UTILIZATION OF UNTAPPED WOOD MATERIALS FOR THE NEXT-GENERATION SUSTAINABLE AGRICULTURE

A Dissertation Submitted to the Shimane University in Partial Fulfillment of the Requirement for the Degree of

Doctor of Philosophy

By

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This dissertation entitled “Utilization of Untapped Wood Materials for the Next-Generation Sustainable Agriculture” by Mohammed Zahidul Islam, has been supervised, examined and accepted as partial fulfillment of the requirement for the degree of Doctor of Philosophy.

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ABSTRACT

Wood was the main source of energy for the world until the middle of the 19th century. Wood continues to be an important fuel in many countries, especially for cooking and heating in developing countries. It has been practiced for thousands of years for both fuel and as a building material. Recently, the new approach using a high C: N ratio organic material such as untapped wood materials was used to establish high-yield and sustainable agricultural production. Every year, a huge volume of wood waste is engendered in Japan; it is approximately 30 million m^3 in every year. Felicitous management of wood waste should be established as quickly as possible to use wood materials properly. The purpose of this study is to present new perspectives and strategies for efficient and effective use of wood wastes to enhance sustainable systems of agriculture.

Worldwide indiscriminate use of agro-chemicals boosts agricultural productivity since the green revolution of 1960s, with the cost of the environment and society. The scientific community all over the world is searching for an "economically viable, socially safe and environmentally sustainable" alternative to the poisonous agro-chemicals. Thus, it is important to find some ways and means to use the natural resources in a manner that does not pollute the environment and at the same time, provides energy and sustainability for plant production.

Based on the characteristics and properties, wood has possibility to be used for sustainable agricultural production. Wood is fundamentally composed of cellulose, hemicelluloses, lignin, and extracts. The chemical composition of wood varies from species to species, but it is approximately 50% carbon, 42% oxygen, 6% hydrogen, 1% nitrogen, and 1% other elements (mainly calcium, potassium, sodium, magnesium, iron, and manganese) by weight. The new approach using a high C: N ratio organic material such as wood that supplies carbon sources exclusively to various fungi, which contribute to the formation of soil aggregation. The aggregate structures, which possess high air and water permeability and water holding capacity, provide essential functions for plants and microorganisms including fungal and bacterial symbionts, and consequently give fast plant growth and high productivity.

For experimental investigation, wood wastes, bamboo wastes, sugi chips, konara chips, biochar as carbon sources, small amounts of oil cake, rice bran, cut weeds as organic sources, and nameko, arbuscular mycorrhizal fungi (AMF), and gliocladium fungi (GF) as fungal sources were applied to vegetable production. Conventional agro materials as nitrogen, phosphorus, or potassium fertilizer, microelements, growth promoters, pH control chemicals, or other agricultural chemicals were not used. Integrated pest management or other conventional methods were not applied to control pests and diseases; only natural defense system was approached. To minimize soil disturbance, weeds were cut by sickle when they began to race with crops. Vegetable crops generally require frequent irrigation, but irrigation was continued for 1 week from seedling day during the whole life cycle of vegetables. In these contexts, our experimental results revealed that combined application of sugi chips, konara chips, oil cake, rice bran, nameko, AMF, GF for cabbage production (Study I), wood wastes, bamboo wastes, cut weeds, AMF, GF for small green pepper production (Study II), and woodchips, biochar, leaf litter, rice bran for sweet corn production (Study III) showed a significant difference in the plant's growth and yield, as compared to plants grown in control. Notable yield was observed in the small green pepper production, the yield was 400 times higher than the yield of control. In the treated soil, levels of soil minerals (N, P, K and Ca) were increased which were significantly higher than the soil minerals of untreated (control) soil. Furthermore, wood materials influenced to grow fungal mycelium enormously, and increased the soil pH and water holding capacity. It was observed that application of wood materials to soil influenced the plant's growth and yield, and soil minerals positively but along with organic and fungal sources enhanced this effect significantly, this new approach is able to achieve higher productivity without adverse environmental impact and without the cultivation of more land, which is called sustainable intensification. Other notable significant results are that the vegetables (cabbage, small green pepper, sweet corn) grown in all treatments contained a very small amount of nitrate, high amount of potassium, calcium and sugar compared to conventional practice. These results can substantially contribute to the nutritional status of vegetables.

This study suggests that wood materials have a potential to be new agricultural sources for the next generation sustainable agriculture.

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I. BACKGROUND

1.1. Wood waste generation in Japan

Wood waste is tree bark, wood shavings, sawdust, low-grade lumber and rejects from sawmills, plywood mills and pulp mills. Wood waste also consists of the refuse from construction and demolition activities, or old furniture and scrap. Approximately 30 million m^3 of wood wastes are engendered every year in Japan (Basic act for the promotion of biomass utilization 2016), this waste wood is excreted from several different sources, including municipal waste, construction, demolition, wood processing and manufacturing, pallets and wooden packaging, and any other way. Wood is an organic material. Wood is fundamentally composed of cellulose, hemicelluloses, lignin, and extracts. The composition of wood waste is approximately 41.20% carbon, 5.03% hydrogen, 34.55% oxygen, 0.24% nitrogen, 0.09% chlorine, 0.07% sulfur, 16.00% moisture, 2.82% ash by weight (Tillman 1991). Felicitous management of wood wastes should be established as quickly as possible to use wood material properly.

1.2. Wood waste management and sustainable agriculture

People have used wood for cooking, for heat, and for light for thousands of years. Wood was the main source of energy for the world until the middle of the 19th century. Wood continues to be an important fuel in many countries, especially for cooking and heating in developing countries. Recently, a promising agricultural approach for utilizing wood wastes has been reported that application of wood waste with arbuscular mycorrhizal fungi (AMF) and gliocladium fungi (GF) achieved approximately 400 times higher yield than untreated soil (Islam and Katoh 2017). Another study has also found that application of a high carbon:nitrogen (C:N) ratio organic material without additional nitrogen fertilizer achieved four times higher productivity than that of conventional farms (Oda et al. 2014). Wood chips are considered slow decomposers, as their tissues are rich in lignin, suberin, tannins, and other complex natural compounds. Thus, wood chips add nutrients slowly to the soil as well as absorb significant amount of water that improve water holding capacity of soil. Studies have found that wood chip to be one of the best promoters in terms of moisture retention, temperature moderation, weed control, and sustainability (Chalker 2007, Bell et al. 2009). In many urban areas, wood chips are available free of charge, making them one of the most economically practical choices. Thermal degradation of wood waste under oxygen-limited conditions produced Biochar. The beneficial effects of biochar on crop production have been known since ancient times. In Amazon basin, pre-Columbian populations developed the "terra preta" soils, also known as "Amazonian dark earth," by repeating cycles of vegetation burning combined with the application of organic amendments including leaf litter, nutrient rich kitchen wastes, and fecal materials (Kammann et al. 2016); however, it is distinguished by its use as a soil amendment (Lehmann and Joseph 2009, Sohi et al. 2009). Many studies have shown the beneficial effects of biochar on soil chemical properties such as cation exchange capacity (Glaser et al. 2002), nutrients availability (Chan et al. 2008), pH (Topoliantz et al. 2007), and nutrients retention (Lehmann et al. 2003).

The U.S. National Research Council (1989) defined sustainable agriculture as "those alternative farming systems and technologies incorporating natural processes, reducing the use of inputs of off-farm sources, ensuring the long term sustainability of current production levels and conserving soil, water, energy, and farm biodiversity”. Sustainable agriculture is considered mainly as an eco-system approach where all the living organisms in soil live in harmony with a well-balanced equilibrium of food chains and their related energy balances. The goal of sustainable agriculture is to sustain significant increase of farm productivity through natural resource management, to ensure the efficient use of land and other resources, to provide better economic returns to farmers, and to contribute to the quality of life and economic development. Thus, Sustainable agriculture integrates three main goals — environmental health, economic profitability, and social and economic equity (Figure 1)

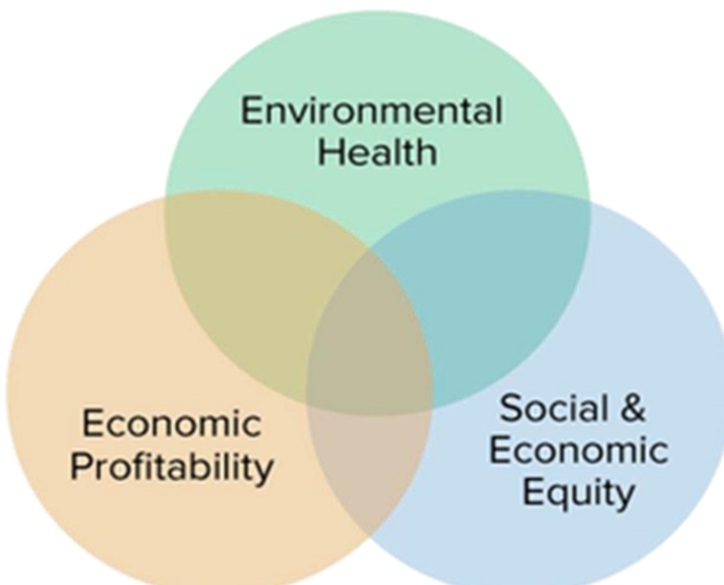

Figure 1. Sustainable agriculture

In this context, the purpose of this study is to present new perspectives and strategies for efficient and effective use of natural resources (wood wastes, bamboo wastes, sugi chips, konara chips, biochar, oil cake, rice bran, cut weeds, nameko, AMF, and GF) to enhance sustainable systems of agriculture.

1.3. Conventional agriculture and environmental concern

After World War II, agriculture has reformed radically. Use of chemical fertilizers in conventional agriculture to boost crop yield worldwide may lead to loss of carbon, nutrient run-off, excessive erosion, acidification, mineral depletion, loss of biodiversity, insect resistance, toxicity and hazard of agrochemicals. In a recent comparison of domestic, industrial, and agricultural sources of pollution from the coastal zone of Mediterranean countries, agriculture was the leading source of phosphorus compounds and sediment (UNEP 1996). Nutrient enrichment, most often associated with nitrogen and phosphorus from agricultural runoff, can deplete oxygen levels and eliminate species with higher oxygen requirements, affecting the structure and diversity of ecosystems. Nitrate is the most common chemical contaminant in the world's groundwater aquifers (Spalding and Exner, 1993), moreover, mean nitrate levels have risen by an estimated 36% in global waterways since 1990 with the most dramatic increases seen in the Eastern Mediterranean and Africa, where nitrate contamination has more than doubled (UNEP 2004). According to various surveys in India and Africa, 20-50% of wells contain nitrate1 levels greater than 50 mg/1 and in some cases as high as several hundred milligrams per liter (FAO 1996).

Thus, food security and maintenance of sustainable ecological balance are major challenges for researchers, conservationists and policy makers. Soil degradation, including decreased fertility and increased erosion, is a major concern for global agriculture (Jianping 1999). Major changes in agriculture management are necessitated to develop more sustainable agriculture system and improve weak rural economies. Soil degradation, soil acidification, soil organic matter depletion, and severe soil erosion are occurred by the long-term cultivation of lands (De Meyer et al. 2011). Moreover, soil organic matter and aggregate stability of soil are decreased (Annabi et al. 2011). It is essential to remediate the degradation of soil by simple and sustainable methods. World population is increasing dramatically; increased human pressure on land has forced the conversion of natural landscapes into agricultural fields while instantaneously depleting the land under agricultural use (Lal 2009). Therefore, there is a crucial need to establish alternate agricultural management practices that not only increase crop production but also prevent the negative environmental impacts of conventional agriculture.

Nitrogen (N), phosphorus (P), and potassium (K) are key nutrients for conventional agriculture that play a major role in crop production on degraded soils. Aforementioned nutrients deficiency is a very common issue for most of the agricultural soils in the world; there will be a high demand of chemical fertilizers to fulfill nutrients deficiency in the agricultural field. According to FAO (2012), by the end of 2020, the global requirement of chemical fertilizers (N, P, K and other macronutrients) is expected to reach 194 million tons (FAO 2012). A huge amount of nonrenewable resources such as energy in the form of oil and natural gas is required for manufacturing of the chemical fertilizers to meet this demand. In addition, soil and air pollution (greenhouse gaseous emissions) as well as water eutrophication in many parts of the world is occurred by the use of excessive chemical fertilizers. Therefore, high-level researches are essential to figure out innovative, alternative, environmentally friendly options to decrease the use of costly and non-environmentally friendly chemical fertilizers.

Thus, it is important to find some ways and means to use the natural resources in a manner that does not pollute the environment and at the same time, provides energy and sustainability for plant production. The studies were conducted through a series of study, which aim to provide solution of present unsustainable agricultural situation by the innovative agricultural materials for the next generation sustainable agriculture.

1.4. Agricultural materials for sustainable agriculture

Experimental investigations were conducted with three elements as carbon (sugi chips, konara chips, wood wastes, bamboo wastes, biochar), organic (oil cake, rice bran, weeds, leaf litter), and fungal (arbuscular mycorrhizal fungi, and gliocladium fungi, nameko) sources (Figure 2). Conventional agro materials such as chemical fertilizers, microelements, growth promoters, pH control chemicals, or other agricultural chemicals were not used.

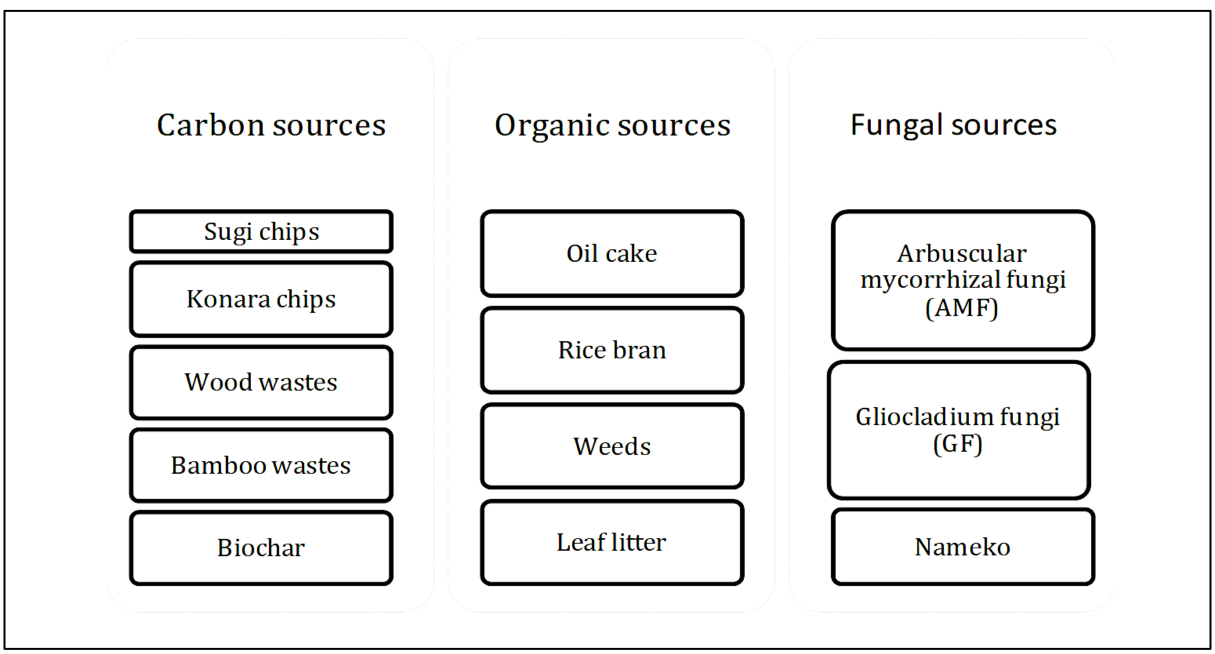

Figure 2. Agricultural materials for sustainable agriculture

The loss of carbon from agricultural soil is a critical issue in conventional agriculture. Fertilizer input generally increases net primary production but does not increase soil carbon content. Thus, the major agricultural component was wood materials (high C:N ratio).

1.5. Key point of this study

Wood is a high C:N ratio organic material in nature, wood supplies high amount of carbon to various fungi. The fungi organize an ideal condition for the growth of plants and mycorrhizas, and suppressing bacterial growth. Fungi perform important functions in the soil in relation to nutrient cycling, disease suppression, water dynamics, and create biodiversity. All of which help plants become healthier and more vigorous without using any chemical fertilizers or insecticides (Figure 3, Figure 4)

Figure 3. Soil, woodchips (carbon source), and micorrhizal development

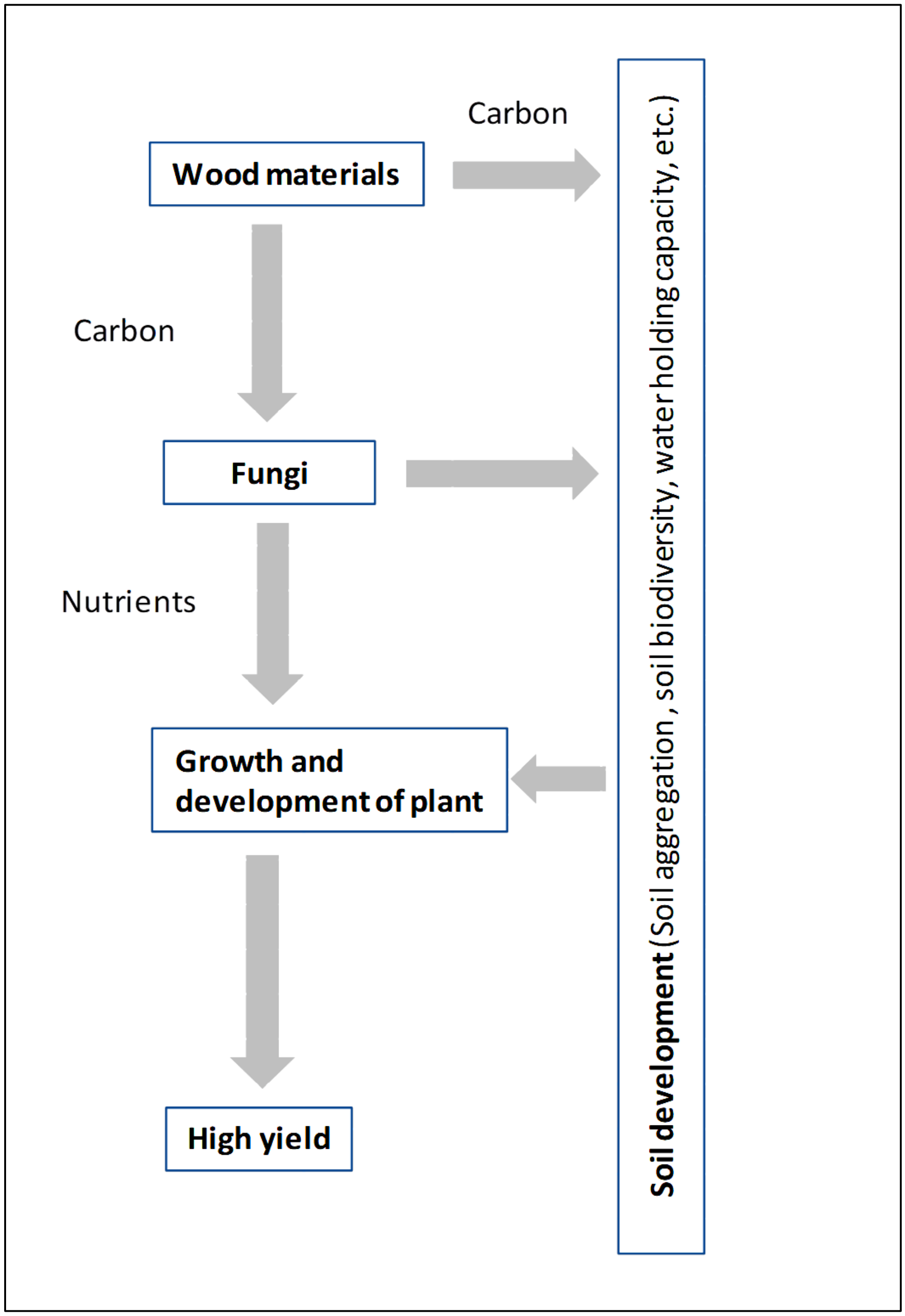

Figure 4. Flow chart of sustainable agricultural technology by the use of wood materials

II. APPLICATION EFFICIENCIES OF WOOD CHIPS (*Cryptomeria japonica* and *Quercus serrate)* ON CABBAGE (*Brassica oleraceae*) PRODUCTION WITHOUT COMPOSTING AND AGRICULTURAL CHEMICALS

STUDY I

2.1 INTRODUCTION

Wood is a porous and fibrous structural tissue found in the stems and roots of trees and other woody plants. It has been practiced for thousands of years for both fuel and as a building material. Wood is fundamentally composed of cellulose, hemicelluloses, lignin, and extracts. The chemical composition of wood varies from species to species, but it is approximately 50% carbon, 42% oxygen, 6% hydrogen, 1% nitrogen, and 1% other elements (mainly calcium, potassium, sodium, magnesium, iron, and manganese) by weight (Jean-pierre et al. 1996). In Japan, approximately 8 million tons of wood wastes are engendered every year. This waste wood is excreted from several different sources, including municipal waste, construction, demolition, wood processing and manufacturing, pallets and wooden packaging, and any other way. Felicitous management of wood wastes should be established as quickly as possible to use wood material properly.

At the same time, numerous researchers have emphasized that organic farming must be reinstated as a sustainable agricultural system that minimizes the global environmental impacts (Verena et al. 2012). However, many reports have concluded that the yields of organic agriculture are typically lower than those of conventional agriculture. Organic farming would therefore need more land to produce the same amount of food as conventional agriculture resulting in adverse environmental impact.

Recently, a promising agricultural approach for utilizing wood wastes has been reported that application of a high carbon: nitrogen (C: N) ratio organic material without additional nitrogen fertilizer achieved four times higher productivity than that of conventional farms (Oda et al. 2014).

The use of organic matter such as animal manures, human waste, and food wastes has long been recognized in agriculture as beneficial for plant growth and yield. The new approach using a high C: N ratio organic material such as wood supplies carbon sources exclusively to various fungi which contribute to the formation of soil aggregation. The aggregate structures, which possess high air and water permeability and water holding capacity, provide essential functions for plants and microorganisms including fungal and bacterial symbionts, and consequently give fast plant growth and high productivity. This condition is also suitable for preventing plant diseases and insect infestation of plants because constructed soil biodiversity does not allow exclusive propagations of specific pathogens and pests. That is to say, chemical fertilizers and insecticides are not required for this approach. Furthermore, it contributes greatly to the mitigation of greenhouse gas, since CO_2 emission at the composting process can be largely reduced. Accordingly, this new approach is able to achieve higher productivity without adverse environmental impact and without the cultivation of more land, which is called sustainable intensification.

Sustainable agriculture, as defined by Farm Bill, the U.S. Department of Agriculture in the 1990, should "Over the long term, satisfy human needs, enhance environmental quality and natural resource base, make the most efficient use of nonrenewable resources and integrate natural biological processes, sustain economic viability and enhance quality of life" (Food, Agriculture, Conservation, and Trade Act. 1990). Thus, this new approach utilizing a raw wood material is exactly sustainable and innovative farming system that can feed human populations and simultaneously improve various environmental issues.

To address the innovative agricultural approach, application efficiencies of carbon source, and combined application of carbon, organic and fungal sources on cabbage (*Brassica oleraceae*) production were investigated.

2.2 MATERIALS AND METHODS

2.2.1 Experimental site

The experiment was carried out at the experimental field of Shimane University, Matsue-shi, Shimane, during the period from 1st May 2015 to 4th August 2015. The experimental site was established on the fallow land. The land had severe limitations which significantly restrict the range of crops and the level of productivity. It was mainly suited to permanent pasture or rough grazing, the application of wood chips (high C:N ratio) was initiated first in the experimental field on April 2011.

2.2.2 Plant material

In this study we considered cabbage (*Brassica oleraceae)* as plant material. Commercially available seedlings were used, 10 plants were transplanted in each treatment and each plant had average 10 cm height at transplanting day. Cabbage was planted regularly without any break, usual crop rotation was ignored, and more than two years land was cultivated in a single crop.

2.2.3 Land preparation, experimental design and treatment combination

The experimental land was first opened on 1st April 2011. Five treated and one control plots were prepared. Each plot site contained 1 ridge (1 ridge =175 cm length × 40 cm width × 20 cm height) and 2 furrows in both side (1 furrow =175 cm length × 40 cm width × 20 cm depth), plot areas are presented in Figure 5. The experimental design was laid out in a Completely Randomized Design (CRD) with 5 treatments namely,

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T_0- control (untreated)

T_1- sugi chips + konara chips (Saninmaruwa),

T_2- konara chips,

T_3- sugi chips + oil cake (NisshinGM) + rice bran (Twinbird) + nameko *(Pholiota microspora*, Nihonnorinsyukin),

T_4- sugi chips + konara chips + oil cake + rice bran + nameko + arbuscular mycorrhizal fungi (Idemitsu) + gliocladium fungi (Idemitsu),

T_5- konara chips + oil cake + rice bran + nameko + arbuscular mycorrhizal fungi + gliocladium fungi.

In these 5 treatments, agricultural materials were carbon, organic, and fungal sources, carbon sources (wood chips) were used enormously. Wood chips (0.28 m^3/furrow), small amounts of oil cake (0.25 kg/furrow), rice bran (0.30 kg/furrow), nameko (0.05 kg/furrow), *Gliocladium sp.* and arbuscular mycorrhizal fungi (5 mg/plant) were used for experimental investigation (Figure 9).

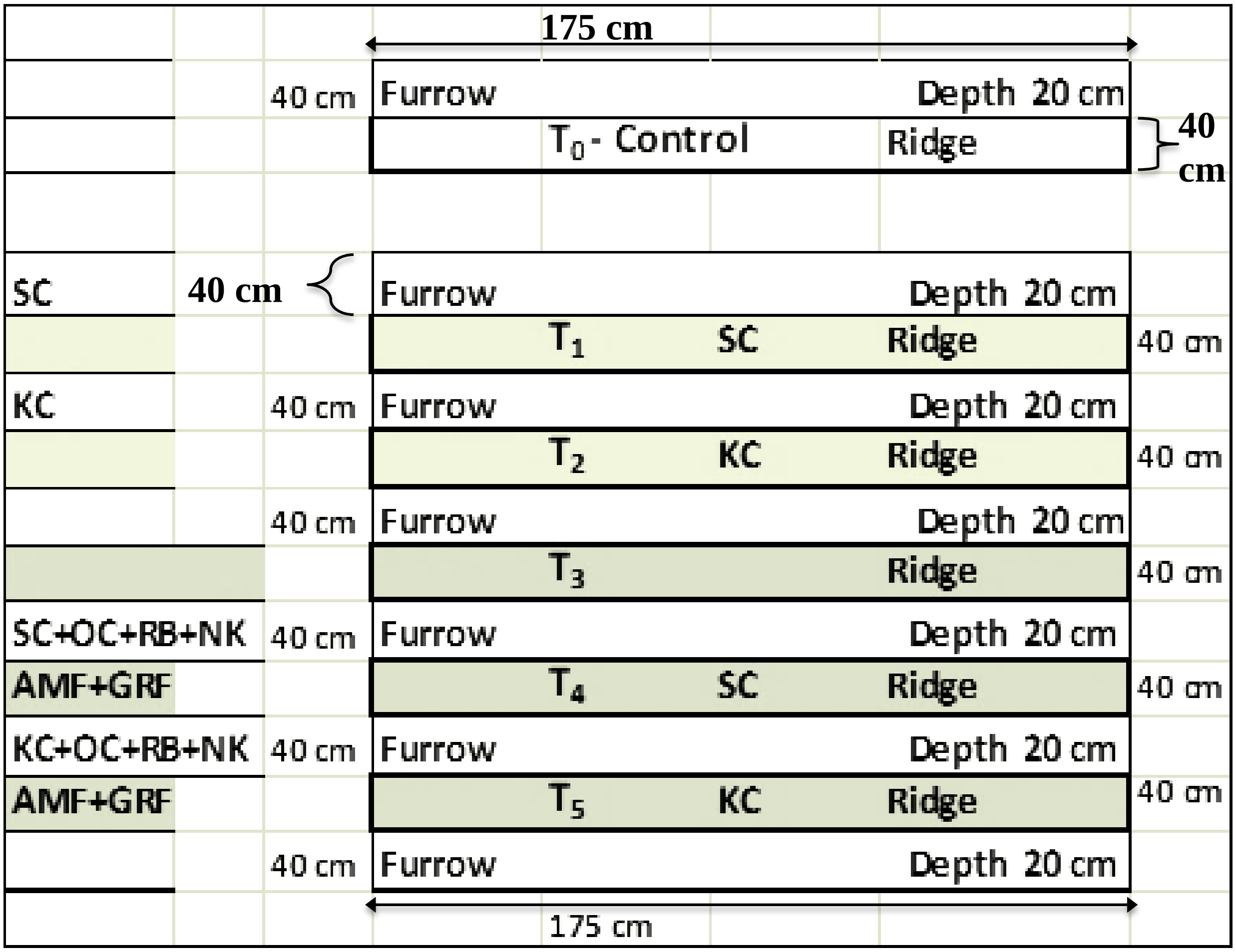

Figure 5. Layout of the experimental site.

Notes: SC= Sugi chips (*Cryptomeria japonica*), KC= Konara chips (*Quercus serrata*), OC= Oil cake, RB= Rice bran, NK= Nameko (*Pholiota microspora* (Berk.) Sacc.), AMF= Arbuscular mycorrhizal fungi, GRF = Gliocladium rhizofungi.

2.2.4 Test crop establishment and management

2.2.4.1 Agrochemicals and compost applications

Experimental investigations were conducted with three elements as carbon, organic and fungal sources. Conventional agro materials as nitrogen, phosphorus, or potassium fertilizer, microelements, growth promoters, pH control chemicals, or other agricultural chemicals were not used. The loss of carbon from agricultural soil is a critical issue in conventional agriculture. Fertilizer input generally increases net primary production but does not increase soil carbon content. Thus, the major agricultural component was wood chips (high C: N ratio).

2.2.4.2 Weed control

To minimize soil disturbance, weeds were cut by sickle when they began to race with crops.

2.2.4.3 Pests and diseases

The big advantage of great social and environmental significance of this method is that it can suppress or eradicate pests and diseases in crops without the application of any pesticides and fungicides. Thus, integrated pest management or other conventional methods were not used; only natural defense system was approached.

2.2.4.4 Irrigation

Vegetable crops generally require frequent irrigation, but irrigation was continued for 1 week from transplanting day during the whole life cycle of cabbage.

2.2.5 Sampling and data collection methods

(1) Cabbage was harvested at 95 days after transplanting. Cabbage yield was calculated on the basis of plot area (area of each plot = 1.4 m^2), and converted the average yield into kg/m^2.

(2) Soil minerals N, P, K and Ca (mg/100 g) and cabbage minerals NO_3^-, PO_4^{3-}, K^+, and Ca^{2+} (mg/L) were measured according to the guideline of RQ flex plus 10 (MERCK), Quantofix (MN) and LAQUA (HORIBA). Conventionally grown cabbages obtained from retail stores were used for mineral analysis and comparative study with experimental field cabbages.

(3) Endophyte colonization in cabbage root was observed by a compound microscope. Fungal structures were stained with blue ink.

(4) Insect damage (%), plant height (cm), and cabbage head diameter (cm) were measured.

2.2.6 Data analysis

The experimental data was conveyed as mean ± Standard Error (SE), One way analysis of variance (ANOVA) and Least Significant Difference (LSD) were carried out by Microsoft Excel to determine the difference between control and the treatments ($P \leq 0.05$).

2.3. RESULTS

2.3.1 Cabbage growth and yield performance

The highest yield of cabbage was 2.05 kg/m^2 produced at T_4, which was approximately, 5 times higher than that of control (T_0), 4 times higher than T_1 and T_2, 2 times higher than T_3 and T_5 (Figure 6). The highest plant height (25.66 cm) and cabbage head diameter (18 cm) were observed at T_4, which were approximately 1.5 and 18 times higher than the control respectively (Figure 7, and 8).

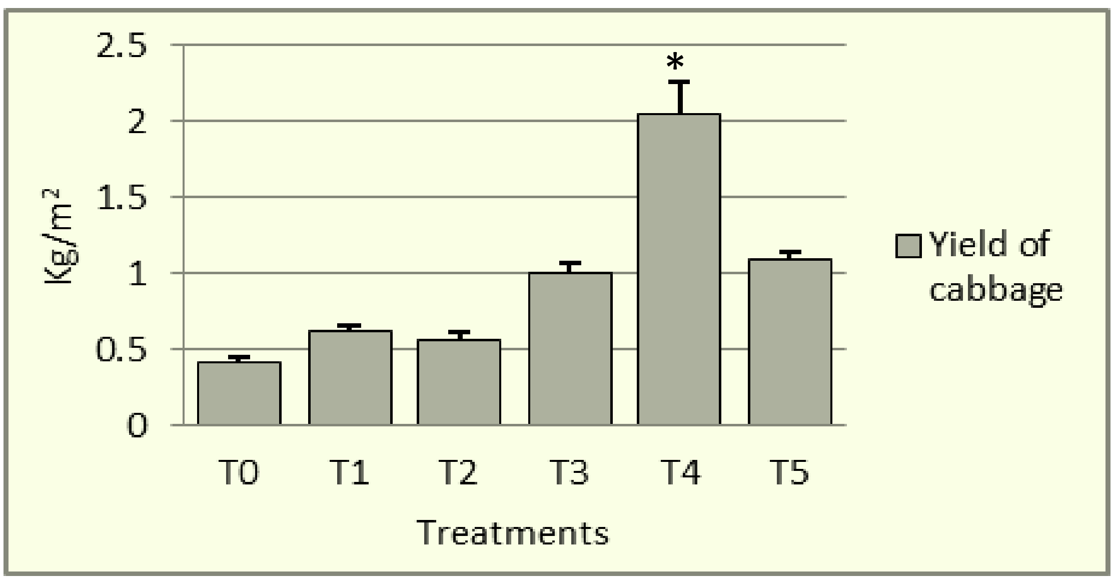

Figure 6. The effect of different treatments on the yield of cabbage. Significant difference is indicated by asterisks (*P<0.05), vertical lines represent SE.

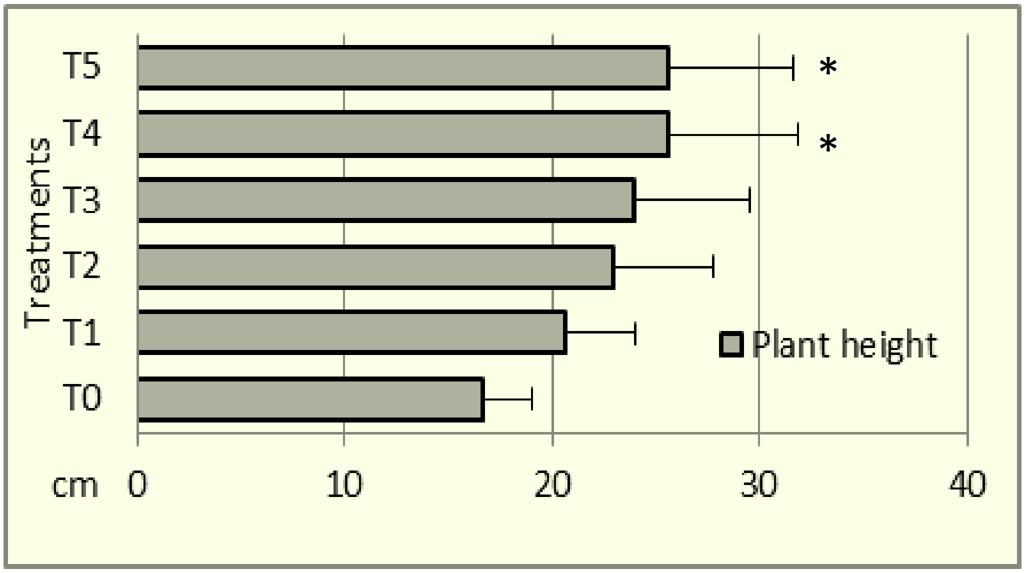

Figure 7. The effect of different treatments on the plant height of cabbage. Significant differences are indicated by asterisks (*$P<0.05$), horizontal lines represent SE.

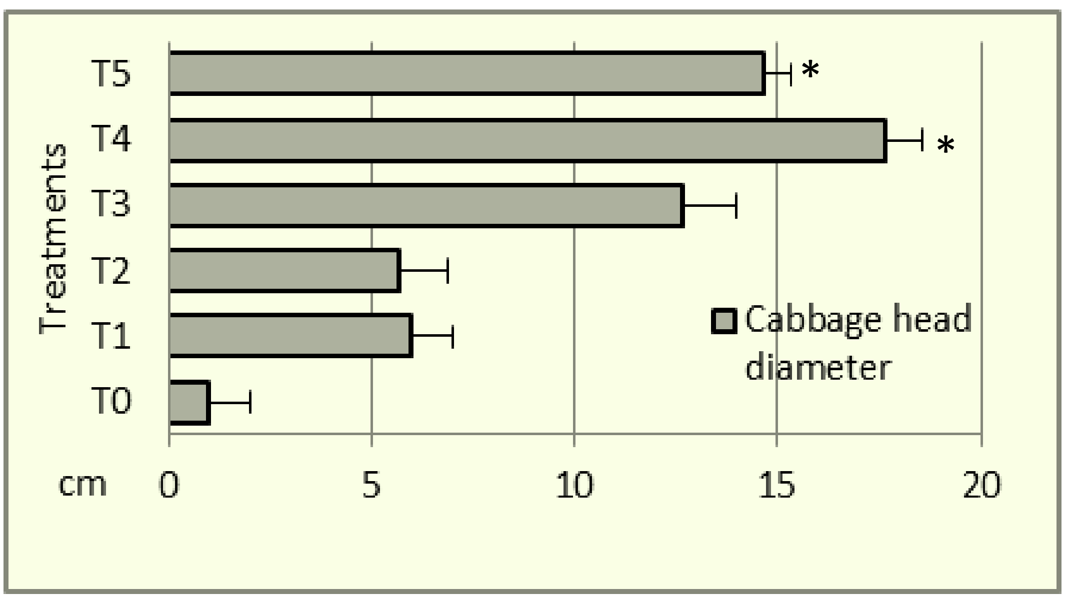

Figure 8. The effect of different treatments on the head diameter of cabbage. Significant differences are indicated by asterisks (*$P<0.05$), horizontal lines represent SE.

Figure 9. Experimental cabbage field.

2.3.2 Pest attack and damage

Damaged leaves of cabbage plants were counted at vegetative stage. Higher infestation density of cabbage leaves were 87%, and 84%, observed at T_3 and control, respectively, and lower were 31%, 36%, 37%, and 38%, observed at T_2, T_5, T_4, and T_1, respectively, at 40 days after transplanting (Figure 10). Use of wood chips could reduce insect infestation without harmful chemical pesticides.

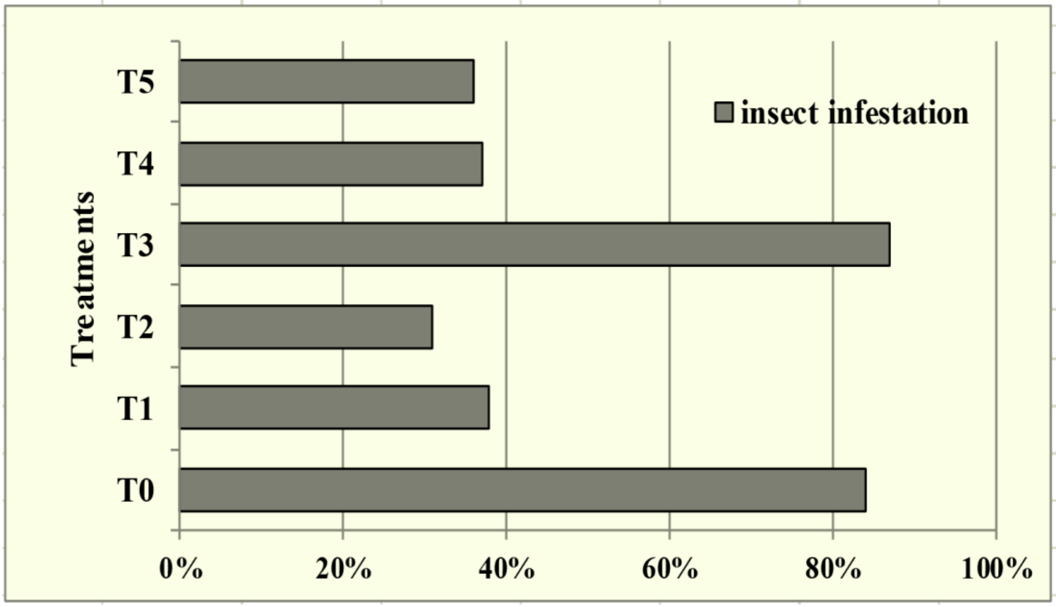

Figure 10. The effect of different treatments on insect infestation of cabbage.

2.3.3 Mineral of cabbage

2.3.3.1 K^+ and Ca^{2+} level

The higher level of K^+ was 5450, 4925, and 4100 (mg/L), produced at T_3, T_5, and T_4, respectively, which were approximately 3 times higher than that of conventionally grown cabbage (chemical based farming) and control. Moreover, the higher Ca^{2+} level of cabbage was 560, and 398 (mg/L) produced at T_2, and T_1, respectively, while conventionally grown cabbage and control were approximately 3 times lower (Figure 11).

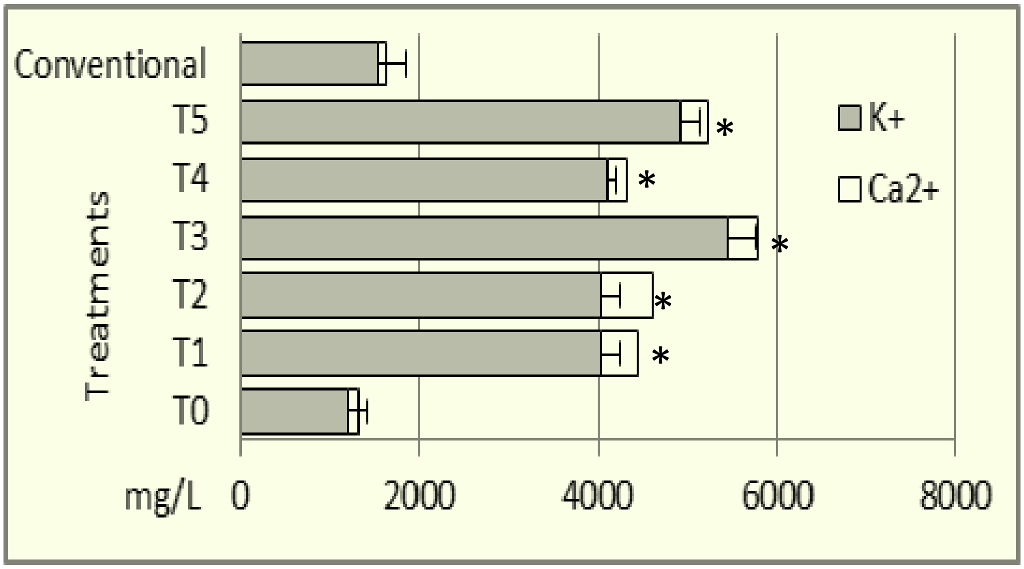

Figure 11. The effect of different treatments on the K^+ and Ca^{2+} levels of cabbage. Significant differences are indicated by asterisks (*P<0.05), horizontal lines represent SE.

2.3.3.2 NO_3^- level

NO_3^- level of cabbage was the most important contributing parameter which was significantly marked in treatments and conventionally grown cabbage. The level of NO_3^- of all treatments was approximately 41 times lower than the conventionally (chemical based farming) grown cabbage (Figure 12).

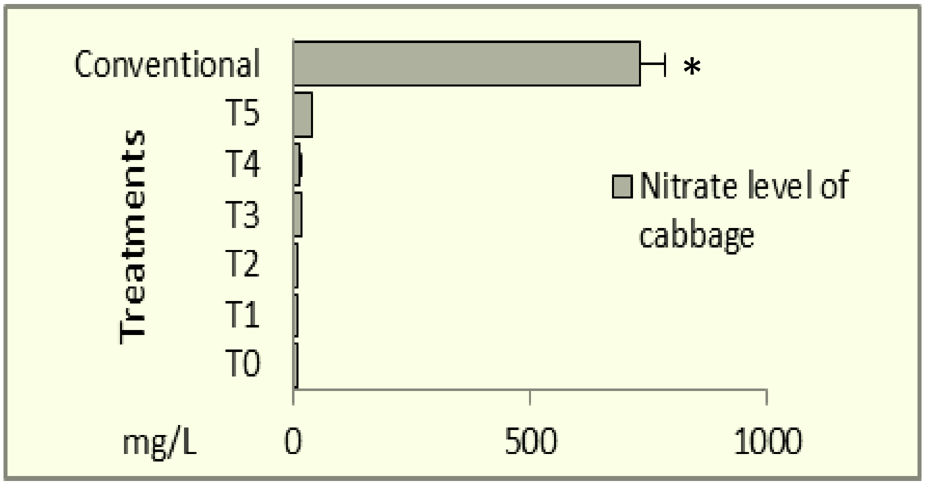

Figure 12. The effect of different treatments on the NO_3^- level of cabbage. Significant difference is indicated by asterisks (*P<0.05), horizontal lines represent SE.

2.3.4 Changes in soil mineral concentration

Mineral concentration of soil was significantly influenced by different treatments. Soil mineral concentration was measured 4 times, the concentration gradually increased after passing a certain time (Figure 13). The highest concentration of N, P, and Ca was 2.03, 5.05, and 48.00 mg/100 g observed at T_4 during harvesting time at after planting period. Moreover, the highest total amount of soil mineral (N, P, K, and Ca) was 83.08 mg/100 g at T_4 during the harvesting time, that was significantly higher than that of all other treatments and control.

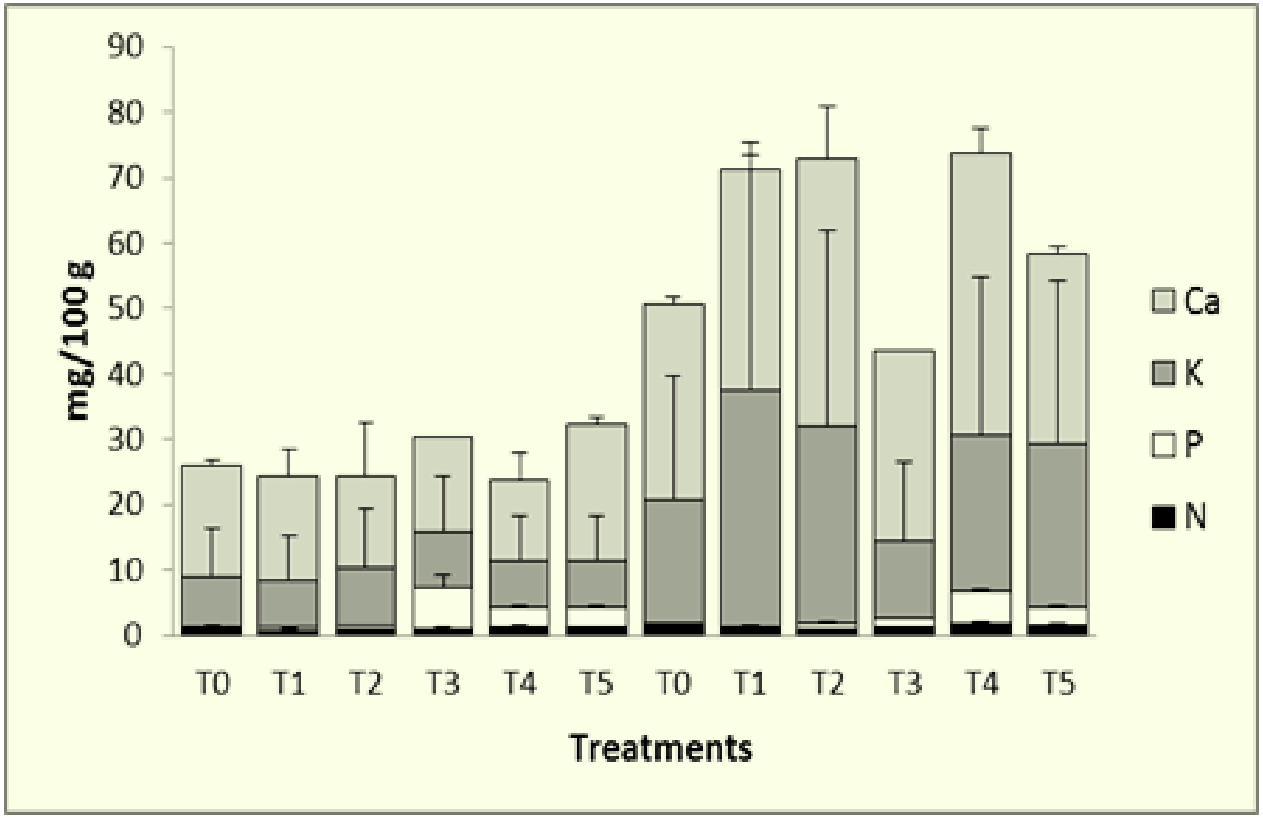

Figure 13. The effect of different treatments on N, P, K and Ca levels of soil.

2.3.5 Endophyte colonization in cabbage root

Compound microscope was used to visualize the colonization of fungi; this type of colonization was only involved in intercellular space of the cortical cells of roots in T_4 and T_5 plants. In this intercellular space, fungal hyphae and spores were frequently observed (Figure 14).

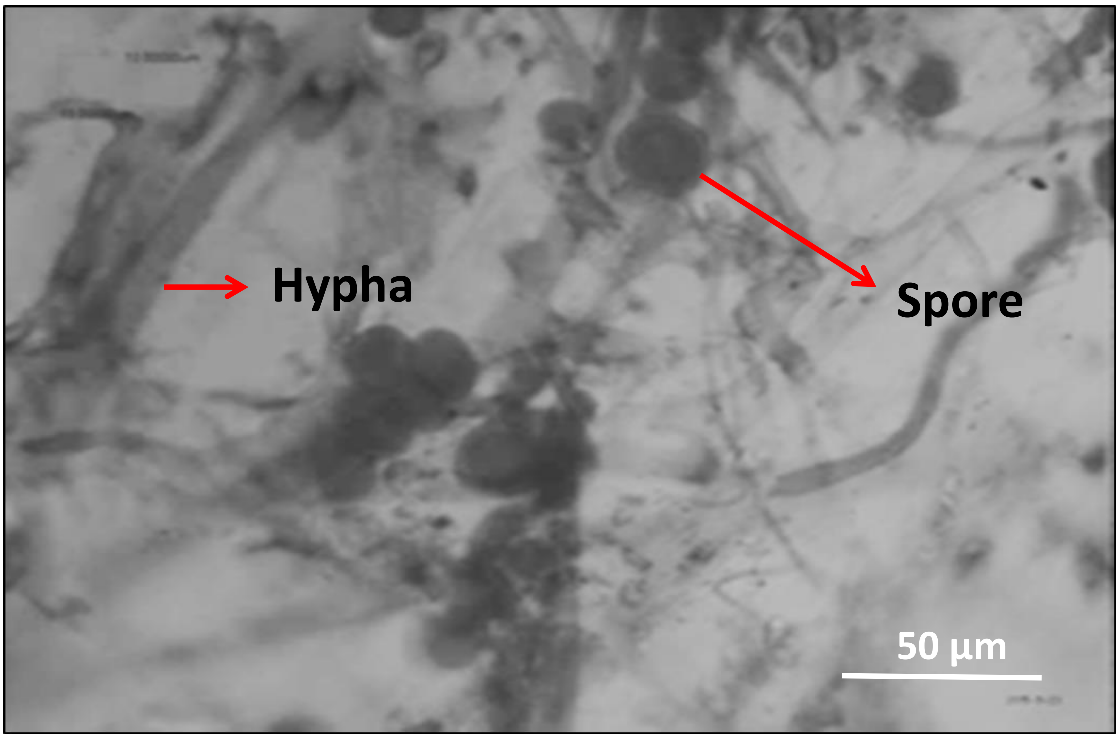

Figure 14. Fungal colonization of cabbage roots

2.4 DISCUSSION

We conducted a research by using a huge volume of wood chips and few amount of organic and fungal sources without using any fertilizers and pesticides to develop the sustainable agricultural system that maintains or enhances soil productivity through the balanced use of carbon, organic, and fungal sources. Combined application of carbon, organic and fungal sources (T_3, T_4, and T_5) is one of the effective method to grow vegetable without using any fertilizers and pesticides. At this time, soil fertility and crop productivity were increased. Another important feature of these treatments (T_3, T_4, and T_5) is that NO_3^- level of the cabbage was approximately 41 times lower than that of conventionally grown cabbage. High level of nitrate in crop has been implicated to cause bladder, ovarian, stomach and liver cancers for human body (Mueller et al. 2001). Excess nitrogen of chemical based farming reduces carbohydrate synthesis, lowers resistance to diseases (rust and downy mildew), lowers resistance to insect and reduces the biological value of plant protein (Hornick 2010). The low rate of insect damage of treated plants was involved in the low NO_3^- level of cabbage. Although, the N concentration of soil T_5 was comparatively higher than the other treatments (1.76 mg/100 g), which was very low than the lower limit concentration in the soil for conventional or general organic farming (20 mg NO_3^-N/ kg) (Breschini and Hartz 2002). The large root system and root zone are required for the absorption of sufficient amount of nutrition under low nitrogen concentration. Endophyte colonization was also observed in intercellular space of the cortical cells of roots in T_4 and T_5 plants. Most of the plants convey fungal endophytes, some of which may enrich host development, nutrient gaining and may progress the plant's propensity to tolerate abiotic pressures, such as drought, and grow resistance to insects, plant pathogens and mammalian herbivores (Cheplick et al. 2009). The greater production of cabbage with the combined application of carbon, organic and fungal sources could be summarized as follows:

1. The highest yield of cabbage was at T_4 (with sugi chips), second highest at T_5 (without sugi chips), and third highest at T_3 (without arbuscular mycorrhizal fungi and gliocladium). Yield of T_4 was approximately, 2 times higher than T_3, and T_5.

2. The application of sugi chips, fungal sources, and organic matter in soil assisted to raise the fungal community at T_4.

3. The presence of fungal community of T_4, generated soil environment for highest yield of cabbage without using any fertilizers and pesticides.

It can be concluded that cabbage production with carbon, organic and fungal sources is a new dimension for world agriculture. Further research is underway to explore more in detail.

III. THE EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI AND GLIOCLADIUM FUNGI ON THE YIELD OF SMALL GREEN PEPPER (*Capsicum annuum*) GROWN BY SUSTAINABLE AGRICULTURE

STUDY II

3.1 INTRODUCTION

Worldwide indiscriminate use of agro-chemicals boosts agricultural productivity since the green revolution of 1960s, with the cost of the environment and society. It kills the valuable soil microorganisms and destroys their natural fertility, reduces the power of biological resistance in crops to make them more susceptible to pests and diseases (FAO 1996, U.S. News and World Report 2008, Pingali 2012). The scientific community all over the world is urgently searching for an 'economically viable, socially safe and environmentally sustainable' alternative to the poisonous agro-chemicals (Sinha et al. 2009). The U.S. National Research Council (1989) defined sustainable agriculture as 'those alternative farming systems and technologies incorporating natural processes, reducing the use of inputs of off-farm sources, ensuring the long term sustainability of current production levels and conserving soil, water, energy, and farm biodiversity'. It is a method of agricultural production, which avoids or largely omits the application of systematically compounded chemical fertilizers and pesticides and promises the utilization of environmentally amicable organic inputs.

By 2050 the world's population will reach 9.1 billion, 34 percent higher than today (FAO 2009). Global demand for agricultural crops definitely emphasizes the necessity to implement eco-friendly agricultural management practices for sustainable agricultural production. It is not adequate to produce sufficient food to feed the civilization, but to engender a high quality of nutritive food which should be safe (chemical free) and protective to human health and the environment, and to engender it in a sustainable manner to deserve food security for all. The difficulties are associated with the consumption of poisonous chemicals, because crop protection, weed control, and soil fertility are getting increasing attention worldwide since pests, diseases, and weeds become resistant to chemical pesticides and environmental pollution and ecological imbalances may occur. So, the engenderment of organic agriculture products without inputs of chemical pesticides and synthetic fertilizers has become more concerned (Marini-Bettolo 1987, Peggy 2000, FAO 2001, Horrigan et al. 2002, Sinha 2008).

Numerous researchers have emphasized that organic firming must be reinstated as a sustainable agricultural system that minimizes the global environmental impacts (Verena et al. 2012). However, many reports have concluded that the yields of organic agriculture are typically lower than those of conventional agriculture. Organic farming would therefore need more land to produce the same amount of food as conventional agriculture resulting in adverse environmental impact (Trewavas 2001, McIntyre et al. 2009, De Schutter 2010).

Recently, a promising agricultural approach for utilizing wood wastes has been reported that application of a high carbon: nitrogen (C: N) ratio organic material without additional nitrogen fertilizer achieved four times higher productivity than that of conventional farms (Oda et al. 2014). The new approach using a high C: N ratio organic material such as wood and bamboo wastes supplies high amount of carbon to various fungi, and fungi perform important functions in the soil in relation to nutrient cycling, disease suppression, and water dynamics, all of which help plants become healthier and more vigorous. Moreover, fungi can promote soil aggregation (Miller and Jastrow 2000), the aggregate soil structures, which possess high air and water permeability and water holding capacity, provide essential functions for plants and microorganisms, including fungi and bacterial symbionts, and consequently give faster plant growth and high productivity. This condition is also suitable for preventing plant diseases and insect infestation of plants because constructed soil biodiversity does not allow exclusive propagations of specific pathogens and pests. Furthermore, it contributes greatly to the mitigation of greenhouse gas, since CO_2 emission in the composting process can be largely reduced. Accordingly, this new approach is able to achieve higher productivity without adverse environmental impact and without the cultivation of more land, which is called sustainable intensification (Pretty and Bharucha 2014). Wood grows naturally, and it is renewable resource. The objective of this study is to present new directions and approaches for effective use of wood and bamboo wastes, weeds, and fungi to develop sustainable systems of agriculture. Large volumes of wood wastes are generated in many ways. Sawdust, chips, planer shavings, bark, slabs, end trims, sander dust, used or scrapped pallets, logs, brush, and branches are very common wood wastes. Every year, approximately 8 million tons of wood wastes are engendered in Japan (Basic Act for the Promotion of Biomass Utilization 2016). Bamboo generates large volumes of wastes, and these wastes are excreted from construction, demolition, furniture and any other way. Felicitous management of wood and bamboo wastes should be established as quickly as possible to use wood and bamboo materials properly.

To address the innovative agricultural approach, application efficiencies of carbon (wood, and bamboo wastes), organic (cut weeds), and fungal sources on small green pepper (SGP, *Capsicum annuum*) production were investigated.

3.2. MATERIAL AND METHODS

3.2.1 Experimental Site

The experiment was carried out in the experimental field of Shimane University, Matsue, Shimane, during the period from 21st April 2015 to 27th November 2015 to assess the integrated effect of carbon, organic, and fungal sources on the growth, yield, and minerals of SGP. Geographically, the site was located between 35°28'27''N and 133°3'11''E. The average temperature, precipitation (rainfall), and relative humidity were 12.5°C to 26.5°C, 140 mm to 280 mm, and 70-80%, respectively, from April to November. The soil type of the experimental area was sandy loam with soil pH of 6.0.

3.2.2 Land Preparation, Experimental Design, and Treatment Combination

The experimental field was cleared, ploughed, harrowed and divided into 4 plots, with 11.20 m^2 areas. 3 treated and 1 control plots were prepared. Each plot site contained 1 ridge (1 ridge = 350 cm length × 40 cm width × 20 cm height) and 1 furrow (1 furrow = 350 cm length × 40 cm width × 80 cm depth), plot area is presented in Figure 15. Wood wastes, bamboo wastes, cut weeds, arbuscular mycorrhizal fungi (AMF), and gliocladium fungi (GF) were applied as agricultural materials. The experimental design was laid out in a completely randomized design with 3 treatments namely,

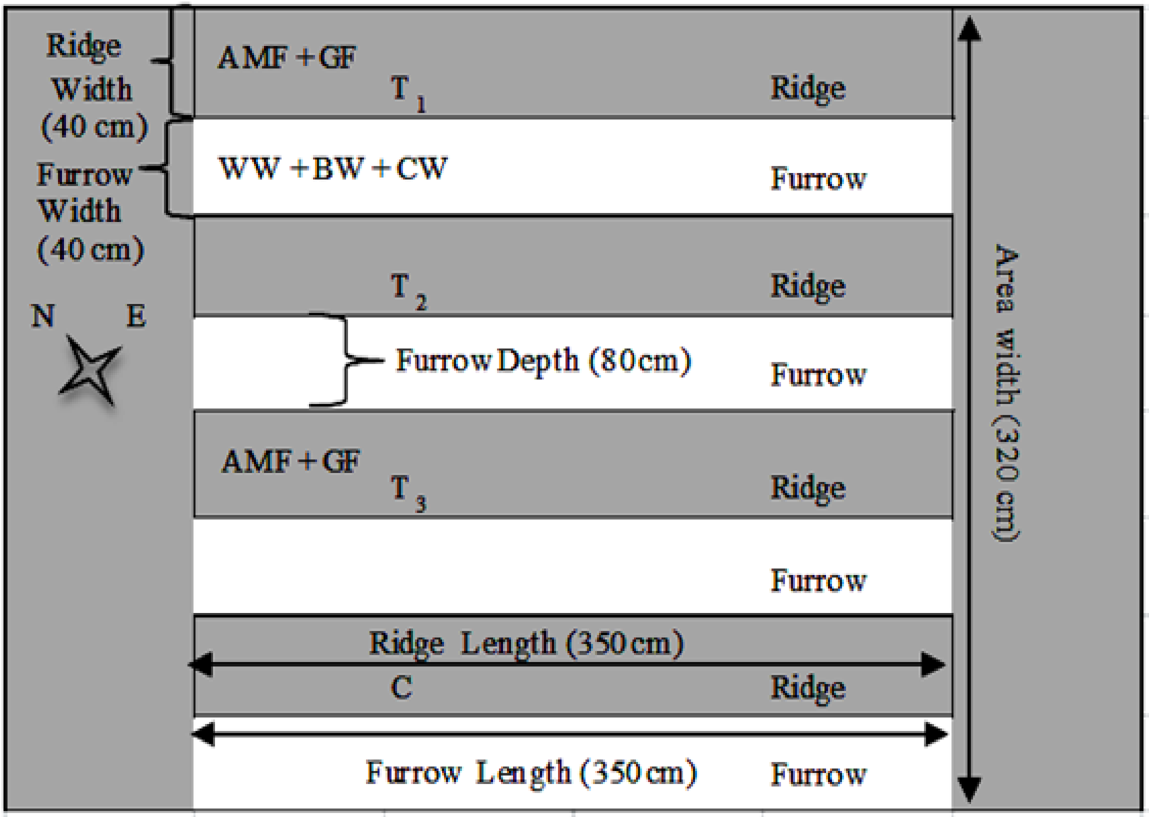

Figure 15. Layout of the experimental site.

Notes: WW = Wood wastes, BW = Bamboo wastes, CW = Cut weeds (meadow grass, couch grass, horsetail, nettle, chickweed, ground elder, etc.), AMF = Arbuscular mycorrhizal fungi, GF = Gliocladium fungi (*Gliocladium* sp.).

T_1- wood wastes + bamboo wastes + cut weeds (meadow grass, couch grass, horsetail, nettle, chickweed, ground elder, etc.) + AMF (Idemitsu) + GF (Idemitsu),

T_2- wood wastes + bamboo wastes + cut weeds,

T_3- AMF + GF,

C- control (untreated)

Wood wastes (0.40 m^3/furrow), bamboo wastes (0.40 m^3/furrow), weeds (0.25 m^3/furrow), AMF and GF (5 mg/plant) were directly used in the ridges and furrows for the experimental investigation.

3.2.3 Plant Material

In the present work, SGP was considered as plant material. Commercially available seedlings were used for the experimental observation, 4 plants were transplanted in each treatment and average plant height was 15 cm at transplanting time.

3.2.4. Test Crop Establishment and Management

3.2.4.1 Application of Agricultural Material

Experimental investigations were conducted with three elements as carbon (wood, and bamboo wastes), organic (cut weeds), and fungal (AMF, and GF) sources. Conventional agro materials such as chemical fertilizers, microelements, growth promoters, pH control chemicals, or other agricultural chemicals were not used. The loss of carbon from agricultural soil is a critical issue in conventional agriculture. Fertilizer input generally increases net primary production but does not increase soil carbon content. Thus, the major agricultural component was wood and bamboo wastes (high C: N ratio). Root, branch, bark, and log of chinaberry (*Melia azedarach*) tree as wood wastes, and stem of bamboo as bamboo wastes were used in the two furrows. Bamboo wastes were generated from demolition work, and wood wastes were collected from fallen tree trunk in the experimental area.

3.2.4.2 Weed Control

To minimize soil disturbance, weeds were cut by sickle and put in the furrow when they began to race with crops.

3.2.4.3 Pests and Diseases

Integrated pest management or other conventional methods were not used; only the natural defense system was approached to control pests and diseases.

3.2.4.4 Irrigation

SGP plant generally requires frequent irrigation, but irrigation was continued for only 1 week from the transplanting day during the whole life cycle of SGP.

3.2.5. Data Collection and Sampling

3.2.5.1 Yield and Vegetative Growth

SGP was collected 28 times, from 73th day to 220th day after transplantation, and yield was calculated based on the plot area, and converted the average yield into kg/m^2. Area of each plot was 2.8 m^2 (area of one furrow + area of one ridge). Shoot length (cm), and stem diameter (cm) were measured at 100th day and 220th day, respectively.

3.2.5.2 Soil Mineral Analysis

Soil minerals NO_3^-, K^+, and Ca^{2+} (mg/L) were measured by LAQUA (HORIBA) and RQ flex plus 10 (MERCK). NO_3^-, K^+, and Ca^{2+} (mg/L) values were converted into N, P, and K (mg/100 g). Soil minerals were measured 4 times in the last 8 months before the transplanting date and 4 times in the next 8 months after the transplanting date of SGP. Every time soil samples were collected from five different places of each treatment, and soil samples of all treatments and control were air-dried for 30 minutes at 105°C. The LAQUA twin Nitrate Ion meter was used to measure NO_3^- concentration in soil samples. Soil extract was prepared by mixing soil samples and distilled water (1: 6), shaken for 1 minute, and centrifuged for 1 minute. LAQUA twin Nitrate Ion meter was calibrated by the standard solution, and 500 µl of soil extract was taken and placed into the sensor. NO_3^- reading was recorded from the extract solution. RQ flex plus 10 (MERCK) was used to measure PO_4^{3-} concentration in soil samples. Soil extract was prepared by mixing 1g soil sample and 50 ml of 1 mmol/L H_2SO_4, shaken for 30 seconds, and centrifuged for 2 minutes. Filter paper and funnel were used for filtration. RQ flex plus 10 (MERCK) was calibrated by the standard solution and measured PO_4^{3-}concentration of the filtrated solution. The LAQUA twin Potassium Ion meter was used to measure K^+ concentration in soil samples. Soil extract was prepared by mixing 1g of air-dried soil and 20ml of 0.01mol/L ammonium acetate, shaken for 1 hour to extract K^+ from the soil. LAQUA twin Potassium Ion meter was calibrated by the standard solution, and 500 µl of soil extract was taken and placed into the sensor. K^+ reading was recorded from the extract solution.

3.2.5.3 SGP Mineral Analysis

SGP minerals NO_3^-, K^+, and Ca^{2+} (mg/L) were measured by Quantofix (MN) and LAQUA (HORIBA). Minerals were measured 3 times. Conventionally grown SGP was collected from 3 different retail stores, and used for mineral analysis and comparative study with experimental field SGP. Each time SGPs were collected from treatments and control plants, and SGPs were blended to take the juice for mineral analysis. Quantofix (MN) was used to measure NO_3^- concentration in SGP samples. It was a nitrite test strip. It measured NO_3^- concentration from 0 to 500 mg/L. The LAQUA twin Potassium Ion meter was used to measure K^+ concentration and LAQUA twin calcium Ion meter was used to measure Ca^{2+} concentration in SGP's juice samples. LAQUA meter was calibrated by the standard solution, and 500 µl of SGP sample was taken and placed into the sensor. K^+, and Ca^{2+}readings were recorded from the SGP samples.

3.2.5.4 Observation of AMF

Arbuscular mycorrhizal (AM) colonization in SGP root was observed with a compound microscope. 10% KOH, 1mol/L HCl, Trypan blue were used for staining the AMF (Phillips and Hayman 1970). Root samples were collected and cut at the size of 1 cm. 500 µl of 10% KOH was added with root samples and then incubated at 95°C for 15 minutes. 750 µl of 1mol/L HCl was added with root samples and discarded the solution. Roots were rinsed several times with tap water and then discarded the water. Two drops of trypan blue were added with root samples and incubated at 95°C for 10 minutes. Root samples were rinsed by lactoglycerol for 2 days and then observed AMF with a compound microscope.

3.2.6. Statistical Analysis

The experiment was conducted with four replications per treatment and data were conveyed as Mean ± Standard Error. Statistical analyses of the data were carried out using SPSS software (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.). The level of significance was calculated from the F value of ANOVA. Mean comparison was achieved by Tukey-test ($P \leq 0.01$).

3.3. RESULTS

3.3.1 Effect of Different Treatments on the Yield of SGP

There was statistically significant difference between treatments (T_1, and T_2) and control (**Figure 16**). Average yield (kg/m^2) of different treatments was in the order as follows: T_1 (1.220) > T_2 (0.290) > C (0.003) > T_3 (0.001). The highest yield was obtained at T_1 (wood wastes + bamboo wastes + cut weeds + AMF + GF), which was, approximately, 400 times higher than control (C), 4 times higher than T_2, 1200 times higher than T_3. The average plant yield did not show significant difference at T_3, and C. Based on the result,

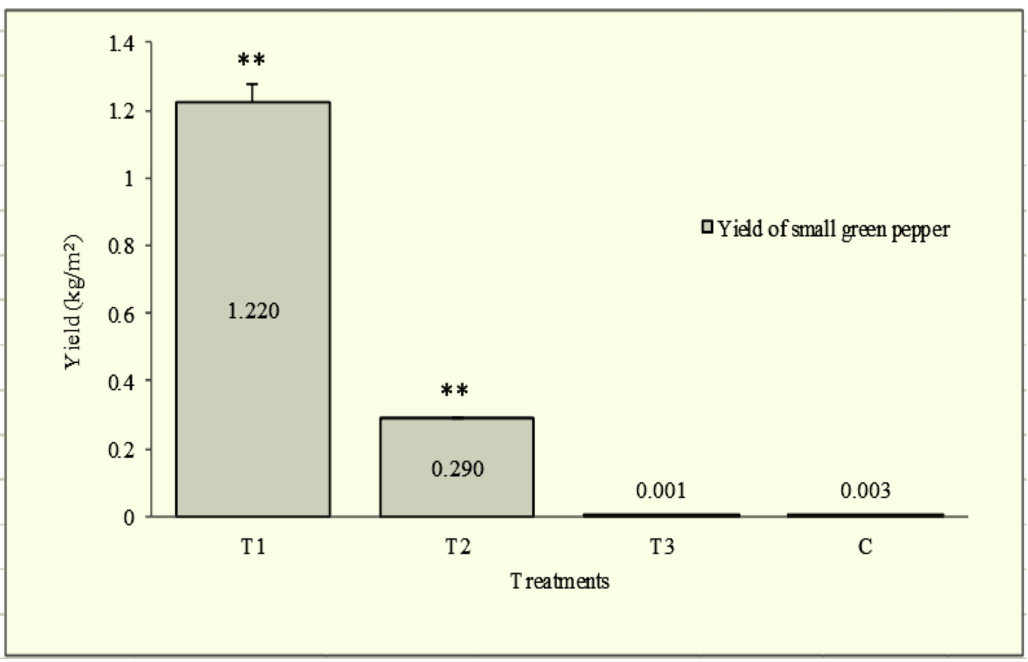

Figure 16. The effect of different treatments on the yield (kg/m^2) of small green pepper. Significant differences are indicated by asterisks ($^{**}P$<0.01), vertical lines represent SE.

noticeable yield of T_1 was influenced by AMF and GF, the application of carbon, organic, and fungal sources increased significantly SGP yield of T_1, and T_2. Several researchers revealed that the AMF have a direct effect on the plant productivity and sustainability (Van der Heijden et al. 1998).

3.3.2 Effect of Different Treatments on Growth Performance

3.3.2.1 Shoot Length

Shoot length is one of the most important parameters to measure plant growth. The experimental results clearly indicate that shoot length was significantly high at T_1. Combined application of carbon, organic and fungal sources had significant effects on shoot length. Average shoot length (cm) for different treatments was in the order as follows: T_1 (59.00) > T_2 (43.25) > T_3 (20.00) > C (17.25) (Figure 17 A, and B). The average shoot length did not show significant difference at T_3 and C. AMF have been reported to produce plant growth hormones that have beneficial effects on plant growth (Iqbal and Ashraf 2013). Several researchers have shown that AMF improve plant rooting and establishment, enhance vegetative growth, and accelerate budding and flowering (Smith and Read 1997).

3.3.2.2 Stem Diameter

Combined application of carbon, organic and fungal sources had significant effects on stem diameter. The stem diameter (cm) of SGP plant was in the order: T_1 (2.00) > T_2 (1.02) > C (0.26) > T_3 (0.25) (Figure 18). The average stem diameter did not show significant difference at T_3 and C. The application of carbon, organic, and fungal sources increased significantly the stem diameter of SGP plant.

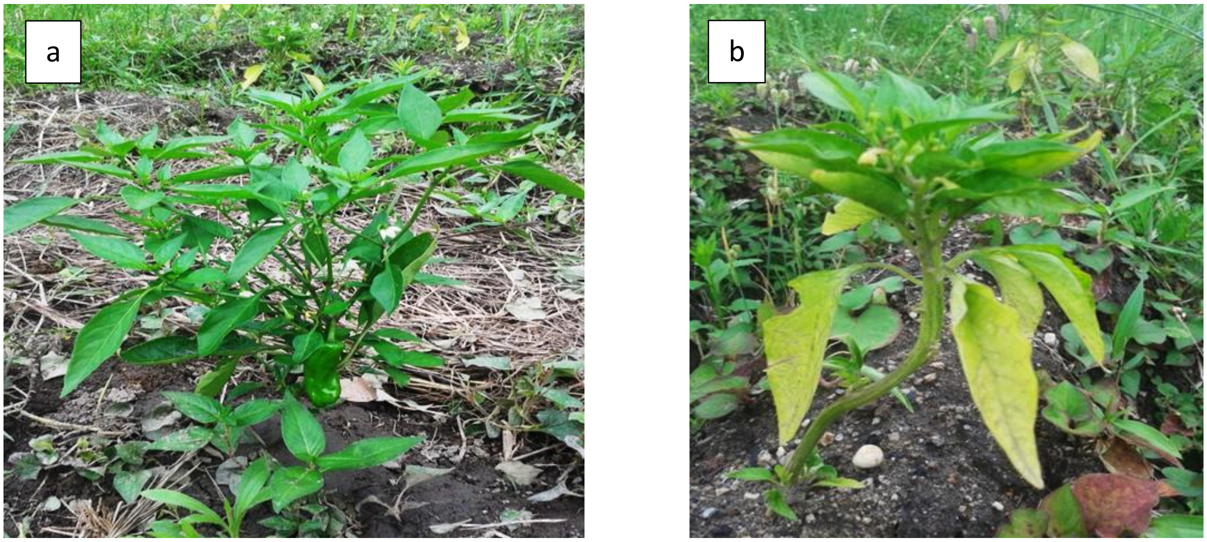

Figure 17(A). Growth and development of small green pepper plant, a: T_1 plot, and b: Control plot.

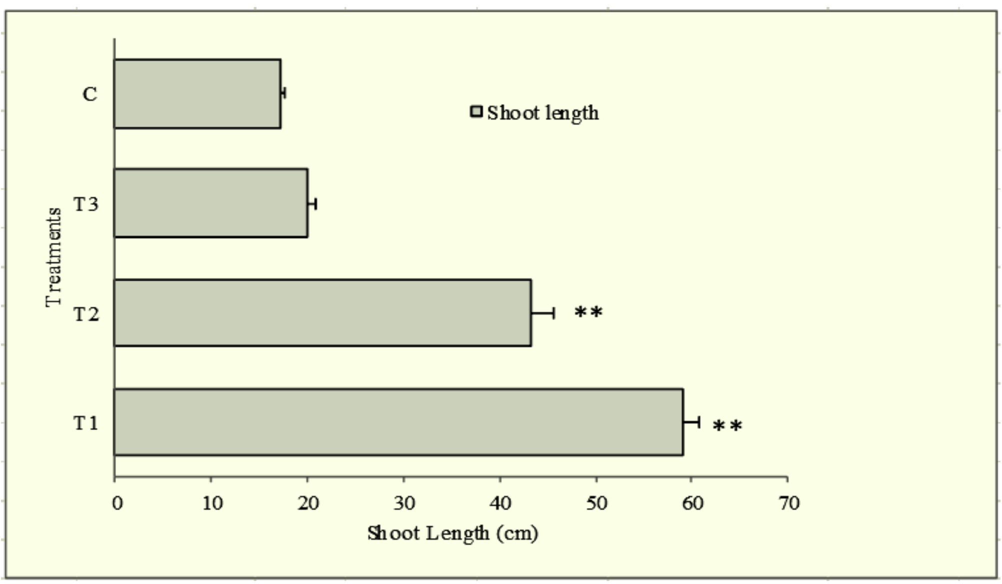

Figure 17(B). The effect of different treatments on the shoot length (cm) of small green pepper plant. Significant differences are indicated by asterisks ($^{**}P$<0.01), horizontal lines represent SE.

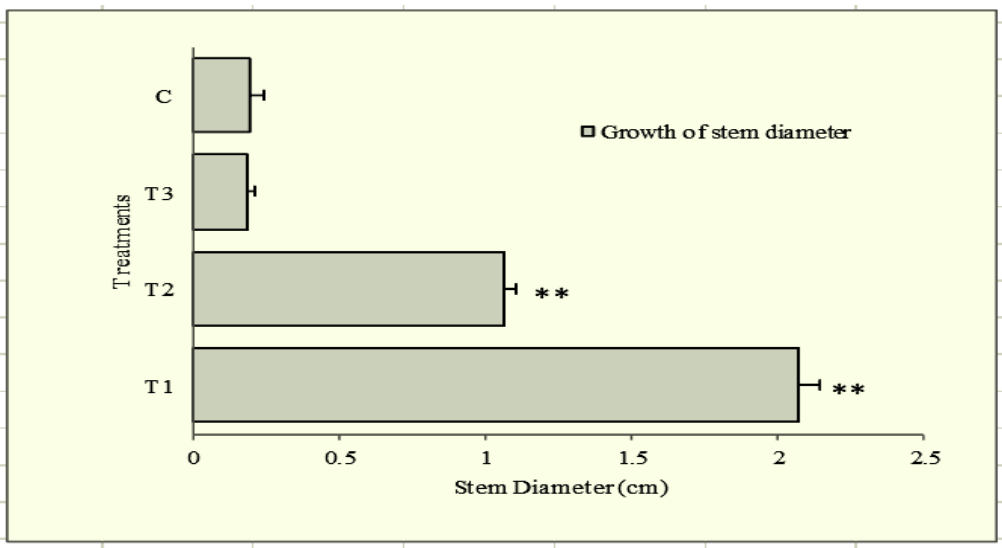

Figure 18. The effect of different treatments on the growth of stem diameter (cm) of small green pepper plant. Significant differences are indicated by asterisks ($^{**}P$<0.01), horizontal lines represent SE.

3.3.3 Changes in Soil Mineral Concentration

Soil mineral nutrients play a vital role in soil fertility. Sixteen minerals are essential for plant growth and reproduction. Mineral nutrients required for plants to complete their life cycle are considered as essential nutrients. Nitrogen (N), phosphorus (P), and potassium (K) are essential plant nutrients. These mineral concentrations of soil were significantly influenced by different treatments. Soil mineral concentration gradually increased after passing of a certain time of SGP transplants (Figure 19). Average mineral (N, P, and K) concentrations of different treatments were in the order: $C>T_1>T_2>T_3$, $T_1>T_2>T_3>C$, and $C>T_3>T_2>T_1$, respectively in the last 8 months before the transplanting date (21st April 2015) of SGP. However, soil mineral (N, P, and K) concentrations in the next 8 months after the transplanting date of SGP were in the order: $T_1>T_2>C>T_3$, $T_1>T_2>T_3>C$, and $T_1>C>T_2>T_3$, respectively. The highest concentration of N, P, and K was observed at the last month (November 2015) of harvesting stage; it was 6.8, 16.3, and 26.7 mg/100 g, respectively, at T_1. The application of minor amount of AMF and GF raised a large AMF community in the soil of T_1, which increased mineral (N, P, and K) concentrations of T_1 soil at the last month of harvesting stage. Several researchers have shown that the AMF colonize in the dead leaves (Rivera and Guerrero 1998, Aristizabal et al. 2004), and they are involved in the closure of nutrient cycles in nutrient-poor ecosystems, and have a direct effect on the ecosystem, as they improve the soil structure and aggregation (Leifheit et al. 2014, Leifheit et al. 2015, Rillig et al. 2015), and increase nutrient uptake. AMF absorb N, P, K, Ca, S, Fe, Mn, Cu, and Zn from the soil and then translocate these nutrients to the plants with whose roots they are associated (Gerdemann et al. 1975, Hayman et al. 1982, Tinker and Gildon 1983, Newsham et al. 1994). Their most consistent and important nutritional effect is to improve uptake of immobile nutrients such as P, Cu, and Zn (Pacovsky 1986, Manjunath and Habte 1988).

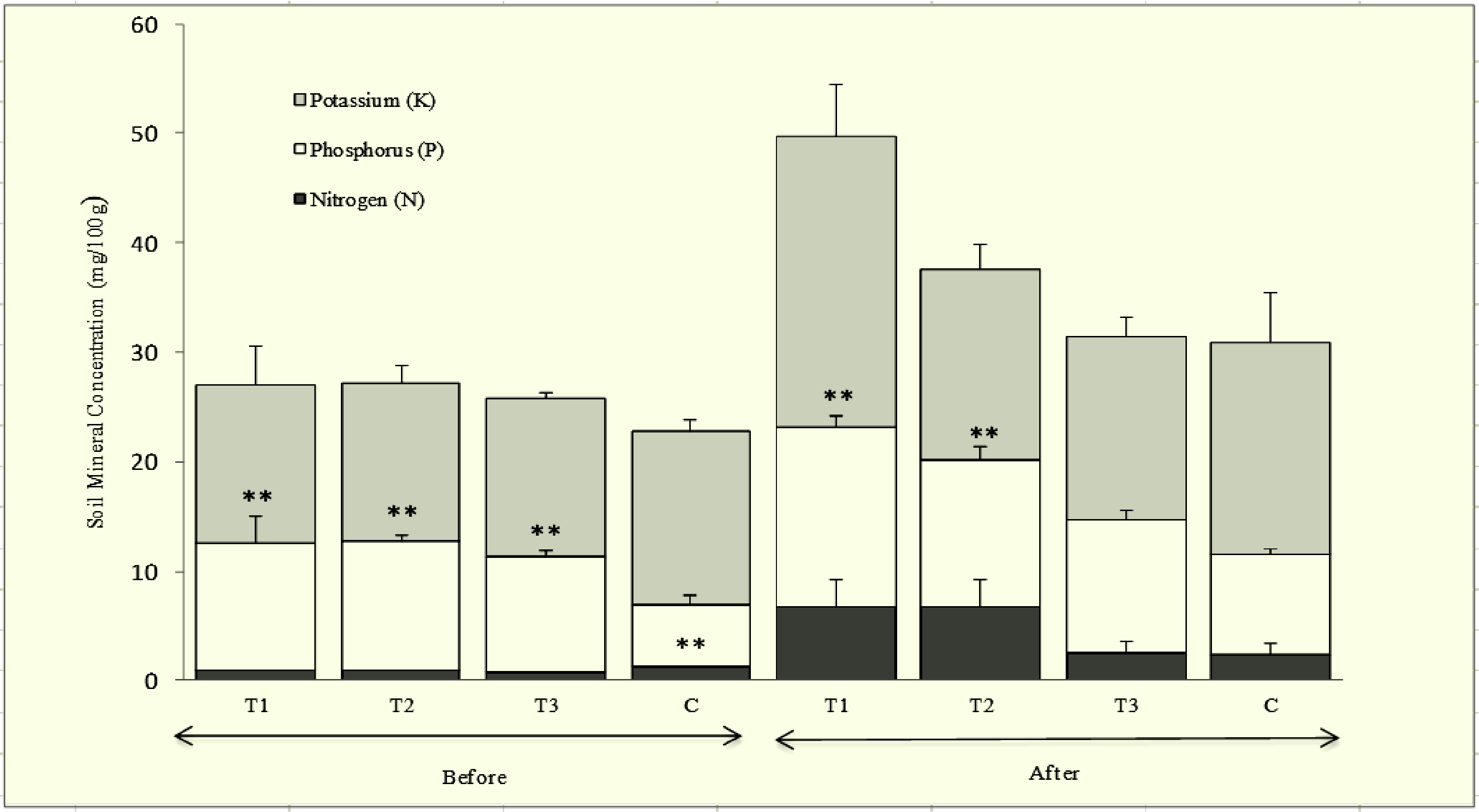

Figure 19. The effect of different treatments on N, P, and K levels of soil. Significant differences are indicated by asterisks (**P<0.01), vertical lines represent SE.

3.3.4 AM Colonization in Roots

AMF are the most common soil microorganisms in natural and agricultural soils (Mohammad and Mittra 2013). Compound microscope was used to visualize the colonization of AMF; this type of colonization was only involved in inter- and intra-cellularly in cortical cells of roots at T_1, and T_2. In these cortical cells of roots, vesicles (Figure 20(E)), arbuscules (Figure 20(C), and 20(D)), and hyphae (Figure 20(B)) were observed frequently. AM hyphae colonized in the root cortex at T_1 and T_2, and formed highly branched bush-like structures (Figure 20(A), 20(C), and 20(D)) within the host cells. Several types of mycorrhizal associations have been found in the plant kingdom geographically, and the endomycorrhizal association of the AM type is the most widespread (Olsson et al. 1999). Mycorrhizal associations provide many benefits to the host plant, such as, increase fixation of soil nutrients, mainly N and P (Grace et al. 2009, Atul-Nayyar et al. 2009), decrease biotic and abiotic stresses, increase photosynthetic rate (Silveira and Freitas 2007), and influence chemical defenses (Gang et al. 2007). AMF represent a key link between plants and soil mineral nutrients at T_1 (wood wastes + bamboo wastes + cut weeds + AMF + GF), and T_2 (wood wastes + bamboo wastes + cut weeds). AMF spread to a large area from the soil of T_1 (source place) to the soil of T_2. Thus, AMF were also observed in the inner cortical cells of roots in both treatment plants (T_1, and T_2). AMF extraradical hyphal length is estimated, it is widely in the field range (Rillig and Allen1999). One of the highest estimates is 111 m/cm^3 of soil for a prairie community, for which a hyphal dry weight of less than 0.5 mg/g is calculated (Miller et al. 1995).

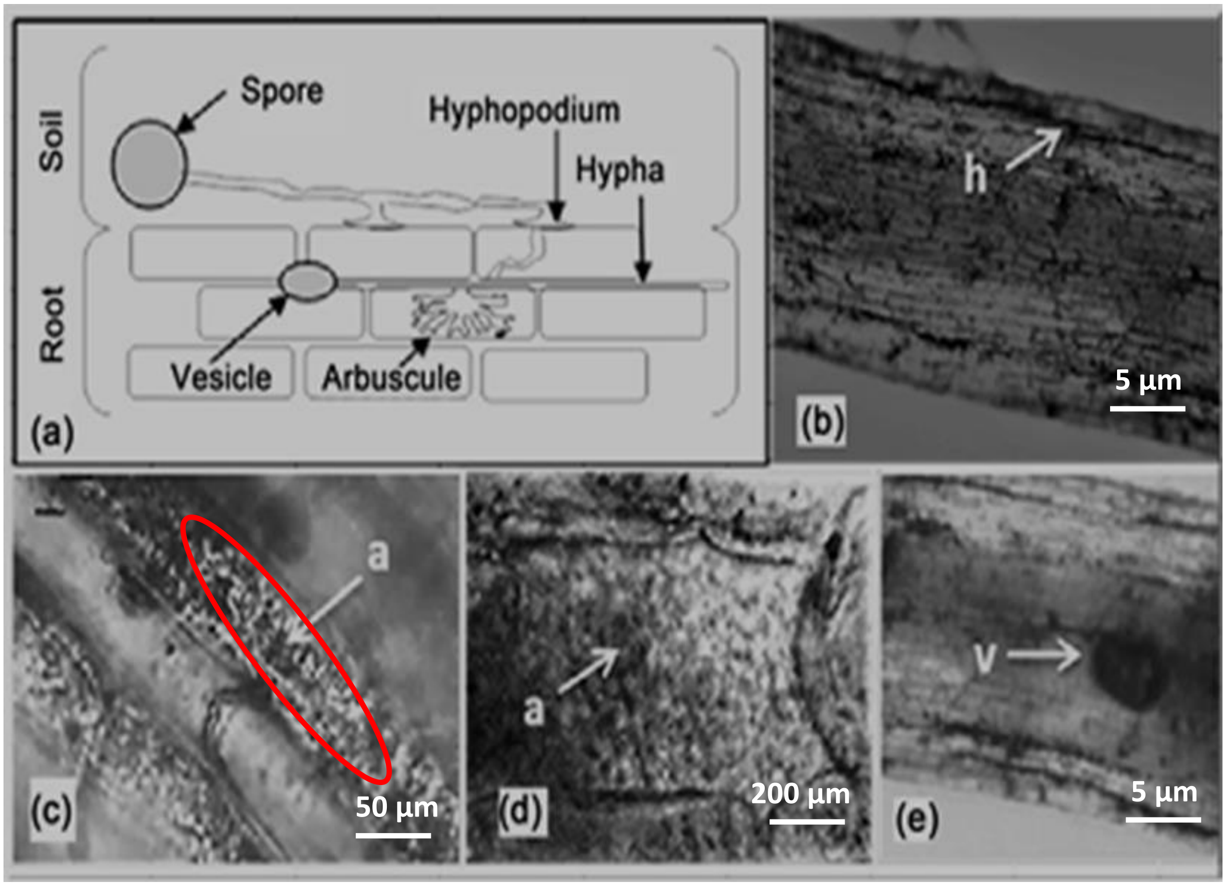

Figure 20. Roots in longitudinal view; all roots were stained with trypan blue and viewed with white light. **A:** Process of colonization. Arbuscular spores germinate and hyphae grow towards the root after perception of strigolactones, the fungus forms hyphopodia on the root surface and invades the plant via rhizodermal cells, the hyphae enter the apoplast when they reach in the root cortex and form arbuscules inside inner cortical cells, vesicles are formed inside the apoplast (Harley and Smith 1983) **B:** *Capsicum annuum* root cortex was colonized by AM hyphae. **C,** and **D:** Arbuscules. **E:** Vesicle. a: arbuscule, h: hypha, v: vesicle.

3.3.5. Mineral of SGP

3.3.5.1 NO_3^- Level

NO_3^- level was the most important contributing parameter, which was significantly marked in conventionally grown SGP and treatments. The level of NO_3^- of all treatments was approximately 16 times lower than the conventionally (chemical based farming) grown SGP (Figure 21). Average NO_3^- level (mg/L) of different treatments was recorded in the order: Conventional (313) > C (20), T_1 (20) > T_2 (15), T_3 (15). Several researchers have revealed that the reason for the high gastric cancer incidence in the Far East may lie in the consumption of specific foods that are high in nitrates (Duncan et al. 1997).

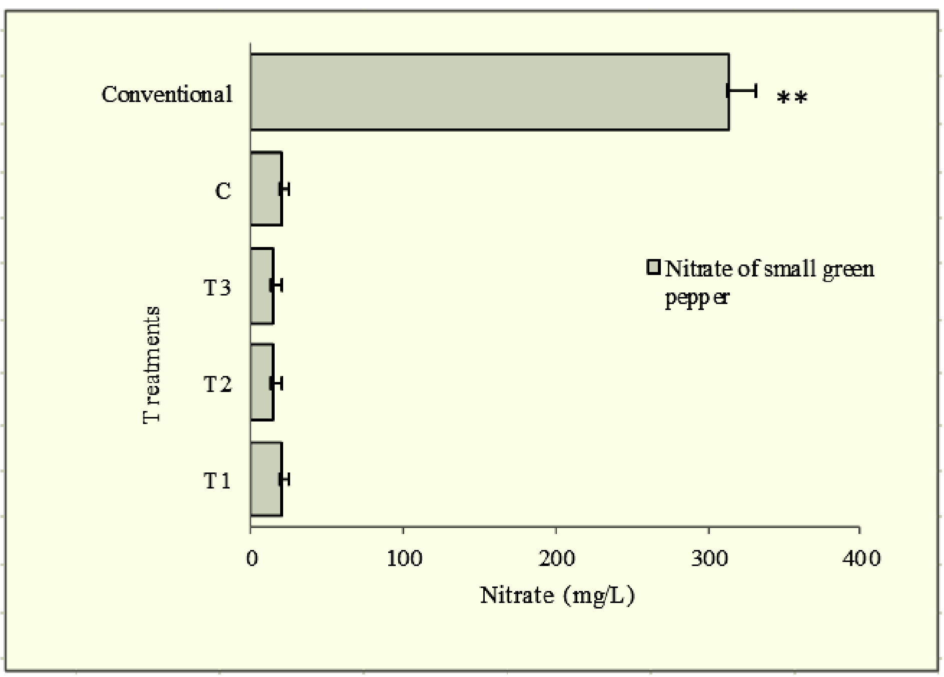

Figure 21. The effect of different treatments on the NO_3^- level of small green pepper. Significant differences are indicated by asterisks ($^{**}P<0.01$), horizontal lines represent SE.

3.3.5.2 : K^+, and Ca^{2+} Level

K^+, and Ca^{2+} level of SGP were significantly influenced by different treatments. K^+, and Ca^{2+} of SGP of different treatments were in the order: T_1>T_2>T_3>C>Conventional, and T_1>T_2>T_3>C>Conventional, respectively. The highest level of K^+, and Ca^{2+} was 5389, and 537 mg/L recorded at T_1 (Figure 22). Several researchers have shown that the diets low in potassium increase risk of hypertension, stroke and cardiovascular disease (D'Elia et al. 2011) and low calcium intake over time, medication interactions that may decrease dietary calcium absorption, and the underlying chronic disease osteoporosis which changes bone formation and strength (Institute of Medicine Standing Committee 1997, National Institutes of Health 2013).

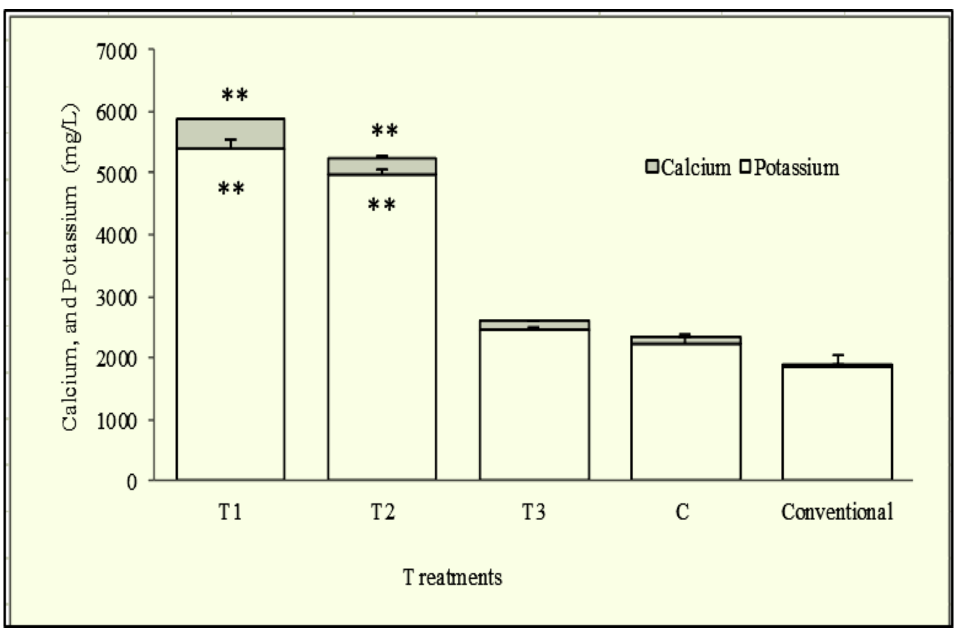

Figure 22. The effect of different treatments on the K^+ and Ca^{2+} levels of small green pepper. Significant differences are indicated by asterisks (**P<0.01), vertical lines represent SE.

3.4 DISCUSSION

Wood and bamboo are rich carbon sources in nature. The chemical composition of wood differs from species to species, but it is approximately 50% carbon, 42% oxygen, 6% hydrogen, 1% nitrogen, and 1% other elements (mainly calcium, potassium, sodium, magnesium, iron, and manganese) by weight (Jean-pierre et al. 1996). The chemical composition of bamboo is similar to that of wood (Smith and Smith 2012). Carbon of wood and bamboo is one of the most useful and active agents introducing suitable chemical, physical and microbiological changes in the soil, and thereby directly increasing the fertility and crop productivity of the soil. This organic carbon is the basis of soil fertility; it promotes the structure, biological and physical health of soil. Several researchers have revealed that the application of high Carbon: Nitrogen material increases nitrogen fixation in tropical agricultural field and enriches the formation of top layer soil (Annabi et al. 2011). In the present work, the growth and yield of SGP showed the great variations between T_1 (carbon sources, and AMF and GF) and T_3 (only AMF and GF) treatment plots whereas the same amount of AMF and GF was given at the both treatments (T_1, and T_3). The experimental results indicate the importance of carbon sources at T_1 treatment. The highest yield was 1.22 kg/m^2 produced at T_1, which was, approximately, 4 times higher than T_2, AMF and GF were the basic differences concerning T_1, and T_2. It must be pointed out that the AMF were observed in the inner cortical cells of roots at the both treatments (T_1, and T_2). Whereas, AMF and GF were used only in T_1, AMF spread to a large area from the soil of T_1 (source place) to the soil of T_2. Several researchers have revealed that the root-external mycelium spreads several centimeters away from root surfaces into the soil, many metres of fungal hyphae may be produced per gram of soil, this result significantly increases surface area for plant nutrient absorption (Oda et al. 2014). Although there is a marked diversity among AM fungal communities belowground, depending on plant species diversity, soil type, and season, or a combination of these factors (Smith and Smith 2012). AMF influenced significantly the shoot length, and the stem diameter of plants at T_1. The stem diameter (cm) of plants was in the order: T_1 (2.00) > T_2 (1.02) > C (0.26) > T_3 (0.25), and average shoot length (cm) for different treatments was in the order: T_1 (59.00) > T_2 (43.25) > T_3 (20.00) > C (17.25). AM symbiosis can rise the leaf area, and delays senescence (Beltrano et al. 2003, Beltrano and Ronco 2008). AMF can increase P absorption in pepper, and proliferate the shoot and root dry weight (Davies et al. 2000). In the present work, weeds (meadow grass, couch grass, horsetail, nettle, chickweed, ground elder, etc) were put in the one furrow (combined furrow of T_1 and T_2) to add organic matter in the soil. Meanwhile, AMF accelerate the organic matter decomposition rates (Zhang et al. 2015) and deliver some raw materials, which raise CO_2 concentration in micro circumstance (Wang et al. 2010, Cheng et al. 2012) and assist soil carbon turnover in the agro-or grassland via CO_2 stimulation effects (Van Groenigen et al. 2011). According to the result of this experiment, AMF played a dynamic role in the presence of carbon and organic materials on the growth, development, and yield of T_1 plants. Plenty of carbon and organic materials, and small amounts of AMF and GF were applied at T_1, whereas AMF and GF at T_2 were not applied at the transplanting date of SGP. The AM symbiosis is the association between fungi of the order Glomales (Zygomycetes) and the roots of terrestrial plants (Sinha 2008). AMF growth can result in enhance decomposition of complex organic material and alter plant N uptake (Hodge et al. 2001, Hodge and Fitter 2010). GF are endophytic fungi, a number of authors have documented that the presence of endophytic fungi provide a protection of the plant hosts against insect herbivore (Clement et al. 2005), parasitic nematodes (Nur 1994, Elmi et al. 2000), and plant pathogens (Dingle and McGee 2003, Wiclow et al. 2005). In the present study, mineral concentration of soil was significantly enhanced from the before transplanting time to the harvesting time at T_1 due to the presence of carbon, organic and fungal sources. Several researchers have shown that the AMF increase soil carbon sequestration in the coalfields in northwest China and AMF are favorable to ecosystems through assisting carbon conservation in coalfield soils (Wang et al. 2016). AMF inoculation deserves a series changes in soil respiration, photosynthesis, and carbon storage via close symbiosis with host plants, leading to increase carbon production and enrich nutritional status against the harmful effects (Moyano et al. 2007, Cavagnaro et al. 2008, Vicca et al. 2009, Kaschuk et al. 2009, Tian et al. 2013, Verbruggen et al. 2013). The highest soil mineral concentration was 6.83 (N), 16.30 (P), and 26.66 (K) mg/100 g analyzed at harvesting time in the T_1 soil. The soil mineral concentration gradually increased after passing of a certain time. The application of carbon, organic, and fungal sources improved soil fertility at after planting period. Several researchers have revealed that the AM symbiosis is being recognized to influence soil development (Miller and Jastrow 1992, Schreiner and Bethlenfalvay 1995). Mycorrhizal hyphae adhere to soil particles, which would improve contact with the soil solution, and can access smaller pores than plant roots and root hairs (O'Keefe and Sylvia 1993). AM association allows plants to explore larger volumes of soil to absorb more water and nutrients uptake and transport, and increases absorption of immobile mineral elements such as phosphorus, provides resistance to soil pathogens and drought, and improves water-use efficiency (Al-Karaki 2000). In the present study, integrated application of carbon and organic sources along with minor amounts of fungal sources not only influenced the growth and production of SGP, but also increased the soil fertility and assured agricultural sustainability. Another important feature of all treatments is that NO_3^- level was significantly marked at the treatments and conventionally grown SGP. It was approximately 18 times lower than the conventional. Based on the data, K^+ and Ca^{2+} level showed the great variation between treatments (T_1, and T_2), and conventionally grown SGP, which indicates the importance of integrated application of carbon, organic and fungal sources (T_1, and T_2). High level of nitrate in the crop has been implicated to cause bladder, ovarian, stomach, and liver cancers for human body (Mueller et al. 2001). Excess nitrogen of chemical based farming reduces carbohydrate synthesis, lowers resistance to diseases (rust and downy mildew), lowers resistance to insect, and reduces the biological value of plant protein (Hornick 2010).

3.5 CONCLUSION

Plants and microorganisms have been coexisting for hundreds of millions of years. Plants conserve a complex interaction with their rhizospheric populations, which is essential for nutrient assimilation, soil development, and activation of defense mechanisms. In the present work, this beneficial interaction is sustainable. SGP and AMF can communicate with each other through the support of slowly degradable wood and bamboo wastes. According to the result of this experiment, the application of carbon, organic, and fungal sources at T_1 (wood wastes + bamboo wastes + cut weeds + AMF + GF) obtained high productivity of SGP, and enhanced plant growth, soil mineral, and AMF activity. The yield was 400 times higher than that of control. Another distinctive result was that the NO_3^- level of all SGP was extremely lower than the conventionally grown SGP. The application of rich carbon sources along with fungal and organic sources (T_1) creates the ideal soil environment, which is suitable for plant growth and development, and increases the populations and diversity of soil organisms. Constructed soil biodiversity does not allow exclusive propagations of harmful pathogens and pests. AMF and GF of T_1 influence both plant and soil functions, and mediate their interactions between above and belowground events. This study suggests that application of carbon, organic, and fungal sources have a potential to be innovative agricultural materials for the next generation sustainable agriculture.

IV. EFFECT OF BIOCHAR ALONG WITH WOODCHIPS ON GROWTH AND YIELD OF SWEET CORN (*Zea mays*) GROWN BY SUSTAINABLE AGRICULTURE

STUDY III

4.1 INTRODUCTION

Use of chemical fertilizers in conventional agriculture to boost crop yield worldwide may lead to loss of carbon, nutrient run-off, loss of biodiversity, toxicity, and hazard of agrochemicals. The present day agriculture is challenged to fulfill with twin objectives of achieving food, fodder, fiber, and fuel security as well as sustainability with emphasis on restoring soil resources, improving water quality, mitigating climate change, and preserving soil and natural resources for long-term use. Recently, a sustainable agricultural approach for utilizing wood wastes has been reported that application of wood waste with arbuscular mycorrhizal fungi (AMF) and gliocladium fungi (GF) achieved approximately 400 times higher yield than untreated soil (Islam and Katoh 2017). However, Rising global population and thus doubling global food demand projected for the next 50 years poses huge challenges for the farmers and sustainability of food production and ecosystems both and the services they provide to society. Therefore, high-level researches are essential to figure out innovative, alternative, environmentally friendly, sustainable options to decrease the use of costly and non-environmentally friendly chemical fertilizers. According to FAO (2012), by the end of 2020, the global requirement of chemical fertilizers (N, P, K and other macronutrients) is expected to reach 194 million. A huge amount of nonrenewable resources such as energy in the form of oil and natural gas is required for manufacturing of the chemical fertilizers to meet this demand. In addition, soil and air pollution (greenhouse gaseous emissions) as well as water eutrophication in many parts of the world is occurred by the use of excessive chemical fertilizers. In this context, biochar has very promising potential for the development of sustainable agriculture production systems. Biochar is a carbon-rich material produced by thermally treating biomass materials in zero or limited oxygen conditions using a process called pyrolysis. When applied to land, biochar is not only a carbon sink, but also can act as a soil improver by increasing the water and nutrient holding capacity of the soil. It may also be effective in reducing greenhouse gas emission from the soil. With the correct calibration, therefore, biochar application could offer considerable benefits in terms of mitigating climate change, improving food security and reducing reliance on chemical fertilizers, all of which could have considerable environmental and economic advantages.

The U.S. National Research Council (1989) defined sustainable agriculture as "those alternative farming systems and technologies incorporating natural processes, reducing the use of inputs of off-farm sources, ensuring the long term sustainability of current production levels and conserving soil, water, energy, and farm biodiversity". Sustainable agriculture is considered mainly as an eco-system approach where all the living organisms in soil live in harmony with a well-balanced equilibrium of food chains and their related energy balances. The goal of sustainable agriculture is to sustain significant increase of farm productivity through natural resource management, ensure the efficient use of land and other resources, provide better economic returns to farmers, and thus contribute to the quality of life and economic development.

The purpose of this study is to present new perspectives and strategies for efficient and effective use of natural resources to enhance sustainable agroecosystems. To address the innovative agricultural approach, application efficiencies of woodchips, biochar, rice bran, leaf litter, AMF, and GF on sweet corn (*Zea mays*) production were investigated.

4.2. MATERIAL AND METHODS

4.2.1 Experimental setup

The experiment was carried out in the experimental field of Shimane University, Matsue, Shimane, during the period from 29 th March 2016 to 17 th August 2016. The soil type of the experimental area was clay with soil pH of 5.5. The experimental field was cleared, ploughed, harrowed and divided into 5 plots, with 15 m^2 areas. 4 treated and 1 control

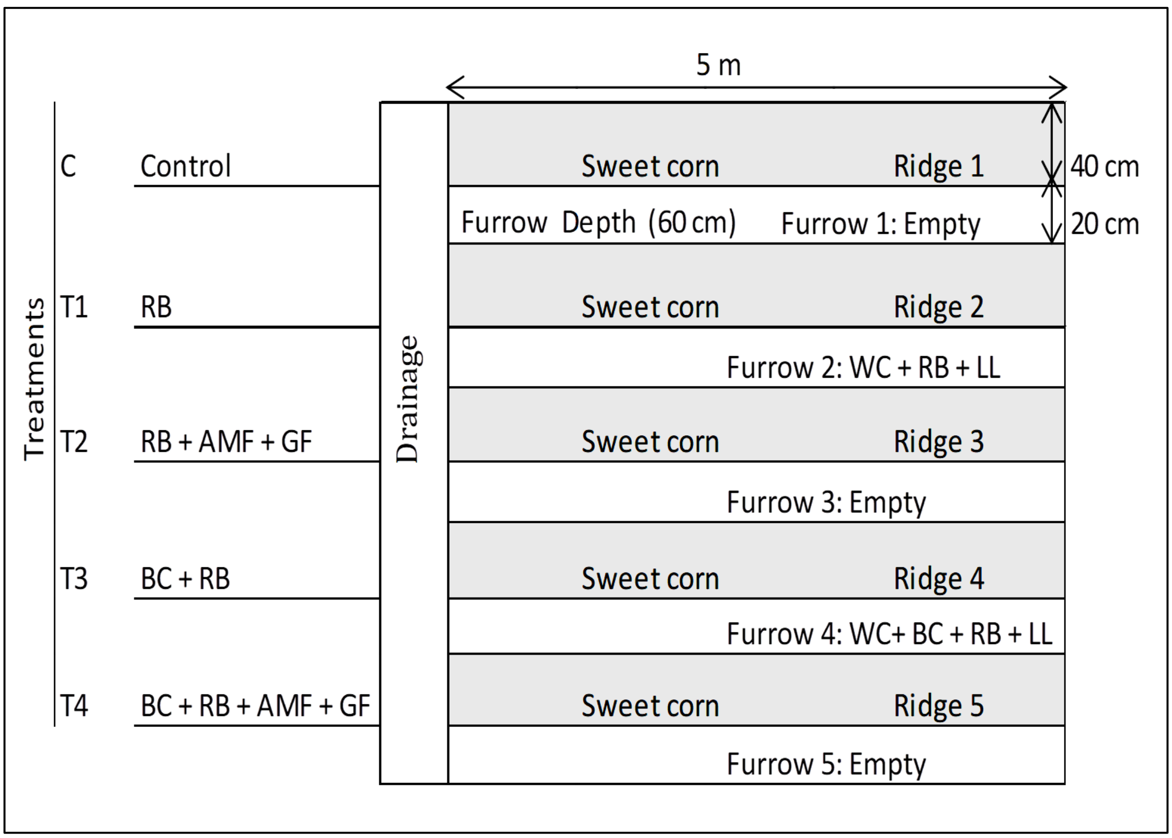

Figure 23. Layout of the experimental site.

Notes: WC = Woodchips, RB = Rice bran, LL= Leaf litter, BC = Biochar, AMF = Arbuscular mycorrhizal fungi, GF = Gliocladium fungi (*Gliocladium* sp.)

plots were prepared. Each plot site contained 1 ridge (1 ridge = 500 cm length × 40 cm width × 20 cm height) and 1 furrow (1 furrow = 500 cm length × 20 cm width × 60 cm depth), plot area is presented in Figure 23. Conifer woodchips, biochar (Yamamoto funtan kogyo, raw material: mainly oak species, pyrolysis temperature: 800°C to 1000°C), rice bran (Twinbird), leaf litter (broadleaves), AMF (Idemitsu) and GF (Idemitsu) were used as agricultural materials. The experimental design was laid out in a completely randomized design with 4 treatments namely,

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T_1 - woodchips + rice bran + leaf litter,

T_2 - woodchips + rice bran + leaf litter + AMF + GF,

T_3 - woodchips + biochar + rice bran + leaf litter,

T_4 - woodchips + biochar + rice bran + leaf litter + AMF + GF , and

C – control (untreated)

Woodchips, rice bran, leaf litter, biochar, AMF and GF were directly used in the ridges and furrows for the experimental investigation (Table 1).

Table 1. Experimental treatment overview.

Treatment	Furrow				Ridge			
	Conifer woodchips (kg/furrow)	Biochar (kg/furrow)	Rice bran (kg/furrow)	Leaf litter (kg/furrow)	Soil (g/pit)	Biochar (g/pit)	Rice bran (g/pit)	AMF and GF (g/pit)
C	0	0	0	0	25	0	0	0
T_1	48	0	3	10	25	0	5	0
T_2	48	0	3	10	25	0	5	5
T_3	48	20	3	10	15	10	5	0
T_4	48	20	3	10	15	10	5	5

4.2.2 Test Crop Establishment and Management

Sweet corn was considered as plant material. Commercially available seeds (Atariya, Honey bantam) were used for the experimental observation, 20 seeds were sown in each treatment on 20th April, 2016, seed-sowing distance (spacing) was maintained 20 cm. Experimental investigations were conducted with conifer woodchips, biochar, rice bran, leaf litter, AMF and GF. Conventional agro materials such as chemical fertilizers, microelements, growth promoters, pH control chemicals, or other agricultural chemicals were not used. Sweet corn has a high water requirement, particularly from tasselling to harvesting but irrigation was continued for only 1 week from the seed-sowing day during the whole life cycle of sweet corn. Pest and diseases are likely to threaten at some stages and can cause major and even total crop losses. In this experiment, only natural defense mechanism was approached to control pests and diseases, integrated pest management or other conventional methods were not used. For weed control, weeds were cut and put in the furrow when they began to race with crops.

4.2.3 Analyses

4.2.3.1 Yield and plant growth

Sweet corn was collected 9 times from 92th day to 119th day after the seed sowing date, yield was calculated based on the plot area, and converted the average yield into kg/m^2. Area of each plot was 3 m^2 (area of one furrow + area of one ridge). Stalk length (cm) was measured 3 times at 69th, 83th, and 97th day, respectively.

4.2.3.2 Minerals and sugar of sweet corn

NO_3^- (mg/L) level of sweet corn was measured by Quantofix (MN), K^+, and Ca^{2+} (mg/L) were measured by LAQUA (HORIBA), and sugar (g/100 mL) was measured by pocket refractometer (ATAGO). Corn minerals were measured 3 times. Conventionally grown sweet corn was collected from 3 different retail stores, and used for mineral analysis and comparative study with experimental sweet corn.

4.2.3.3 Soil Minerals

NO_3^-, K^+, and Ca^{2+} (mg/L) of soil were measured by LAQUA (HORIBA), and PO_4^{3-} was measured by RQ flex plus 10 (MERCK). NO_3^-, PO_4^{3-}, K^+, and Ca^{2+} (mg/L) values were converted into N, P, K, and Ca (mg/100 g).

4.2.3.4 Soil pH and water holding capacity

Soil pH was measured by SHINWA digital soil acidity meter. Soil water holding capacity was measured by the following formula, i) Volume of water retained = Volume of water poured - Volume of water collected in cylinder (6 hours) ii) Water holding capacity = [(volume of water retained/ volume of water required) × 100].

4.2.4 Statistical analysis

The experiment was conducted with 20 replications per treatment and data were conveyed as Mean ± Standard Error. Statistical analyses of the data were carried out using SPSS software (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.). The level of significance was calculated from the F value of ANOVA. Mean comparison was achieved by Tukey-test ($P \leq 0.01$).

4. 3. RESULTS

4.3.1 Yield and plant growth

Average yield (kg/m²) of corn was in the order as follows: T_3 (0.963) > T_4 (0.632) > C (0.598) > T_2 (0.540) > T_1 (0.377) (Figure 24). Significant ($p < 0.01$) effect on the yield was observed at T_3. Corn yield did not show significant difference at T_1, T_2, T_4, and C. Stalk length is one of the most important parameters to measure plant growth [Figure 26(a)]. Stalk length was significantly higher at T_3 and T_4 (Figure 25). Combined application of

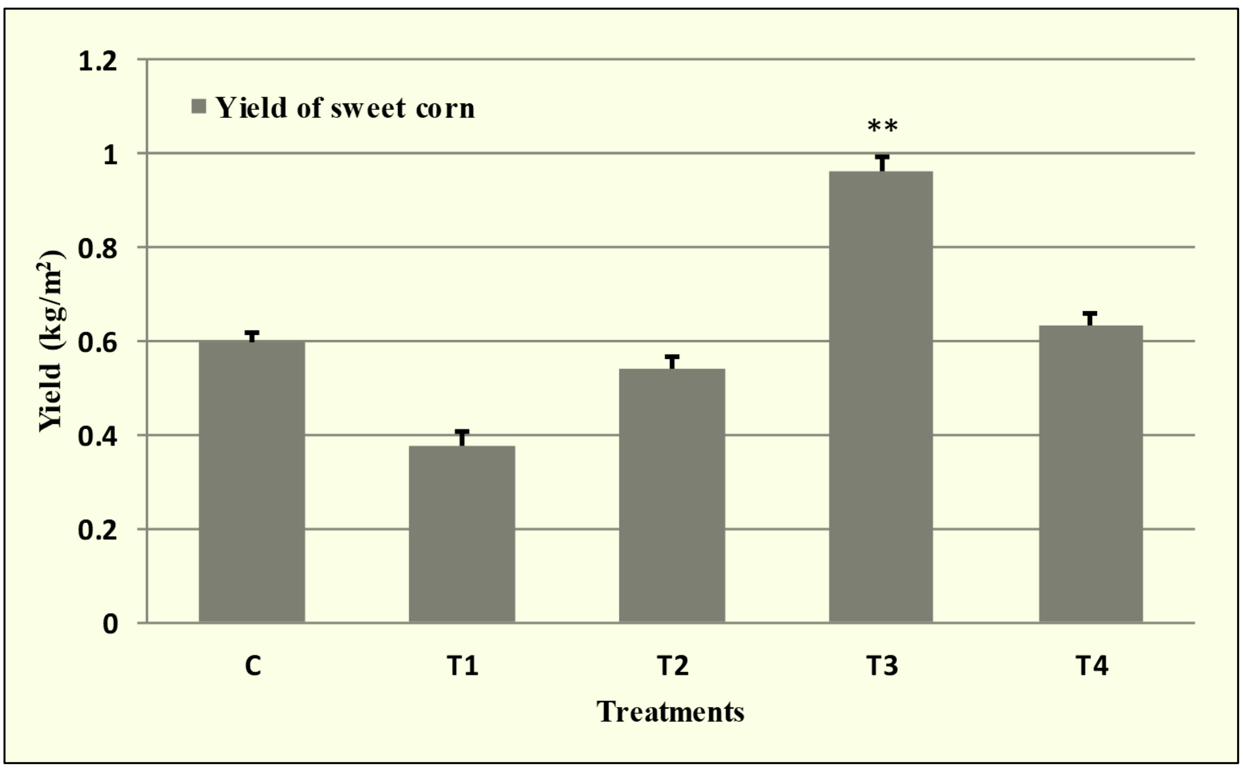

Figure 24. The effect of different treatments on the yield (kg/m²) of sweet corn. Significant differences are indicated by asterisks (**$P < 0.01$), vertical lines represent SE.

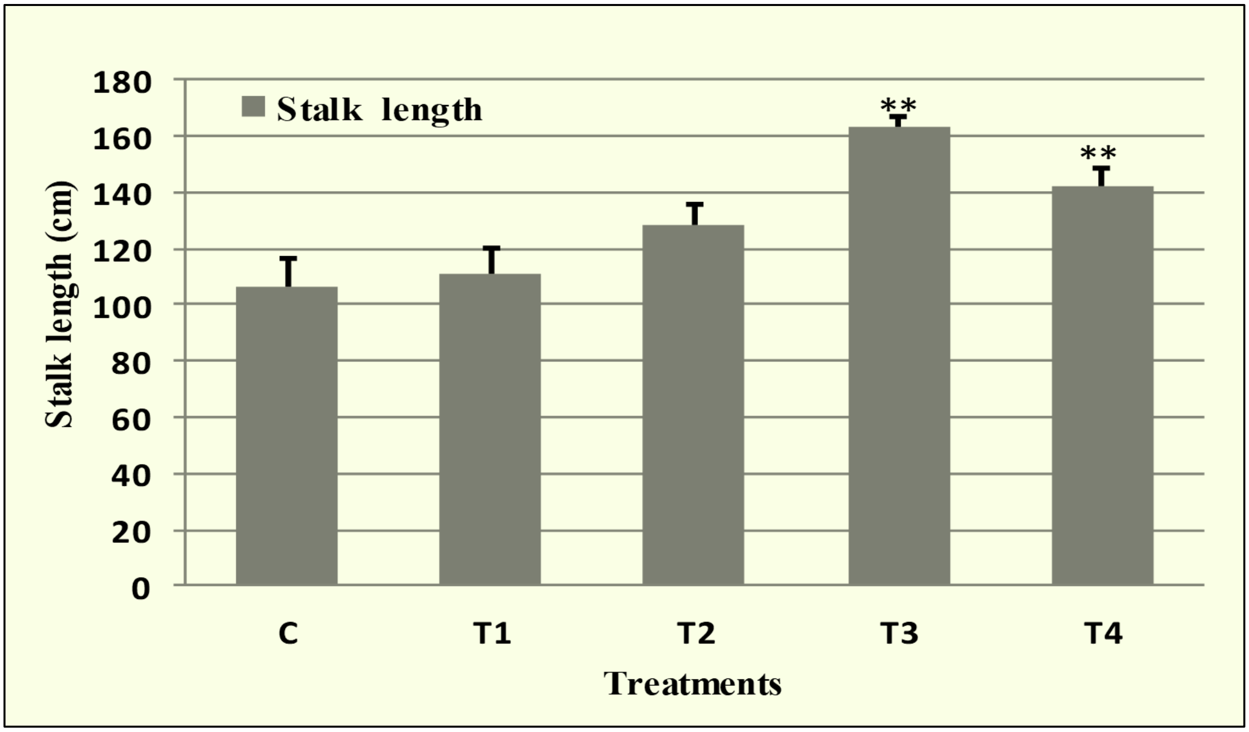

Figure 25. The effect of different treatments on the stalk length (cm) of sweet corn. Significant differences are indicated by asterisks (**$P < 0.01$), vertical lines represent SE.

Figure 26. (a) Experimental field of sweet corn after 90 days of plant growth **(b)** Corn ear from T_3 treatment.

woodchips, biochar, rice bran, leaf litter, AMF and GF (T_4) had significant effects on stalk length, but application of biochar along with woodchips, rice bran, and leaf litter (T_3) obtained the highest stalk length. Average stalk length (cm) of corn plants was in the order as follows: T_3 (163) > T_4 (142) > T_2 (128) > T_1 (111) > C (106). It is reported that plant growth can also be effected by biochar-induced changes in soil nutrients condition, particularly the cycling of P and K (Dempster et al. 2012a, Dempster et al. 2012b, Taghizadeh-Toosi et al. 2012), and It has been suggested that application of biochar enhances plant growth, retains nutrients, and improves soil physical and biological properties (Downie et al. 2009).

4.3.2 Minerals of sweet corn

In this study, NO_3^- Level was the most important contributing parameter, NO_3^- level (mg/L) of sweet corn was recorded in the order: Conventional (83.33) > C (6.66), T_1 (6.66), T_2 (6.66), T_3 (6.66), T_4 (6.66) (Figure 27). No significant difference ($p \leq 0.01$) was found among treatments and control. However, Significant difference ($p \leq 0.01$) was observed in conventionally grown sweet corn. Many studies of organic plant raw materials indicate that organic plant contain less nitrates and pesticide residues, but more dry matter, vitamin C, secondary substances, total sugars, certain mineral components and essential amino acids, but less β-carotene (Zadoks 1989, Rembiałkowska 2000, Worthington 2001). Several researchers have revealed that the reason for the high gastric cancer incidence in the Far East may lie in the consumption of specific foods that are high in nitrates (Duncan et al. 1997).

K^+ level (mg/L) of sweet corn was recorded in the order: T_3 (2900) > T_2 (2833) > C (2800) > T_4 (2766) > T_1 (2600) > Conventional (1966) (Figure 28). Significant differences ($p \leq 0.01$) of K^+ level (mg/L) of sweet corn were found at T_2, T_3, T_4, and C. Several researchers have shown that the diets low in potassium increase risk of hypertension, stroke and cardiovascular disease (D'Elia et al. 2011).

The highest level of Ca^{2+} in sweet corn was found at T_4 and the lowest was found in the conventional, T_2, and T_3 (Figure 29). Ca^{2+} level (mg/L) of sweet corn was recorded in the order: T_4 (7.33) > T_1 (6.00) > C (5.00) > T_2 (4.66), T_3 (4.66), and Conventional (4.66). Significant difference ($p \leq 0.01$) was not found among the treatments, control and conventional.

Sugar level (g/100 mL) of sweet corn was recorded in the order: T_1 (17.06) > T_4 (16.86) > T_2 (16.76) > T_3 (15.86) > C (13.73) > Conventional (8.33). Significant differences ($p \leq 0.01$) were found at T_1, T_2, T_3, T_4, and C (Figure 30).

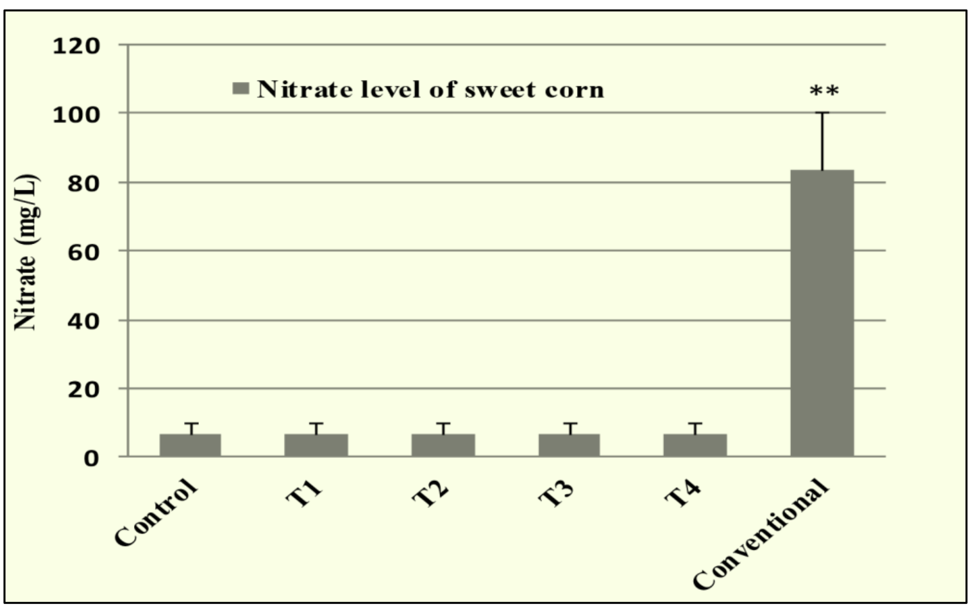

Figure 27. The effect of different treatments on NO_3^- (mg/L) levels of sweet corn. Significant differences are indicated by asterisks (**P < 0.01), vertical lines represent SE.

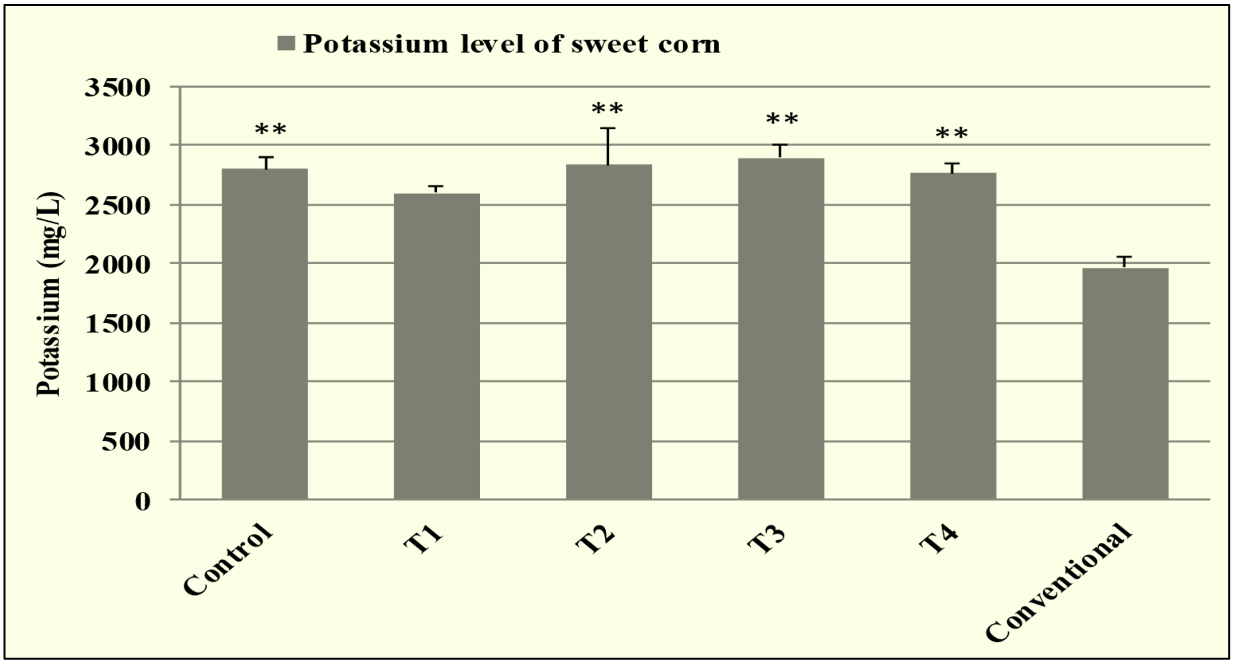

Figure 28. The effect of different treatments on K^+ (mg/L) levels of sweet corn. Significant differences are indicated by asterisks (**P < 0.01), vertical lines represent SE.

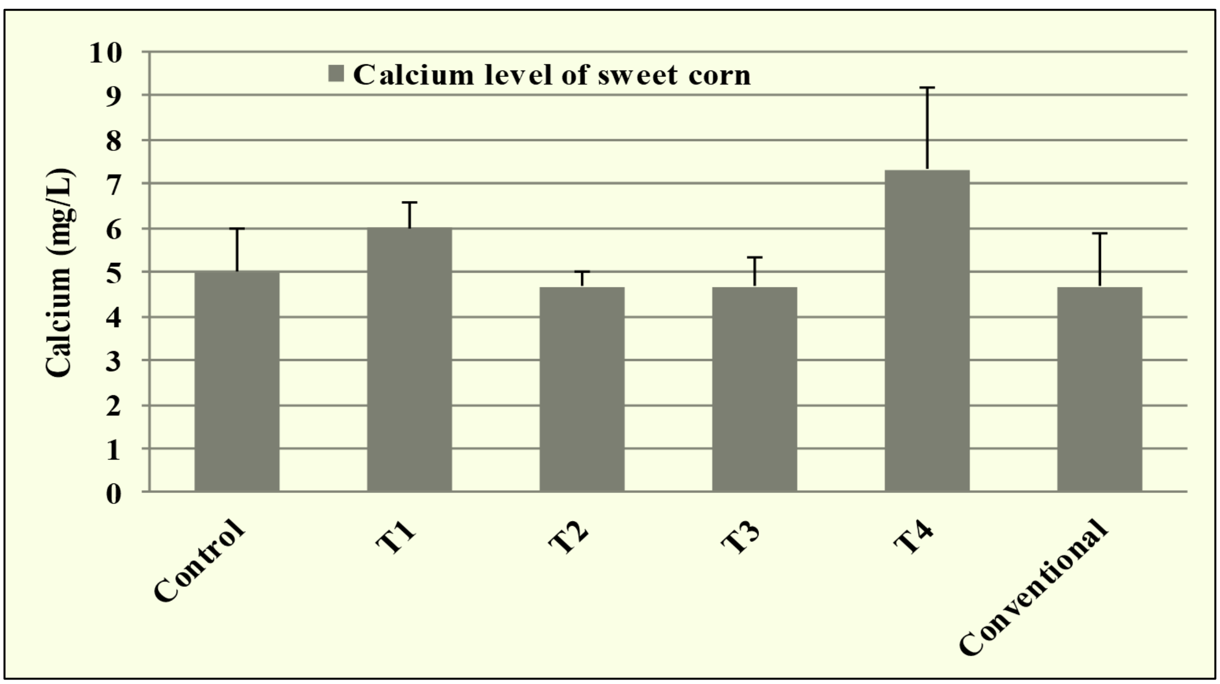

Figure 29. The effect of different treatments on Ca^{2+} (mg/L) levels of sweet corn. Significant differences are indicated by asterisks (**P < 0.01), vertical lines represent SE.

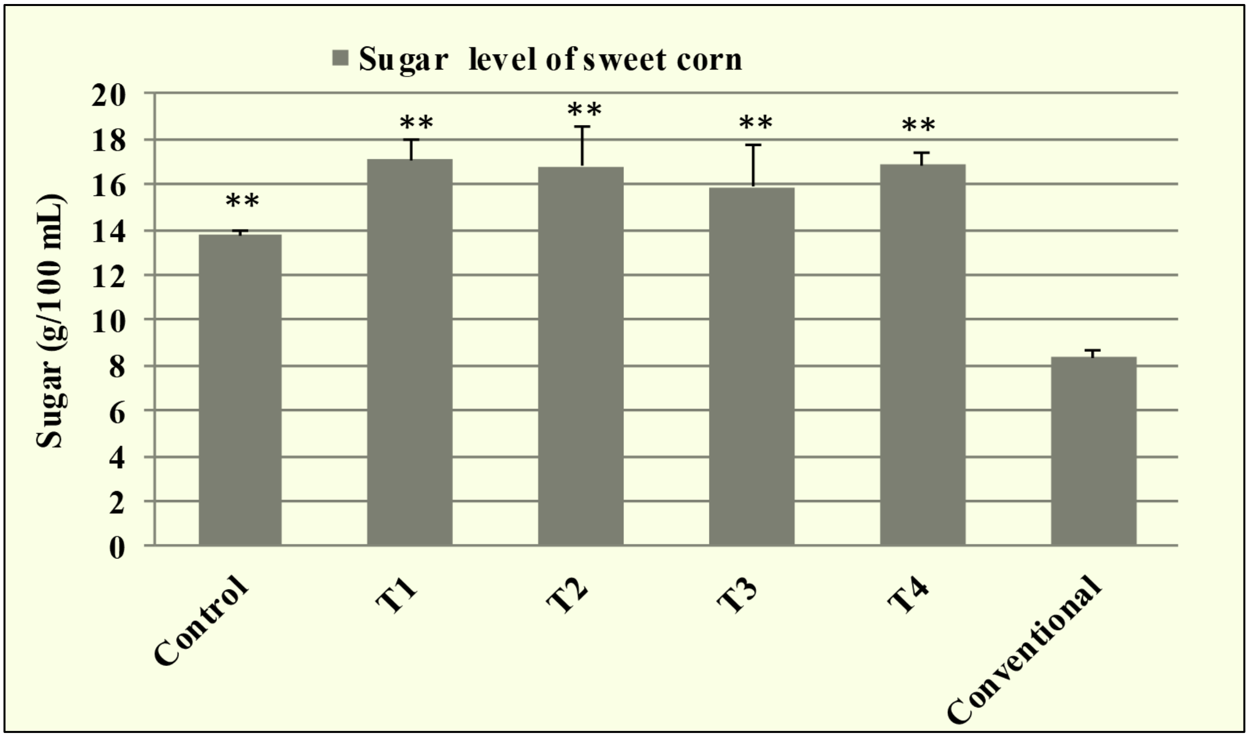

Figure 30. The effect of different treatments on Sugar (g/100 mL) levels of sweet corn. Significant differences are indicated by asterisks (**P < 0.01), vertical lines represent SE.

4.3.3 Soil Minerals

N, P, K, and Ca (mg/100g) levels of untreated soil (before treatment) of all treatments were recorded 2 times, and average levels were found in the order: T_2 (2.91) > C (2.84) > T_3 (2.64) > T_4 (2.50) > T_1 (2.44), T_2 (16.30) > C (16.05) > T_4 (13.52) > T_3 (13.12) > T_1 (12.79), C (22) > T_1 (19), T_2 (19), T_4 (19) > T_3 (17), and T_1 (48) > C (45) > T_3 (41) > T_2 (40) > T_4 (39), respectively. N, P, K, and Ca (mg/100g) levels of treated soil (after treatment) of all treatments were recorded 3 times, and average levels were found in the order: T_4 (13.78) > T_3 (12.38) > T_2 (7.91) > T_1 (7.41) > C (2.89), T_4 (21.30) > T_3 (19.23) > T_2 (10.05) > T_1 (8.69) > C (3.91), T_3 (41) > T_4 (36) > T_2 (32) > T_1 (26) > C (15), and T_4 (56) > T_3 (43) > C (42) > T_2 (34) > T_1 (33), respectively [Figure 31. (a), (b), (c), (d)]. Higher levels of soil

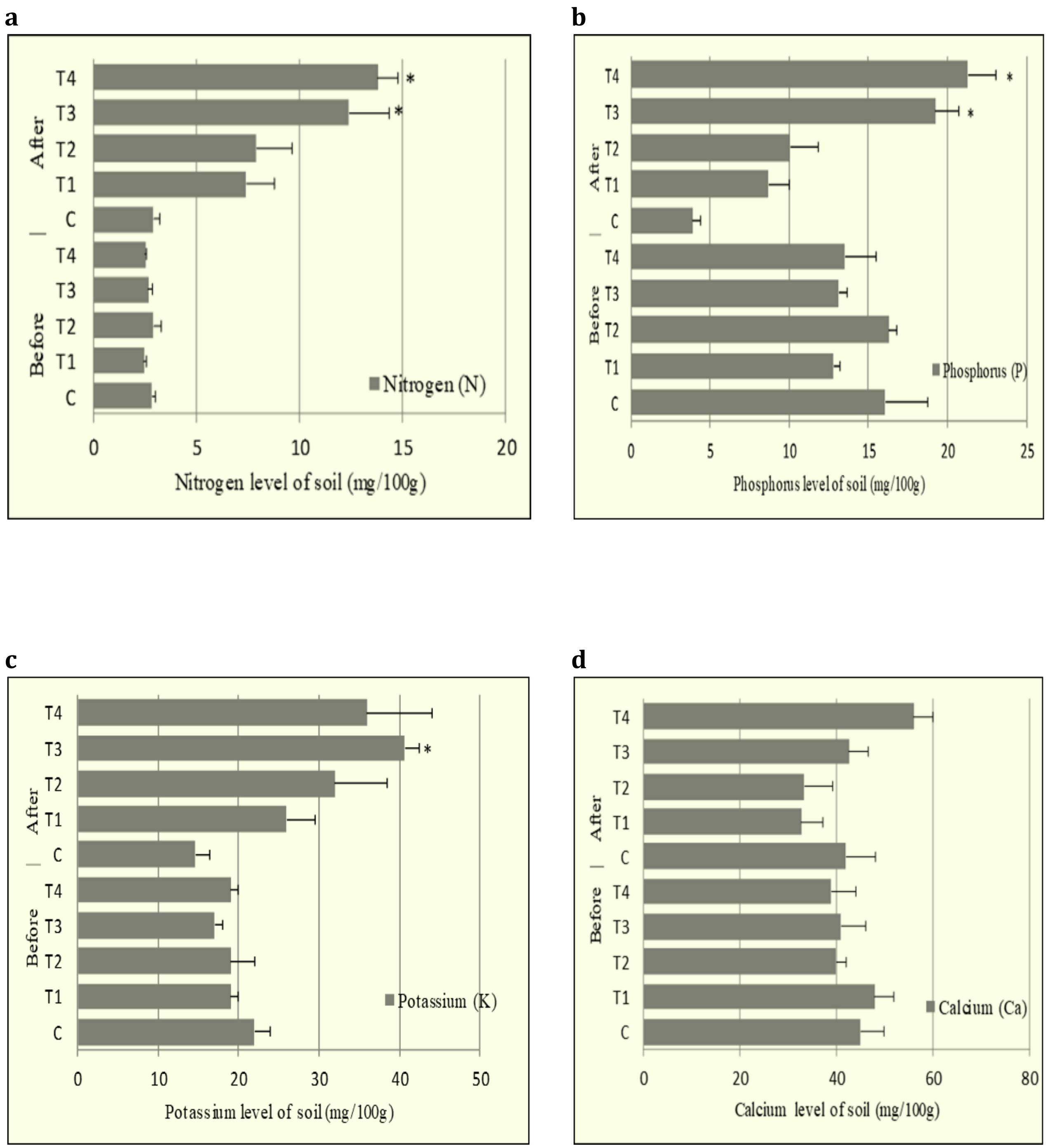

Figure 31. (a) N (mg/100g), **(b)** P (mg/100g), **(c)** K (mg/100g), and **(d)** Ca (mg/100g) levels of soil at before and after treatment. Significant differences are indicated by asterisks (**P < 0.01), horizontal lines represent SE.

minerals (N, P, K, and Ca) were observed at T_3 and T_4, which implied the influence of biochar (T_3 and T_4), and AMF and GF (T_4) along with woodchips. For N and P, significant difference ($p \leq 0.01$) was found at T_3, and T_4. For K, significant difference was found at T_3. For Ca, no significant difference was found at treatments and control. Several researchers have shown that biochar enhances soil productivity and improves soil fertility, which decreases fertilizer requirements (Lehmann et al. 2006, Sohi et al. 2010, De la Rosa et al. 2014, Zhao et al. 2014). Another way biochar may affect soil nutrients is through the reduction in leaching losses (Laird et al. 2010a). Biochar is a promising resource for soil's fertility management. Biochar loaded with ammonium, nitrate, and phosphate could be also proposed to be a slow-release fertilizer to enhance soil fertility (Spokas et al. 2012, Xu G et al. 2014, Schmidt et al. 2015, Kammann et al. 2015).

4.3.4 Soil pH and water holding capacity

Soil pH was recorded in the order: T_3 (5.8), T_4 (5.8) > T_1 (5.7), T_2 (5.7) > C (5.2) (Figure 32), and water holding capacity (WHC, %) of soil was recorded in the order: T_4 (40) > T_3 (35) > T_1 (30) > T_2 (27) > C (22) (Figure 33). Although, no significant difference ($p \leq 0.01$) was found among the treatments and control. Positive impacts of biochar application on the relationship between plant and water are due to increased availability of water for the plants have been reported (Baronti et al. 2014). Several researchers report that finer charcoal works better for elevating the pH of soils (Tryon 1948).

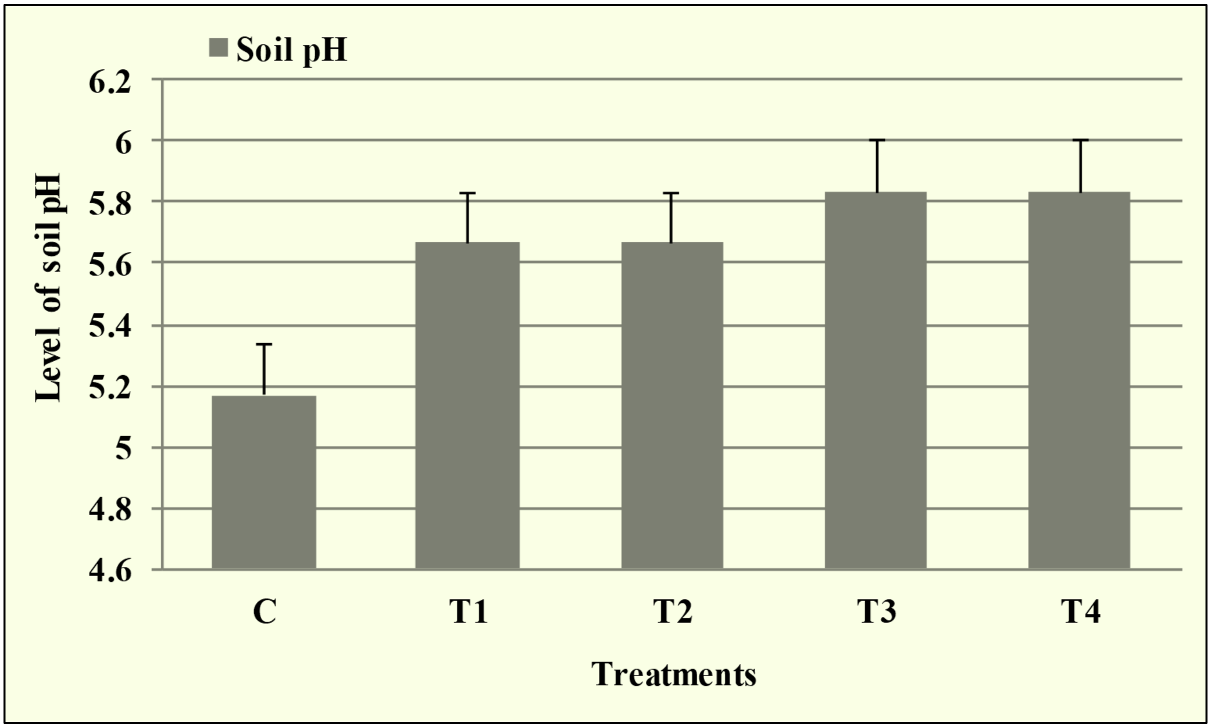

Figure 32. The effect of different treatments on soil pH. Vertical lines represent SE.

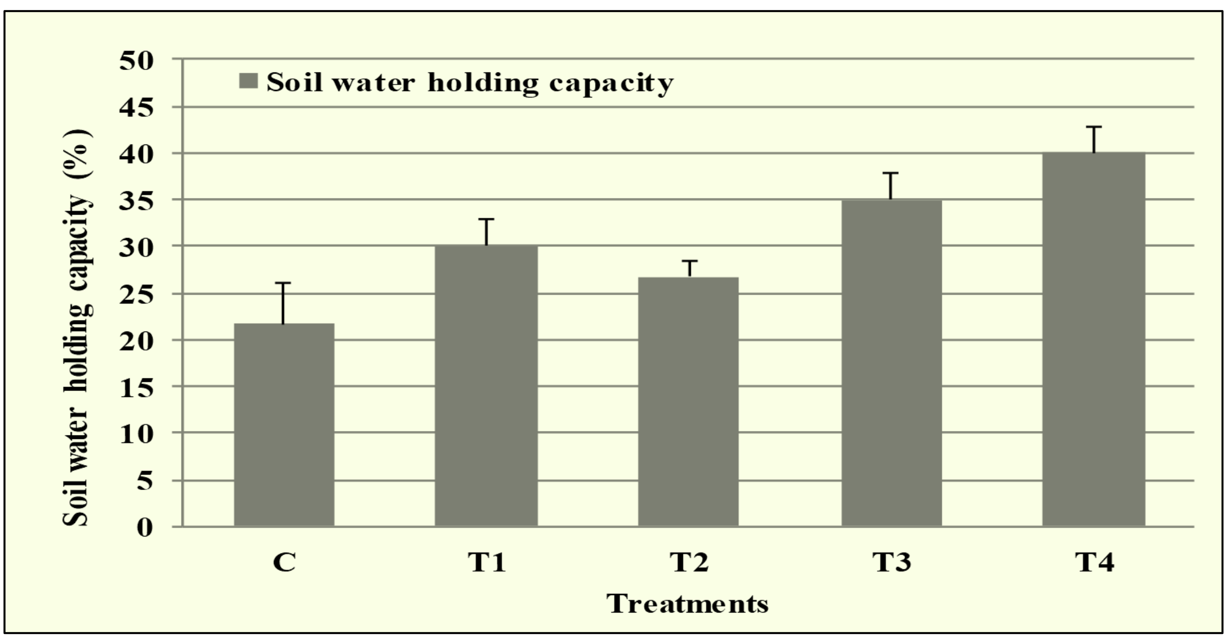

Figure 33. The effect of different treatments on water holding capacity of soil. Vertical lines represent SE.

4.3.5 Fungal mycelium observation

Fungal mycelia were observed enormously in woodchips at T_1 and T_2 [Figure 34(A)], T_3 and T_4 [Figure 34(B)]. Mycelia were not observed at control. Application of woodchips had positive effects to grow fungal mycelia at all treatments. It was observed that fungal mycelia grew especially on woodchips surface at T_1, T_2, T_3, and T_4. The body of fungi, called mycelium, consists of strands that form massive 'string networks' within the soil and have tremendous surface area contact with the soil, this fungi network is more efficient than plant's roots for gathering water and nutrients. Mycelium serves an important function in the decomposition of wood chip, and leaf letter at T_1, T_2, T_3, and T_4. Fungal mycelium represents an important pool of organic matter in forest litter and soil (Baldrian et al. 2013b). Several researchers have demonstrated that mycelia act as a filter, removing ground water contaminants and pollutants, having mycelium in the soil is a sign of a good and healthy soil (Soudzilovskaia et al. 2015). There are many benefits of having mycelium in soil. Mycelium holds water, helping the soil with water retention, It Improves root growth and strength, and adds to the soil nutrients by releasing nitrogen, phosphate, and other micronutrients during the breakdown of organic matter, mycelium enables plants to more readily absorb nutrients.

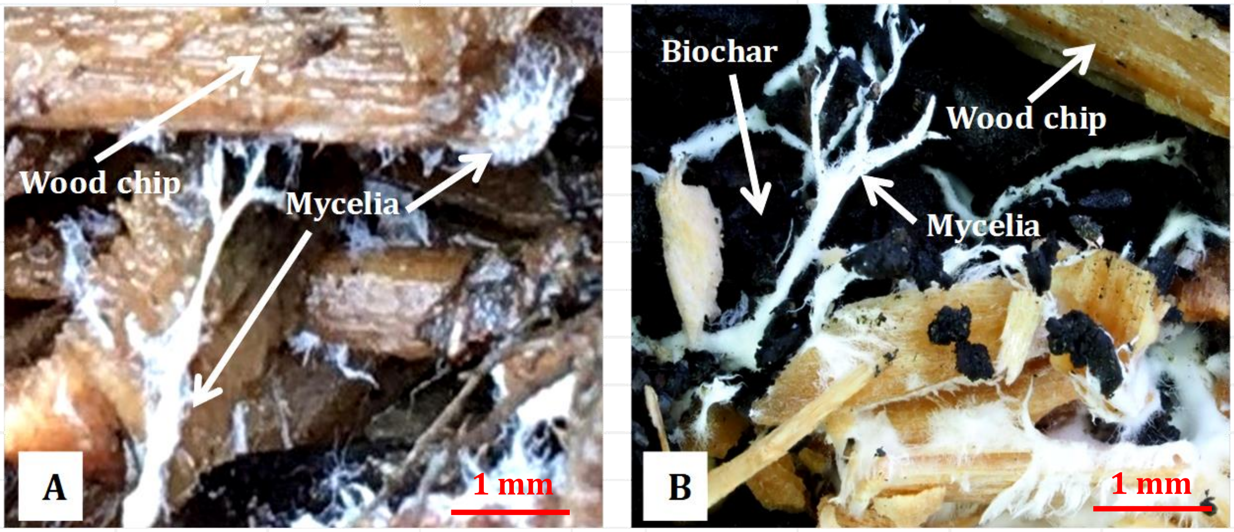

Figure 34: Fungal mycelia in woodchips at T_1, T_2, T_3, and T_4 treatments. The white patches and strands are the fungal mycelia, it is in close contact with the chips and it binds loose chips together in clumps, **(A)** Fungal mycelia in woodchips without biochar (T_1 and T_2), and **(B)** Fungal mycelia in wood chips with biochar (T_3 and T_4).

4.3.6 Pest management

Natural biological control was observed at T_1, T_2, T_3, T_4 and control. Corn leaf aphid (CLA, *Rhopalosiphum maidis*) is a major insect pest for sweet corn. It was observed that natural enemies reduced CLA numbers drastically over the entire experimental field. Selective insecticides are used to control CLA in conventional agriculture. CLAs [Figure 35(A)] were identified at the vegetative stage (after 106 days of growth) of corn plants. After 109 days of plant growth, larvae of aphid midge *(Aphidoletes aphidimyza)* were observed on the stalk of sweet corn [Figure 35(B)], and CLAs fed the larvae of aphid midge [Figure 35(C)]. Natural enemies of agricultural pests may offer a sustainable solution to pest problems, and the control they provide is valued at $13 billion per year in the United States (Losey and Vaughan 2006).

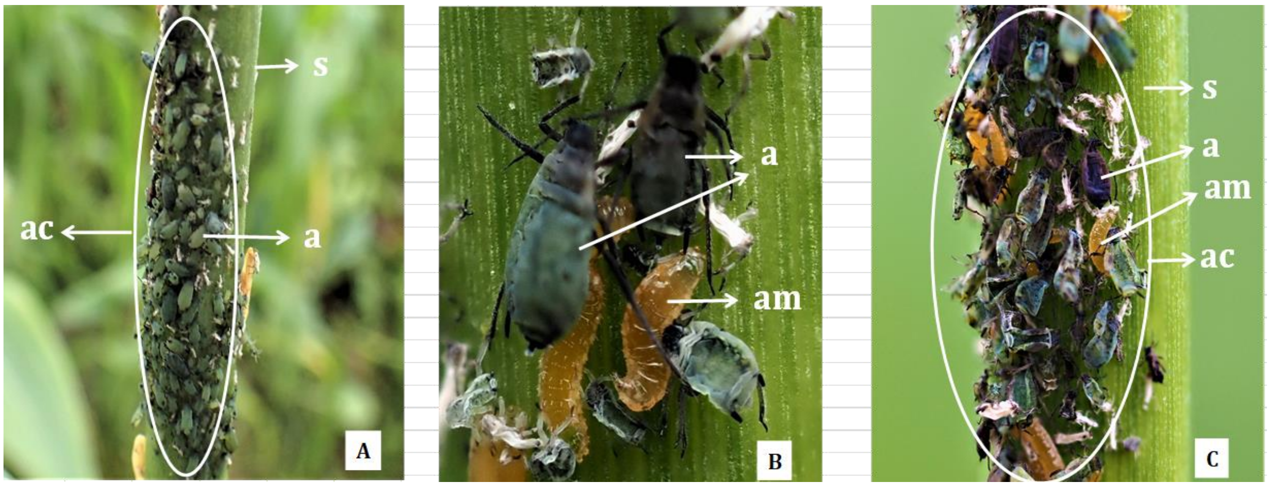

Figure 35. Natural biological control of CLA. **(A)** CLAs colonized on corn stalk, **(B)** Aphid midge larvae (shiny orange) in CLA colony, larvae of aphid midge inject a toxin into aphid's leg joint to paralyze them and then suck out the aphid body contents through a hole bitten in the thorax, it can kill on over 75 different aphid species (Harris 1973), **(C)** Dead CLAs. ac: aphid colony, s: stalk, a: corn leaf aphid, am: aphid midge.

4. 4. DISCUSSION

Our experimental results revealed that the combined application of biochar and woodchips (T_3, and T_4) had positive effects on the sweet corn yield, stalk length, and soil minerals. For experimental investigation, woodchips and biochar were applied at T_3, and T_4. At T_1 and T_2, biochar was not applied and woodchips was applied to grow sweet corn, the highest corn yield and stalk length were observed at T_3 and the second highest were observed at T_4. Moreover, significant ($p < 0.01$) effect on soil minerals (N, P, and K) was observed at T_3, and T_4. Thus, application of biochar along with woodchips had significant effects on the sweet corn yield, stalk length and soil minerals. Several researchers have revealed that sweet corn yield increased by 98–150% as a result of biochar (Uzoma et al. 2011), and application of biochar enhances plant growth, retains nutrients, improves soil physical and biological properties (Downie et al. 2009), and biochar could release large amount of N (23–635 mg/kg), P (46–1664 mg/kg), K (711 mg/kg), and Ca (5880 mg/ kg) in soil (Mukherjee and Zimmerman 2013, Zheng et al. 2013). The highest level of soil pH was measured at T_3 and T_4, and the highest level of WHC was observed at T_4. Soil pH and WHC were influenced by the combined application of biochar and woodchips at T_3 and T_4. Several researchers have reported that biochar works better for elevating the pH of soils (Tryon 1948), and biochar amendment in soil can increase WHC of soil (Laird et al. 2010). The application materials (woodchips, biochar, rice bran, leaf litter, AMF, and GF) of different treatments had significant effects on the nutritional status of the sweet corn. NO_3^- level of experimental sweet corn (T_1, T_2, T_3, T_4) was extremely lower than that of conventionally grown sweet corn. Cultivating edible crops with high nitrate content may lead to poisonous for human health (Mensinga et al. 2003). However, higher level of K^+, Ca^{2+}, and sugar were found in the experimental sweet corn of T_1, T_2, T_3, T_4, and the lowest was found in conventionally grown sweet corn. Previous studies indicate that cultivating edible crops with high K^+ and Ca^{2+} content is beneficial for human health (D'Elia et al. 2011). The content of total sugars in plant raw materials is an important element of technological quality (Zadoks 1989). Fungal mycelium was observed at T_1, T_2, T_3, and T_4. Mycelium was not observed at control where woodchips was not given. According to the results, woodchips influenced to grow fungal mycelium. Mycelium in the soil is a sign of good and healthy soil (Soudzilovskaia et al. 2015). Natural biological control of CLA was observed over the entire experimental field. A female aphid midge lays 100 to 250 eggs among aphid colonies. Larva feeds for three to seven days, consuming up to 65 aphids a day (Rice Mahr et al. 2001). In this study, reproductive behavior and feeding propensity of aphid midge are the key factors for controlling CLA. Several researchers have reported that microbial abundance was increased by 5–56 % with the increase of biochar rates (from 0 to 14 %)(Domene et al. 2015). It is suggested that the application of woodchips, biochar, rice bran, leaf litter, AMF, and GF influenced biodiversity at all treatments. The successful control of pests and pathogens by naturally occurring biological agents is of key economic and ecological importance (Lewis et al. 1997).

4. 5. CONCLUSION

Combined application of biochar and woodchips not only enhances crop growth and yield but also helps prevent the present unsustainable agricultural situation. To the best of our knowledge, this is the first report to study the effect of biochar along with woodchips. The results of this study may provide useful information to farmers and policymakers. However, subsequent field studies are planned to be carried out in future, especially long-term experiments.

V. SUMMARY AND CONCLUSION

5.1 SUMMARY

Every year, a huge volume of wood waste is engendered in Japan; it is approximately 30 million m^3 in every year. Felicitous management of wood waste should be established as quickly as possible to use wood materials properly. Wood is fundamentally composed of cellulose, hemicelluloses, lignin, and extracts. The composition of wood waste is approximately 41.20% carbon, 5.03% hydrogen, 34.55% oxygen, 0.24% nitrogen, 0.09% chlorine, 0.07% sulfur, 16.00% moisture, 2.82% ash by weight.

This study has been chosen sustainable agriculture as methods to recycle unused wood waste for addressing environmental problems, and ensure the efficient utilization of natural resources. Based on the characteristics and properties, this biomass has possibility to be used for sustainable agricultural production. Thus, we selected wood waste for vegetable production. Recently, a promising agricultural approach for utilizing wood wastes has been reported that application of a high carbon: nitrogen (C:N) ratio organic material without additional nitrogen fertilizer achieved four times higher productivity than that of conventional farms.

In this study I, I conducted a research by using a huge volume of wood chips and few amount of organic and fungal sources without using any fertilizers and pesticides to develop the sustainable agricultural system. At this time, soil fertility and crop productivity were increased. Another important feature of the treatments is that NO_3^- level of the cabbage was approximately 41 times lower than that of conventionally grown cabbage. The results of this study suggest that untapped raw wood chips have a potential to be new agricultural sources for the next generation sustainable agriculture.

In study II, to address the innovative agricultural approach, application efficiencies of carbon (wood, and bamboo wastes), organic (cut weeds), and fungal sources (AMF, and GF) on small green pepper production were investigated. The combination of carbon, organic, and fungal sources obtained high productivity of SGP. The yield was 400 times higher than control (untreated). Another notable significant result is that all the treatments contained a very small amount of nitrate compared to conventional practice. The results of this study strongly suggest that combination of carbon (wood, and bamboo wastes), organic, and fungal sources has a potential to be innovative agricultural materials for the next generation sustainable agriculture.

The addition of biochar to agricultural soils has recently received much attention due to the apparent benefits to soil quality and enhanced crop yields, as well as the potential of gaining carbon credits by carbon sequestration. The aim of study III is to determine the effect of biochar along with woodchips on growth and yield of sweet corn (*Zea mays*) to enhance sustainable systems of agriculture. Biochar, woodchips, rice bran, leaf litter, arbuscular mycorrhizal fungi, and gliocladium fungi were applied separately and conjointly in the four experimental plots for sweet corn production. Combined application of biochar, woodchips, leaf litter, and rice bran showed a significant difference in the growth, yield, and soil minerals as compared to plants grown in control. Application of woodchips, rice bran, and leaf litter influenced the growth, yield, and soil minerals positively. Furthermore, addition of biochar to the mixed material enhanced this effect more significantly. Another notable significant result is that the sweet corn grown in all treatments contained a very small amount of nitrate and high amount of sugar compared to conventional practice. This study suggests that combination of biochar, woodchips, leaf litter, and rice bran could be a good treatment for sustainable agriculture.

5.2 CONCLUSION

Untapped wood materials are one of the biomass categories that can be utilized for the next generation sustainable agriculture. The application of wood biomass along with fungal and organic sources creates the ideal soil environment, which is suitable for plant growth and development, and increases the populations and diversity of soil organisms. Constructed soil biodiversity does not allow exclusive propagations of harmful pathogens and pests. Thus, it is a method of agricultural production, which avoids or largely omits the application of systematically compounded chemical fertilizers and pesticides and promises the utilization of environmentally amicable organic inputs.

This study suggests that untapped wood materials addition in soil is an alternative to chemical fertilizers and pesticides and it could be a good treatment for sustainable agriculture.

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