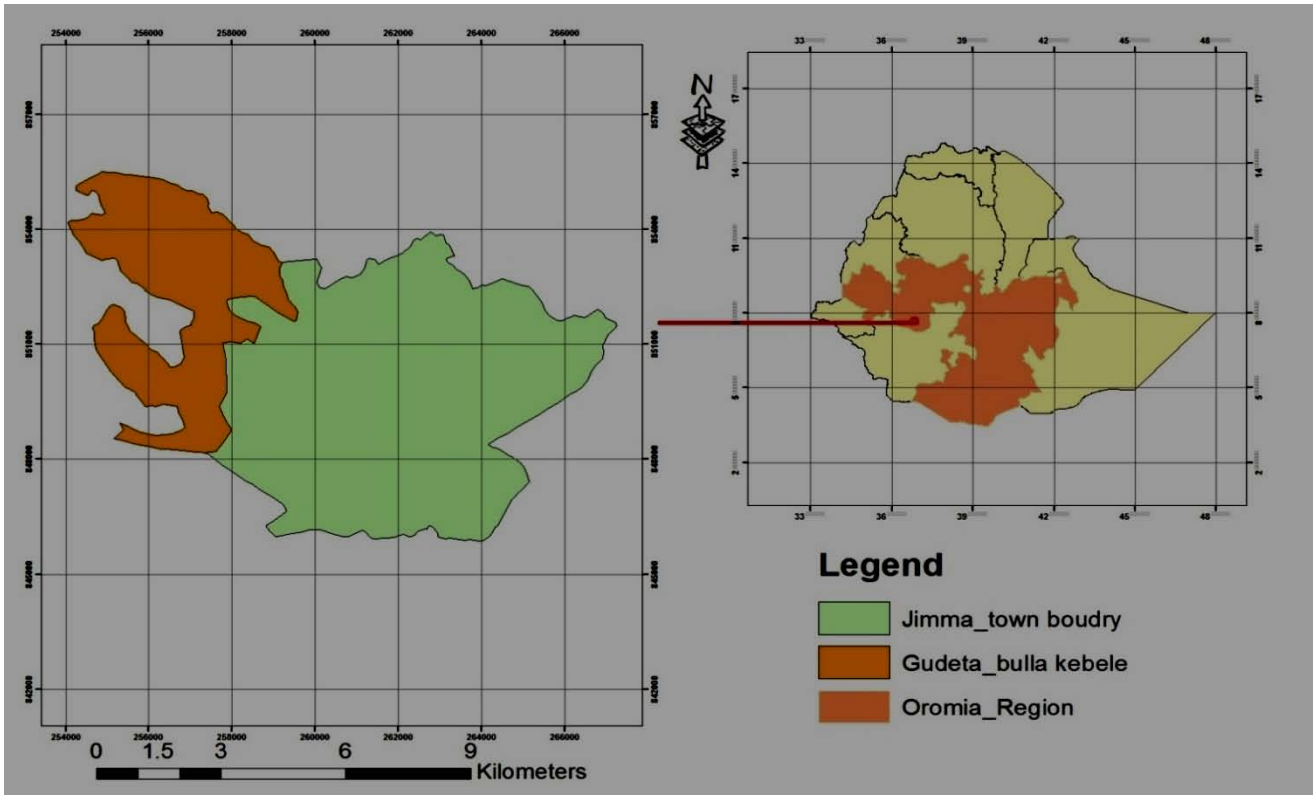

Figure 1 Study site Gudeta Bulla Kebele Source: JTAO (2015)

3.2. Experimental Materials

A high yielding tomato seed variety-Marglobe which is adapted to the agro-ecology of the area, was used for this study. It is one of the most successful varieties released by Melkassa Agricultural Research center in 1976. It has a wider adaptability and grows well at altitudes ranging from 700 to 2000 meters above sea level with annul precipitation of 1000 to 1500 mm. It needs about 110-120 days for maturity and performs better under suitable condition and good soil conditions. Variety selection criteria were based on characteristics such as fruit shape, size, colour, productivity level, shape of plant, vitality and resistance to pests and diseases.It has indeterminate growth habit , good performance and high yield. (FAO,2004).

3.3. Treatments and Experimental Design

The experiment consisted of five rates of biochar (0, 6, 12, 36, and 72 ton /ha supplemented with of the recommended and half recommended fertilizers rates. N fertilizer was applied at rates, 48 and 96 kg/ha and phosphorus was also applied at rates 46, and 92 P_2O_5 kg/ha and with out N and P supplement.The source of these nutrients were Urea and DAP for N and P, respectively. The exepermental design used was laid out in randomized complete block design (RCBD) with four replication (Table 1).

Table 1 . Experimental Treatments

Treatments	Description
T1	Control
T2	96 N and 92 P_2O_5 kg/ha
T3	6 t/ha biochar only + 48 N and 46 P_2O_5 kg/ha
T4	12 t/ha biochar only + 48 N and 46 P_2O_5 kg/ha
T5	36 t/ha biochar only + 48 N and 46 P_2O kg/ha
T6	72 t/ha biochar only + 48 N and 46 P_2O_5 kg/ha
T7	6 t/ha biochar only + 96 N and 92 P_2O_5 kg/ha
T8	12 t/ha biochar only + 96 N and 92 P_2O_5 kg/ha
T9	36 t/ha biochar only + 96 N and 92 P_2O_5 kg/ha
T10	72 t/ha biochar only + 96 N and 92 P_2O_5 kg/ha
T11	6 t/ha biochar only
T12	12 t/ha biochar only
T13	36 t/ha biochar only
T14	72 t/ha biochar only

3.4. Experimental Procedure

3.4.1. Biochar Production

Biochar from coffee husk was prepared at JUCAVM by using pyrolysis unit at temperature of 350°C and for 3 hours of residence time as suggested by Lehmann (2007). After the pyrolysis it was watered to cool down. It was then air dried and put in to sacks. The resulting biochar was grounded to small granules and pass through 2mm sieve in order to have the same particle size as that of the soil (Bayu, 2015).Selected physical and chemical characteristics of the biochar was determined.

3.4.2. Green-house activities

An experiment was conducted with fifty six Pots, each were prepared and tagged for identification purpose. The diameter of each pot was 20 cm; radius of pot was 10 cm and height of pot 20 cm.The net volume of pot 0.00628m^3. The soil was air dried for 24 h, weighed and crushed finely then mixed thoroughly before lightly packing the pots. The pots were filled with the biochar and to 200 g of smaller stones graves were put at base to improve drainage. A total of 56 pots were initially established for each treatment which were treated under similar conditions. Another experiments 56 pots were prepared and kept for replacement of missed plants.

The green house used for this experiment did not have the required facilitate to control weather condition inside. It is simply a screen-house with shelters from its top and sides. Its could not block wind /air movement through the main door.

The N and P for each pot was given by two different recommended and half recommended rates. Accordingly, 100% and 50% N (96, 48 N kg), and 100% and 50% P (92, 46 P_20_5 kg) respectively. Half dose of N and full dose of P_2O_5 were applied at the time of planting while remaining half N was applied after 30 days of planting (EIAR, 2004).Three tomato seeds planting per pot was done on January 2016. To ensure the provision of seedlings, three seeds were planted per pot, quantifying the number of germinated seeds in each pot after seven

days. In order to have one seedling per pot, poorly emerged and extra number of seedlings were removed. Pot orientation was changed weekly to avoid excessive tilting of the stems.

Each pot was provided of water, two times per day as required depending on the prevailing weather conditions. All pots were stood on raised plat forms allowing drainage and a suspended net was used to reduce the sunlight. Fruits were picked twice a week during the harvest time of orange to red maturity stage. They were counted and sorted out; subsequently, total fruit yield and number of fruits per plant were counted.

3.4.3. Soil and Biochar Samples Analysis

Soil samples were collected from Eladale Research site of Jimma University College of Agriculture in a diagonal pattern from 0-20 cm depth before planting. These samples were when composited and replicated three times samples per collected soil samples were prepared for determination. The soil samples were cleaned from root and other duster; air dried thoroughly, ground using a pestle and a mortar and allowed to pass through a 2 mm sieve before laboratory analysis. Working samples were obtained from each submitted samples and analyzed for selected soil chemical and physical properties such as soil texture, organic matter, organic carbon, EC, pH, and amount of phosphorus (P), nitrogen (N) and cation exchange capacity. Soil samples were also taken after harvesting from each pots of the experimental and soil chemical properties after harvest was determined.

pH, OC, %TN, Av.p, EC, and CEC were analyzed for both soil and biochar at Jimma University College of Agriculture and Veterinary Medicine soil laboratory. pH of the sample was measured with 1: 2.5 soil water ratio method (Reeuwijk, 2002). For the soil-water ratio methods, 25 ML of distilled water was added to 10 g of soil. The solution was stirred for one minuets and left for 1 hour to rest. Then, the soil suspension was stirred and measured by using glass electrode pH meter. The soil organic carbon was determined by Walkley –Black oxidation method (Nelson and Summers, 1996) and with potassium dichromate ($K_2Cr_2O_7$) in sulfuric acid solution and titrated with 0.5 N ferrous sulfate solution (Walkley and Black, 1934). Total Nitrogen of the soil was determined thorough digestion distillation and titration procedure of wet digestion by semi-micro Kjeldhal digestion procedure (Bremmer,1996)

were by the ammonia evolved collected in boric acid solution in the presence of indicators (methyl red and bromocresol green) and titrated with 0.1N H_2SO_4 to pink end colour (Sahlemedhin and Taye,2000).

Available phosphorus (P) in the soil determined using the Bray II method extraction method as described by (Bray and Kurtz, 1945).Thus; 2 g of soil was mixed with 14 ML extracting solution Bray, containing 0.03 MNH_4F and 0.025 MHCL.The solutions was shaken for 1 minute and filtrated through Whatman filter paper. The 2 ML of the sample was pipette into a test tube and 8 ML boric acid as well as 2 ML mixed reagent was added. Solution was left for about 1 hour to develop the blue color. Absorbance was measured at 882 nm with UV/VIS Spectrophotometer.

Texture Particle was determined by hydrometer method (Van Reeuwijk, 1992) after destroying OM using hydrogen peroxide (H_2O_4), sodium carbonate (Na_2co_3) was used as soil dispersing agent two drops of amyl alcohol was used for foam reduction. The soil texture classes were determined using the international soil science society system (Yong and Warkentin, 1966), triangular guideline. EC was estimated by conductivity meter in 1:5 soils to water ratio. Soil cation Exchange **(**CEC**)** determined by using 1N ammonium acetate method and then estimated titrimetrically by distillation of ammonium that was displaced by sodium (Gaskin *et al*., 2008).

Coffee husk biochar sample was collected one composite sample after pyrolysis (350^0c) before application to pot. Biochar samples were evaluated for chemical properties including pH, EC, CEC, OC, OM, TN and available phosphorus (Av.p). 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). Biochar organic carbon content was determined by the Walkley-Black method and Total Nitrogen (TN) by the Kjeldahl method (Chintala *et al*., 2014). Available phosphorous (P) was determined by using the Olsen extraction method (Shaheen *et al*., 2009).

Table 2. Initial physicochemical properties of soil and biochar

Parameter	Soil	Biochar
pH	5.03	9.72
OC (%)	2.06	15.45
TN (%)	0.2	1.21
Av.p.(ppm)	4.7	15
EC(dsm	0.21	4.58
CEC (Cmol)	17	37
Texture		
Sand%	23	ND
Clay%	52	ND
silt%	25	ND
Textural class	Clay	ND

Where Cmol = Cent mole, pH = hydrogen power, % OC = percent of organic carbon, %TN = Percent of total nitrogen, Av.p. ppm = available phosphorus in parts per million, EC (ds) m = Electrical conductivity in dessicemen, CEC = Cation exchange capacity, % = percent, ND = not determined.

3.4.4. Plant tissue sampling and analysis

At physiological maturity, a total of forty two selected plant samples were harvested from pot per plant and partitioned in to fruit and haulm. Fruit and haulm samples were separately oven dried at 70 ºC for 48 hours at constant weight, grinded to pass 1 mm sieve and saved for tissue analysis of fruit and haulm. Plant sample were ashed in porcelain crucibles for 5 hours at 550^0c. Total N in fruit and haulm sub-samples were quantitatively determined by a Kjeldahl procedure, (Bremer and Mulvarey. 1982). Where by the ammonia evolved was collected in a

boric acid solution in the presence of innicaters (methyl red and bromoceresol green) and titrated with 0.1N H_2S0_4 to pink end color (Sahlemedhin and Taye,200).

Phosphorus in fruit and haulm sub-samples were determined by using Metavandate method (NSL, 1994). Plant Samples were accustomed in the furnace for 24 hours at 450^0C and the ash was dissolved in 20% nitric acid (HNO_3) to liberate organic phosphorus. The P in solution was determined colorimetrically by using Molybdate and Metavandate for color development. Plant phosphorus was converted to orthophosphates during digestion.These orthophosphates react with 10 ml Molybdate and vanadate and give yellow colored unreduced vanado-mollybdo phosphoric heteropoly complex in acid medium. The yellow color attributed to a substititution of oxyvandaium radicals for the oxgen of phosphate.The reading of phosphorus was made at 460 nm in spectrophotometer(Khair *etal*.,2002).

3.4.5. Determination of N and P contents in fruit and haulm

N content in fruit was determined after multiplying N content of the fruit with fruit yield. Similarly, haulm N content was determined by multiplying nitrogen content in the haulm with haulm yield. P uptake by fruit and Haulm was determined from the phosphorus content of the respective parts after multiplying with the fruit yield and haulm yield, respectively. Total P uptake was then calculated as the summation of fruit and haulm uptake described by Albrizio *et al*. (2010)

N uptake =N concentration% x Dry matter

P uptake = P concentration % x Dry matter

Total N uptake =Fruit N uptake + Haulm N uptake

Total P uptake =Fruit P uptake + Haulm P uptake

3.4.6. Staining root samples

Forty two Root samples were collected by uprooting starting from the tap root and working out towards the fine roots. Roots were brought into the laboratory. After carefully washing with tap water, fine roots were cut and maintained in 95% alcohol (FAA).The Staining Root samples were analyzed the Addis Ababa University, laboratory of applied microbiology**.** Staining of mycorrhizal roots was made according to Brundrett *et al. (*1996). The stored root samples were washed carefully with tap water and transferred to labeled tube. Having cleaned samples, clearing was done adding 10% KOH solution in an autoclave liquids cycle of 15-20 minutes at 121°C. The roots were then treated with 1% HCl (v/v) for 15-20 minutes at room temperature and finally stained with 0.05% w/v try pan blue in lacto glycerol (1:1:1; lactic acid, glycerol and water)**.** With the exception of the HCl treatment, samples were drained and washed thoroughly with distilled water at the end of every step. The root samples were then left overnight in the lacto glycerol destaining solution (1:1:1; lactic acid, glycerol and water) in a dark room to remove coloration from root cells. Finally, roots were mounted in PVLG mountant on microscopic slides and covered with 40×22 mm cover slips.

3.5. Data collection

3.5.1 Growth Parameters

Plant heights (Cm): Data was taken on per pots in each replication plant with in a pot at full flowering the plants in a plot by using tape meter from collar region to the apex and mean value was determined as mean plant height.

Number of leaves per plant: This was determined as the total number of leaves and was recorded at physiological maturity.

Number of primary branches per plant: The actual number of primary branches on the Stem was recorded at 100% flowering.

Days t**o flowering:** The number of days from planting to full flowering was recorded from each pot.

Days to first harvest: The number of days from planting to first picking of fruits from the plants per pot.

Arbuscular mycorrhizal fungi (AMF) colonization: AMF root colonization rate were quantified using the magnified intersections method of McGonigle *et al.* (1990). The analyses were done at Addis Ababa University, applied microbiology laboratory.

Nitrogen (N) uptake: N uptake was assessed by determining the N concentration (%) and then multiplying it by the dry matter.

Phosphorus (P) uptake: P uptake was assessed determining the P concentration (%) and then multiplying it by the dry Matter.

3.5.2. Yield Parameters

Number of fruit per plant: Total number of fruit harvested was counted individually and mean values expressed.

Fresh fruit Yield per plant (**kg**): All the ripen fruits per plant were collected, weighed individually using sensitive balance and the mean value was record.

3.6. 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 version 9.2 (SAS Inst., 2008).The difference between treatments means were compared using Least Significant Difference (LSD) at 5% level of significance. Pearson's correlation analysis was done to observe the relationship between different parameters.

4. RESULT AND DISCUTION

4.1. Effect of Biochar on Plant Growth Parameter

4.1.1. Plant height

The analysis of variance showed that the biochar treatments had significant effect ***(P<0.01)*** on the plant height (Appendix Table 1). The mean comparison showed that the highest plant height of (110.75cm) was recorded from 36 t/ha biochar + 96 N and 92 P_2O_5 kg/ha, which was statistically on par with plant height (99.75 cm) that was recorded at 72 ton biochar + 96 N and 92 P_2O_5 kg/ha, and the shortest plant height of (56 cm) was recorded from negative control plot. When the biochar application rate was increased from control to 36 ton biochar + 96 N and 92 P_2O_5 kg/ha, plant height increased by 98% compared to the negative control treatment.

Significant increase in plant height as a consequence of biochar addition could have resulted from increased pH, EC and soil fertility leading to better nutrient absorption. Similar results were reported by Hoshi, (2001) in tea increase in plant height obtained by the application of biochar and inorganic fertilizers could be due to better nutrition for increase plant height and vegetative growth. The shortest plant height at the negative control and sole biochar treatments might be due to the result of low soil nutrient that decrease the rate of photosynthesis and plant growth. According to Zavalloni *et al*. (2011), most biochar materials are not substitutes for fertilizer, so application biochar without necessary amounts of nitrogen and other nutrients cannot be expected to provide improvements to plant growth (Rondon *et al*., 2007).

4.1.2. Leaf per plant

The analysis of variance showed that treatments had significant effect ***(P<0.01)*** on plant leaf number (Appendix Table1). The highest leaf number of (159) was recorded from 36 ton

biochar + 96 N and 92 P_2O_5 kg/ha followed by 72 ton biochar + 96 N and 92 P_2O_5 kg/ha that gave (138). The lowest leaf number was obtained from the negative control, 96 N and 92 P_2O_5 kg/ha and biochar alone treatments, respectively. Application of biochar amended with fertilizer also significantly increased plant leaf number by 33% over the negative control.

The increase in leaf number of tomato might be due to biochar effect that enhanced the activity of beneficial fungi and bacteria in the soil, enhancing special some other organisms that infect roots and help to observe more nutrients from the soil (Yamato *et al.*, 2006). Saarnio *et al.* (2013) who showed that biochar application integrated with to fertilizer increase activity of beneficial fungi and bacteria in the soil, enhancing special fungi that infect a plant's roots and help to increase in plant leaf number.

On the other hand, the lowest leaf number of tomato at the negative control and sole biochar treatments might be due to the lowest application rate of biochar. The current finding is in agreement with Kishimoto and Sugiura (1985) who reported reductions the leaf number and vegetative growth of tomato by 37% and 71% when biochar was applied at 15t/ ha and 5t /ha respectively, that attributed to macro and micronutrient deficiency.

4.1.3. Number of primary branches per plant

A highly significant ($P<0.01$) treatment effect was observed for the number of primary branches per plant (Appendix Table 1). The highest branch number per plant of (8.25) was recorded from tomato plants grown by application of 36 ton biochar addition + 96 N and 92 P_2O_5 kg/ha but was not statistically different from the 12 ton biochar addition + 96 N and 92 P_2O_5 kg/ha.The lowest number of primary branch was observed from the control, which was not statistically different from the treated with 96 N and 92 P_2O_5 kg/ha and 6 ton biochar only (Table 3).

The highest branch number per plant as a result of application of biochar along with inorganic fertilizers could be attributed to increased uptake of nutrients in the plants. Prabhu *et al*. (2003) also reported that the bigger canopy diameter as a result of increased branch number in

biochar and inorganic fertilizers treated plots was attributed to increased uptake of nutrients by the plants due to increased soil pH leading to enhanced carbohydrate synthesis which have resulted in increased cell division and primary branch.

On the other hand the observed lowest primary branches per plant might be due to low nutrients availability and high acid soils which results from low application of biochar. These results is in agreement with Steiner *et al*. (2008) who reported in acidic soil aluminum reduce root growth while manganese disrupts photosynthesis and other functions of growth and agriculture is limited by low P availability.

4.1.4. Days to flowering

Analysis of variance indicated that the treatment effect was significantly *($P< 0.01$*) different on days to flowering (Appendix Table 1). Accordingly, plants grown by negative control treatment,and sole treatements of 6 ton biochar and 12 ton biochar took relatively shortest days to reach to flowering with respective days of 61, 62.7 and 64.7.The average days to reach flowering was relatively higher for plants grown by treatments of 36 ton biochar addition + 96 N and 92 P_2O_5 kg/ha, 72 ton biochar + 96 N and 92 P_2O_5 kg/ha that were 74.75 days and 72.25 days, respectively. As biochar rate increased from control to 36 ton biochar + 96 N and 92 P_2O_5 kg/ha, days to flowering extended by 22.54% as compared to the control treatment (Table 3).

The shortest days for flowering might be due to the deficiency of the most limiting nutrients for plant growth. Although soil may contain higher amount of either nutrients, most of them were not readily available for the plant use and low soil organic matter. Gill *et al*. (1974) found that plant receiving low amount of biochar with inorganic fertilizer (N) dose produced flower buds earlier than in plants with high amount of biochar with inorganic fertilizer (N) in sweet pepper.

The delay days to reach flowering of tomato might be due to the fact that the optimum amount of biochar with inorganic fertilizer (N) promoted optimum amount vegetative growth which in turn extended days to flowering. This result is in agreement with Gnanakumari and

Satyanarayana (1971) who reported sufficient amount of biochar with inorganic fertilizer (N) supply to the tomato crops result in high vegetative material production leading to extended days to flowering of flower bud formation and hence delayed flowering of the plants.

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4.1.5. Days to First Harvest

Days to first harvest was significantly affected *($p<0.01$)* by the different treatments. Treatments with the highest rates of biochar 12, 36 and 72 ton/ha along with 96 N and 92 P_2O_5 kg/ha resulted in longest time to reach to the first harvest with respective days of 123.5, 125.5 and 124.25. The negative control treatment and the application of 6 ton biochar alone, took relatively shorter periods to reach to first harvest with respective days of 106.7 and 108.7 (Table 3). Plants in negative the control treatment mature earlier, and plants at the highest biochar rate integrated with fertilizer(N) rates showed delayed maturity dates.The result on the untreated plant early maturity might be due to most limiting nutrients for plant growth and soil may contain vast amount of either nutrients, most of them are not readily available for the plant use and low soil origanic matter thereby reduced vegetative growth of plants which in turn enhanced flower formation early. Gill *et al*. (1974) found that plant receiving low biochar with inorganic fertilizer (N) dose produced flower buds earlier than in plants with high biochar with inorganic fertilizer (N) in sweet pepper.

The delaying in tomato maturity might be due to the accumulation of more heat units (thermal time) up to flowering, fruit bearing and physiological maturity with optimum amount biochar with (N) fertilizer rates. These results are corroborated by those of Akbar *et al*. (2002) who reported that the maturity days of tomato increase as raise of biochar and Nitrogen Fertilizer optimized.

Table 3. Effect of biochar and Inorganic fertilizers application on plant height, Number of leaves per plant, Number of primary branch per plant, Days to flower and Days to harvest of tomato at Jimma, 2016 cropping season

Treatments	Plant height (Cm)	Number leaves per Plant	Number branch Per plant	Days to flowering	Days to first harvest
Control	56^{h}	81.25g	3.75^{g}	61^{g}	106.75^{j}
96 N and 92 p_2O_5 kg/ha	65^{efgh}	97fg	4.25^{g}	65.75^{de}	111^{hi}
6 tone biochar +48 N and 46 P_2O_5 kg/ha	70.5^{efg}	110^{cdef}	5.5^{ef}	69^{c}	114.25^{fg}
12 t/ha biochar +48N and 46 P_2O_5 kg/ha	73.5^{def}	109.5^{cdef}	5.75^{de}	69.75^{bc}	116^{ef}
36 t/ha biochar +48Nand 46 P_2O_5 kg/ha	83.5^{cd}	114.25^{cde}	6^{cde}	70.25^{bc}	118^{de}
72 t/ha biochar +48N and 46 P_2O_5 kg/ha	88^{bc}	116.25^{cd}	6^{cde}	71^{bc}	119.75^{cd}
6 t/ha biochar +96 N and 92 P_2O_5 kg/ha	89^{bc}	114^{cde}	$7b^{c}$	71.25^{bc}	122.25^{bc}
12 t/ha biochar +96 Nand 92 P_2O_5 kg/ha	89.25^{bc}	123.5^{bc}	7.5^{ab}	72^{b}	123.5^{ab}
36t/ha biochar +96 N and 92 P_2O_5 kg/ha	110.75^{a}	158.5^{a}	8.25^{a}	74.75^{a}	125.5^{a}
72 t/ha biochar+96 N and 92 P_2O_5kg/ha	99.75^{ab}	137.5^{b}	6.75^{bcd}	72.25^{ab}	124.25^{ab}
6 t/ha biochar only	59.5^{gh}	94.25^{fg}	4.5^{fg}	62.75^{fg}	108.75^{ji}
12 t/h biocharonly	62^{fgh}	96.25^{fg}	5.5^{ef}	64.75^{fg}	110.25^{hi}
36 ton biocharonly	68.75^{efgh}	99.75^{ef}	5.75^{de}	66^{d}	112.75^{gh}
72 ton biocharonly	77^{cde}	104.25^{def}	6.25^{cde}	69.75^{bc}	115^{fg}
Mean	78.07	111.16	5.91	68.28	116.32
CV%	11.53	9.98	11.86	2.8	1.754
$LSD_{0.01}$	12.87	15.88	1.003	2.74	2.91

DAP = Di-Ammonium Phosphate, CV=coefficient of variations, LSD=List significant difference Means followed by the same letters within a column are not significantly different at $P < 0.01$.

4.2. Effect of Biochar on Arbuscular Mycorrhizal Fungi (AMF) Colonization

AMF colonization was significantly (*P<0.05)* affected by the treatments (Appendix Table 2). The AM fungal colonization pattern showed significant heterogeneity among the roots of the tomato plant. The tomato roots had higher mycorrhization percent with the typical fungal structures like (arbuscules, hyphae and vesicles). Hyphae (HC) root colonization varied from 13 to 76.9% (Table 4). The highest HC colonization of (76.9%), Arbuscules colonization (AC) of (17.3 %) and vesicle colonization (VC) (13.1%) was found at the sole application of 72 ton biochar per hectar followed by sole application of 36 ton biochar per hectar. The negative control treatment showed the lowest root mycorrhization and was not significantly different from the treatment of 96 N and 92 P_2O_5 kg/ha (Table 4). In general, it was observed that percent mycorrhizal root colonization was at a lower rate when biochar was added along with DAP and Urea compared to colonization rate when biochar was added alone. The decrease in root colonization by AMF in fertilizer amended treatments may have resulted from an increase in plant available soil P that was in the range of 7.06 - 12.75 ppm (Table 8) compared to the initial plant available soil P which was 4.7 ppm (Table 2). It has been argued that biochar amendments could increase AMF root colonization in plants grown on acidic soils (Ezawa *et al*. 2002; Matsubara *et al*. 2002; Yamato *et al*., 2006). Some Previous reports confirmed that as the rate of P fertilization was very high, decrease AMF abundance was recorded (Warnock *et al*., 2010). The present result is in agreement with the later argument. AMF root colonization in plants depends on availability of P in the soil as low P soils showed good colonization (Warnock *et al*., 2007). According to Steiner *et al*. (2009), nutrient additions in the form of fertilizers could reduce the enhancing effect of biochar on microbial reproduction rates.

Table 4. Effect of biochar and Inorganic fertilizers application on Percentage of the Mycorrhizal root colonization of tomato at Jimma 2016 cropping Season.

Treatments	%Arbuscules	%Vesicles	%Hyphae
Control	2.57^{i}	2.0367^{h}	13.4^{h}
96 N and 92 P_2O_5	3.02^{hi}	2.46^{gh}	13.71^{h}
6t/ha biochar + 48 N and 46 P_2O_5kg/ha	4.19^{fgh}	3.28^{fgh}	25.26^{f}
12 t/ha biochar +48 N and 46 P_2O_5kg/ha	4.32^{fg}	4.95^{e}	27.1^{ef}
36 t/ha biochar +48 N and 46 P_2O_5kg/ha	3.2^{ghi}	8.45^{cd}	21.2^{g}
72 t/ha biochar+48 N and 46 P_2O_5kg/ha	5.76^{de}	7.14^{d}	31.3^{d}
6 t/ha biochar +96 N and 92 P_2O_5kg/ha	3.45^{ghi}	2.78^{fgh}	21.46^{g}
12 t/ha biochar+96 N and 92 P_2O_5kg/ha	6.1^{de}	3.76^{efg}	33.06^{d}
36 t/ha biochar +96 N and 92 P_2O_5kg/ha	4.99^{ef}	3.31^{fgh}	30.1^{de}
72 t/ha biochar +96 N and 92 P_2O_5kg/ha	3.62^{ghi}	2.66^{gh}	22.1^{g}
6 t/h biocharonly	6.52^{d}	4.34^{ef}	38.5^{c}
12t/h biocharonly	11.6^{c}	8.766^{c}	40.26^{c}
36t/h biocharonly	13.1^{b}	14.4^{b}	63.05^{b}
72t/ biocharonly	17.36^{a}	17.1^{a}	76.9^{a}
Mean	6.417	6.1	32.68
CV%	11.32	15.56	5.597
$LSD_{0.05}$	1.22	1.59	3.07

%AC= Percentage of arbuscular colonization, %VC= percentage of vesicular colonization and %HC=hyphal colonization or Total colonization.DAP = Di-Ammonium Phosphate, CV=coefficient of variations, LSD=List significant difference Means followed by the same letters within a column are not significantly different at $P < 0.01$.

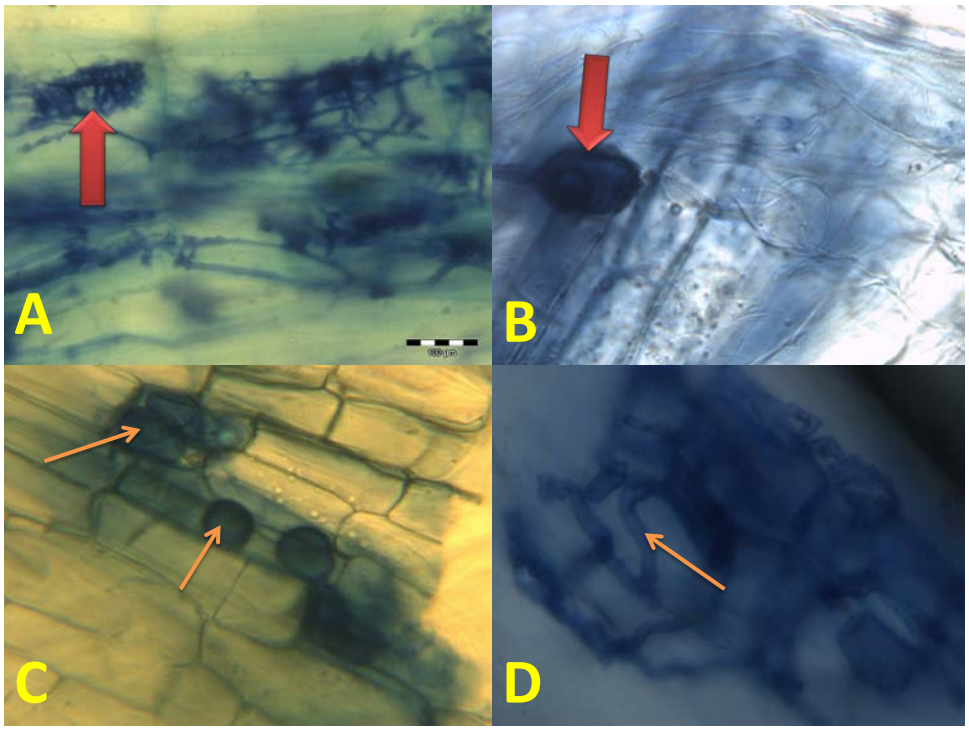

Figure. 2 . Photos depicting the effect of mycorrhizal colonization on tomato root, at 72 t/ha biochar only and 36 t/ha biochar only. Where, Fig. **A** shows Arbuscules and **C** Hyphae, vesicles and arbuscules together from sample from72t/ha biochar only, Where, Fig **B** Vesicles and **D** Hyphae coils sample from 36 t/ha biochar only.

4.3 Effect of Biochar on Nitrogen Up-take by Tomato

Analysis of variance showed that the treatment effect was significant *(P<0.05*) for N uptake by haulms and highly significant *(P< 0.01*) for fruits and total plant N uptake (Appendix Table 3). The highest N uptake by haulms of (7.47 g/plant) , fruits of (1.4 g/plant) and total plant of (8.87 g/plant) were recorded from the treatment 36 ton biochar + 96 N and 92 P_2O_5 kg/ha. Besides, **N** uptake for the haulms, fruits and total plant was low at the negative control and 6 ton biochar alone. (Table 5). It was observed in general that N uptake increased with an increase in the biochar and fertilizer rates. All the treatments with biochar only showed lower N uptake compared to biochar treatments along with 96 N and 92 P_2O_5 kg/ha. The improved N uptake due to biochar along with inorganic fertilizer application could be attributed to improvements in soil pH which can help in improved N uptake at the modified pH. High dry matter production potential due to fertilizer application can also facilitate plant N uptake

(Lehmann, 2007). Chan *et al*. (2008) also suggested that biochar application to a soil can inhibit nitrate transformation so that plant N uptake was improved.

Table 5.Effect of biochar and Inorganic fertilizers application on N uptake by fruit, N uptake by haulm and total uptake nitrogen of tomato at Jimma 2016 cropping Season.

Treatments	Nitrogen up-take by Haulm (g/plant)	Nitrogen up-take by fruit (g/plant)	Total Nitrogen uptake (g/plant)
Control	0.545^{i}	1.322^{h}	1.876^{j}
96 N and 9_2 P_2O_5	0.795^{h}	1.387^{h}	2.183^{i}
6t/ha biochar +48N and 46 P_2O_5 kg/ha	$0.85g^{h}$	1.627^{g}	2.477^{gi}
12 t/ha biochar +48N and 46 P_2O_5 kg/ha	0.959^{fg}	3.63^{e}	4.59^{e}
36 t/ha biochar +48N and 46 P_2O_5 kg/ha	1.022^{ef}	4.457^{d}	5.476^{d}
72 t/ha biochar +48N and 46 P_2O_5 kg/ha	1.111^{de}	4.582^{d}	5.69^{d}
6 t/ha biochar +96 N and 92 P_2O_5 kg/ha	1.178^{cd}	5.278^{c}	6.457^{c}
12 t/ha biochar +96 N and 92 P_2O_5 kg/ha	1.267^{bc}	5.99^{b}	7.258^{b}
36t/ha biochar +96 N and 92 P_2O_5 kg/ha	1.4a	7.473^{a}	8.875^{a}
72 t/ha biochar $_+$ 96 N and 92 P_2O_5 kg/ha	1.33^{ab}	6.08^{b}	7.41^{b}
6 t/ha biochar only	0.594^{i}	1.331^{h}	1.916^{j}
12 t/h biocharonly	0.782^{h}	1.455^{gh}	2.237^{hi}
36 t/h biocharonly	0.886^{gh}	1.637^{g}	2.523^{g}
72 t/h biocharonly	0.955^{fg}	2.894^{f}	3.848^{f}
Mean	0.977	3.51	4.487
CV%	7.81	3.67	3.19
$LSD\%_{0.05}$	0.128	0.216	0.24

DAP = Di-Ammonium Phosphate, CV=coefficient of variations, LSD=List significant difference Means followed by the same letters within a column are not significantly different at $P < 0.01$

4.4. Effect of Biochar on Phosphorous Up-take by Tomato

Analysis of variance showed that the treatment effect was significant *(P<0.05)* for P uptake by haulms and fruits and highly significant *(P< 0.01*) for total plant P uptake (Appendix Table 3). The highest P uptake by tomato haulms of (1.4 g/plant), fruits (4.9 g/plant) and total plant (6.2 g/plant) was achieved in the treatment 36 ton of biochar +96 N and 92 P_2O_5 kg/h. P uptake for the haulms, fruits and total plant was low at the negative control and 6 ton biochar only treatments (Table 6). In the same manner as in N uptake by tomato, P uptake increased with an increase in the biochar and fertilizer rates. All the sole treatments of biochar showed lower P uptake compared to biochar treatments along with either 50% or 100% N and P. The increased P uptake due to biochar application could be attributed to the high P content in the biochar and the improvements in soil pH which can help in improved P uptake at the modified pH. 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 as a result of biochar addition could also be related to microbial activity in that biochar can offer a habitat for AMF. The positive association between AMF hyphal colonization and total plant P uptake (r=0. 86***) was positive and significant in the present study which may justify that AMF colonization helped in P uptake (Warnock *et al*., 2007).

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Table 6. Effect of biochar and Inorganic fertilizer application on P uptake by fruit, P uptake Haulm and total uptake Phosphorus of tomato at Jimma 2016 cropping Season.

Treatments	Phosphorus uptake Fruit (g/plant)	Phosphorus uptake Haulm (g/plant	Total phosphorus uptake (g/plant)
Control	1.32^{e}	0.54^{i}	1.87^{h}
96 N and 92 P_2O_5	1.38^{e}	0.79^{h}	2.1^{fgh}
6t/ha biochar 48 N and 46 p_2O_5	1.62^{e}	0.85^{gh}	2.47^{f}
12 t/ biochar + 48 N and 46 p_2O_5	2.63^{d}	0.95^{gh}	3.59^{e}
36 t/ biochar + 48 N and 46 p_2O_5	3.45^{c}	1.02^{ef}	4.47^{d}
72 t/ biochar + 48 N and 46 p_2O_5	3.71^{c}	1.1^{de}	4.82^{c}
6 t/ biochar + 96 N and 92 P_2O_5	4.27^{b}	1.17^{cd}	5.45^{b}
12 t/ biochar + 96 N and 92 P_2O_5	4.45^{b}	1.26^{bc}	5.7^{b}
36t/ biochar + 96 N and 92 P_2O_5	4.9^{a}	1.4^{a}	6.2^{a}
72 t/ biochar+ 96 N and 92 P_2O_5	4.4^{b}	1.3^{ab}	5.7^{b}
6 t/ biochar only	1.33^{e}	0.59^{i}	1.91^{gh}
12 t/ biocharonly	1.45^{e}	0.78^{h}	2.23^{fg}
36 t/ biocharonly	1.63^{e}	0.88^{gh}	2.52^{f}
72 t/ biocharonly	2.36^{d}	0.95^{fg}	3.31^{e}
Mean	2.7	0.97	3.75
CV%	6.82	7.8	5.48
$LSD_{0.05}$	0.31	0.128	0.345

DAP = Di-Ammonium Phosphate, CV=coefficient of variations, LSD=List significant difference. Means followed by the same letters within a column are not significantly different at $P < 0.01$.

4.5. Effect of Biochar on yield components and yield

4.5.1. Number of Fruit per Plant

The analysis of variance showed that application of biochar had significantly *($p<0.05$*) affected a number of fruit yield (Appendix Table 1). Significantly highest fruit number of (28) was recorded from 36 ton, biochar + 96 N and 92 P_2O_5 kg/ha and the lowest fruit number (17)was record from negative control. When the biochar application rate was increased from control to 36 ton biochar + 96 N and 92 P_2O_5 kg/ha fruit number increased by 65%.(Table 7)

The increased number of fruit from 36 ton/ha biochar application along with N and P could be due to the beneficial effects of biochar on soil chemical and microbial properties. It positively affect the uptake of available nutrients in the soil by the plants leading to the development of adequate photosynthetic structures which in turn can increase the synthesis of carbohydrates and subsequent accumulation in the fruits leading to the high fruit number. Laird *et al.* (2010) concluded that the application of biochar resulted in significantly highest fruit yield per hectare that was mainly attributed to increased uptake of available nutrients present in the soil. Chan *et al*. (2008) also reported that 96% increase in radish yields from application of biochar in a greenhouse experiment and the further argued that the increment was largely due to the ability of biochar to increase N availability.

The lowest fruit number from negative control plots could be explained by the fact that essential plant nutrients are deficient that can limit plant growth, flower number, fruit setting and development. Chan *et al*. (2007) found that tomato fruit number decreased when biochar was applied at lower rates of 10 t/ha but increased when the biochar was integrated with N and P fertilizers.

4.5.2. Total Fresh Tomato Yield per Plant

Significant *($P<0.05$)* difference was observed on total fresh tomato yield due to the effect of treatments. Significantly highest fresh tomato yield of (2.88 kg/plant) was recorded from plots that received 36 ton biochar + 96 N and 92 P_2O_5 kg/ha. This was not significantly different from the yield obtained at 72t/biochar + 96 N and 92 P_2O_5 kg/ha that gave 2.75 kg/plant.The

lowest yield of 0.76 kg/plant) was obtained from the negative control, 96 N and 92 P_2O_5 kg/ha and 6t/ha biochar with 48 N and 46 P_2O_5 and 96 N and 92 P_2O_5 kg/ha produced similar yield of 0.86 kg/plant (Table 7). The highest fruit yield weight might be improved by biochar which had a potential of activating soil micro organisms and increasing the water retention capacity of the soil. As a result it caused increasing photosynthetic rate and consequent increase in growth of tomato. Similar finding was reported by Premsek and Rajashree (2009), biochar integrated with inorganic fertilizer treatments to attributed to bigger plant canopy size and higher number of fruit. These could the attributed to viable pollen germination and stem growth, which ultimately increase the fruit set. The higher fruit set due might have been to higher percentage of productive flowers (Lehmann *et al.*, 2003).

Most of plant nutrients in the control plot were found low due to soil organ matter and strong soil acidity effect. Major nutrients were found insufficient as a result as a tomato plant grown properly could not bear fruit. Sainju *et al*. (2002) reported that, tomato requires at least twelve essential plants nutrients for normal growth and result and productive flowering and better fruit setting.

Table 7. Effect of biochar and inorganic fertilizer application on Number o**f** fruit per plant and fresh tomato yield (kg) per plant of tomato at Jimma 2016 cropping season

	Number of fruit per plant	Fresh tomato yield(kg)per plant
Control	16.5i	0.76f
96 N and 92 P_2O_5	18^{h}	0.86^{f}
6t/ha biochar +48 Nand 46 P_2O_5 kg/ha	19.5^{fg}	0.93^{f}
12 t/ha biochar +48 Nand 46 P_2O5 kg/ha	21.5^{e}	1.93^{e}
36 t/ha biochar +48 Nand 46 P_2O_5 kg/ha	$22d^{e}$	2.3^{d}
72 t/ha biochar +48 Nand 46 P_2O_5 kg/ha	23^{cd}	2.47^{cd}
6 t/ha biochar +96 N and 92 P_2O_5 kg/ha	24^{c}	2.54^{c}
12 t/ha biochar +96 N and 92 P_2O_5 kg/ha	25.5^{b}	2.58^{bc}
36t/ha biochar +96 N and 92 P_2O_5 kg/ha	28.75^{a}	2.88^{a}
72 t/ha biochar +96 N and 92 P_2O_5 kg/ha	26.25^{b}	2.75^{ab}
6 t/h biochar only	17.5^{hi}	0.83^{f}
12 t/h biocharonly	18.75^{gh}	0.85^{f}
36 t/h biocharonly	19.75^{fg}	0.88^{f}
72 t/h biocharonly	20.75ef	1.8e
Mean	21.48	1.743
CV%	4.63	7.76
$LSD_{0.05}$%	1.45	0.194

DAP = Di-Ammonium Phosphate, CV=coefficient of variations, LSD=List significant difference Means followed by the same letters within a column are not significantly different at $P < 0.01$.

4.6. Effect of Biochar Application on Soil pH, EC and CEC Content

The post-harvest soil result indicated significant *($p<0.01$)* difference a mean value of soil pH, EC and CEC due to the application of biochar**.** The effect of biochar application on pH, EC and CEC values are given (Table 8). The highest value of pH 6.3 and EC 0.296 were observed in soils treated with 72 t/ha biochar + 96 N and 92 P_2O_5 kg/ha and it was not statistically different from plots treated with 36 ton/ha biochar + 96 N and 92 P_2O_5 kg/ha. The lowest mean values were recorded at the negative control (0 t/ha) but was not statistically different from 96 N and 92 P_2O_5 and 6 ton/ha biochar alone. When the biochar application rate was increased from negative control to 72 ton biochar + 96 N and 92 P_2O_5, soil pH values raised by 25.24%.

The raise in soil pH and EC values were be due to the addition of biochar and generally could be attributed to ash carbon accumulation as ash residues and that might generally dominated by carbonates and variable amounts of phosphates and small amounts of organic and inorganic N. In agreement with this, Arocena and Opio, (2003), also reported the capacity of ashes to neutralize the acidic soil. Another reason for the raise in soil pH due to application of biochar could be because of high surface and porous nature of biochar that increases the cation exchange capacity (CEC) of the soil. Thus, there could be a chance for Al and Fe to bind with the exchange site of the soil. The decrease in exchangeable Al and pH soluble Fe in biochar amended soils was also reported by Agusalim *et al*. (2010) who showed that the decrease in Al and soluble Fe in biochar amended soil was due to the increase in CEC.

The increase in CEC due to application of biochar could be resulted from the inherent characteristics of biochar. Biochar has high surface area, highly porous variable charge organic material that has the potential to increase soil cation exchange capacity (CEC), surface sorption capacity and base saturation when added to soil (Glaser *et al*., 2002). Available evidence also suggested that on a mass basis, the intrinsic CEC of biochar is consistently higher than that of whole soil, clays or soil organic matter (Peitikainen *et al*., 2000). Therefore, it is quite logical that soil applied with biochar had the highest CEC. Agusalim *et al*. (2010) also revealed the increase in soil cation exchange capacity after the

application of biochar. One potential mechanism for enhanced nutrient retention and supply following biochar amendment is increasing CEC by up to 50% as compared to unamended soils (Lehmann, 2003 and Liang, 2006).

4.7. Effect of biochar application on soil Organic C, Total N and Available P content

Biochar addition significantly *(P<0.01*) increased the mean values of soil organic C and total N content (Appendix Table 4). The highest mean values of organic carbon and total nitrogen were observed in soils amended with the highest rate 72 ton/ha biochar + 96 N and 92 P_2O_5 kg/ha and followed by 36t/ha biochar + 96 N and 92 P_2O_5 kg/ha , and the lowest mean values were recorded at the control (0 t/ha).

The increase in organic carbon and total nitrogen content due to addition of biochar could be resulted from the presence of high amount of carbon and nitrogen in the biochar. High organic carbon in soils treated with biochar has been also reported by Lehmann, (2007), Solomon *et al. (*2007) and Liang *et al. (*2006) who showed the higher organic C and total N at the ancient *terra preta* compared with adjacent soils.

Amount of available phosphorous was also significantly *(P<0.01)* increased by application of biochar. The highest mean values of available phosphorous was observed in soils amended with the highest rate 72 ton/ha biochar + 96 N and 92 P_2O_5 kg/ha , and the lowest mean values were recorded at the negative control (0 t/ha).

The observed increase in available phosphorus due to application of biochar could be due to the presence of high phosphorus in the biochar. The increase in soil pH and CEC, that reduce the activity of Fe and Al, could also contribute for the highest values of available phosphorous in soils treated with biochar. According to Kleineidam *et al*. (2002), the increased available P content of the soil with the application of biochar and inorganic fertilizers could be attributed to release of P from complexes of Al and Fe under increasing soil pH, the higher sorption affinity of biochar for organic and inorganic compounds and higher nutrient retention ability of biochar.

Generally, the chemical properties of the soil as illustrated by post harvest analysis, showed increased in percent of organic matter, organic carbon, total nitrogen, available phosphorus,CEC and increased in pH and electro conductivities. This was in agreement with Glaser *et al*. (2002) that the increase in the pH of the soil after the application of biochar could be attributed to the high pH level of the biochar and carbonate concentration which had a liming effect on the soil. The pH of the experimental soil at the start of the experiment was 5.03 compared to a range of 5.03- 6.3 obtained after 120 days after application of the biochar.

Table 8. Effect of biochar and inoriganic fertilizer application on the soil pH, EC, CEC, Organic carbon, Total Nitrogen and available Phosphorus from post harvest samples.

	PH-H_2o	EC(mmhos)	CEC	OC%	T/N%	Av.p(Ppm)
Control	5.03^{g}	0.21^{g}	17^{g}	2.06^{l}	0.2^{i}	4.76^{k}
96 N and 92 $p2O_5$ kg/ha	5.13^{g}	0.22^{g}	21.6^{f}	2.36^{j}	0.23^{ghi}	5.6^{j}
6t/ha biochar+48N and 46 $P2O_5$ kg/ha	$5.2f^{g}$	0.23^{f}	24.3^{e}	2.76^{h}	0.24^{gh}	7.06^{h}
12 t/ha biochar +48N and $46P_2O_5$kg/ha	5.5^{e}	0.236^{f}	26^{d}	3.03^{h}	0.26^{fg}	8.3^{g}
36 t/ha biochar +48N and 46 P_2O_5 kg/ha	5.6^{de}	0.246^{d}	27^{cd}	$3.1f^{g}$	0.28^{ef}	8.5^{g}
72 t/ha biochar +48N and $46P_2O_5$ kg/ha	5.8^{cd}	0.256^{c}	27.6^{c}	3.3^{e}	0.33^{cd}	9.2^{f}
6 t/ha biochar +96 N and 92 p_2O_5 kg/ha	5.9^{bc}	0.26^{c}	29^{b}	3.4^{d}	0.35^{bc}	9.6^{e}
12 t/ha biochar +96 N and 92 p_2O_5 kg/ha	6.03^{bc}	0.273^{b}	29.6^{b}	3.56^{c}	0.36^{bc}	10.3^{d}
36t/ha biochar +96 N and 92 p_2O_5 kg/ha	6.1^{ab}	0.28^{ab}	31.3^{a}	3.76^{b}	0.41^{ab}	11.4^{c}
72 t/ha biochar +96 N and 92 p_2O_5 kg/ha	6.3^{a}	0.29a	32.3^{a}	3.86^{a}	0.42^{a}	12.75^{a}
6 t/ha biocharonly	5.1^{g}	0.22^{g}	21.3f	2.23^{k}	0.22^{hi}	5.7^{j}
12t/ha biocharonly	5.2^{fg}	0.23^{f}	24.6^{e}	2.256^{i}	0.23^{ghi}	6.5^{i}
36t/ah biocharonly	5.4^{ef}	0.24^{de}	26.6^{cd}	2.73^{h}	0.31^{de}	10.2^{d}
72t/ha biocharonly	5.8^{cd}	$o.26^{c}$	30^{b}	3.16^{f}	0.38^{b}	12.2^{b}
Mean	5.61	0.248	26.3	2.29	0.3	8.75
CV%	2.57	2.1	2.1	1.96	6	2.58
$LSD_{0.05}$%	0.24	0.008	1.29	0.098	0.031	0.379

PH; Power of hydrogen, EC; Electric conductivity, CEC: Cation exchange capacity, OC: Organic carbon, TN: Total nitrogen and Av. P: Available phosphorus.

4.8. Correlation Analysis

Table 9 indicates correlation coefficients among major variables such as growth, yield, total nitrogen, Av.p.ppm,of organic carbon, soil pH, total phosphorus uptake, total Nitrogen uptake and typical structure of mycorrhizae colonization reaction. Plant height was very highly significant and positively correlated with Av.p.ppm (r=0.679**) organic carbon (r=0.819**), nitrogen uptake (r=0.85**), Cation exchange capacity (r=0.74**). Mycorrhizae hyphal colonization highly significantly and positively correlated with total phosphorus uptake (r=0. 86**), fruit yield (r=0884**) and branch number=(0.844**). The correlation matrix also showed a positive and significant relationship between soil pH and CEC. These results therefore indicate that biochar could be used as a substitution for lime materials to increase the pH of acidic soils.

There was an indication of positive correlation between percentages of organic carbon and hyphal colonization that was similar to the work of Lingfei *et al*. (2005) and some previous results conifirm positive effect of biochar was observed on mycorrhiza abundance (Warnock *et al*., 2007).

From the result of the study it could be concluded that some of the growth parameters assessed in this experiment, such as primary branch, plant height and fruit number per plant, Av.p.ppm, total nitrogen, organic carbon and total Arbuscular colonization were directly related to the fruit tomato yield per plant. The positive association between AMF hyphal colonization and total plant P uptake (r=0. 86***) was positive and significant in the present study which may justify that AMF colonization improved the P uptake. Moreover, significant

and positive correlation was also observed between total N and P uptake with number of fruit and mycorrhizae hyphal colonization (AMF).

Table 9. Pearson correlation

	Pht	BN	FN	FG	PH	AV.P	OC	T/N	T/N/up	CEC	AC	VC	HC	T/P/UP
Pht	1	0.764**	0.844**	0.884**	0.8118**	0.679**	0.819**	0.755**	0.85**	0.74**	-0.19	-0.181	0.76*	0.86**
BN		1	0.808*	0.718**	0.795**	0.755**	0.811**	0.729**	0.74**	0.809**	0.093*	0.054*	0.181	0.78**
FN			1	0.934**	0.864**	0.742**	0.948**	0.84**	0.909**	0.843**	0.165	0.163	0.067	0.965**
FG				1	0.884**	0.71**	0.927**	0.803**	0.94**	0.802**	0.22	0.148	0.124	0.978**
PH					1	0.852**	0.92**	0.84**	0.91**	0.87**	0. 01	0. 01*	0.081*	0.889**
AV.P						1	0.858**	0.918**	0.837**	0.919**	0.375*	0.4	0.48*	0.72**
OC							1	0.906**	0.95**	0.93**	-0.046	0.020*	0.76**	0.94**
T/N								1	0.89**	0.905**	0.176*	0.139*	0.261*	0.825**
T/N/up									1	0.87**	-0.0639	0.007*	0.033*	0.945**
CEC										1	0.205*	0.211*	0.296**	0.82**
AC											1	0.87**	0.95**	0.72**
VC												1	0.88**	-0.18
HC														0.71**
T/P/up														

Pht= Plant height, BN=primary branch number, FN=Fruit number, FG=Fresh number per k/g, PH= Power of hydrogen, Av.p=Available of phosphorus, OC=organic, T/N=Total nitrogen, T/N/up=Total nitrogen uptake, CEC= Cation exchange capacity, AC=Arbuscular colonization, VC=Vesicle, HC=Hyphal colonization. NS= non significant, * = Correlation is significant at the 0.05 level. **=Correlation is significant atthe0.01 level.

5. SUMMARY AND CONCLUSSION

Tomato is among the most important vegetable crops in Ethiopia. It is the most profitable crop providing with a higher income to small scale farmers than any other vegetable crops. However,current a tomato production in Ethiopia is highly constrained by acidic soils and low soil Nitrogen and Phosphorus content thuse,Its yield potential is found at low level.

Therfore a study was proposed to investigate effect of biochar and inoriganic ferilizer application addition on Nitrogen and Phosphorus uptake and arbuscular mycorrhizal fungi (AMF) root colonization of tomato plant on dominate soil type (Nitisol) at Jimma area. Biochar was produced from coffee husk at pyrolysis temperature of 350 °C. Different biochar rates were evaluated with integrating inorganic fertilizers.Tomato fruit yield and yield components, N and P uptake and AMF root colonization were studied in pots raised in greenhouse condition.

The results showed that biochar was beneficial for N and P uptake, and mycorrhizal colonization by tomato. Yield parameters like number of fruits per plant and fruit weight, fruit yield per plant were also significantly influenced by biochar application. In the present study, 36 t/ha biochar supplemented with 96 N and 92 P_2O_5 kg/ha produced better tomato yield and yield componets than the rest of treatements. Biochar applied at 72 t/ ha could reduce tomato yields below those obtained in the 36 t/ ha plots.

In addition, the chemical properties of the soil sample collected at post harvest and analytied results showed improvements in percent of organic matter, organic carbon, total nitrogen, available phosphorus, CEC and an improved pH. There fore,our current result suggested that coffee husk biochar can be used as an alternative source material for soil amendment in tomato production in Jimma areas.

Correlation analysis indicated that plant height was highly significantly and positively correlated with primary branch number, fruit number per plant, fresh fruit yield. Available P was also highly significantly and positively correlated with organic carbon, N uptake, and Cation exchange capacity. More importantly, mycorrhizae hyphal colonization was highly significantly and positively correlated with total P uptake. Moreover, arbuscular colonization was strongly correlated with vesicular and hyphal colonization. AMF root colonization in tomato depends on availability of P in the soil as low P soils showed good colonization.

In general, results of the present study indicated that biochar application substantially improved yield and yield components of tomato. Addition of 36 t/ha biochar supplemented with 96 N and 92 P_2O_5 kg/ha had better effect on N and P uptake and producing better tomato fruit yield. Moreover , future study under production field condition to a determine the N and P use efficiency in the presence of biochar needs greater attention to benefit small scale farmers and other producers interest in tomato production.

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APPENDIX

Apendex 1. Mean squares of ANOVA for analysis of variance showing mean squares for fruit fresh weight biological yield, and fruit number of tomato supplied with different Biochar rate.

		Mean squire						
Source of variance	df	PH	LNPP	BN	DF	DH	FNPP	FWKg
REP	3	183.76	449.4	1.35	59.57	16.8	2.01	0.182
TRT	13	1024.3**	1512.6**	6.1**	822.42**	148**	47.6**	2.862*
ERROR	39	123.3	80.99	0.49	3.7	4.1	0.99	0.0183

Where; df = degrees of freedom; PH=plant height, N/L/P/P= Leaf No. Leaf per plant; BN=Branch of per plant DF=Day of flower, DH=Days to harvest, F/N/P/P= Fruit No of per Plant and F/F/W/K/g Fruit fresh weight kilo gram; Asterisks*, and =NS= non-significant, significantly different at 5%, 1%.

Apendex 2. A analysis of variance showing mean squares for Arbuscular colonization, Vesicular colonization, Hyphal colonization or Total colonization weight biological yield, and fruit number of tomato supplied with different Biochar rate.

Source ofvaration	df	Meansquars %AC	%VC	%HC
REP	2	1.54	0.872	3.61
TRT	13	59.19*	65.1**	961.3**
ERROR	26	0.52	0.9	3.3479

Where; df = degrees of freedom, %AC: Percentage of arbuscular colonization, %VC: percentage of vesicular colonization, %HC: hyphal colonization or Total colonization Asterisks*, and =NS= non-significant, significantly different at 5%, 1%,

Apendex 3. Mean squares of ANOVA for analysis of variance showing mean squares for fruit fresh weight biological yield, and fruit number of tomato supplied with different Biochar rate

		Mean square					
Source of variance	dF	Haulm Nitrogen uptake	Fruit Nitrogen Up-take	Total nitrogen Up-take	Fruit phosphorus up-take	Haulm phosphorus up-take	Total phosphorus Up-take
REP	2	0.00725	0.00251	0.01576	0.00787	0.00725	0.0234
TRT	13	0.203*	13.69**	17.05**	5.51*	0.2032*	7.72*
ERROR	26	0.0058	0.0166	0.0205	0.0358	0.00583	0.042

Where; dF = degrees of freedom; *, ** and *** = non-significant, significantly different at 5%, 1%.

Apendex 4. Mean squares of ANOVA for Biochar Application on the soil EC , PH, Organic carbon, total nitrogen and available phosphorus.

Source of variance	df	Mean squares EC	PH	OC%	N%	Avpp	CEC
REP	2	0.0001738	0.0716	0.0216	0.002	0.088	5.3
TRT	13	0.00203**	0.0536**	0.95**	0.017**	19.1**	54.4**
ERROR	26	0.0000276	0.0208	0.00346	0.000347	0.051	0.59

df= degree of freedom ns, non significant, **, significant at $p \leq 0.01$.