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THE INFLUENCE OF BIOCHAR ON CROP GROWTH AND THE COLONIZATION OF HORTICULTURAL CROPS BY ARBUSCULAR MYCORRHIZAL FUNGI

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Abstract

Variation in crop growth and mycorrhizal colonization within and between crops can be influenced by several factors. Soil plays a major role in this variation and sustainable management practices are suggested to improve soil fertility and crop productivity. Among the sustainable approaches, biochar is gaining interest world-wide for carbon sequestration and improving crop productivity. Studies on the comparative influence of biochar from different sources and their application rates on growth of vegetable crops and their colonization by arbuscular mycorrhizal fungi are inadequate.

Pot experiments were conducted in a glasshouse to determine the growth pattern of lettuce, true potato seedlings (TPS) and single node cuttings of TPS in response to five application rates (0, 10, 30, 50 and 100 t ha^{-1}) of Green Waste biochar. The results showed that biochar had significant effect on growth of lettuce but no consistent influence on TPS and single node cuttings. Among the biochar rates, 30 t ha^{-1} had the greatest influence on overall growth of lettuce. The pH and electrical conductivity increased as the biochar rates increased. Application of Sugarcane Trash and Green Waste A biochar was also beneficial for cabbage growth in a separate pot trial.

Two pot experiments in sand compared the influence of Sugarcane Trash, Green Waste A and Green Waste B biochars on onion and tomato at rates of 10, 30, 50, 100 t ha^{-1} with a control. This study confirmed biochar was beneficial for growth parameters and mycorrhizal colonization. Onion roots had greater colonization than tomato roots. Among the application rates, 30 t ha^{-1} of each biochar had greater effect on onion in terms of morphological growth and colonization of roots while 50 t ha^{-1} was more effective on tomato.

Effect of zinc (Zn), copper (Cu) and mycorrhizal rates were tested in a calcareous soil amended with Sugarcane Trash biochar at the rate of 30 t ha^{-1} in a glasshouse study. These were compared with controls such as soil, soil plus biochar and soil plus biochar plus mycorrhizae. Soil plus biochar plus mycorrhizae was more beneficial than soil and soil plus biochar. Soil plus biochar was more effective than soil. Mycorrhizal colonization in this highly alkaline calcareous soil confirmed mycorrhizal tolerance to alkalinity. Lower rates (50 mg kg^{-1} of soil) of Zn and Cu had greater positive effect on plant growth and colonization.

In two separate pot trials, ferrosol and podsol soils were balanced for pH by biochar and lime. Lime + biochar (L + B), lime + biochar + Nitrogen at a rate of 110 kg ha^{-1} (L + B + N) and lime + Nitrogen + Phosphorus and potassium equivalent to biochar (L + N + PK) were tested for growth of

tomato. The treatment L + B + N was beneficial over L + B application. The application of L + N + PK had the greatest positive effect on performance of tomato in both soils.

In two separate pot experiments for ferrosol and podsols soils, Sugarcane Trash and Green Waste A biochars at a rate of 30 t ha^{-1} and mycorrhizal inoculum rates 0 and 5 g kg^{-1} of soil were tested for growth and colonization of onion. Sole effects of biochar and mycorrhizal rates were prominent but there were little effects of their interactions. In ferrosol soils, Sugarcane Trash biochar and mycorrhizal rates of 5 g kg^{-1} produced more growth than other treatments.

In a pot trial, podsol and ferrosol soils were balanced for pH in which lime (L), lime + washed biochar (L + W), lime + washed biochar + mycorrhizae (L + W + M), lime + extracted nutrients (L + E), lime + extracted nutrients + mycorrhizae (L + E + M), lime + P and K equivalent to biochar (L + PK) and P and K equivalent to biochar + mycorrhizae (L + PK + M) were tested. The effect of treatments in ferrosol was greater than in podsol for most of the parameters. Mycorrhizae had greater positive effect than L. L + E had more beneficial effect compared to L + W. The L + PK + M had greater effect on plant growth.

In a field trial, Sugarcane Trash and Green Waste A biochar rates of 10, 20 and 30 t ha^{-1} plus control and nitrogen at a rate of 110 kg ha^{-1} were tested in ferrosol soil. The results confirmed that the biochar application was beneficial for shallot growth and colonization. The rate of 10 t ha^{-1} was considered as optimum for plant growth and mycorrhizal colonization in ferrosol soil while the rate of 30 t ha^{-1} was best for the crops grown in sand in pot trials. This difference was considered to be due to higher nutrient levels in the field even though pot trials were fertilized with nutrient solution on a regular basis.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

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Publications during candidature

Peer-reviewed paper

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Conference paper

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Upadhyay, KP, George, D, Swift, RS, and Galea, V 2013. Growth response of cabbage (*Brassica oleraceae var. capitata*, Cv. Hearty) to two biochars in a pot trial. Conference abstract of the International Symposium on Soil Organic Matter Dynamics, Nanjing, China, May 5-10, 2013.

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Contributor	Statement of contribution
Kalika Prasad Upadhyay	Designed experiments and wrote the paper (85%)
Doug George	Edited paper (5%)
Victor Galea	Edited paper (5%)
Roger Swift	Edited paper (5%)

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Keywords

Biochar, arbuscular mycorrhizal fungi, heavy metals, colonization, plant growth, soil fertility

Australian and New Zealand Standard Research Classifications (ANZSRC)

070601 Horticultural crop growth and development	50%
070306 Crop and pasture nutrition	50%

Fields of Research (FoR) Classification

0706 Horticultural production	50%
0703 Crop and pasture production	50%

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Abbreviations/Acronyms	Description
%	Per cent
µg g^{-1}	Microgram per gram
µM	micrometre
^0C	Degree Celsius
40x	40 times
AM	Arbuscular mycorrhizae
AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of variance
AusAID	Australian Alliance for International Development
B	Boron
C	Carbon
C/N ratio	Carbon Nitrogen ratio
Ca	Calcium
Ca(NO3)2.4H2O	Calcium nitrate tetra hydrate
$CaCO_3$	Calcium carbonate
Cd	Cadmium
CEC	Cation exchange capacity
Cl	Chlorine
cm	Centimetre
cmol or $cmol_C$	Centimol
CO_2	Carbon dioxide
CO_2-C	Carbon in the form of CO_2
CO_2-Ce	CO_2-C equivalent
C-sequestration	Carbon sequestration
Cu	Copper
$CuSO_4.5H_2O$	Copper sulphate pentahydrate
cv	Cultivar
CV	Coefficient of variation
d	Days
dS m^{-1}	deciSimens per metre

Abbreviations/Acronyms	Description
DTPA	Diethylene triamine pentaacetic acid
EC	Electrical conductivity
EC_{50}	Concentration of a drug that gives half-maximal response
F- Probability	Fisher–Snedecor's continuous probability distribution
FAO	Food and Agriculture Organization of the United Nations
Fe	Iron
Fe EDTA	Iron-ethylenediaminetetraaceticacid
Fe-P	Soil inorganic phosphorus fraction as Iron phosphate
g	Gram
g kg^{-1}	Gram per kilogram
GA	Gibberellic acid
GA_3	A type of Gibberellic acid
Gt	Gigatonne
Gt C	Gigatonne of carbon
GWA	Green Waste A
GWB	Green Waste B
h	Hour
H_3BO_3	Boric acid
HM	Heavy metal
K^+ or K	Potassium ion or potassium
K_2SO_4	Potassium sulphate
KCl	Potassium chloride
kg	Kilogram
KH_2PO_4	Monopotassium phosphate
KNO_3	Potassium nitrate
KOH	Potassium hydroxide
kPa	Kilopascals
L	Litre
LSD (α0.05)	Least significant difference at the level of 0.05
m	Metre
m^2	Square metre
Mg	Magnesium
$MgSO_4.7H_2O$	Magnesium sulphate heptahydrate

Abbreviations/Acronyms	Description
min	Minute (s)
mL	Millilitre
Mn	Manganese
$MnSO_4.H_2O$	Manganese sulphate monohydrate
Mo	Molybdenum
Mycorr/Myc/Myco	Mycorrhizae
MAI	Mycorrhizal Applications International
N	Nitrogen
N	Number of observations
N_2 fixation	Nitrogen fixation
Na+	Sodium ion
$Na_2MoO_4.2H_2O$	Sodium molybdate dihydrate
NaOH-Pi	Sodium hydroxide- inorganic phosphorus
N-fixers	Nitrogen fixers
NH_4+	Ammonium
NH_4NO_3	Ammonium nitrate
Ni	Nickel
$NiSO_4.6H_2O$	Nickel sulphate hexahydrate
nm	nanometer
ns	non-significant
P	Phosphorus
$p<0.05$	Probability value less than 0.05
Pb	Lead

Chapter 1. General introduction

Increasing crop productivity is of global concern, and will require the development of new technologies. Inputs to optimize crop productivity can be applied through soil and water and a crop will thrive if all inputs are optimal. To achieve optimum crop productivity, the soil should be fertile because plants absorb nutrients from this source. The continuing need for increased crop productivity dictates increasing demands on soil fertility world-wide (Wild 2003). Thus, the soil is one of the most important considerations for plant growth and development. However, to keep soil fertile is highly technical and requires thorough knowledge of soil quality and health; this refers to the soil's fitness to support crop growth without becoming degraded or otherwise harming the environment (Acton & Gregorich 1995).

The government of Queensland has clearly defined that soil fertility decline generally occurs when the quantities of nutrients applied to the soils are less than those removed by crops at harvest (https://www.qld.gov.au/environment/land/soil/soil-health/fertility-decline/). This type of fertility decline has been a great issue all over the world (Harden 2001; Lal 2001; Tiwari et al. 2010), but this problem is quite serious in the soils of Hindu Kush Himalayan region (FAO 1994, 1999), where Nepal lies. Soil fertility decline has become a great threat in the hills of Nepal due to nutrient losses through various ways (Tripathi et al. 1999; Gardner et al. 2000; Tripathi et al. 2000; Paudyal 2001); for example, about 1.3 million tonnes of plant nutrients are estimated to be displaced from soils of Nepal annually (Atreya et al. 2005) due to several reasons including erosion.

Sustainable soil fertility management has been suggested as essential to the prosperity of many households in the mid-hills of Nepal (Pilbeam et al. 2005). Therefore, sustainable management of soil using inputs such as compost, cattle manure, poultry manure, microorganisms and biochar, and their known crop performance benefits are matters of interest.

Most soils of South Asia have extremely low levels of carbon (C) ranging from 8 to 10 g kg^{-1} and the potential of carbon sequestration in Nepal has been estimated to be about 0.7-1.2 Tg C yr^{-1} (Lal 2004). This information demands the inputs of carbon in degraded soils of the developing countries. Among the carbon sequestering inputs, biochar is one of the major concerns of the scientists.

Soils of South and South East Asian countries in which cereals are extensively grown are invariably deficient in nitrogen (N) and organic matter (De Datta & Hundal 1984). Similarly, soil fertility is

declining in Nepal (Gardner et al. 2000; Tripathi et al. 2000; Gami et al. 2001; Panthi 2010), associated largely with the loss of organic matter, declining soil N (Pande 2005) and widespread phosphorous (P) deficiencies. Nepal is divided into three eco-regions: high altitude mountains, mid hills and southern plains or terai; and each region and cropping system has unique soil fertility issues. For example, total N, available phosphorus, available potash and organic matter content decrease with increases in altitude in Nepal (Panthi 2010). In the mid hills, phosphorus deficiencies are widespread because the bedrock is generally low in P and soils are acidic, hence limiting P availability to plants (Sharpley et al. 1993). Significant amounts of N, phosphorus, organic matter and other nutrients have been lost from mid hill soils under a maize based cropping system (Atreya et al. 2005) while rice based cropping systems on Nepal's plains tend to be deficient in organic matter, N, P and K (Mandal 2002).

One way to improve soil fertility in both intensive and marginal agriculture is to apply amendments to increase soil organic matter content and health (Abington 1992; Sherchan & Karki 2005; Tiwari et al. 2010). In addition, loss of organic C from cultivated agricultural soils has generated interest in C-sequestration (Gami et al. 2009). Traditionally, soil amendments have included farm yard manure (Bista et al. 2010), composted manure (Shrestha et al. 2000), poultry manure (Uddin et al. 2009) and cattle manure (Abington 1992). When combined with beneficial microbes such as arbuscular mycorrhizal fungi (Young et al. 1986), they have improved plant performance by enhancing decomposition of organic matter and phosphorus availability.

One novel technology gaining interest world-wide is the application of biochar as a soil amendment for carbon sequestration and improved crop production (Glaser et al. 2000; Skjemstad et al. 2002; Lehmann & Rondon 2006; Rondon et al. 2007; Chan et al. 2008a; Brandstaka et al. 2010; Luostarinen et al. 2010). However, to date, very little research has been conducted to identify combined effects of crops, biochars from different sources, biochars with varying level of heavy metal concentration and biochar application rates in different soils and ecological systems. Comparative interaction of vegetable crops, biochar types and application rates has been inadequately studied. If these combinations are found to be beneficial for mycorrhizal association, crop growth and development, their usefulness in developing countries such as Nepal may be discovered.

Recently, biochar effects on crop performance (Carter et al. 2013), microorganisms (Warnok et al. 2007), heavy metals (Kochanek et al. 2014) and soil nutrients stock (Brandstaka et al. 2010) have been intensively studied. However, the influence of biochar on colonization of horticultural crops

by arbuscular mycorrhizal fungi in different soil types including sand-biochar medium has been inadequately studied. Therefore, the experiments under this study aim to answer the following questions:

1) Biochar effects on crop growth: Do crops tested for biochar effects show similar responses? Do the biochars produced from different feedstocks have similar effects? What is the appropriate application rate of biochar for optimum plant growth?

2) Biochar effects on colonization of crops by arbuscular mycorrhizal fungi: Do the biochars produced from different feedstock have different effects on mycorrhizal colonization of the same crop? What is the difference in mycorrhizal colonization from different application rates of biochar? Is there any interaction effect between biochar types and their application rates? Do the crops differ in colonization due to the effect of biochar types and application rates?

3) Zinc (Zn) and copper (Cu) effects on mycorrhizal colonization in biochar amended soil: How do Zn and Cu influence mycorrhizal colonization when they are amended in biochar added soil?

4) Effects of biochar-added soil and soil balanced for nutrients available in biochar: Is there any difference between the effect of biochar (with associated nutrients) on plant growth and that caused by the equivalent amount of nutrients alone? Is N contained in biochar available in soil for plant growth? Is the effect different in different biochars and soil types?

5) Comparative effects of soil types, biochar types and mycorrhizal inoculum: Is there any difference in plant growth between inoculated and non-inoculated soils? Is the colonization and plant growth similar in different biochars added to the same soil? Do plants respond differently to different soils and different biochar types?

6) Effects of washed biochar and extracted nutrients of biochar: Is there any difference in the performance and colonization of onion plants by mycorrhizae when pure biochar and extracted solutions of biochar are added to different soils?

7) Verification of biochar effects in the field condition: Are the results of glasshouse experiments similar to those in the field? Which is the most effective biochar for optimum plant growth and colonization? What is the appropriate application rate of biochar for plant growth and colonization in the field?

These questions are addressed in the following sections. Chapter 2 reviews the literature which provides information on research work undertaken so far and gaps in knowledge needed to be filled by research. Chapters 3 to 9 answer the questions asked above. Chapter 10 summarizes the research finding by drawing conclusions on how the biochars influence plant growth and mycorrhizal colonization. The implication and future research goals arising from these findings are also discussed in this chapter.

Chapter 2. Literature review

The investigations on the effects of biochar on soil, plant and environment are being undertaken worldwide. However, the results are not uniform. A logically organized complete study of the effects of biochars from different feedstock on different crops, their interaction with microorganisms and agriculturally important heavy metals such as Zn and Cu could confirm to what extent biochar is beneficial for crop lands.

The literature review section evaluates biochar effects on crop, soils and arbuscular mycorrhizal fungi, the effects of AM fungi on crop, soil fertility and heavy metal remediation, and the interaction effects of biochar and mycorrhizae on crops and soil nutrients.

This section also emphasizes why and how this study was understood to be a researchable issue. This section ends with the need for this thesis research, explaining the research gap that this thesis aims to fill.

2.1 Biochar

2.1.1 What is biochar?

Biochar has been defined in similar ways by several authors. It is a 'black carbon manufactured through pyrolysis of biomass' (Lehmann et al. 2006); 'the high carbon materials produced from the slow pyrolysis (heating in the absence of oxygen) of biomass' (Chan et al. 2007); and 'a fine-grained and porous substance, similar in its appearance to charcoal produced by natural burning or by the combustion of biomass under oxygen-limited conditions' (Sohi et al. 2009). In fact, it is a product of biomass obtained from heating in a suitable temperature regime in the absence of oxygen (the process of fast or slow pyrolysis) or from a gasification system.

2.1.2 Origin of biochar

The occurrence of charcoal in '*Terra Preta*' in some soils of the Amazon basin was reported by Sombroek (1966) who indicated the presence of black carbon derived from incomplete combustion of cooking fires (Glaser et al. 2001). This soil is also called as *'terra preta do indio'* or 'black-earth-like' anthropogenic soil which was possibly made by the several activities of pre-Columbian residents with their slash and char activities (Taylor 2010). It was a soil management practice of these ancient Amerindians of the Amazon region (Petersen et al. 2001; Lehmann & Joseph 2012). These highly fertile soils have up to 70 times more black carbon than the surrounding soils and also

contain high level of N, P, K, calcium, organic matter and aromatic humic substances (Glaser et al. 2001).

The term ‘biochar’ was invented by the late Peter Read, a New Zealander, by describing it as a soil amendment for agricultural purpose but it was called ‘agrichar’ until an Australian Company trademarked it and it was the first ingredient in the Terra Preta recipe (Bates 2010). Lehmann and Joseph (2012) have distinguished the term biochar from charcoal in that it is charred organic matter that is applied to soil not only to improve soil properties but also to promote soil remediation or other environmental services while the charcoal is used as fuel or source of heat, as a filter, as a reductant in iron-making or as a colouring agent in industry or art.

2.1.3 Sources of biochar

Biochars can be produced from many organic materials and under different conditions resulting in products of varying properties (Baldock & Smernik 2002; Nguyen et al. 2004; Guerrero et al. 2005). It can be produced from a wide range of biomass sources, for example, woods and barks, agricultural wastes such as olive husks, corncobs and tea waste (Demirbas 2004; Ioannidou & Zabaniotou 2007), greenwaste (Chan et al. 2007), animal manures and other waste products (Downie et al. 2007; Chan et al. 2008a; Lima et al. 2008). Biochar is a mixture of char and ash with the major part (70 – 95%) carbon (C) (Brandstaka et al. 2010; Luostarinen et al. 2010). It can also be produced from poultry litter (Revel et al. 2012), sewage sludge (Khan et al. 2013), rice-husk (Carter et al. 2013; Lu et al. 2014), wheat straw (Junna et al. 2014) and several other materials.

2.1.4 Biochar and carbon sequestration

The relatively stable nature of biochar allows for carbon sequestration value (Lehmann et al. 2006). Lehmann et al. (2006) estimated that about 5-10 Gt C is sequestrated per year which is the equivalent or more than the present global emissions from fossil fuel use. In addition, biochar carbon added almost 40% of the carbon to soil (Glaser et al. 2000; Skjemstad et al. 2002). Lehmann (2007a) predicted that the retention times of carbon in biochar would be at least hundreds, but more likely thousands of years. In addition, as a pyrolysed product, biochar is protected from rapid microbial degradation and is able to securely sequester carbon, contributing to mitigation of greenhouse gas emissions (Lehmann et al. 2006). Day et al. (2004) emphasized that using biochar to sequester carbon in soil to mitigate climate change could only be economical if the sequestered C has beneficial soil amendment and/or fertilizer value.

2.1.5 Biochar and climate change

Some authors have indicated the effectiveness of biochar in mitigating climate change due to the greenhouse effect. Lehmann (2007b) mentioned that greenhouse gas emission can be reduced by sequestering carbon as biochar that stores carbon for hundreds of years or more with its relative recalcitrance against microbial decay and slower return of carbon as carbon dioxide to the atmosphere. Rondon et al. (2005) tested charcoal in a soybean crop and *Brachiaria humidicola* and found that the net fluxes of methane and nitrous oxide from pots cropped were significantly reduced by the addition of charcoal. Woolf et al. (2010) estimated that biochar can reduce annual net emissions of carbon dioxide (CO_2), methane and nitrous oxide by a maximum of 1.8 Pg CO_2-C equivalent (CO_2-C_e) per year (12% of current anthropogenic CO_2-C_e emissions; 1 Pg=1 Gt), and total net emissions over the course of a century by 130 Pg CO_2-C_e. The nitrous oxide production from the top soil layer could be reduced by increasing soil pH following the application of biochar (Deng 2013).

Literature shows varying effect of biochar on carbon sequestration and greenhouse gas emission in different soil conditions and regions. For example, Lehmann et al. (2006) have emphasized that about 50% of the initial carbon is sequestered by biochar compared to 3% by burning. Elimination of carbon emission in the form of methane (CH_4) has also been explained by Renner et al. (2007). Biochar may suppress methane (CH_4) and nitrous oxide (N_2O) emissions from soil (Sohi et al. 2009). In an experiment, no significant increase in N_2O and CO_2 emissions and CH_4 soil consumption and production was observed in char-treated plots (Castaldi et al. 2011). However, soil N_2O production was suppressed by 49% with biochar within 48 h of wetting (Case et al. 2012). By addition of biochar, the efflux of N_2O and CO_2 increased in bare soil in dry conditions while N_2O efflux increased from vegetated wet soil and decreased from vegetated dry soil (Sarino et al. 2013) indicating the role of soil moisture in greenhouse gas emission. When manure-derived biochar was added to the soil in temperate conditions, it increased N_2O and CO_2 emission but did not change CH_4 emission (Troy et al. 2013).

2.1.6 Effects of biochar on soils

Some authors have reported that biochar can be beneficial as soil amendment for improving the quality of agricultural soils (Glaser et al. 2002; Lehmann et al. 2003). Currently, very little biochar material is being used in agriculture in Australia and elsewhere, due to the fact that its agronomic values in terms of crop response and soil health benefits are inadequately quantified (Chan et al. 2007). Some literature on its effects on soil is described in this section.

Biochar application in soils has positive influences on improving soil quality and plant growth (Chan et al. 2007; Chan et al. 2008a). The general effects of biochar on soil have been listed by Brandstaka et al. (2010). They characterize it as beneficial for sequestration of carbon, improvement of cation exchange capacity, durability of soil aggregates, microbial activity, bioenergy production and water retention capacity; reduction of nitrous oxide and methane emissions from soils, leaching, soil erosion and need of fertilization and thereby enhancement of soil fertility and crop yields (Brandstaka et al. 2010). Other authors have also described its value for reduction of greenhouse gas emissions (Yanai et al. 2007; Van Zwieten et al. 2010b) and adsorption of anions and cations to prevent leaching of applied nutrients (Major et al. 2009). According to Chan et al. (2008a), biochar produced from green waste by pyrolysis significantly increased soil pH, organic carbon, and exchangeable cations with a substantial decrease in tensile strength at higher rates of biochar application (>50 t ha^{-1}) in alfisol soil. Similar results were observed when biochar produced from poultry litter was tested (Chan et al. 2008b). Van Zwieten et al. (2010a) tested two biochars produced from the slow pyrolysis of paper mill waste, in two agricultural soils in a glasshouse and found that the biochars differed slightly in their liming values (33% and 29%), and carbon content (50% and 52%). The thermal processing of wastes into biochar has been identified as an opportunity to destroy contaminants (Glover 2009), making beneficial land application possible. Since extracts from biochar derived from poultry litter increased microbial growth but that from pine timber inhibited it (Das et al. 2008), the effect of biochar on microbes may depend on its feedstock.

The biochar effects on soil aggregation depend on soil and biochar types (Herath et al. 2013). Hardie et al. (2014) incorporated biochar in soil and found after thirty months observation that it had no significant effect on some soil properties. Tammeorg et al. (2014) evaluated 0, 5, 10, 20 and 30 t ha^{-1} of biochar with or without inorganic fertilizer or meat bone meal for two years, and found biochar improved nitrate N content, water retention capacity, soil organic carbon and K content. Biochar derived from wheat straw decreased soil bulk density and increased soil field capacity, dissolved organic carbon and available P (Alburquerque et al. 2014).

Reports are also available for the effect of biochar on nutrient availability. A biochar produced from corn cobs increased nitrate N in the first ten days of crop growth and thereafter it decreased; while it decreased P content when biochar was applied solely and increased it after addition of nitrogenous or phosphate fertilizer (Nelson et al. 2011). This finding indicates the use of biochar combined with application of other sources of fertilizers could be beneficial for improving plant growth and soil nutrient status.

The pyrolysis method could play an important role in soil properties. For example, mineralization of N could be enhanced by application of biochar produced from slow pyrolysis rather than fast pyrolysis (Bruun et al. 2012). Similarly, there are varied responses of soils to biochar for the leaching of nutrients and the sorption of nutrients on biochar (Yao et al. 2012). In a three-year field experiment, there was no difference between biochar added and not-added soil but reapplication of biochar after three years significantly increased available P, exchangeable K and calcium, dissolved organic carbon, soil moisture and electrical conductivity (Quilliam et al. 2012).

2.1.7 Biochar effects on crops

There are varied responses of crops to biochar (Chan et al. 2008a). Van Zwieten et al. (2010a) tested two biochars produced from the slow pyrolysis of paper mill waste, in two agricultural soils in a glasshouse and found that they significantly increased biomass in wheat, soybean and radish in ferrosol soil but reduced wheat and radish biomass in calcaresol, amended with fertilizer in both soils. A significant decrease in dry matter content of radish was obtained when biochar was applied at 10 ton ha^{-1} (Chan et al. 2008a). In a separate experiment, there was no significant effect of biochar rates (0, 7 and 15 tons ha^{-1}) on turnip, wheat, rape and faba bean yields (Brandstaka et al. 2010).

Asai et al. (2009) showed that biochar increased rice grain yields at sites with low P availability, which might be due to improved saturated hydraulic conductivity of the top soil, xylem sap flow of the plant and response to N and NP chemical fertilizer treatments. Limiting soil N content by biochar application in N deficient soils could be due to the high C/N ratio, hence it might reduce crop productivity temporarily (Lehmann et al. 2003). However, some biochars contain considerable amount of micronutrients. For example, pecan-shelled biochar contained greater amount of copper (Cu), magnesium (Mg) and zinc (Zn) than the soil (Novak et al. 2009). In a separate experiment, concentrations of heavy metals including Cu and Zn increased in sewage sludge biochar but those of available heavy metals decreased (Lie et al. 2014). Furthermore, poultry litter biochar was also rich with considerable amounts of Zn, Cu and manganese (Mn) (Inal et al. 2015). Thus, it is essential to compare its effect solely and in combination with other nutrient sources. Some authors (Verheijen et al. 2009; Brandstaka et al. 2010) have emphasized the need for further research on potential benefits of biochars as well as their economics. However, their interactions with other organic sources as well as microbes and release of nutrients from them are insufficiently assessed.

Biochar at the rates of 20 and 40 t ha^{-1} without N fertilization in a carbon poor calcareous soil of China increased maize yield by 15.8% and 7.3% while the rates with 300 kg ha^{-1} N fertilization

enhanced the yield by 8.8% and 12.1%, respectively (Zhang et al. 2012). In addition, biochar application in a nutrient-poor, slightly acidic loamy sand soil had little effect on wheat yield in the absence of mineral fertilization but when applied with the highest rate of mineral fertilization, it produced yield 20–30 % more than mineral fertilizer alone (Alburquerque et al. 2014).

The yield of tomato fruit was significantly higher in beds with charcoal than without charcoal (Yilangai et al. 2014). Biochar application increased vegetable yields by 4.7-25.5% as compared to farmers' practices (Vinh et al. 2014). In another work, biochar did not increase annual yield of winter wheat and summer maize but the cumulative yield over four growing season was significantly increased in a calcareous soil (Liang et al. 2014). Biochar of maple was tested at different concentrations for root elongation of pea and wheat but no significant difference was observed (Borsari 2011), possibly due to little effect of biochar in the short-term. The wood chip biochars produced at 290^0C and 700^0C had no effect on growth and yield of either rice or leaf beet (Lai et al. 2013). A biochar significantly increased growth and yield of French bean as compared to no biochar (Saxena et al. 2013). A rice-husk biochar tested in lettuce-cabbage-lettuce cycle increased final biomass, root biomass, plant height and number of leaves in comparison to no biochar treatments (Carter et al. 2013).

An oak biochar derived from a slow pyrolysis process was tested for four years at 0 t ha-^{1}, 5 t ha^{-1} and 25 t ha^{-1} with 100% and 50% of N fertilizer on a maize -soybean rotation in an alfisol soil, resulting in an overall positive trend in total above-ground biomass and grain yield (Hottle 2013). A poultry-litter biochar derived from slow pyrolysis tested in cotton showed that a higher level (3000 kg ha^{-1}) with urea produced better cotton growth than the lower rate (1500 kg ha^{-1}) which, in turn, did better than the control (Coomer et al. 2012) .

2.2 Soil microorganisms

Microorganisms are considered as the driving force for basic metabolic processes involving specific enzyme activities in the soil (Nannipieri et al. 2003). Several authors (Linderman 1992; Glick 1995; Kennedy 1998; Bowen & Rovira 1999; Barea et al. 2005) have indicated that soil-borne microbes interact with soil constituents as well as plant roots at the root–soil interface. Kennedy (1998) argued that the differing physical, chemical, and biological properties of the root-associated soil, compared with those of the root-free bulk soil, are responsible for changes in microbial diversity and for increased numbers and activity of micro-organisms in the rhizosphere micro-environment. The release of root exudates and decaying plant material provide sources of carbon compounds for heterotrophic soil biota (Werner 2001). Microbial activity in the rhizosphere affects rooting patterns

and the supply of available nutrients to plants, thereby modifying the quality and quantity of root exudates (Bowen & Rovira 1999; Barea 2000; Gryndler 2000; Barea et al. 2005).

Soil microorganisms are important for various functions in the soil environment. Among them, the major ones are the decomposition and transformation of organic materials, which are mostly derived from above and below-ground plant residues, thereby contributing to carbon cycling, nutrient turnover, or the production of trace gases (Araújo et al. 2009). Microbial respiration can be used as a soil quality indicator (Brendecke et al. 1993) by using it to quantify their activity (Alef & Nannipieri 1995). The soil environment around the root system is a centre of attraction for the microbes due to the presence of root exudates and rhizodeposits (Smalla et al. 2006; Hartmann et al. 2008). All microorganisms are not equally active for plant systems, for example, some of them may be neutral or deleterious to plant growth while other microbes may support the host plants (Raaijmakers et al. 2002). In fact, various factors can influence the soil microbial community and function; among them, organic matter quality, nutrient input, soil types, vegetation, management systems, and soil contamination are very important (Bending et al. 2002; Johnson et al. 2003; Renella et al. 2004).

Beneficial microorganisms are also known as plant growth promoters. They can not only stimulate plant growth and yield but also reduce pathogen infection as well as biotic or abiotic plant stress without conferring pathogenicity (Welbaum et al. 2004; Loon & Bakker 2006; Lugtenberg & Kamilova 2009). Plant beneficial microorganisms have been given due interest for their application either as biofertilizers or as pesticides or for phytoremediation applications (Sturz et al. 2000; Berg 2009; Lugtenberg & Kamilova 2009; Weyens et al. 2009). Their biomass and activity are greater in soils amended with organic fertilizers than with conventional ones (Drinkwater et al. 1995). However, in many cases, some of them fail to induce the desired effects when applied in the field. This might be due to inadequate rhizosphere environment and/or plant colonization, which is an important step required for exhibiting beneficial effects (Lugtenberg et al. 2001). Therefore, not only mechanisms responsible for plant growth promotion need to be investigated, but also a thorough understanding of all steps involved in plant colonization is required to improve the efficiency and reliability of inoculant strains (Compant et al. 2010).

2.2.1 Effect of nutrient management practices on soil microbial activity

The effect of soil nutrient management on microbial activity and functional diversity was the concern of various studies (Bulluck et al. 2002; He et al. 2006). Mader et al. (2002) showed that soils under an organic management system had higher microbial functional diversity than those under conventional farming systems. This could be due to soil amendments of crop residues or

other organic materials establishing an environment favourable for the microbial community (Bending et al. 2002). It was suggested that different fertilizer treatments could affect soil microbial properties and the use of soil microbial indicators to assess soil quality change needs to be further studied (Kong et al. 2008). Araujo et al. (2009) indicated that soil microbial activity, biomass and bulk density were significantly improved in organic management compared with the conventional system.

2.2.2 Microorganisms versus conventional fertilizers

Conventional inputs are manmade or synthetic products, for example, effects of chemical fertilizer on microbes have been investigated in several ways. Kong et al. (2008) compared the effect of various combinations of conventional fertilizers (NPK-fertilizer treatments) on microbial activity and found variation in soil microbial biomass C ranging from 94 to 145 mg kg^{-1}, where the NK treatment showed a lower biomass whereas functional diversity of soil microbes ranged from 4.13 to 4.25 mg kg^{-1} increasing from control to double and triple-fertilizer treatments. However, the soil microbial biomass and its functional diversity and evenness did not show any significant differences. This result suggests that the long-term application of chemical fertilizers would not result in significant changes in microbial characteristics of vertisol soil. Nevertheless, particular chemical fertilizers have specific effects on soil microorganisms, for example, ammonium sulphate is a very strong biocide that hinders N fixation and kills nematodes and earthworms whereas superphosphate has a negative effect on free-living N-fixing bacteria (Primavesi 1990). According to Barabasz et al. (2002), high rates of N fertilization caused significant changes in microbiocenoses resulting in the decline of some beneficial microbes as well as the occurrence of carcinogenic nitrosamines in soil. A side-effect of the conventional nutrient supply system through incorrect agrotechniques such as improper fertilization is that it reduces soil fertility of arable soils and hinders microbial activity and qualitative selection of their community (Jenkinson 1982; Doran et al. 1996; Barabasz et al. 2002).

2.2.3 Micro-organisms as biofertilizers

Biofertilizers are considered as organic as well as natural sources of plant nutrients. The role and importance of biofertilizers in sustainable crop production and soil have been documented (Wani & Lee 1995). However, progress in the field of biofertilizer production technology is greater in Asia because the deficit of synthetic fertilizer could be fulfilled by their addition, they are cheaper than chemical fertilizers for farmers in lower socio-economic regions and they provide a sustainable way of improving soil fertility without environmental hazards (Sheraz Mahdi et al. 2010).

2.2.4 Characteristics of microbial fertilizers

Microbial fertilizers have specific characteristics like N fixation, P solubilization, S oxidation and organic matter decomposition; they are also called bio-inoculants (bacterium or fungus) and supply nutrients to plants to improve their growth and yield (Deshmukh 1998). These bio-inoculants influence plant growth by colonizing plant surfaces, forming endophytic associations, or interacting with other microbes in the rhizosphere or phyllosphere. For example, N-fixers increase soil fertility and crop yields (Kachroo & Razdan 2006; Subashini et al. 2007) by improving soil biota and reducing the use of chemical fertilizers (Subashini et al. 2007). Some examples of N fixing and phosphate solubilizing organisms and their effects on soil and crops are mentioned below.

2.2.5 Microbial inoculants

There are several microbial inoculants identified for fixing N and P. Rhizobial inoculants applied to leguminous plants and arbuscular mycorrhizal (AM) fungi to muskmelon were effective in reducing chemical fertilizer amounts by one third to half (Young et al. 2003). Rhizobial strain inoculation alone or in combination with AM fungi increased N_2 fixation and soybean yield (from 5 to 134%) and was suggested as an efficient biological fertilizer (Young et al. 1988). Inoculation of Rhizobium improved yield and yield components of green gram over the control (Bhat et al. 2010).

Since P is relatively insoluble and thus less available in the root zone, phosphate solubilising bacteria are helpful for this purpose. The bacterial genera that can solubilize phosphate are *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Microccocus*, *Aereobacter*, *Flavobacterium* and *Erwinia* (Sheraz Mahdi et al. 2010).

2.2.6 Effects of microbial inoculants on crops

Phosphate solubilising bacteria improved both growth and quality of crops but drastically reduced the usage of chemical fertilizers (Young et al. 2003). *Azotobacter chrooccocum* + P fertilizer showed the highest alkaline phosphate activity in peach root (Godara et al. 1995). Application of *Glomus fasciculatum* + *A. chrooccocum* + 50% of the recommended dose of P fertilizer resulted in the greatest root length, plant height, bulb girth, bulb fresh weight, root colonization and P uptake in onion (Mandhare et al. 1998). In a separate experiment, inoculation with *Azotobacter* + *Rhizobium* + VAM with rock phosphate as a P fertilizer significantly increased straw and grain yield of wheat (Elgala et al. 1995).

Azotobacter in combination with *Bacillus* enhanced tolerance to salinity reflecting the better performance of growth, dry matter accumulation, and yields of wheat (Mahmoud & Mohamed 2008).

2.3 Arbuscular Mycorrhizal (AM) Fungi

The term 'mycorrhiza' means 'fungus roots' and is characterized by a symbiotic association between host plants and a certain group of fungi at the root system, in which the fungus is benefited by receiving carbon compounds from photosynthates of the host plant and the host is benefited with required and inaccessible nutrients such as P, Ca, Cu, and Zn with the help of ramifying fine absorbing hyphae of the fungus (Sheraz Mahdi et al. 2010).

According to their association types, Brundrett et al. (1996) have classified mycorrhizae as follows:

Vesicular-arbuscular mycorrhizae: These types of mycorrhizae are called VAM which are the members of the Zygomycetes fungi. Their name 'vesicular arbuscular' is derived from their characteristic structures, 'arbuscules', which occur in cortical cells and vesicles are found within or between the cells. These classes are those studied in this thesis and are further described in the following section.

Ectomycorrhiza: These are the members of Basidiomycetes. They form a mantle around roots and a hartig net between root cells.

Orchid mycorrhizae: These types of mycorrhizae are associated with orchid roots. They produce hyphal coils within roots or stems.

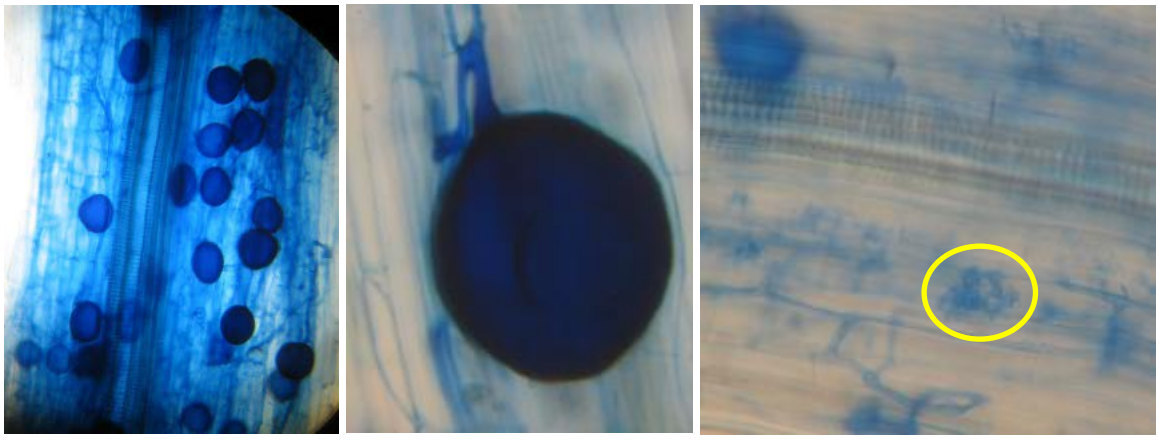
Ericoid Mycorrhiza: These mycorrhizae are found in plants members of Ericales such as tea, persimmon, brazil nut, azalea, kiwi fruit, blueberry, cranberry, and rhododendron. They develop hyphal coils in epidermal cells of root hairs.

Ectendo-, arbutoid and motropoid mycorrhizae are similar to ericoid mycorrhizae but they have a more specific anatomy.

Among the endo-mycorrhizae, vesicular-arbuscular mycorrhizae (VAM) occur in a range of environments from desert to aquatic (Mosse et al. 1981). There are six genera of fungi that contain species, which are known to produce symbiotic relationships with plants; among them two (*Glomus* and *Sclerocytis*) produce chlamydospores while *Gigaspora, Scutellospora, Acaulospora and Entrophospora* develop spores similar to azygospores (Sheraz Mahdi et al. 2010).

The oldest and most popular of these associations are the AM fungi symbioses and have been estimated to have evolved about 400 million years ago coinciding with the appearance of the first land plants while crop domestication began about 10,000 years ago (Sawers et al. 2008).

A few members of this fungi form sporocarps with a little amount of sterile mycelium, the majority (80%) of the species form both vesicles and arbuscules while the remaining (~20%) do not form vesicles; therefore, those should be called 'arbuscular mycorrhizal fungi' (Brundrett et al. 1996). The images shown in Plate 2.1 represent how *Glomus* mycorrhiza colonize and develop in plant tissues.



A **B** **C**

Plate 2.1 Glomus mycorrhizal network in root cells, B. fully developed single vesicle C. arbuscule marked by oval outline. Images captured during the present study**.**

2.3.1 Role of AM fungi in agricultural soils

The fungi are mostly abundant in agricultural soils accounting for 5-50% of soil microbes (Olsson et al. 1999). Biomass of their hyphae was estimated to be about 54-900 kg ha^{-1} (Zhu & Michael Miller 2003). Their by-products may account for 3000 kg ha^{-1} (Lovelock et al. 2004). Rillig et al. (2001) estimated that the pools of organic carbon such as glomalin produced by them might exceed soil microbial biomass by a factor of 10-20. Jakobsen & Rosendahl (1990) found that the external mycelium attained about 3% of root weight. These fungi can develop mycelium of a length of 10-100 m per cm of root length (McGonigle & Miller 1999).

The fungi were found to improve soil structure by binding soil particles together, forming micro-aggregates, meshing of micro-aggregates and binding carbon resources to the soils (Miller & Jastrow 2000). AM fungal hyphae are small in size; thus they can penetrate organic material better than plant roots and decompose and compete for the recently mineralized N so that they capture

simple organic N compounds thereby shortening the N cycle (Hodge 2003). Another important role of AM fungi in soils is in water economy of plants such that their association improves the hydraulic conductivity of the root at lower soil water potentials contributing to better water uptake by plants (Sheraz Mahdi et al. 2010).

The impacts of AM fungi on development of aluminium tolerance in plants have been outlined by Seguel et al. (2013). They have concluded that AM fungi provide biosorption of aluminium to their hyphae probably by glomalin and organic acids exudated from the roots where they are colonized but they have accepted that this mechanism is not fully understood.

Aroca et al. (2013) suggested that AM symbiosis can alleviate salt stress. They have proposed a mechanism whereby the fungi alter hormonal profiles and affect plant physiology in which the plant produces strigolactone in salt stress conditions and strigolactone promotes symbiosis to cope with the stress. Porcel et al. (2012) reviewed the literature and concluded that the tolerance of mycorrhizal plants to salt stress is possibly mediated through increased K^+: Na^+ ratios, accumulation of proline, glycine, betaine or soluble sugars, photosynthetic and water use efficiency and antioxidant enzymes in plant tissues. They have also proposed that the involvement of cation proton antiporters and cyclic-nucleotide-gated channels could be a mechanism of salt tolerance of mycorrhizal plants but it is yet to be investigated.

2.3.2 Responses of crops to AM fungi

Inoculation increased soybean yields by 7-45% over the non-inoculated treatments (Young et al. 1986). Chang & Young (1992) observed that tea cuttings inoculated with AM had significantly increased growth. It was demonstrated in Mexico that in a soil with low phosphate availability, wheat growth capacity was highest in AMF inoculated plants reducing the need for phosphate fertilizer applications indicating that the mycorrhiza-mediated growth for plant dry weight, number of grains per spike, and 1000 grain weight was higher at 5 and 10 kg than at 20 kg P ha^{-1}. (Mohammad et al. 2004). Further, onion, sweet potato, tomato and cassava were highly dependent on mycorrhiza for their growth and development (Khasa et al. 1992). The AMF reduced the effects of drought stress in peanut (*Arachis hypogaea*) (Quilambo et al. 2005). Some positive results of rice seedling inoculation with AMF species on biomass, plant tissue mineral content, and grain yield of the Prakash cultivar of cowpea were found in inundated fields of India (Secilia & Bagyaraj 1992), and in the Pusa Basmati-1 cultivar sown in soils deficient in P and Zn (Purakayastha & Chhonkar 2001). According to Krishna et al. (2006), a mix of AMF strains resulted in increased survival percentage, shoot length, fresh and dry weight, leaf area, and photosynthetic rate of grape vines.

Trindade et al. (2006) reported that a total of 24 different AMF species colonized papaya (*Carica papaya*) roots.

2.3.3 Mechanisms of nutrient uptake

VAM can improve uptake of P and other nutrients. Bolan et al. (1987) argued that the fungi could break the bond between iron (Fe) and P (Fe-P form is stable and unavailable) but did not explain the mechanism. The mechanism behind the P uptake by mycorrhizal fungi includes the production of glomalin that contains very substantial amounts of iron (up to 5% of the glomalin pool) assuming that this iron was derived initially from unavailable Fe-P forms in the NaOH-Pi fraction; the destabilization of this bond could have released P that was taken up (Lovelock et al. 2004). The life time for hyphae varied from days to months (Staddon et al. 2003) and could range from 6 to 42 years for glomalin secretion (Rillig et al. 2001). Even under relatively favourable conditions for decomposition, 40% of AM fungi hyphae and 75% of total glomalin could be extracted from the soil 150 days after being separated from their host (Steinberg & Rillig 2003). Apart from this, VAM fungi increase the efficiency and uptake of several micronutrients, for example, Zn, Cu and Fe through the secretion of nutrient mobilizing enzymes and organic acids (Sheraz Mahdi et al. 2010).

2.3.4 Mechanisms of AMF tolerance to heavy metals (HM)

HM-tolerant species of AMF

Recent reports claim that the effect of heavy metals is specific to AMF species. For example, *Glomus etunicatum* was more sensitive to Cd, Pb and Zn than was *Glomus intraradices* (Pawlowska & Charvat 2004). Similarly, *Glomus sps* and *Glomus mosseae* were more sensitive than *Glomus claroideum* (del Val et al. 1999). The degree of infection of onions with *Glomus mosseae* was reduced when Zn, Cu, Ni or Cd were added to the soil medium (Gildon & Tinker 1983). The infection rates of *Glomus caledonium* were the highest but its sporulating ability was the poorest among the three AMF when their response to heavy metals (Cu and Cd) was tested (Liao et al. 2003) In a separate experiment, *Glomus lamellosum, Glomus intraradices* and *Glomus proliferum* exhibited tolerance to 5 ppm lead (Khade & Adholeya 2008). *Glomus intraradices* showed a heavy metal tolerance in a variety of plants in diverse heavy metal soils under optimum fertilization (Hildebrandt et al. 1999; Kaldorf et al. 1999). However, some reports indicated that the high concentrations of heavy metals had adverse effects on AMF (Leyval et al. 1997).

HM-tolerant plant species

Heavy metal tolerance also depends on plant species which can cope with adverse effects of metals; these type of plants are called metallophytes (Hildebrandt et al. 2007). Protection by AMF that

colonize plant roots and reduce the uptake of heavy metals into plant cells could be a mechanism that allows metallophytes to thrive on polluted soils (Weissenhorn et al. 1995; Leyval et al. 1997; Kaldorf et al. 1999; Berreck & Haselwandter 2001; Ouziad et al. 2005; Vogel-Mikus et al. 2005). Maize grown in heavy metal soils had more of the essential elements such as K, P and Mg but less of the heavy metals such as Ni, Fe, Zn, or Cu when symbiotically grown with *G. intraradices* (Kaldorf et al. 1999).

Adaptation of spores to HM-rich conditions

Spores and presymbiotic hyphae are generally sensitive to heavy metals in the absence of plants (Göhre & Paszkowski 2006). EC_{50} values (effective concentration reducing germination or hyphal growth to 50%) vary with the strain, but overall effect of heavy metals such as Zn, Pb and Cd is negative; however, spores from polluted soils were more tolerant than the spores from non-polluted soils (Shalaby 2003). This result indicates the adaptation of strains in contaminated environments. The interaction of heavy metal themselves can play a role in the degree of sensitivity of spores and hyphae to heavy metals. For instance, addition of Zn plays an antagonistic role on the toxicity of Pb and/or Cd on pre-symbiotic hyphal growth, while Pb and Cd acted synergistically (Shalaby 2003)

Sensitivity to HM types

HM have specific effects on colonization of AMF. In soils with 8% Zn and 863 µg g^{-1} Cd, 35% of clover roots were colonized (Gildon & Tinker 1981). In fact, VAM can decrease Zn toxicity in grasses growing in Zn-polluted soils (Dueck et al. 1986). Similarly, colonization of AMF in *Agrotis capillaris* was significantly higher in Zn and Cd-polluted soils (Griffioen et al. 1994). However, their infection was lower in Zn and Pb polluted soil where HM levels were lower (Diaz & Honrubia 1993). Controversially, some authors (Hildebrandt et al. 1999; Audet & Charest 2006) have proposed that mycorrhizal colonization of the roots increased with increasing heavy metals in soils, but others (Gildon & Tinker 1981; Graham et al. 1986; McGee 1987; Chao & Wang 1991) indicated some inhibition of AMF colonization by heavy metals occurs. In fact, most reports suggest that mycorrhiza have some degree of metal tolerance.

Metal binding mechanism

AMF can bind HM in two processes: they either release glomalin in soil that binds metals outside the rhizosphere (Gonzalez-Chavez et al. 2004; Göhre & Paszkowski 2006) or metals are bound to chitin of hyphal cell wall to reduce their local concentrations in the soil (Zhou 1999; Göhre & Paszkowski 2006). On average, 28 mg Cu per gram of glomalin is sequestered by *Gigaspora rosea* (Joner et al. 2000) while up to 0.5 mg Cd is bound per mg biomass of fungal hyphae (Joner et al.

2000). Gonzalez-Chavez et al. (2004) found that up to 4.3 mg Cu, 0.08 mg Cd and 1.12 mg Pb can be extracted from a gram of glomalin.

2.3.5 Phytoremediation by AMF

The roles of AM fungi in HM tolerance in plants can be summarized as phytoremediation of soil pollutants (Wang et al. 2007). Based on the type of pollutants, different strategies such as phytostabilization, phytodegradation, and phytoextraction can be used (Barea et al. 2005). AM can help phytoremediation activities, particularly in phytostabilization (Gonçalves et al. 1997; Leyval et al. 1997; Orłowska et al. 2002; Regvar et al. 2003; Turnau et al. 2006). Phytostabilization involves preventing the spread and leaching of HM into soil; this can be attained through resistant plant and AMF species, metal binding process and chelation in the fungus cell wall (Gaur & Adholeya 2004; Göhre & Paszkowski 2006) These reduce both soil erosion and transfer of heavy metals to aquifers, thus minimising their dispersion by wind while phytoextraction takes advantage of the ability of plants to hyper-accumulate metals (Turnau et al. 2006).

Phytoextraction involves removing metals through plant harvest, phytomining, combustion for energy or storage at low volume after drying (Kramer 2005; Peuke & Rennenberg 2005). Among the possible mechanisms by which AM fungi improve the resistance of plants to heavy metals is the ability of the AM fungi to sequester heavy metals through the production of chelates or by absorption (Salt & Kramer 2000). AM plants translocate less heavy metals to their shoots than the corresponding non-AM hyper-accumulating plants (Barea et al. 2005). Although AM fungi do not necessarily stimulate phytoextraction, the potential to increase the biomass of the plants, to enhance nutrient and water uptake and to improve soil conditions are important reasons to include AM fungi in further research (Barea et al. 2005; Turnau et al. 2006).

The mechanisms of HM detoxification or degradation during the symbiosis are listed by Gaur & Adholeya (2004) and can be achieved by the following processes: chelates such as histidine, organic acids from plants and glomalin from fungus, which take part in metal binding; binding of metals by cell wall components of symbionts; selective entry of metals by plasma membrane of symbionts, presence of metal transporters in plasma membrane, chelates in the cytosol (metallotheins, organic acids, aminoacids and metal specific chaperones), sequestration of HM in vacuoles, transport of HM in fungal hyphae and metal export from arbuscules to the plants (Adholeya 2004).

2.3.6 AMF-Biochar interactions

Some reports emphasize that biochar amendments can increase AMF % root colonization in plant roots (Elmer & Pignatello 2011) grown in acidic soils (Ezawa et al. 2002; Matsubara et al. 2002; Yamato et al. 2006), while others show decrease in AMF abundance (Warnock et al. 2010). Improved colonization was also found due to decreased infection of plant pathogens after addition of AMF and char as activated carbon similar to biochar (Matsubara et al. 2002). Inhibited colonization after char amendment might be due to improved availability of P (Warnock et al. 2007). Addition of biochar increased ecto-mycorrhizal (ECM) colonization (Harvey et al. 1976; Harvey et al. 1978; Harvey et al. 1979; Mori 1994).

Alteration of mycorrhizal abundance under biochar amended conditions has been explained by four mechanisms: biochar changes soil nutrient availability, alters activity of other micro-organisms, detoxifies allelochemicals or alters plant-AMF signalling processes and serves as a refuge from hyphal grazers (Warnock et al. 2007). Availability of N, P and metal ions (Tryon 1948; Lehmann et al. 2003; DeLuca et al. 2006; Gundale & DeLuca 2006; Warnock et al. 2007) increased or decreased pH, increased CEC, enhanced water holding capacity and decreased bulk density (Tryon 1948), and addition of small amount of nutrients contained in biochar (Lehmann et al. 2003; Topoliantz et al. 2005; Gundale & DeLuca 2006; Yamato et al. 2006) are examples of changes in soil properties by biochar. Decrease in N uptake in plants after biochar addition has also been reported by some authors (Lehmann et al. 2003).

2.4 Role of biochar on signalling process of mycorrhizae

Signalling between AMF and plants occurs in the rhizosphere (Bais et al. 2004; Harrison 2005; Bais et al. 2006; Paszkowski 2006). Plants secrete CO_2, flavonoids, sesqueterpenes and strigolactones that favour AMF colonization (Bécard & Piché 1989; Nair et al. 1991; Xie et al. 1995), hyphal branching and spore germination (Gianinazzi-Pearson et al. 1989; Akiyama et al. 2005). Provided that the function of flavonoid compounds could be inhibitory or stimulatory on micro-organisms due to the change in pH (Angelini et al. 2003), addition of biochar increases pH which may have some stimulatory effects on AMF abundance, because biochar is a reservoir of both signalling and inhibitory compounds (allelochemicals) (Warnock et al. 2007). The activated carbon adsorbs AMF signalling compounds (strigolactones); after desorbing strigolactones with acetone, strigolactones stimulate hyphal branching and growth of *Gigaspora margarita* (Akiyama et al. 2005). Actually, water plays an important role in desorbing signalling molecules and makes them available for hyphal stimulation; if the water continues to remove these signalling compounds from biochar permanently, there will be a net decrease in the number of signal molecules resulting in decreased

spore germination, hyphal growth and fungal abundance (Warnock et al. 2007). In addition, activated carbon can absorb phenolic compounds, which are toxic to AMF (Vaario et al. 1999; Herrmann et al. 2004).

2.5 Protection of mycorrhizae by biochar against soil predators

Biochar particles can protect AMF from soil predators (Saito 1990; Pietikäinen et al. 2000; Ezawa et al. 2002) such as mites, collembola, large protozoans and nematodes (Warnock et al. 2007) providing shelter for them, including in its pores (<16µm) (Kawamoto et al. 2005; Glaser 2007; Hockaday et al. 2007) which are of suitable size (cell diameter of bacteria 1-4 µm, hyphal size 2-64 µm but the majority <16µ) (Swift et al. 1979).

2.6 Current research issues

Warnock et al. (2010) have suggested that research activities should cover the potential effects of biochar feedstock properties, production conditions and application rates on AMF because functional relationships between biochar application, improved soil fertility and AMF are not clear; some biochars may decrease AMF abundance and biochar properties can differ with their feedstock identities. Solaiman et al. (2010) identified the need of comparative evaluation of a range of biochar sources. The future research should be focussed on efficacy of different chars in different ecosystems because their impact relies on the source of biochars, production conditions, application regimes, target molecules and site specific parameters (Ennis et al. 2011). For any objective, for example, disease suppression, interaction of biochar rates and different biochar types on different crops and in different soils should be a priority of study (Elmer & Pignatello 2011). Different biochar types and basic manipulative experiments should be carried out to identify the interactions between biochar and soil microorganisms (Lehmann et al. 2011).

The composition of mycorrhizal species is important to mycorrhizal functioning (Van Der Heijden et al. 1988). Sharif & Moawad (2006) identified the occurrence, distribution and identification of indigenous VA mycorrhiza in various field crops, their interactions with other soil micro-organisms and management through agronomic practices as matters of investigation. Others felt lack of sufficient information on experimental biochar such as source material, production temperature, application rate, application method, and materials used in controls could be a matter of research (Warnock et al. 2007). Similarly, they recognized that the examination of the management context of biochar application on AMF, and negative effects of biochar (quality and application rate) on environment (soil nutrient content, plant species) as important research needs. Yet, data on response of mycorrhizal fungus species to biochar are not readily available (Warnock et al. 2007).

Some research needs were proposed for AMF-heavy metal relationships, for example, the relationship between AM infection and metal concentration (Gildon & Tinker 1983), the extent to which AMF can alleviate metal toxicity in plants under field conditions (Leyval et al. 1997), development of AMF ecotypes tolerant to different stress situations (del Val et al. 1999), interaction between AMF and HM and molecular mechanisms of HM tolerance in AMF (Leyval & Joner 2001; Martin et al. 2001), assessment of efficiency of AMF on phytoremediation (Göhre & Paszkowski 2006), the relationship among AMF, P nutrition and plant growth as well as mechanisms of absorption and transportation of HM by AMF and comparison between sterile and HM rich media with AMF inoculation (Wang et al. 2007). Other general research needs are survey of mycorrhizosphere under various environmental stresses (Audet & Charest 2007), identification of metal toxicity and its mechanism (Khade & Adholeya 2008), and identification of appropriate combination of plants and soil microbes to effectively control the stress of heavy metals (Miransari 2011).

To fully assess the interaction of biochar fertilizers, farming systems and microbes, several bioassays at different times throughout the cropping season should be undertaken (Solaiman et al. 2010). Recently, Lehman et al. (2011) reviewed biochar effects on several micro-organisms and classified research areas such as microbial abundance and root abundance as low priority, microbial community and faunal community as medium, and faunal abundance, microbial function, root function, biochar inoculants, biochar enzyme interaction, biochar pathogen control and environmental risk as high priority.

2.7 Crops of interest

To evaluate the effect of biochar on growth performance, lettuce (leafy), potato (tuber), cabbage (brassica) and onion (bulbous) and tomato (fruit vegetable) were assessed to represent different classes of vegetable crops. Among them, onion and tomato were selected for further mycorrhizal assessment based on their response to mycorrhizae representing highly responsive (onion) and moderately responsive (tomato). From the literature review, it can be confirmed that mycorrhizal association is more prominent in onion (bulb crop) than tomato. Therefore, a good comparison can be performed on these two crops for biochar types.

2.8 Conclusions and future goals

Long-term soil fertility management has been a great challenge to developing countries because of continued soil fertility degradation due to several natural factors and inappropriate management practices. We cannot modify or change the natural factors but we can apply certain sustainable management practices that can reduce their adverse effects on soil fertility and crop growth. For example, in Nepalese soils, N and P are the most limiting factors for crop yields which are lower than yields of other developed countries. Many research reports have focussed on the role that poor soil management practices play in reducing crop yields. Considering this fact, many research works were undertaken on soil fertility management in the past. Research findings on the effect of organic manures such as compost, cattle manure and poultry manure on soil quality and fertility have been documented. Similarly their influence on performance of various crops has also been published. In combination with organic manures, the effect of inoculation of some micro-organisms such as AM fungi was also evaluated on various crops and soil environments and the evidence was reported. Yet, there are limited research reports available on long-term fertility maintenance practices in the field that promote carbon sequestration, N release at the appropriate time and amount, and P uptake especially in acidic soils. Considering these research issues, the present study aims to test biochar types, and their application rates for AMF association in partially (tomato) and highly (onion) mycorrhyzal crops. In conclusion, the study will determine the interactive effect of biochar-AMF on soil and crop systems. The future goal of this study is to increase global yields of organic foods and vegetables. The purpose is to reduce poverty, hunger and malnutrition and improve personal health with the increased consumption of healthy organic foods. Thus the research addresses many millennium goals such as environmental sustainability. The outputs of the study will be combinations of crop, biochar and rate of application for optimum AMF association for sustainable management of soil fertility and improved plant growth.

Chapter 3. Biochar effects on crop growth

3.1 The influence of biochar on growth of lettuce and potato

3.1.1 Abstract

Pot experiments were conducted in a glasshouse to determine the growth pattern of lettuce, true potato seedlings (TPS) and single node cuttings of TPS in response to biochar. The treatments were arranged in a randomized complete block design with 5 treatments (0, 10, 30, 50 and 100 t ha^{-1}) of biochar from green waste with 5 replications in lettuce, 10 in TPS and 5 in single node cuttings of TPS. The observations recorded on growth parameters showed that biochar had significant effect on growth of lettuce but no consistent effect on growth of TPS and single node cuttings. Among the biochar rates, 30 t ha^{-1} had the greatest influence on overall growth of lettuce. The pH and electrical conductivity increased as the biochar rates increased in all experiments. These results provide an avenue for soil management systems by using biochar as an amendment in horticultural crops. However, their verification in the field is important for specific recommendations.

3.1.2 Introduction

Biochar is described as a 'black carbon manufactured through pyrolysis of biomass' (Lehmann & Rondon 2006); 'the high carbon materials produced from the slow pyrolysis (heating in the absence of oxygen) of biomass' (Chan et al. 2007); and 'a fine-grained and porous substance, similar in its appearance to charcoal produced by natural burning or by the combustion of biomass under oxygen-limited conditions' (Sohi et al. 2009). It can be produced from a wide range of biomass sources, for example, woods and barks, agricultural wastes such as olive husks, corncobs and tea waste (Demirbas 2004; Ioannidou & Zabaniotou 2007), green waste (Chan et al. 2007), animal manures and other waste products (Downie et al. 2007; Chan et al. 2008a; Lima et al. 2008). Biochar is a mixture of char and ash with the major part (70 - 95%) carbon (C) (Brandstaka et al. 2010; Luostarinen et al. 2010). The relatively stable nature of biochar allows for carbon sequestration value such that the amount sequestrated per year by natural processes is equivalent to or more than the present global emissions from fossil fuel use (Lehmann et al. 2006). In addition, biochar carbon constitutes almost 40% of the carbon in some soils (Glaser et al. 2000; Skjemstad et al. 2002). Lehmann (2007a) predicted that the retention times of the carbon in biochar would be at least hundreds, but more likely thousands of years. As a pyrolysed product, biochar is protected from rapid microbial degradation and is able to securely sequester carbon, contributing to mitigation of greenhouse gas emissions (Lehmann et al. 2006). Day et al. (2004) emphasized that using biochar

to sequester carbon in soil to mitigate climate change would only be economical if the sequestered C has beneficial soil amendment and/or fertilizer values. Some authors have reported that it can improve the quality of agricultural soils (Lehmann et al. 2003). Currently, very little biochar is being used in agriculture in Australia and elsewhere, due to the fact that its agronomic value in terms of crop response and soil health benefits are inadequately quantified (Chan et al. 2007). Thus, this paper aims to report the influence of biochar application on growth of lettuce, true potato seedlings (TPS) and single node cuttings of TPS.

3.1.3 Materials and methods

Pot trials were conducted in a glasshouse at the nursery unit of The University of Queensland, Gatton Campus Australia in the winter season of 2011. Lettuce and potato were selected for the target crops to compare biochar effects on crops from different families (leafy and tuber). Lettuce was selected because it was confirmed from other works undertaken in UQ as a more responsive crop to nutrients. Similarly, potato was selected because it was of special research interest. A randomized complete block design with five treatments consisting of 5 replications for lettuce, 10 for TPS and 5 for single node cuttings of TPS was used. The replications were more for TPS than lettuce to allow more power to detect any real variations among treatments for genetically variable seedlings (Major et al. 2009). The treatments were 0, 10, 30, 50 and 100 t ha^{-1} of green waste biochar. Biochar was thoroughly mixed with sand. The biochar was produced through rapid-slow pyrolysis using a kiln which was truck-mountable with interrupted combustion within a direct flaming pyrolysis system at the temperatures of 400-700°C. Core granular sand with the bulk density of 1.22 g cm^{-3} was used for the trial. It was highly porous with pH 5.2 and thoroughly washed to remove clay particles before using. The proportion of sand and biochar was calculated considering the pot area derived by the diameter (12 cm^2) of the pots.

Lettuce seeds of variety Archangels Nr were obtained from South Pacific Seeds and were sown directly in the sand-biochar mix on 21 July and harvested on 21 September. True potato seeds from a randomly pollinated open field variety were collected from a field of New South Wales (an unknown variety) and were germinated in Agar + Gibberellic acid (GA_3) medium on 4 July 2011; seedlings were then transferred to a seed germination tray filled with potting mix on 11 July 2011. They were transplanted into the sand biochar medium by uprooting on 21 July. Shoots with single nodes were cut from these plants on the same day and planted in the sand-biochar medium. Both TPS and single node cuttings were harvested on 21 September 2011 (after eight weeks). For germination of TPS, 10 g of agar was added to 500 mL of distilled water and microwaved for three minutes until all dissolved. Once dissolved, it was topped up to 1 L with cold water and mixed well.

Then 0.1 g GA_3 was added to 250 mL of distilled water and poured into agar after cooling. Previous observation showed that the GA had a significant effect on breaking dormancy of TPS (data not shown). After one week of seed placement in light, there was 46% germination in seeds treated with GA whereas it was only 4% in agar without GA. Similarly, after nine days, germination increased sharply reaching 85% in GA while it was only 16% in agar without GA. In the dark, 65% seeds germinated in agar with GA while only 15% seeds germinated in agar. Two hundred seeds were placed in 10 Petri dishes with Agar + GA medium; as radicles protruded from the seedcoat, seedlings were transferred to Petri dishes with agar but without GA. When enough seeds germinated, they were transplanted into 500 mL pots filled with sand biochar mix and placed in the glasshouse.

Observations were recorded on plant height, number of leaves, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, total fresh weight, total dry weight, root length, root width and shoot to root ratio for lettuce. For TPS and single node cuttings, plant height, number of leaflets per plant, number of tubers per plant and number of stolons per plant were recorded. Plant heights and number of leaves were noted weekly and other parameters were recorded after harvest for all experiments. Root lengths were measured from collar line to the tip of the longest root and root width was assessed at the widest region of root system when spread on the table after washing and oven drying. The pH and electrical conductivity (EC) were analyzed for lettuce and TPS grown medium only. The pH and EC were determined by pH meter and conductivity meter using standard procedures recommended by Rayment and Higginson (1992). Statistical analysis was done through two-way analysis of variance in Minitab 16, version 4.0 (Minitab 2005) and graphs were plotted using Sigma Plot technique, version 12.0 (Systat Software 2007).

3.1.4 Results

Lettuce

Significant differences among treatments were observed in lettuce for weekly plant height (Table 3.1). Biochar at a rate of 30 t ha^{-1} and 50 t ha^{-1} had similar effect and produced the maximum height in the first week. From second to seventh week the influence of 30, 50 and 100 t ha^{-1} was similar but significantly greater than 0 t ha^{-1} (Plate 3.1). In all weeks the control had the least influence. Differences were also significant for the weekly number of leaves in third, fourth and fifth weeks after seeding (Table 3.2). Biochar at a rate of 30 t ha^{-1} produced more leaves in the third, fourth and fifth weeks than that of 0 t ha^{-1}. The rates of 30 and 50 t ha^{-1} had similar effects on number of leaves in third and fifth weeks. In all weeks, the control treatment had the lowest value. The coefficient of variation (CV) was greater (>20%) in the initial two weeks; thereafter, it was below 20% for plant

height and number of leaves indicating the maintenance of growth uniformity by lettuce plants as they became older.

The treatment effect was also significant for shoot fresh weight (Figure 3.1), root fresh weight (Figure 3.2), shoot dry weight (Figure 3.3), total fresh weight (Figure 3.4), total dry weight (Figure 3.5) and root length (Figure 3.6). Differences in treatments were non-significant for root dry weight, root width and shoot root ratio. The influence of a rate of 30 t ha^{-1} was significantly greater than that of 0, 10 and 100 t ha^{-1} for shoot fresh weight; however, it was at a par with that of 50 t ha^{-1}. For root fresh weight, the effect of 30, 50 and 100 t ha^{-1} was similar while 30 t ha^{-1} was superior to 0 and 10 t ha^{-1}. Similarly, the rates of 10, 30 and 50 t ha^{-1} had similar influence on shoot dry weight but 30 t ha^{-1} had significantly greater effect than 0 and 100 t ha^{-1}. Total fresh weight was significantly increased by biochar rates up to 30 t ha^{-1} but was not different to 50 t ha^{-1} (Figure 3.4). Similarly, the effect of 30 t ha^{-1} was also superior to 0 and 100 t ha^{-1} for total dry weight (Figure 3.5). Up to 10 t ha^{-1}, root length was similar to the control but it was significantly greater for higher rates (Figure 3.6). For those observations recorded after harvest, the CV was below 20% indicating the uniform performance of individual plants across replications. The addition of biochar to sand increased the pH significantly ranging from 7.6 for 10 t ha^{-1} to 8.7 for 100 t ha^{-1} biochar (Table 3.3). Biochar also increased electrical conductivity, from 0.290 dS m^{-1} in 10 t ha^{-1} to 1.207 dS m^{-1} in 100 t ha^{-1}.

Table 3.1 Weekly plant height as influenced by the application rates of biochar

Biochar rates in sand (t ha^{-1})	Plant height (cm) over weeks						
	1st	2nd	3rd	4th	5th	6th	7th
0	0.92b	2.76b	4.54c	8.16c	9.54c	9.96c	11.04b
10	1.16ab	3.10b	5.02bc	9.16bc	12.16b	12.50b	12.58ab
30	1.84a	3.70ab	6.34ab	11.12a	12.74ab	12.90ab	13.04a
50	1.78a	4.40a	7.48a	10.88ab	13.50ab	13.66ab	13.76a
100	1.34ab	3.72ab	6.74ab	10.92ab	13.58a	14.00a	14.16a
F-Probability	**	**	**	**	**	**	**
LSD ($\alpha_{0.05}$)	3.302	0.448	0.729	0.755	0.522	0.530	0.808

** = Significant at $\alpha_{0.01}$ level of significance
The values that do not share the same letters are significantly different at $\alpha = 0.05$ level of significance

Table 3.2 Weekly numbers of leaves per plant as influenced by the application rates of biochar

Biochar rates in sand (t ha^{-1})	Number of leaves per plant over weeks						
	1st	2nd	3rd	4th	5th	6th	7th
0	4	4.2	5.2b	8.0b	9.6b	11.4	11.8
10	4	4.0	6.0ab	7.8b	10.6ab	12.4	12.6
30	4	4.4	6.6a	10.0a	11.6a	12.8	13.0
50	4	4.6	6.8a	9.4ab	11.4a	12.8	13.2
100	4	4.0	6.2ab	9.0b	10.6ab	12.6	13.4
F-Probability	ns	ns	**	*	**	ns	ns
LSD ($\alpha_{0.05}$)	-	-	0.413	0.819	0.552	-	-

ns = non-significant, * = Significant at $\alpha_{0.05}$ level of significance, ** = Significant at $\alpha_{0.01}$ level of significance; the values that do not share the same letters are significantly different at $\alpha = 0.05$ level of significance.

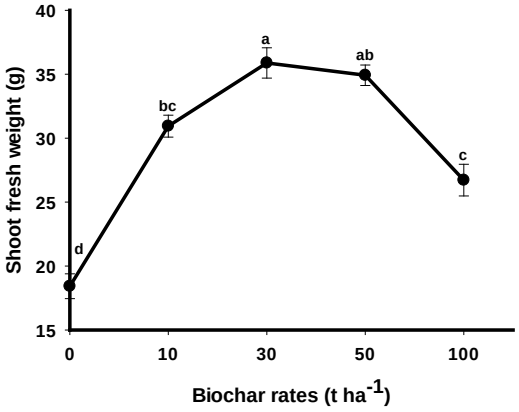


Figure 3.1 Shoot fresh weight of lettuce as influenced by application rates of green waste biochar. Data with the different letters are significantly different at α=0.05 level of significance. Vertical bars represent the standard error (SE) of means.

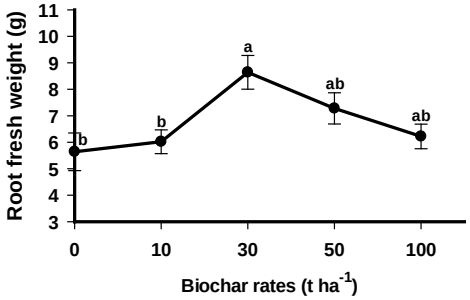


Figure 3.2 Root fresh weight of lettuce as influenced by application rates of green waste biochar. Data with the different letters are significantly different at α=0.05 level of significance. Vertical bars represent the standard error (SE) of means.

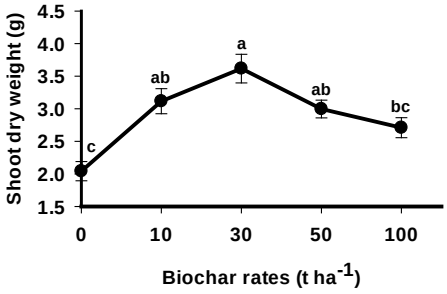


Figure 3.3 Shoot dry weight of lettuce as influenced by application rates of green waste biochar. Data with the different letters are significantly different at α=0.05 level of significance. Vertical bars represent the standard error (SE) of means.

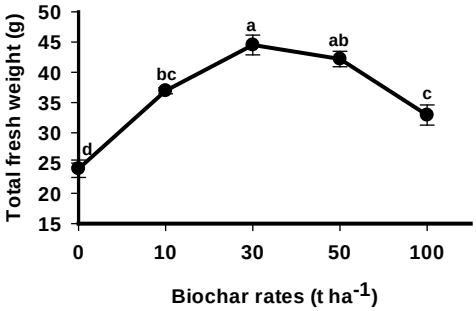


Figure 3.4 Total fresh weight of lettuce as influenced by application rates of green waste biochar. Data with the different letters are significantly different at α=0.05 level of significance. Vertical bars represent the standard error (SE) of means.

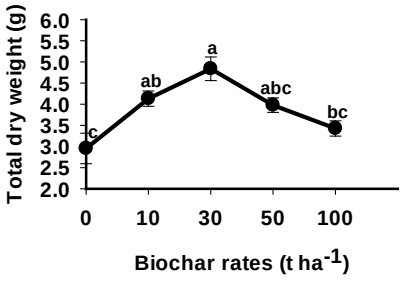


Figure 3.5 Total dry weight of lettuce as influenced by application rates of green waste biochar. Data with the different letters are significantly different at α=0.05 level of significance. Vertical bars represent the standard error (SE) of means.

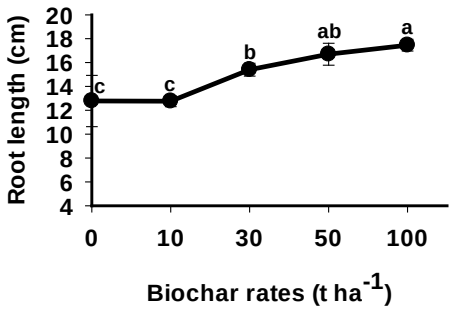


Figure 3.6 Root length of lettuce as influenced by application rates of green waste biochar. Data with the different letters are significantly different at α=0.05 level of significance. Vertical bars represent the standard error (SE) of means.

Table 3.3 Mean values for pH and electrical conductivity of sand-biochar mix for pots with lettuce and TPS

Crop	Biochar rates in sand (t ha^{-1})														
	0			10			30			50			100		
	EC (dS m^{-1})	Salt %	pH	EC (dS m^{-1})	Salt %	pH	EC (dS m^{-1})	Salt %	pH	EC (dS m^{-1})	Salt %	pH	EC (dS m^{-1})	Salt %	pH
Lettuce	0.27	0.092	7.43	0.29	0.099	7.67	0.55	0.187	8.53	0.63	0.214	8.63	1.21	0.411	8.7
TPS	0.25	0.085	7.77	0.29	0.098	8.43	0.53	0.180	8.83	0.67	0.228	8.83	1.04	0.354	8.93

Plate 3.1 Biochar rate effects on lettuce, from left to right 0, 10, 30, 50 and 100 t ha^{-1} showing little growth in 0 and 100 t ha^{-1}, greater height in 30 and 50 t ha^{-1}

True potato seedlings

No significant differences were observed between treatments for weekly plant heights except in the third week after transplanting (data not shown due to non-significant results). The CV>20% was high indicating great variation in growth between individual plants over the measurement period. Similarly, no significant effect of treatments was revealed in all parameters. For example, plant height increased until the 6th week when level of biochar was increased up to 30 t ha^{-1}. A similar trend was seen in number of leaflets. There was no positively greater effect on plant parameters when the application rates of biochar were increased from 0 to 100 t ha^{-1}. Biochar also increased pH and electrical conductivity, an indicator of salt concentration, from 0.297 dS m^{-1} in 10 t ha^{-1} to 1.04 dS m^{-1} in 100 t ha^{-1}.

Single node cuttings of TPS

The results showed no significant differences among the treatments in all observations except in weekly plant height during the first week of growth (data not shown). However, the results showed a general trend of increment in plant height up to a certain rate of biochar application, and then a decrease at higher rates. Plant performance was so erratic that the variation between individual plants was great (CV>20%) over the replications.

3.1.5 Discussion

The overall response of lettuce plants to biochar rates up to 30 t ha^{-1} indicates its sensitivity whereas higher rates had no further effect. Number of lettuce leaves was similar during early and late stages of growth. This pattern indicated the maximum number of leaves was produced when lettuce plants were vegetatively more active. Lettuce plants showed greater uniformity than TPS. However, some variation was still present for plant height in the initial weeks of growth but it attained uniformity over time.

Addition of biochar was beneficial for increasing leaf number in lettuce but only up to 30 t ha^{-1}. This may be due to sensitivity of lettuce to salts as indicated by electrical conductivity analysis (Table 3.3). The erratic root width values could be a possible cause of non-significant differences between biochar rates for shoot to root ratio.

Other reports have also explained the beneficial effect of biochar on lettuce growth. For example, a rice-husk biochar produced by gasification process increased final biomass, root biomass, plant height and number of leaves compared to no biochar application (Carter et al. 2013). Even in alkaline soil, biochar application increased growth of lettuce (Gunes et al. 2014). Significant increase in biomass yield of lettuce was also observed at 2% pine forest waste biochar application

rate in alkaline loamy sand soil (Artiola et al. 2012). Though the effect of biochar was positive on lettuce, it may be different for other crops. In a report, no effect of biochar amendment was observed for growth of sweet pepper and geranium while coriander shoot growth was increased and lettuce shoot biomass decreased when a biochar was amended with potting soil (Gravel et al. 2013). In fact, the influence of biochar may vary depending on feedstock of biochar, pyrolysis temperature and test crops as indicated by Olszyk et al. (2014). They have reported that biochars from different feedstock (pine chips, poultry litter, swine solid and switch grass) produced by pyrolysis at 350^0C, 500^0C and 700^0C had different influences on corn, soybean, lettuce and carrot when it was added to soil at 1% application rate by weight.

TPS were tested to record the degree of variation in plant growth and to determine whether they would be suitable for further experiments on biochar. The results showed great variation between individual plants over replications and within treatments. Thus, they could not be used for further experiments. The variation was also evident when nodal cuttings of these plants were treated with the same biochar rates. TPS-derived plants showed phenotypic variation (Sharma et al. 2007) as a result of genetic variation. In potato, the biochar rates did not influence growth parameters uniformly over the weeks. For example, increased plant height was greater due to biochar at 30 t ha^{-1} in the third week while it was greater due to 50 t ha^{-1} from the fourth to the seventh week. However, the results indicate that application of biochar is beneficial to plant height after four weeks when plants need more nutrients as they start growing vigorously. The single node cuttings of TPS, although they were vegetatively propagated, showed genetic variation among individual plants because they were isolated from separate mother plants.

In this exploratory experiment, plant growth was better at the high rate (30 t ha^{-1}) but the economy of this may be questioned. This rate was appropriate for soil-less sand medium; however, the recommendation for different soils may vary. From this experiment, it was concluded that further experiments on biochar rates should be limited; considering 30 t ha^{-1} as an appropriate upper level of biochar application.

Addition of biochar increased pH of the medium which indicated its liming value (Table 3.3). According to (Chan et al. 2008a), biochar produced from green waste by pyrolysis significantly increased soil pH and exchangeable cations in alfisol soil. Similar results were observed when biochar produced from poultry litter was tested (Chan et al. 2008b). Van Zwieten et al. (2010a) tested two biochars produced from slow pyrolysis of paper mill waste in two agricultural soils in a

glasshouse, and found that they differed slightly in their liming values by 33% and 29% respectively.

As the responses of lettuce, TPS and single node cuttings of TPS varied, this supported (Chan et al. 2008a) on the differential influence of biochar on different crops. A significant decrease in dry matter content of radish was obtained when biochar was applied at 10 t ha^{-1} but the cause was unclear (Chan et al. 2008a). There was no significant effect of biochar rates (0, 7 and 15 t ha^{-1}) on turnip, wheat, rape and faba bean (Brandstaka et al. 2010). Van Zwieten et al. (2010a) tested two biochars produced from the slow pyrolysis of paper mill waste in two agricultural soils in a glasshouse and found that they significantly increased N uptake in wheat and biomass in wheat, soybean and radish in ferrosol soil but reduced wheat and radish biomass in calcaresol soil, amended with fertilizer in both soils. Thus, the influence of biochar may also be dependent on soil types. Therefore, the further focus of research should be given to test various biochars in different soils and crop species.

3.1.6 Conclusion

The influence of biochar on plant growth varied between plant species and plant materials such as seedlings and cuttings. Biochar from green waste was tested on lettuce, TPS and single node cuttings of TPS as pot trials showed varying effects. Biochar application affected growth parameters significantly greater than that of control in lettuce. For lettuce, 30 t ha^{-1} was most effective for optimal growth in sand culture. For potato, no conclusive results could be obtained but the trend in plant growth indicated some influence of biochar. Further investigation of several biochars in different crop species is important to understand their influence on growth.

3.2 Growth response of cabbage (*Brassica oleraceae* var. *capitata,* cv Hearty*)* to two biochars in a pot trial

3.2.1 Abstract

A pot trial was conducted in a glasshouse to compare growth response of cabbage (*Brassica oleraceae var. capitata,* cv. Hearty) to two biochars. A 2 x 4 + 1 factorial arrangement with six replications was used. The factors 'Biochar Type' with two levels ('Sugarcane Trash' and Green Waste A') and their 'Application Rates' with four levels (10, 30, 50 and 100 t ha^{-1}) were compared with a control (No biochar). Three-week-old uniform seedlings were transplanted into 1.6 litre pots and irrigated with half strength Hoagland's solution at a rate of 30 mL per pot three times a week followed by irrigation flush 24 hours after application. Observations were recorded on growth

parameters and pH and electrical conductivity (EC) of growth medium. Presence of biochars significantly increased overall growth as compared to the control. These results represent the growth response of cabbage to biochars in a sand medium; however verification using different soils is necessary before general recommendations can be made.

3.2.2 Introduction

Carbon deficiency is a crucial issue in degraded agricultural soils. Carbon is basically supplied through composts and manures at the farm level but they are decomposed rapidly and are either used by the crops or lost through several means including through regular cropping without replenishment of carbon sources. The carbon deficient soil cannot produce the crops with optimum production. This issue demands improving crop production as well as soil condition in the long term by adding to the soil relatively stable carbon sources, one of which is biochar.

Biochar has recently created international attention for use as a soil amendment. Biochar is a black carbon manufactured through pyrolysis of biomass (Lehmann et al. 2006; Chan et al. 2007; Chan et al. 2008a). It is similar in its appearance to charcoal produced by natural burning (Sohi et al. 2009) but differs in structure and composition. This is a rich source of carbon but is very different in composition and behaviour compared to soil organic matter. Different feedstocks can be used to produce biochar resulting in varying properties depending upon the nature of the material and production conditions (Guerrero et al. 2005). Several feedstocks have been identified as sources to produce biochar, for example, woods, barks, agricultural wastes such as olive husks, corncobs and tea waste (Ioannidou & Zabaniotou 2007), green waste (Chan et al. 2008a), animal manures and other waste products (Downie et al. 2007; Chan et al. 2008a; Lima et al. 2008). It is noteworthy to mention that biochar is a mixture of char and ash with the major part (70 - 95%) carbon (C) (Brandstaka et al. 2010; Luostarinen et al. 2010). Presumably, these authors are referring to the char particles only and not the ash.

The potential effects of feedstock properties, production conditions and application rates of biochar need to be determined before its widespread application (Warnock et al. 2010). Adequate study on quantification of agronomic value of biochars in terms of crop responses is very important (Chan et al. 2007; Chan et al. 2008a). Solaiman et al. (2010) identified the need for comparative evaluation of a range of biochar sources. The efficacy of different chars in different ecosystems is also an important research issue (Ennis et al. 2011) as ecosystems have varied soil conditions. Similarly, the interaction of biochars and their application rates should be determined (Elmer & Pignatello 2011) to know the influence of biochar on crop performance and soils. In this context, the present

paper aims to compare the effects of two biochars, their application rates and the interactions of two biochars with the rates of cabbage growth.

3.2.3 Materials and methods

A pot trial was conducted in a glasshouse of the University of Queensland, Gatton Campus, Australia to study the response of cabbage (*Brassica oleraceae capitata*, cv Hearty) to two biochars. A 2 x 4 + 1 factorial arrangement with six replications was used. The factors 'Biochar Type' with two levels ('Sugarcane Trash' and Green Waste A') and their 'Application Rates' with 4 levels (10, 30, 50 and 100 t ha^{-1}) were compared with a control (No biochar).

The biochar rates were calculated on the basis of pot surface area. The pH of sand was 5.2 before use and was increased up to 7.0 for control pots by adding dolomite. The biochars were thoroughly mixed in coarse sand and used to fill 1.6 litre pots. Before filling, fabric cloth was placed in the base of each pot to moderate drainage from the bottom holes. Three-week-old, uniform seedlings were transplanted into the pots.

The roots of the seedlings were washed with tap water before transplanting to remove the propagation mix and nutrients from the root surface. The plants were irrigated with modified Hoagland's solution (Hoagland & Arnon 1950; Epstein & Bloom 2005; Mattson & Lieth 2008) (half strength of original) (Appendix 2) at a rate of 30 mL per pot three times a week. Pots were irrigated 24 hours after Hoagland's solution application to leach the salts deposited by the solution. Biochars were analyzed by several methods for other studies (Kochanek et al. 2014) (Appendix 1).

Observations were recorded on plant parameters such as: length of the longest outer leaf (for six weeks), number of leaves per plant, root fresh weight, shoot fresh weight, root dry weight and shoot dry weight. The pH (Appendix 4) and EC (Appendix 3) of the growth medium (sand + biochar) were determined by pH meter and conductivity meter using standard procedures of 1:5 water (Rayment & Higginson 1992). The EC values were adjusted using a 4% correction factor by adding 2% for each degree of temperature below 25^0C recommended by the same authors as the ambient room temperature was 23^0C during the observation.

Table 3.4 a. One Way ANOVA, b. General Linear Model for factorial analysis, c. Combined ANOVA from one-way ANOVA and general linear model

a

Source	Df
Replication (r)	(r-1), 6-1 = 5
All treatments (t)	(t-1), 9-1= 8
Error	(T-t), 54-9 = 45
Total (T)	(tr-1), 9 x 6-1= 53

b

Source	Df
Replication (r)	(r-1), 6-1 = 5
Biochar (b)	(b-1), 2-1= 1
Application rates (a)	(a-1), 4-1= 3
Biochar x Application rates	(b-1) (a-1), (1)(3)= 3
Error	ba(r-1), 2 x 4 (6-1) = 40
Total (T)	(T-1), 54-1= 53

c

Source	Df
All treatments (t)	(t-1), 9-1= 8
Biochar (b)	(b-1), 2-1= 1
Application rates (a)	(a-1), 4-1= 3
Biochar x Application rates	(b-1) (a-1), (1)(3)= 3
Factorial subset (f)	(b-1) + (a-1) + (b-1) (a-1), 1 + 3 + 3 = 7
Extra treatment	[(t-1) –f], 8-7 = 1
Error	(T-t), 54-9 = 45
Total (T)	(T-1), 54-1= 53

Statistical analysis was carried out by using the one-way analysis of variance for all treatments, General Linear Model for factorial subset in Minitab 16, version 4.0 (Minitab 2005) and these two analyses were combined in Microsoft Excel 2010, version 14.0 (Microsoft 2010) to get a complete analysis as given in Table 3.4. Grouping of treatments was carried out by using Tukey's range test in Minitab.

3.2.4 Results

Effects of Biochar types

Significant differences ($p<0.05$) for biochar type were found for leaf length in fifth and sixth weeks of planting, number of leaves per plant, root fresh weight and soil pH. Among the parameters, leaf length (Figure 3.7), number of leaves and root fresh weight (Table 3.5) were greater in Sugarcane Trash while the soil pH (Table 3.5) was higher in Green Waste biochar applied plants. When these biochar applied plants were compared with a control (no biochar treatment), they had significantly greater performance.

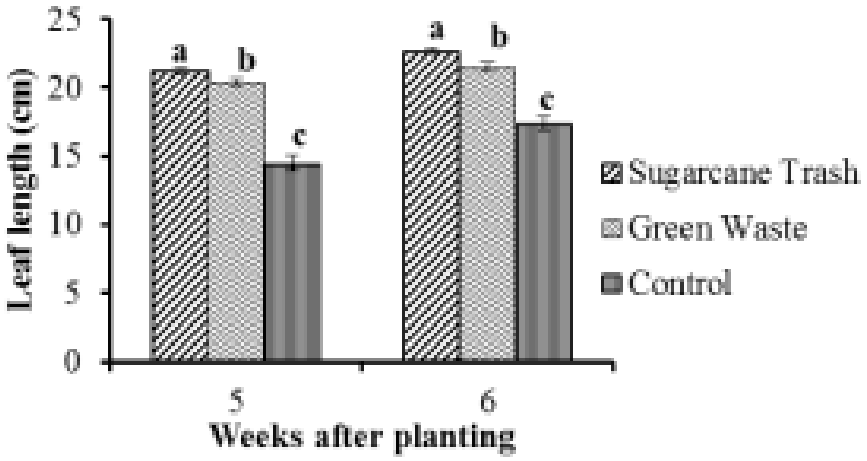


Figure 3.7 Leaf length of the longest outer leaf of cabbage as influenced by biochar types. Different letters in the same block indicate significant differences ($P<0.05$) between the treatment means (N = 24 for biochar types, 6 for control, n was different due to the structure of design, 2 x 4+1 in 6 replications) in a single season. The bars represent the standard error of the means.

Table 3.5 Mean values for number of leaves, root fresh weight and soil pH in response to Sugarcane Trash and Green Waste biochar. Different letters in the same column indicate significant differences ($p < 0.05$) between the treatment means (N = 24 for biochar types, 6 for control, n was different due to the structure of design, 2 x 4+1 in 6 replications) in a single season. The ± values are the standard error of the mean.

Biochar types	No of leaves	Root fresh weight	Soil pH
Sugarcane Trash	13.0 ± 0.23a	12.55 ± 0.19a	8.2 ± 0.4b
Green Waste	12.1 ± 0.16b	11.18 ± 0.34b	8.6 ± 0.06a
Control	10.3 ± 0.21c	7.22 ± 0.55c	7.4 ± 0.16c

Effects of Application rates

For application rates, there was statistical difference for leaf length at fifth week after planting (Figure 3.8). However, overall data showed that the application of any biochar rate was beneficial over no application (Plate 3.2). The differences were also significant for number of leaves, shoot fresh weight, shoot dry weight (Table 3.6), root fresh weight, root dry weight, shoot to root ratio, soil pH and soil electrical conductivity (Table 3.7).

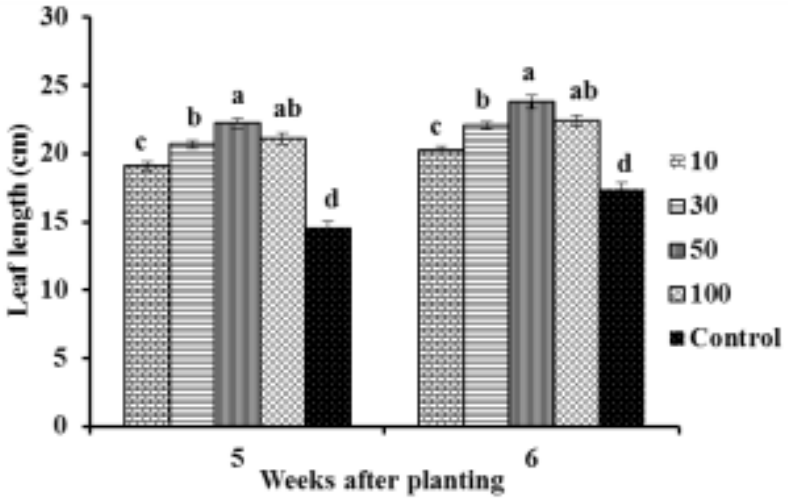


Figure 3.8 Leaf length of the longest outer leaf of cabbage as influenced by application rates of biochar (control, 10, 30, 50 and 100 t ha^{-1}). Different letters in the same block indicate significant differences between the treatment means at α=0.05 level of significance (*N* = 12 for application rates, 6 for control, *N* was different due to the structure of design, 2x4+1 in 6 replications) in a single season. Vertical bars on the blocks represent standard error of the means.

Table 3.6 Mean values for number of leaves, shoot fresh weight and shoot dry weight in response to biochar application rates. Different letters in the same column indicate significant differences between the treatment means (*N* = 12 for biochar application rates, 6 for control, *n* was different due to the structure of design, 2x4+1 in 6 replications) at α=0.05 level of significance in a single season.

Biochar rates (t ha^{-1})	No of leaves	Shoot fresh weight (g)	Shoot dry weight (g)
10	12.0 ± 0.35c	65.5 ± 3.18c	12.02 ± 0.43c
30	12.3 ± 0.18b	80.04 ± 2.82b	13.8 ± 0.29b
50	13.0 ± 0.28ab	91.05 ± 3.22ab	15.3 ± 0.0.21a
100	12.9 ± 0.27a	101.13 ± 4.59a	15.9 ± 0.41a
Control	10.3 ± 0.21d	38.04 ± 3.92d	8.75 ± 0.62d

In most of the growth parameters, the effect of 50 and 100 t ha^{-1} was similar but greater than lower rates except in shoot to root ratio. The ratio was similar for 10, 30 and 50 t ha^{-1} but these ratios were different from that of 0 and 100 t ha^{-1}. Increasing rates of biochar enhanced the soil pH and soil EC indicating their increased liming value and salt contents. Application of biochar had significantly more positive effect than the control.

Table 3.7 Mean values for root fresh weight, root dry weight and shoot to root ratio, soil pH and electrical conductivity (EC). Different letters in the same column indicate significant differences between the treatment means (N = 12 for biochar application rates, 6 for control, n was different due to the structure of design, 2x4+1 in 6 replications) at α=0.05 level of significance in a single season.

Biochar rates (t ha^{-1})	Root fresh weight (g)	Root dry weight (g)	Shoot to root ratio	Soil pH	Electrical conductivity (dS m^{-1})
10	10.82 ± 0.35b	1.08 ± 0.04b	11.27 ± 0.59ab	7.96 ± 0.08c	0.23 ± 0.01d
30	12.10 ± 0.34ab	1.34 ± 0.05a	10.39 ± 0.35b	8.34 ± 0.04b	0.29 ± 0.01c
50	12.56 ± 0.36a	1.51 ± 0.06a	10.26 ± 0.26b	8.58 ± 0.08ab	0.40 ± 0.02b
100	11.99 ± 0.44ab	1.33 ± 0.06a	12.14 ± 0.40a	8.80 ± 0.07a	0.51 ± 0.02a
Control	7.22 ± 0.55c	0.67 ± 0.06c	13.24 ± 0.46a	7.40 ± 0.16d	0.22 ± 0.04e

a **b**

Plate 3.2 Differential growth of cabbage due to application rates of biochar after six weeks of planting, a. Sugarcane Trash, b. Green Waste. Application rates for both plates from left to right: 0, 10, 30, 50, 100 t ha^{-1}. Plants with 0 t ha^{-1} biochar showing poor (1st plant from the left in both plates) performance compared to other treatments in both plates.

Effects of Interactions of biochar type and application rates

Interaction of biochar type and their application rates showed significant differences for the leaf length in 2nd, 3rd, 4th, and 5th weeks (Table 3.8), and soil EC (Figure 3.9). Initially, Sugarcane Trash at the rate of 10 t ha^{-1} had greater effect on leaf length than the other interactions of the same

biochar while the interactions of Green Waste and application rates were similar indicating little effect of biochar in initial stages of growth and possibly less requirement of food materials in the plant system. The interactions showed greater effects on these parameters than the control after the 3rd week, indicating the application of biochar was beneficial.

Table 3.8 Mean values for length of longest outer leaf over weeks. Different letters in the same column indicate significant differences between the treatment means (N = 6) at α=0.05 level of significance in a single season.

Biochar type x Application rate (t ha^{-1})	Leaf length (cm) over weeks			
	Second	Third	Fourth	Fifth
Sugarcane Trash x 10	11.9 ± 0.28a	15.2 ± 0.16a	20.1 ± 0.21a	20.4 ± 0.50ab
Sugarcane Trash x 30	10.3 ± 0.31b	13.4 ± 0.23abc	19.0 ± 0.45ab	21.5 ± 0.46ab
Sugarcane Trash x 50	9.8 ± 0.18b	12.8 ± 0.38bc	18.1 ± 0.52abc	22.2 ± 0.29a
Sugarcane Trash x 100	9.2 ± 0.23b	12.0 ± 0.50c	17.4 ± 0.51bc	20.5 ± 0.53ab
Green Waste x 10	9.9 ± 0.60b	12.4 ± 0.52bc	16.4 ± 0.56c	17.9 ± 0.42c
Green Waste x 30	10.6 ± 0.44ab	13.2 ± 0.36bc	18.1 ± 0.56abc	19.8 ± 0.30bc
Green Waste x 50	10.5 ± 0.19ab	14.0 ± 0.52ab	20.0 ± 0.38a	22.2 ± 0.69a
Green Waste x 100	10.5 ± 0.30ab	13.4 ± 0.47abc	18.7 ± 0.23ab	21.6 ± 0.60ab
Control	8.9 ± 0.43b	10.1 ± 0.33d	11.9 ± 0.71d	14.5 ± 0.56d

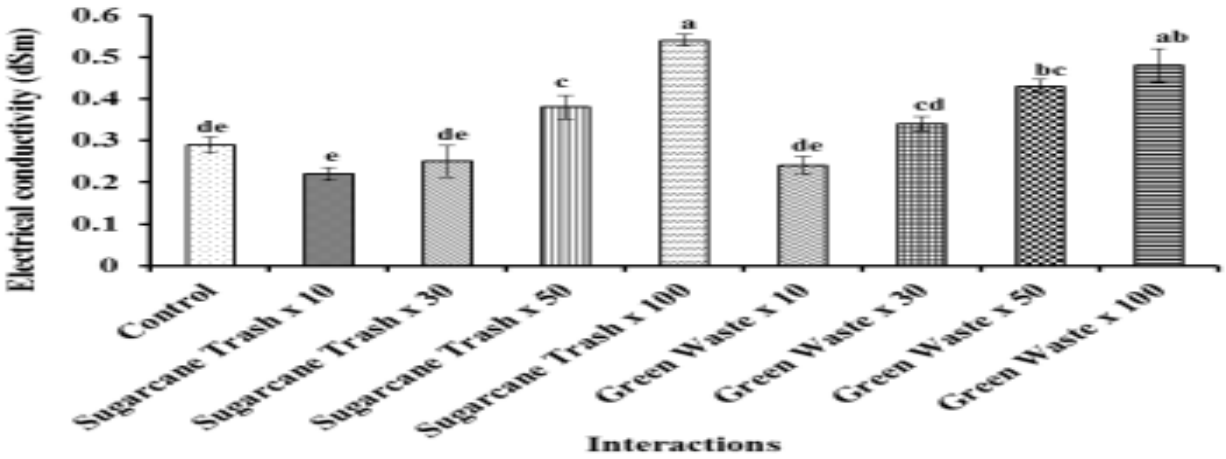


Figure 3.9 Electrical conductivity of the growth medium (sand amended with biochar) as influenced by the interaction of biochar type with application rates of biochar (control, 10, 30, 50 and 100 t ha^{-1}). Data with the different letters are significantly different at α=0.05 level of significance (N = 6). Vertical bars represent the standard error (SE) of means.

Biochar properties

Biochars were analyzed for their nutrients and other properties (Appendix 1). Acid neutralizing capacity and potassium levels were higher in Green Waste than Sugarcane Trash while N and phosphorus were higher in Sugarcane Trash.

3.2.5 Discussion

Poor response of cabbage plants to biochar type indicates little immediate effect on plant growth. However, all parameters showed beneficial effect of biochar application over control. The significant effect of biochar on leaf length at the later stage of growth showed that the biochar may influence growth in the long-term. Generally, the significant increase in leaf length and number of leaves occurred 4-12 weeks after sowing (Olaniyi & Ojetayo 2011), but the period may vary when the growth rate is modified through transplanting as occurred in the present study.

The positive influence of biochar produced from food wastes and bamboo on cabbage growth was also reported by Fujiia et al. (2011). Tayxayngavong (2008) showed that biochar increased germination rate in all soils tested, enhanced plant height and green biomass yield of maize in peaty and clay soils but did not affect it in loam. In the present study, in coarse sand with biochar amendment, cabbage growth increased with increased rate of biochar but was statistically non-significant until the fourth week. In addition, there was no evidence of reduced growth of cabbage at high pH values of media for biochar-treated plants.

Root fresh weight was greater in Sugarcane Trash-treated plants than Green Waste-treated ones. Most likely the fine structure, lower pH and salt content of Sugarcane Trash biochar was more favourable for root growth. Less fresh weight produced by Green Waste could also be linked to its salinity (2.69 dS m^{-1}). In fact, cabbage can tolerate salinity of 1.2 dS m^{-1} but yield reduction of 10% can occur when it reaches 2.2 dS m^{-1} (Evans 2006).

The increased growth in biochar treated plants as compared to control plants could be associated with the porosity of the biochar that retained more water in pots. Another possibility might be additional nutrients, especially phosphorus, which would be available to biochar treated plants due to the ash content of the chars despite the regular irrigation flush.

The greatest value for shoot fresh and dry weights was due to the largest rates (50 and 100 t ha^{-1}) of biochars. However, the experiment was not designed to continue until harvest of cabbage heads. That root fresh weight was similar at high rates such as 50 t ha^{-1} and 100 t ha^{-1} might be associated

with small pot size (1.6 L). Van Zwieten et al. (2010a) showed that the biochars increased biomass in soybean, wheat and radish in ferrosol but reduced biomass of wheat and radish in a calcaresol. A significant decrease in dry matter content of radish was obtained when biochar was applied at 10 ton ha^{-1} (Chan et al. 2008a) but the cause was unclear. In a separate experiment, there was no significant effect of biochar rates (0, 7 and 15 tons ha^{-1}) on turnip, wheat, rape and faba bean (Brandstaka et al. 2010).

Shoot to root ratio was greater in control but values for shoot and root dry weight were smaller than for treatments. This result does not confirm that the control is better because both shoot and root growths were very poor.

The interaction of biochar type and application rate was significantly positive for leaf length at second, third, fourth and fifth weeks of planting. However, the effect was more prominent in later weeks. It confirmed the view that the effect might take longer to be noticeable. When the interactions were compared with the control, all interactions had greater effect on the observed parameters over control. This positive effect of interactions confirmed that the application of biochar was beneficial for cabbage growth.

Chan et al. 2008a found significantly higher soil pH, organic carbon, and exchangeable cations at higher rates (>50 t ha^{-1}) of green waste biochar in alfisol soil. Similar results were observed when poultry litter biochar was tested (Chan et al. 2008b). Van Zwieten et al. (2010a) tested two biochars produced from slow pyrolysis of paper mill waste in two soils amended with fertilizer and found that they differed in liming values (33% and 29%) and carbon content (50% and 52%).

The highly alkaline nature of biochars (pH $\geq$ 8) may be a problem in neutral soils for crops that prefer near neutral or moderately acidic soils. In the present study, the regular application of modified Hoagland's solution may have negated the reduced availability of nutrients due to increased pH.

As an agronomic input, the nutrient solution was applied regularly but most of the nutrients were leached due to the irrigation of the following day. This practice of nutrient application constrained the analysis of the amount of nutrients used by plants and availability of nutrients in the growth medium. However, it was important to know the nutrient uptake of plants and availability in soil. The temperature range in the glasshouse was considerably favourable for cabbage but the pot size might have been too small because the roots were coming out from the bottom holes of the pot

indicating insufficient space for root development. Even though planting was done at the appropriate time, no additional granular fertilizer was applied. The increased pH due to biochar applications was probably associated with less availability of some micronutrients responsible for optimum growth and development. Plants were harvested before head formation during juvenile growth period, therefore, optimum performance, nutrient availability and other parameters could not be observed.

Biochars were added to sand medium at different rates but due to the mixed results, no conclusion could be drawn as to which specific type, rate and interaction was best. However, the results indicated that both biochars were similar for major parameters, rates >30 t ha^{-1} were beneficial for increased growth and any rate was better for cabbage growth than none. Previously, the use of biochar with fertilizer has been shown to have a positive influence on crops in semi-arid soils of Australia (Chan et al. 2008a). Therefore, it should be used as a long-term fertility management tool in combination with other types of fertilizers. Mature crop yield data were not obtained in this work as it was a short-term trial in small pots. It would be very important to observe yield and post-harvest parameters to draw conclusions. However, it can be suggested that cabbage is more tolerant to high rates of biochar than other vegetable crops such as lettuce and potato. In previous experiments, lettuce was found to be less tolerant to rates >30 t ha^{-1} of Green Waste biochar (Upadhyay et al. 2014).

3.2.6 Conclusion

Biochars made from Sugarcane Trash and Green Waste were tested for growth responses of juvenile cabbage. The overall effect of biochar application on cabbage growth was greater than that of the control. Cabbage was found tolerant to high levels of biochar. To find the optimum rate of biochar application, further investigation of biochars and application rates in different crops and soils is important before a general recommendation can be made.

Chapter 4. Biochar effects on colonization of crops by arbuscular mycorrhizal fungi

4.1 Abstract

Two pot trials were conducted on onion and tomato to investigate the effects of biochar types and their application rates on growth and colonization of the crops by arbuscular mycorrhizal fungi. The trials were conducted in 3x4+1 factorial arrangement consisting of 13 treatment combinations of 3 types of biochar, 4 application rates and a control. The plants were grown in sand medium mixed with biochar. The rates of biochar were calculated based on pot area. Growth parameters and mycorrhizal colonization were recorded for analysis. One Way ANOVA for overall effect, General Linear Model for factorial analysis and combined ANOVA for complete effect were applied. The results showed biochar as a beneficial soil amendment for growth parameters of both crops as well as for enhancing soil pH, electrical conductivity and mycorrhizal colonization. All biochars had similar effects on growth parameters when compared at the same rates. Onion roots had more colonization than tomato. Among the application rates, 30 t ha^{-1} of each biochar had better effect on onion while 50 t ha^{-1} was more effective on tomato in terms of morphological growth and colonization pattern of roots.

4.2 Introduction

Waste management has been a great challenge to agriculture, industries, municipalities and other various sectors. Agriculturists have given attention to developing appropriate technologies by using wastes produced from agricultural fields. Among the technologies, biochar has been given due interest as a way to manage carbon and to use it as a soil amendment. Some of the effects of biochar on crop and soil have been documented. For example, biochar application in soils has positive influences on improving soil quality and plant growth (Chan et al. 2007; Chan et al. 2008a). The general effects of biochar on soil have been listed by Brandstaka et al. (2010). Other authors have also described its value for reduction of greenhouse gas emissions (Yanai et al. 2007; Van Zwieten et al. 2010b) and adsorption of anions and cations to prevent leaching of applied nutrients (Major et al. 2009). Chan et al. (2008a) found biochar produced from green waste promising for increasing soil pH, organic carbon, and exchangeable cations with a substantial decrease in tensile strength at higher rates (>50 t ha^{-1}) in alfisol. Similar results were observed when biochar produced from poultry litter was tested (Chan et al. 2008b). Van Zwieten et al. (2010a) tested two biochars produced from the slow pyrolysis of paper mill waste, in two agricultural soils in a glasshouse and found that the biochars differed slightly in their liming values (33% and 29%), and carbon content

(50% and 52%). The thermal processing of wastes into biochar has been identified as an opportunity to destroy contaminants (Glover 2009), making beneficial land application possible. Since extracts from biochar derived from poultry litter increased microbial growth but that from pine timber inhibited it (Das et al. 2008), the effect of biochar on microbes depends upon its feedstock source.

There are varied responses of soils and crops to biochar (Chan et al. 2008a). Two biochars produced from the slow pyrolysis of paper mill waste and tested in a glasshouse on two agricultural soils amended with fertilizer significantly increased biomass of wheat, soybean and radish in ferrosol soil but reduced biomass of wheat and radish in calcaresol (Van Zwieten et al. (2010a). Biochar rates affect plant performance (Jeffery et al. 2011; Jones et al. 2012); however it depends on crops selected (Brandstaka et al. 2010), for example, a significant decrease in dry matter content of radish was obtained when biochar was applied at 10 t ha^{-1} (Chan et al. 2008a) while there was no significant effect of biochar rates (0, 7 and 15 t ha^{-1}) on turnip, wheat, rape and faba bean (Brandstaka et al. 2010). Biochar increased rice grain yields at sites with low P availability, which might be due to improved saturated hydraulic conductivity of the top soil, xylem sap flow of the plant and response to N and NP chemical fertilizer treatments (Asai et al. 2009).

Some of the influences of biochar on beneficial microbes have also been documented. Among the beneficial microbes, arbuscular mycorrhizal fungi has been given due emphasis by scientists. Yet reports are controversial. Some reports emphasize that biochar amendments can increase AMF % root colonization (Elmer & Pignatello 2011) in acidic soils (Ezawa et al. 2002; Matsubara et al. 2002; Yamato et al. 2006), while others show decrease in AMF abundance (Warnock et al. 2010). Alteration of mycorrhizal abundance under biochar amended conditions has been explained by four mechanisms: changes in soil nutrient availability, alteration of other micro-organisms, detoxification of allelochemicals and provision of a refuge from hyphal grazers (Warnock et al. 2007).

Some recent reports have encouraging findings on relationship of AM fungi and biochar. For, example, AM fungi can mediate plant P uptake from microsites of biochar that are too small (<10 μm) for plant roots to enter (Hammer et al 2014). Improved uptake of P and growth of maize by combined application of AM fungi and biochar was also reported by Mau and Utami (2014). Furthermore, the relationship between AMF, biochar and plant performance may be dpendent on composition of media as indicated by Conversa et al (2015) that the best plant performance was achieved when AMF inoculation was applied in 30% biochar amendment. Increase in plant dry weight and P uptake was also reported by Momayezi et al (2015). However, it may depend upon

quality of biochar, which is related to the chemical constituents of its feedstock, and nutrient status of soil. Thus, it is essential to compare biochar effects on mycorrhizal colonization solely and in combination with other nutrient sources. The comparative influence of biochars from different feedstock source on colonization of crop roots by mycorrhizal fungi has not been adequately assessed. In this paper effects of three biochars on colonization of onion and tomato roots by vesicular arbuscular mycorrhizal fungi are compared.

4.3 Materials and methods

4.3.1 Experimental Site and Environment

The trials were conducted in winter season in 2012 and 2013 in a glasshouse at the University of Queensland, Gatton Campus, Australia. The first year trials were conducted in a bay of a small glasshouse with fluctuating temperature and light. There was an effect from the shade cloth on the roof and from adjacent bays. The daily temperature range was 17-33^{0}C during the first week of planting and 11-19^{0}C during the last week of observation. Following the experience of the first year, subsequent trials were conducted in a larger glasshouse where the plants had full access to sunlight and temperature was controlled.

4.3.2 Biochar types

Three types of biochar were used for the experiment *viz.* Sugarcane Trash, Green Waste A and Green Waste B. Sugarcane Trash and Green Waste A were produced by rapid-slow pyrolysis method. The kiln was truck mountable, interrupted combustion within a direct flaming pyrolysis system with temperature at 400-700^{0}C. Green Waste B was produced by slow, continuous pyrolysis. The kiln was a fixed, non-relocatable unit, no oxygen ingress, indirectly heated with highest heating temperature 550°C. General characteristics of the biochars are given in Appendix 1.

4.3.3 Mycorrhizal inoculum and species

The mycorrhizal inoculant MycoApply (Mycorrhizal Applications International (MAI), Australia) was applied at a rate of 10 g per pot just before planting. There were seven species of mycorrhiza among which four species (*G. aggregatum, G. intraradices, G. mosseae, G. etunicatum*) were endo- and three species were ecto-mycorrhizas.

4.3.4 Seed source and seedlings

Onion (variety: Rio Red Rock) and tomato (variety: Rebel F1) seeds were received from South Pacific Seeds, New South Wales, Australia.Seedlings were raised in sterilized propagation mix and planted after emergence of two true leaves. Plant roots were washed gently to remove the mix before planting. Coarse sand was autoclaved at 121^{0}C with a pressure of 105 kp afor an hour to kill

other micro-organisms. This autoclaving system was available at preparation room of soil science laboratory at the University of Queensland, Gatton Campus.

4.3.5 Biochar calculation and mixing

Biochar rates were calculated based on the area of the pots. The calculated amount of biochar was mixed with the sand by a mixer as shown in plate 4.1.

Plate 4.1 Mixer mixing the biochar with sand. The mixer was wiped thoroughly with a piece of cloth between two biochar rates to minimize the mixing of residual biochar from the previous rate.

4.3.6 Pots and nutrients

Plastic pots of 1.6 L volume were used. Nutrients were supplied through modified Hoagland's solution (Hoagland and Arnon 1950; Mattson and Lieth 2008; Epstein and Bloom 2004). This solution was modified with 25% P (Appendix 2) to minimize the negative effect on mycorrhizal infection and was applied at a rate of 50 mL per pot.

4.3.7 Experimental design and treatments

The experiments were continued for seven weeks as mycorrhizal associations can be formed within this period (Brundrett et al. 1996). These glasshouse experiments were conducted in a 3x4+1 (3 biochars, 4 application rates, 1 control) design with factorial arrangements in randomized complete blocks (Factor A: biochar type, Factor B: application rates, plus a control) with 3 replications and 13 treatments for each crop (Table 4.1).

Table 4.1 Treatment composition for the experiments

Treatments	Factor A (Biochar type)	Factor B (Application rates t ha^{-1})
1	Sugarcane Trash	10
2	Sugarcane Trash	30
3	Sugarcane Trash	50
4	Sugarcane Trash	100
5	Green Waste A	10
6	Green Waste A	30
7	Green Waste A	50
8	Green Waste A	100
9	Green Waste B	10
10	Green Waste B	30
11	Green Waste B	50
12	Green Waste B	100
13	Control	0

4.3.8 Observation

Observations were recorded on plant height up to seven weeks after planting, shoot fresh weight, shoot dry weight, root fresh weight, soil pH, soil electrical conductivity, root length, percent of root length colonized, colonized root length, P and Zn content of onion and tomato plants.

Plant height was recorded from the ground level to the base of the fully developed terminal leaf of tomato and the tip of the longest leaf of onion. Shoot fresh weight was taken immediately after harvest while shoot dry weight was recorded after drying at 65^0C for two weeks. Root fresh weight was recorded after absorbing moisture by tissue paper as given in Plate 4.2. The tissue paper was changed several times to maximize the moisture absorbed.

Plate 4.2 Moisture absorbing from root by tissue paper

Root length was determined by WinRhizo software (Regents Instruments Inc., Canada) and Epson 1600 scanner (Seiko Epson Corporation, Japan) as shown in the plate 4.3. Soil pH, EC and nutrient contents were determined by applying the standard techniques given in Appendix 4, 3, 5, 6, 7 and 9, respectively.

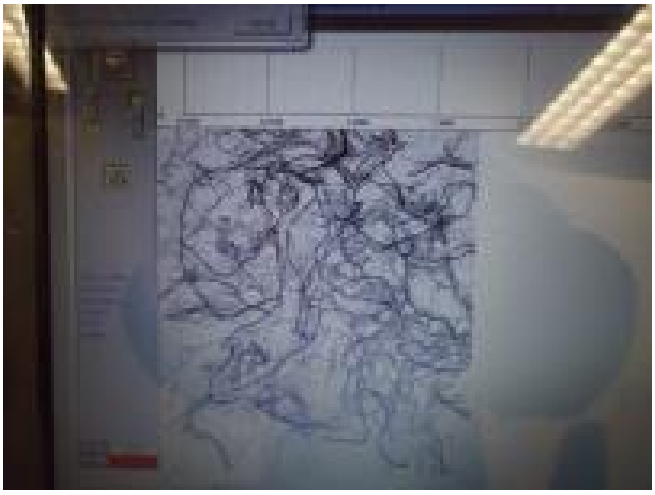


Plate 4.3 Root length measurement through WinRhizo software

4.3.9 Mycorrhizal care and Analysis

Procedures for mycorrhizal inoculation and analysis were adapted from Brundrett et al. (1995). Sand was sterilized with steam at 121^0C with a pressure of 105 kPa for 1 hour and biochar for 30 minutes. This autoclaving system was available at preparation room of soil science laboratory at the University of Queensland, Gatton Campus. Pots were sterilized with 2% household bleach and cleaned; a fine mesh cloth (<40µm pore size) was placed on the bottom of the pots to block the large holes to allow water to drain but not lose mycorrhizal spores. Pots were filled with sand biochar mix and watered to field capacity (approx. 10% w/w) with Hoagland's nutrient solution. At the centre of the filled pot, a 3-4 cm hole was made in which 10 g of mycorrhizal inoculum was placed. Five seedlings were transplanted into a pot. Hoagland's solution was applied for nutrition (modified to 25% P, Appendix 2) every alternate day and irrigated on the following day. Plants were grown for seven weeks as mycorrhizal associations can form within this period (Miyasaka et al. 2003).

Roots were examined for mycorrhizal associations and other relationships. Roots were uprooted and washed avoiding loss of fine laterals, then immersed in a bucket of water and agitated gently. They were cleaned vigorously with a hose over a 2 mm pore size screen ensuring finest laterals were not lost. Root samples were stored in 50% ethanol after taking root fresh weight. Roots were chopped into 2 cm long segments and a 2 g sample was taken for examination. Roots were put in falcon

tubes (50 mL) and autoclaved in KOH 10% (w/v) at 121^0C for 25 minutes and then rinsed in a fine sieve. Roots were stained in trypan blue 0.05% w/v in lactoglycerol solution (1:1:1 lactic acid, glycerol and water) (Bevege 1968; Phillip & Hayman 1970; Kormanik & McGraw 1982) by autoclaving for 25 minutes at 121^0C. To prepare this solution, trypan blue was dissolved in water and equal volumes of lactic acid and glycerol were added. Roots were rinsed on a fine sieve after staining. Then roots were stored in plastic vials with tight-sealing lids containing 50% glycerol. The colonization was measured by the gridline intersection method (Giovannetti & Mosse 1980) (Plate 4.4) in which cleared and stained roots were randomly dispersed with a fine forceps and a dissecting needle in a 8 x 8 square cm Petri plate with gridlines where horizontal and vertical lines were followed. The colonized or non-colonized portions of roots on the line intersects were counted separately. The number of root segments colonized and spread on each vertical and horizontal intersect was counted and divided by the total number of segments. The root length occupied by mycorrhizae (proportion) was observed under the microscope and images were captured as shown in Plate 4.5.

Plate 4.4 Petri plate with 8x8 square cm used for counting colonized roots by mycorrhiza by gridline intersect method.

Plate 4.5 Capturing image of mycorrhizae through microscope. The blue lid Falcon tubes were used for storage of the roots

4.3.10 Statistical analysis

Statistical analysis was done using Minitab 16, version 4.0 (Minitab 2005) statistical package. The data were analysed in three steps. Firstly, all data (including control) were analyzed (Table 4.2) by one-way ANOVA to obtain experimental error variance and all treatment variance. Secondly, the factorial subset was analysed through General Linear Model of 'biochar type' 'application rates' biochar type*application rates. Thirdly, the results of the two analyses were combined to get final results including comparison with the control in Microsoft Excel 2010, version 14.0 (Microsoft 2010). The individual standard errors of the means were derived from the standard deviation of the mean (SD) and number of observations (*N*). The Figures were plotted in Microsoft Excel software. The mycorrhizal data were transformed by log-transformation for analysis and then values were back-transformed.

Table 4.2 a. One Way ANOVA for all treatments, **b.** General Linear Model for factorial analysis, combined ANOVA from one-way ANOVA and general linear model

a.

Source	Df
Replication (r)	(r-1), 3-1 = 2
All treatments (t)	(t-1), 13-1= 12
Error	(T-t), 38-12 = 26
Total (T)	(tr-1), 13 x 3-1= 38

b

Source	Df
Replication (r)	(r-1), 3-1 = 2
Biochar (b)	(b-1), 3-1= 2
Application rates (a)	(a-1), 4-1= 3
Biochar x Application rates	(b-1) (a-1), (2)(3)= 6
Error	ba(r-1), 3 x 4 (3-1) = 24
Total (T)	(T-1), 36-1= 35

c

Source	Df
All treatments (t)	(t-1), 13-1= 12
Biochar (b)	(b-1), 3-1= 2
Application rates (a)	(a-1), 4-1= 3
Biochar x Application rates	(b-1) (a-1), (2)(3)= 6
Factorial subset (f)	(b-1) + (a-1) + (b-1) (a-1), 2 + 3 + 6 = 11
Extra treatment	[(t-1) –f], 12-11 = 1
Error	(T-t), 39-13 = 26
Total (T)	(T-1), 39-1= 38

4.4 Results

4.4.1 Onion trial

Effect of biochar types

Significant differences were observed for plant height in the second year due to the effect of biochar type (Table 4.3) but no differences were found in the first year. In the second year, the grouping of means overlapped until the second week after planting. In the third week and onwards, Sugarcane Trash and Green Waste A biochar had similar and greater positive effect than the Green Waste B

biochar. All biochars increased plant height as compared to the control. At the end of the observation period (7th week of planting), the plant height increased by >100% due to Sugarcane Trash and Green Waste A while it increased by >75% by Green Waste B compared to the control indicating all biochars were positively influencing growth.

Table 4.3 Mean values for weekly plant height as influenced by biochar types in the second year.

Biochar types	Plant height (cm) in weeks						
	1st	2nd	3rd	4th	5th	6th	7th
Sugarcane Trash	9.24 ± 0.16a	15.76 ± 0.26ab	18.01 ± 0.41a	21.72 ± 0.48a	24.56 ± 0.72a	28.48 ± 0.72a	36.44 ± 0.94a
Green Waste A	9.00 ± 0.25ab	16.26 ± 0.18a	17.84 ± 0.13a	21.81 ± 0.34a	24.21 ± 0.38a	28.46 ± 0.63a	35.40 ± 1.03a
Green Waste B	8.30 ± 0.18b	15.17 ± 0.22b	16.43 ± 0.31b	19.74 ± 0.57b	21.55 ± 0.51b	24.83 ± 0.65b	30.86 ± 0.82b
Control	7.80 ± 0.17b	12.43 ± 0.67c	14.10 ± 0.49c	14.43 ± 0.62c	14.48 ± 0.72c	15.99 ± 1.20c	17.67 ± 1.15c

Different letters in the same column indicate significant differences between the treatment means (N = 12 for biochar types, 3 for control) at α=0.05 level of significance. The ± values indicate standard error of the means.

Biochars were significantly different for root length in the second year only (Table 4.4) where Sugarcane Trash had greater influence on root length which was at a par with Green Waste A. Green Waste A and Green Waste B were also at a par but Sugarcane Trash and Green Waste B were statistically different. The root length of biochar applied plants was at least double that for the control plants indicating the effectiveness of biochar.

Table 4.4 Mean values for weekly plant height as influenced by biochar types in the second year.

Biochar types	Root length (cm)
Sugarcane Trash	705.3 ± 44.03a
Green Waste A	605.0 ± 23.82ab
Green Waste B	578.2 ± 28.38b
Control	280.8 ± 12.87c

Different letters in the same column indicate significant differences between the treatment means (N = 12 for biochar types, 3 for control) at α=0.05 level of significance. The ± values indicate standard error of the means.

Table 4.5 Mean values for colonized percent of root length. Log 10 values followed by back transformed mean of original mean in parenthesis

Biochar types	Colonized % root length		
	Year 1	Year 2	Mean
Sugarcane Trash	1.06 (10.97) ± 0.04b	1.31 (21.8) ± 0.23a	16.4
Green Waste A	1.26 (17.6) ± 0.02a	1.22 (17.5) ± 0.03b	17.6
Green Waste B	1.18 (15.92) ± 0.04a	1.19 (16.1) ± 0.13b	16.01
Control	0.37 (1.38) ± 0.07c	0.73 (6.7) ± 0.21c	4.04

Different letters in the same column indicate significant differences between the treatment means (N = 12 for biochar types, 3 for control) at α=0.05 level of significance. The ± values indicate standard error of the means.

Significant differences were also observed for colonized percent of root length, colonized root length and their logarithmically transformed values (Table 4.5 and Table 4.6) in each year of observation. In both years, Green Waste A and Green Waste B had similar effect on the percent of colonized root length while Sugarcane Trash had less colonization in first year and greater in second year than the other biochars. Interestingly, all biochars had significantly higher colonization than the control. It was notable that the difference for average colonization of two years between the least and the most colonizing biochars was only about 1% while the difference was about four times greater in biochar applied roots than in control.

Table 4.6 Mean values for colonized root length. Log 10 values followed by back transformed mean of original mean in parenthesis

Biochar types	Colonized root length		
	Year 1	Year 2	Mean
Sugarcane Trash	1.80 (70.57) ± 0.02b	2.14 (164.48) ± 0.04a	117.5
Green Waste A	2.04 (120.54) ± 0.02a	1.95 (103.38) ± 0.02b	112.0
Green Waste B	1.89 (107.56) ± 0.05b	1.95 (104.8) ± 0.03b	106.2
Control	0.53 (3.64) ± 0.12c	1.18(18.15) ± 0.19c	10.9

The difference in biochar types was statistically significant for electrical conductivity and soil pH in 2nd year only (Table 4.7). Sugarcane Trash and Green Waste A were at a par and the two green wastes were similar. However, there was a difference between Sugarcane Trash and Green Waste B. These results were not solely reliant on the concentration of salts in biochar because the EC was determined after harvesting of plants and several applications of Hoagland's nutrient solution during the plant growth.

The pH of soil was greatest in sand amended with Green Waste A, reflecting its high acid neutralizing capacity (Table 4.7). Green Waste B and Sugarcane Trash had similar effect on soil pH. Treatments with biochar showed significantly greater pH than the control.

Table 4.7 Mean values for soil Electrical conductivity and pH.

Biochar types	Electrical conductivity ($dS\ m^{-1}$)	Soil pH
Sugarcane Trash	1.9 ± 0.11a	7.3 ± 0.03b
Green Waste A	1.6 ± 0.16ab	7.9 ± 0.09a
Green Waste B	1.3 ± 0.05b	7.3 ± 0.06b
Control	0.8 ± 0.06c	6.7 ± 0.07c

Different letters in the same column indicate significant differences between the treatment means (N = 12 for biochar types, 3 for control) at α=0.05 level of significance. The ± values indicate standard error of the means.

Effect of biochar application rates

Significant differences were observed between application rates for several parameters in both years. The trend for plant height was to increase as application rates of biochar increased (Table 4.8). Overall height was less in the first year than in the second. Rates of 30 t ha^{-1} and greater were more effective for increasing height than lower rates. At the seventh week after planting in the second year, 50 and 100 t ha^{-1} had similar effect on height followed by 30 t ha^{-1}while 30 t ha^{-1} was highest in the first year. Average data showed a great difference between control and 10 t ha^{-1}, 10 t ha^{-1} and other high levels and control versus all treatments. As a result of less difference between the means for 30, 50 and 100 t ha^{-1}, 30 t ha^{-1} was likely to be a preferable rate for onion plants. Differences in plant growth were clearly distinguished (Plate 4.6).

Table 4.8 Mean values for weekly plant height of onion. a. 2nd and 3rd week, b. 4th and 5th week, c. 6th and 7th week of planting. Different letters in the same column indicate significant differences between the treatment means (N = 9 for biochar rates, 3 for control) at α=0.05 level of significance. The ± values indicate standard error of the means.

a. 2nd and 3rd week

Application rates (t ha^{-1})	Plant height (cm) in weeks					
	2nd week			3rd week		
	1st year	2nd year	Mean	1st year	2nd year	Mean
10	8.42 ± 0.21b	14.96 ± 0.28b	11.69	11.31 ± 0.28c	15.68 ± 0.27c	13.50
30	13.12 ± 0.24a	16.01 ± 0.22ab	14.57	18.03 ± 0.32a	17.53 ± 0.32b	17.78
50	13.17 ± 0.13a	15.44 ± 0.31ab	14.31	16.11 ± 0.39b	17.42 ± 0.46b	16.77
100	13.28 ± 0.18a	16.51 ± 0.21a	14.90	15.17 ± 0.33b	19.08 ± 0.26a	17.13
Control	5.38± 0.12c	12.43 ± 0.67c	10.91	8.32± 0.32d	14.10 ± 0.49c	11.21

b. 4th and 5th week

Application rates (t ha^{-1})	Plant height (cm) in weeks					
	4th week			5th week		
	1st year	2nd year	Mean	1st year	2nd year	Mean
10	12.86 ± 0.34d	17.23 ± 0.51c	15.05	14.75 ± 0.36c	18.86 ± 0.70c	16.81
30	21.63 ± 0.38a	20.70 ± 0.47b	21.17	25.81± 0.42a	22.70 ± 0.62b	24.26
50	19.31 ± 0.45b	22.21 ± 0.76ab	21.76	23.16 ± 0.66ab	24.88 ± 0.70ab	24.02
100	17.51 ± 0.37c	24.22 ± 0.40a	20.87	21.81 ± 1.11b	27.31 ± 0.47a	24.56
Control	9.63 ± 0.37e	14.43 ± 0.62d	12.02	11.90 ± 0.45c	14.48 ± 0.72d	13.19

c. 6th and 7th week

Application rates (t ha^{-1})	Plant height (cm) in weeks					
	6th week			7th week		
	1st year	2nd year	Mean	1st year	2nd year	Mean
10	17.92 ± 0.36d	20.52 ± 0.97c	19.22	20.89 ± 0.399d	25.39 ± 1.11c	23.14
30	30.78 ± 0.36a	26.76 ± 0.90b	28.77	35.77 ± 0.23a	32.70 ± 1.44b	34.24
50	27.78 ± 0.57b	29.89 ± 0.85ab	28.84	31.59 ± 0.22b	38.76 ± 1.00a	35.18
100	25.08 ± 0.33c	31.87 ± 0.38a	28.48	29.33 ± 0.12c	40.04 ± 0.75a	34.70
Control	14.80 ± 0.65e	15.99 ± 1.19c	15.40	17.73 ± 0.87e	17.67 ± 1.15d	17.7

A **B** **C**

Plate 4.6 Effect of biochar application rates (control, 10, 30, 50 and 100 t ha^{-1} from left to right in each plate) on growth of onion after 7 weeks of planting, from left to right A. Sugarcane Trash, B. Green Waste A and C. Green Waste B.

Shoot fresh weight was greater in the second year than in the first (Figure 4.1). In the first year, the shoot weight increased up to 100 t ha^{-1} but in the second year, it declined after 30 t ha^{-1}. The weight in the second year was four times more than in the first year when biochars were applied at 30 t ha^{-1}. When they were applied at 10, 50, 100 t ha^{-1} and control, the weight was three times more in the second year than in the first year. However, the average weight was similar in 30, 50 and 100 t ha^{-1}. All application rates showed a greater shoot fresh weight than the control. The great difference between the years was associated with the weight of more leaves and bulbs formed in the second year in an environment quite different from the first year.

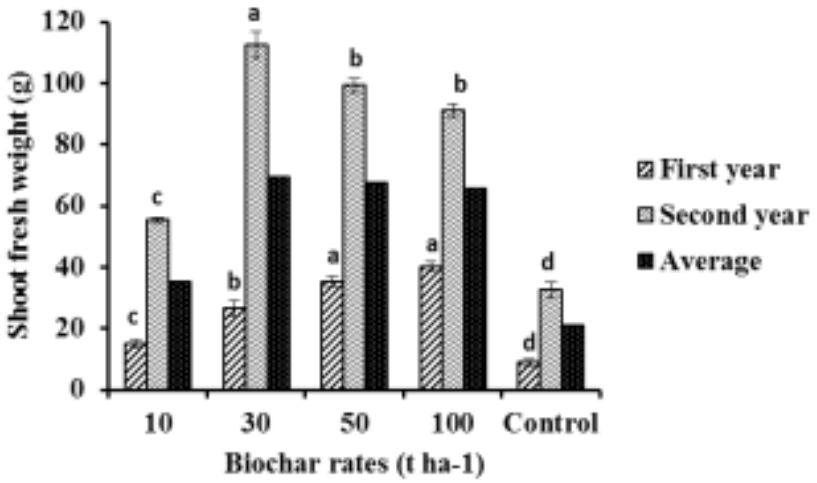


Figure 4.1 Shoot fresh weight of onion as influenced by the biochar application rates. The different letters in the same data series indicate significant differences between treatment means (*N* = 9 for biochar rates, 3 for control) at α=0.05 level of significance. The bars represent the standard error of the means.

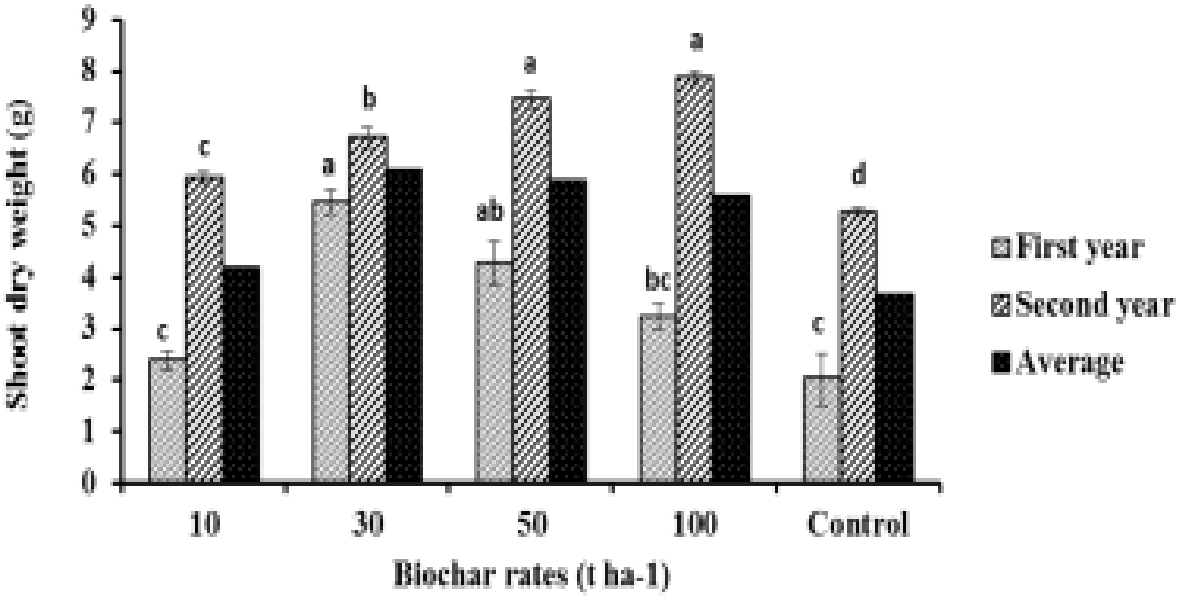


Figure 4.2 Shoot dry weight of onion as influenced by the biochar application rates. Different letters in the same series indicate significant differences between the treatment means (N = 9 for biochar rates, 3 for control) at α=0.05 level of significance. The vertical bars indicate standard error of the means.

The shoot dry weight increased up to 100 t ha^{-1} but it was not five times or more than 10 t ha^{-1} (Figure 4.2). There was no difference between the means of shoot dry weight at 50 and 100 t ha^{-1} in both years. In the second year, no clear difference in weight was observed between 30, 50 and 100 t

ha^{-1}. There was some difference in weight between 10 t ha^{-1} and control but they were similar in the second year. The average dry weight increased up to application of 30 t ha^{-1} biochar and thereafter declined. This variation in dry weight over the years was mainly due to their growth in different environments and the duration of drying. They were dried for 48 hours at 65^0C.

Treatment means were significantly different for root fresh weight in the second year only (Table 4.9). Root fresh weights produced by 30, 50 and 100 t ha^{-1} were statistically similar but they were different from 10 t ha^{-1} and control. Control and 10 t ha^{-1} produced similar root fresh weight. The highest root fresh weight was recorded for 30 t ha^{-1} biochar followed by 50 and 100 t ha^{-1}. The rate of 30 t ha^{-1} and more produced two times more weight than the control.

Table 4.9 Mean values for root fresh weight of onion as influenced by biochar rates in the second year.

Application Rates (t ha^{-1})	Root fresh weight (g)
10	2.37 ± 0.10b
30	4.86 ± 0.40a
50	4.46 ± 0.43a
100	4.07 ± 0.43a
Control	2.33 ± 0.18b

Different letters in the same column indicate significant differences between the treatment means (N = 9 for biochar rates, 3 for control) at α=0.05 level of significance. The ± values indicate standard error of the means.

Means of electrical conductivity and pH of growth medium increased as rate of biochars increased in the second year (Figure 4.3). Data showed no difference between treatments in the first year. In the second year, the EC and pH were greatest in the sand amended with 100 t ha^{-1} biochar. Sand showed neutral pH when amended with 10 t ha^{-1}. However, pH of the medium treated with 30 and 50 t ha^{-1} was statistically similar. As the values are antilogarithms, differences were higher when they were converted into positive logarithmic value.

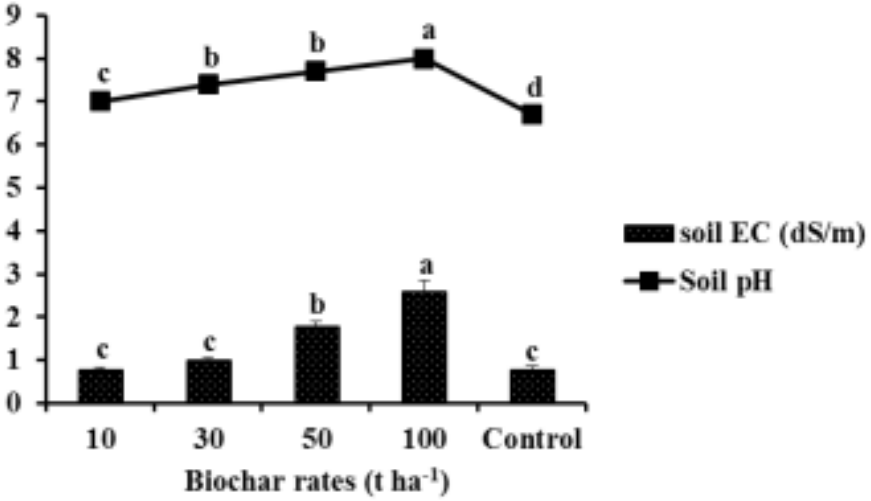


Figure 4.3 Soil electrical conductivity (EC) and pH as influenced by biochar rates in the 2nd year. Different letters in the same column indicate significant differences between the treatment means (N = 9 for biochar rates, 3 for control) at α=0.05 level of significance. Vertical bars indicate standard error of the means.

There were no differences among 30, 50 and 100 t ha^{-1} for root length of onion in both years (Table 4.10). The rates of 10 t ha^{-1} and control were also similar for root length. Average data showed that 30 t ha^{-1} produced root length more than 2.5 times compared to the control. The average data also revealed that the root length increased up to 30 t ha^{-1} and thereafter decreased.

Table 4.10 Mean values for root length of onion as influenced by biochar rates

Application Rates (t ha^{-1})	Root length (cm)		
	1st year	2nd year	Mean
10	362.7 ± 32.43b	336.9 ± 23.63b	349.8
30	743.8 ± 27.60a	748.1 ± 39.70a	746.0
50	754.2 ± 44.03a	716.6 ± 42.93a	735.4
100	719.1 ±59.93a	716.5 ± 41.90a	717.8
Control	264.1 ± 18.65b	280.8 ± 12.87b	272.5

Different letters in the same column indicate significant differences between the treatment means (N = 9 for biochar rates, 3 for control) at α=0.05 level of significance. The ± values indicate standard error of the means.

Colonization of root length significantly increased by addition of biochar in both years (Table 4.11). In the first year, 30 t ha^{-1} increased the percent of colonization by 13.8 times over the control. Likewise, the colonization percentage increased by about 6.9, 12.6, and 9.2 times over the control with 10, 50 and 100 t ha^{-1} biochar. Means of 30, 50 and 100 t ha^{-1} statistically overlapped indicating 30 t ha^{-1} had similar colonization percent to 100 t ha^{-1}.

In the second year, no difference was observed between the means of control and 10 t ha^{-1} but they were less than the means for 30, 50 and 100 t ha^{-1}. The means of these three treatments were similar. The average of the two-year means showed that application of 10, 30, 50 and 100 t ha^{-1} biochar could increase colonization percent by 2.4, 5.2, 4.7 and 4.0 times, respectively over the control. The colonization patterns are shown in the Plate 4.7.

Increasing the application rates of biochar had significantly positive effect on the colonized root length in both years (Table 4.12). In the first year, the increment was 9.8, 39.2, 36.5, 25.0 times greater than control with 10, 30, 50 and 100 t ha^{-1} respectively. The means of 30 and 50t ha^{-1} were statistically similar but greater than the mean of 100 t ha^{-1}. In the second year, the colonized length was 2.1, 9.5, 8.0 and 7.8 times more than the control for 10, 30, 50 and 100 t ha^{-1} respectively. The length was similar with the application of 30, 50 and 100 t ha^{-1}. On average, colonization was greatest with 30 t ha^{-1} biochar which was 14.4 and 4.3 times more than the control and 10 t ha^{-1}, respectively.

Table 4.11 Mean values for colonized precent of root length of onion as influenced by biochar rates. Different letters in the same column indicate significant differences between the treatment means (N = 9 for biochar rates, 3 for control) at α=0.05 level of significance. The ± values indicate standard error of the means.

Application rates (t ha^{-1})	Colonized % of root length		
	Year 1	Year 2	Mean
10	1.00 (9.6) ± 0.04c	1.03 (11.7) ± 0.03b	10.7
30	1.29 (19.3) ± 0.04a	1.35 (22.7) ± 0.01a	21.0
50	1.25 (17.6) ± 0.03ab	1.30 (20.1) ± 0.02a	18.9
100	1.12 (12.9) ± 0.03bc	1.29 (19.9) ± 0.02a	16.4
Control	0.37 (1.4) ± 0.07d	1.07 (6.7) ± 0.21b	4.05

Table 4.12 Mean values for colonized root length of onion as influenced by biochar rates. Different letters in the same column indicate significant differences between the treatment means (N = 9 for biochar rates, 3 for control) at α=0.05 level of significance. The ± values indicate standard error of the means.

Application rates ($t\ ha^{-1}$)	Colonized root length (cm)		
	Year 1	Year 2	Mean
10	1.48 (35.4) ± 0.03c	1.55 (37.7) ± 0.12b	36.6
30	2.13 (141.2) ± 0.03a	2.22 (172.2) ± 0.03a	156.7
50	2.10 (131.5) ± 0.03a	2.14 (144.9) ± 0.03a	138.2
100	1.93 (90.1) ± 0.03b	2.14 (142.1) ± 0.03a	116.1
Control	0.53 (3.6) ± 0.12d	1.18 (18.2) ± 0.19c	10.9

Table 4.13 Mean values for root fresh weight and colonized root length of onion as influenced by interaction of biochar types and application rates. The different letters in the same column indicate significant difference between interactions (*N* = 3) at α=0.05 level of significance. The ± values represent the standard error of the means.

Biochar types	Biochar rates ($t\ ha^{-1}$)	Root fresh weight (g)	Log colonized root length
Sugarcane Trash	10	2.26 ± 0.48ab	1.48 (30.4) ± 0.04cd
	30	1.83 ± 0.47b	2.01 (103.2) ± 0.02ab
	50	2.44 ± 0.61ab	1.97 (93.3) ± 0.04ab
	100	2.42 ± 0.38ab	1.74 (55.4) ± 0.03bc
Green Waste A	10	2.31 ± 0.70ab	1.77 (59.1) ± 0.03bc
	30	2.09 ± 0.04ab	2.23 (171.7) ± 0.04a
	50	5.87 ± 1.67a	2.18 (152.6) ± 0.04a
	100	2.43 ± 0.49ab	1.99 (98.7) ± 0.04ab
Green Waste B	10	2.03 ± 0.70b	1.20 (16.83) ± 0.11d
	30	3.29 ± 0.99ab	3.16 (148.8) ± 0.09a
	50	1.95 ± 0.27b	2.15 (148.6) ± 0.10a
	100	1.88 ± 0.80b	2.05 (116.1) ± 0.09ab
Control		1.04 ± 0.43b	0.53 (3.64) ± 0.12e

Interaction effects

The interaction of biochar types and application rates was not significant for any parameter except root fresh weight and colonized root length in the first year and soil EC and pH in the second year. The details of the parameters are shown in Tables 4.13 and 4.14. These data could indicate that some interactions had significantly positive effect on root fresh weight, colonized root length, EC and pH over the control.

Table 4.14 Mean values for soil EC and pH of onion as influenced by interaction of biochar types and application rates. The different letters in the same column indicate significant difference between interactions (N = 3) at α=0.05 level of significance. The ± values represent the standard error of the means.

Biochar types	Biochar rates (t ha^{-1})	EC (dS m^{-1})	pH
Sugarcane trash	10	0.8 ± 0.00c	7.1 ± 0.05de
	30	1.1 ± 0.17c	7.3 ± 0.09cde
	50	2.1 ± 0.16abc	7.5 ± 0.08cde
	100	3.6 ± 0.55a	7.6 ± 0.06bcd
Green waste A	10	0.8 ± 0.18c	6.9 ± 0.09e
	30	0.9 ± 0.12c	7.9 ± 0.28bc
	50	1.8 ± 0.32bc	8.2 ± 0.18ab
	100	2.6 ± 0.69ab	8.7 ± 0.14a
Green waste B	10	0.8 ± 0.06c	6.9 ± 0.09e
	30	1.0 ± 0.07c	7.1 ± 0.10de
	50	1.5 ± 0.24bc	7.4 ± 0.12cde
	100	1.7 ± 0.12bc	7.7 ± 0.18bcd
Control		0.8 ± 0.06c	6.7 ± 0.07e

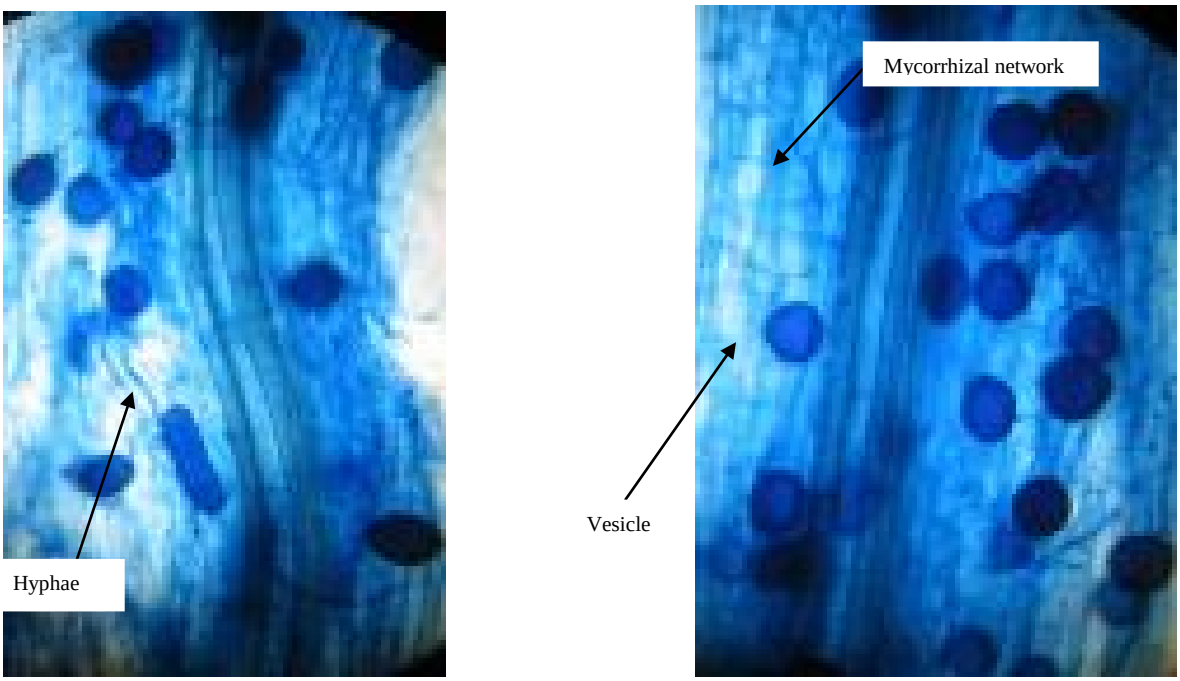


Plate 4.7 Patterns of mycorrhizal colonization of onion roots observed at 40x in microscope

4.4.2 Tomato trial

Effect of biochar type

Table 4.15 Mean values for root fresh weight of tomato as influenced by biochar types in the first year. The different letters in the same column indicate significant difference between treatments (N = 12 for biochar types and 3 for control) at α=0.05 level of significance. The ± values represent the standard error of the means.

Biochar types	Root fresh weight (g)
Sugarcane trash	6.28 ± 0.19b
Green waste A	7.93 ± 0.45a
Green waste B	6.44 ± 0.40b
Control	3.31 ± 1.00c

Statistically, all biochar types were similar for most of the parameters in first year of observation except root fresh weight (Table 4.15). Mean root fresh weight for Green Waste A was 1.65 and 1.49 g more than for Sugarcane Trash and Green Waste B respectively. However, the weight was two times greater in the plants treated with biochars than control.

In the second year, means of soil EC and colonized percent of root length were different between biochar types (Table 4.16). EC was greater in soil treated with Green Waste A than the Sugarcane Trash biochar. The two green waste biochars showed similar ECs. These data are not a result of sole effect of biochars because additional salts were applied through Hoagland's nutrient solution to fulfil the plant requirement for nutrients.

The colonized percent of root length was significantly higher in biochar treated roots than the control. The percentages were 3.9, 3.3 and 3.2 times more than the control for Sugarcane Trash, Green Waste A and Green Waste B respectively. Green Waste A and B were similar for the percentage but had lower root length values than Sugarcane Trash.

Table 4.16 Mean values for colonization levels of root length and colonized root length of tomato as influenced by biochar types in the second year. The different letters in the same column indicate significant difference between treatments (N = 12 for biochar type and 3 for control) at α=0.05 level of significance. The ± values represent the standard error of the means.

Biochar types	EC (dS m^{-1})	Colonized % root length
Sugarcane trash	0.48 ± 0.02b	1.10 (12.91) ± 0.02a
Green waste A	0.59 ± 0.03a	1.04 (11.02) ± 0.02b
Green waste B	0.56 ± 0.02ab	1.02 (10.58) ± 0.01b
Control	0.23 ± 0.03c	0.50 (3.30) ± 0.10c

The pH was similar in the sand amended with Sugarcane Trash, Green Waste B and Control (Figure 4.4). Yet, they tended to increase as expected for the acid neutralizing capacity of the respective biochar. The average data of two years showed that the pH was maintained around neutral in control and it was beyond 8 in Green Waste A treated sand. However, the difference is small in the Figure; the actual difference is higher because it is antilogarithmic value of hydrogen ion concentration that could differentiate by a value of 10.

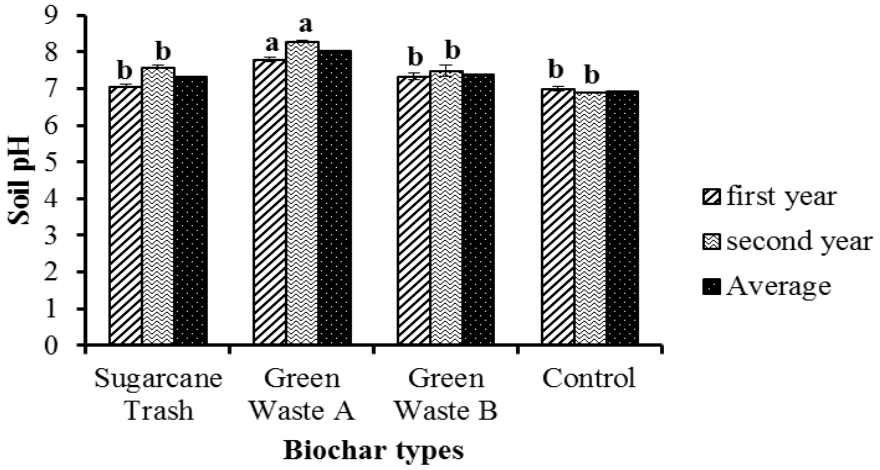


Figure 4.4 Soil pH over years as influenced by biochar types. The different letters in the same series indicate significant difference between treatments (N = 12 for biochar type and 3 for control) at α=0.05 level of significance. The vertical bars represent the standard error of the means.

Effect of application rates

Significant differences between application rates for plant height were observed after the fifth week of planting (Table 4.17) in both years. The morphological difference in plant architecture due to different rates of biochar has been shown in Plate 4.8. Overall growth was greater in the second year than in the first. In the fifth week, plant height was similar for 30, 50 and 100 t ha^{-1} in the second year, while 30 and 50 t ha^{-1} had greater plant height than 10, 100 t ha^{-1} and control in the first year. The average of two years' data showed that addition of 50 t ha^{-1} biochar resulted in 2.3, 1.5, 1.1 and 1.2 times greater plant height than control.

In 6^{th} week, height was about 2 times more in 2^{nd} year than the 1^{st} year. The rate of 50 t ha^{-1} produced the greatest height in 2^{nd} year while it was at a par with that for 30 t ha^{-1} in 1^{st} year. In 1^{st} year, all biochar rates produced greater height than control. On average, height produced by 50 t ha^{-1} was 2.5, 1.5, 1.2 and 1.2 times of that generated by control, 10, 30 and 100 t ha^{-1} respectively.

In the seventh week, all biochar rates produced greater plant height than the control in both years. In the second year, application of 50 t ha^{-1} biochar produced the greatest plant height which was 2 times the height of the control, 1.5 times 10 t ha^{-1} and 1.2 times 30 and 100 t ha^{-1} rates. In the first year, 30 and 50 t ha^{-1} produced similar heights but greater than control and 10 t ha^{-1}. On average, the height created by 50 t ha^{-1} biochar was 2.4, 1.5, 1.1 and 1.2 times that of control, 10, 30 and 100 t ha^{-1} respectively.

Table 4.17 Mean values for weekly plant height as influenced by biochar rates. The different letters in the same column indicate significant difference between treatments (*N* = 9 for biochar application rates and 3 for control) at α=0.05 level of significance. The ± values represent the standard error of the means.

Application rates (t ha^{-1})	Plant height (cm) in weeks								
	5th week			6th week			7th week		
	1st year	2nd year	Mean	1st year	2nd year	Mean	1st year	2nd year	Mean
10	14.4 ± 1.29c	32.8 ± 0.65b	23.6	20.5 ± 1.65c	39.6 ± 0.99c	30.0	24.3 ± 1.19c	42.5 ± 0.87c	33.4
30	20.7 ± 0.53ab	41.7 ± 1.38a	31.2	28.2 ± 0.91ab	49.7 ± 1.45b	39.0	32.5 ± 0.82ab	53.7 ± 1.44b	43.1
50	22.6 ± 0.66a	46.7 ± 1.75a	34.7	30.0 ± 0.72a	59.7 ± 0.56a	45.0	34.3 ± 0.62a	63.5 ± 0.69a	48.9
100	17.2 ± 1.02bc	42.6 ± 0.62a	29.9	24.2 ± 1.35bc	50.8 ± 1.04b	37.5	28.8 ± 1.11b	53.8 ± 1.03b	41.3
Control	9.4 ± 1.05c	20.2 ± 3.22c	14.8	13.1 ± 1.18d	22.8 ± 4.64c	18.0	16.5 ± 1.26d	25.1 ± 3.92d	20.8

A **B** **C**

Plate 4.8 Effect of biochar application rates (control, 10, 30, 50 and 100 t ha^{-1} from left to right in each plate) on growth of tomato after six weeks of planting, A. Sugarcane Trash, B. Green Waste A and C. Green Waste B

Shoot fresh weight was greater in the second year than in the first. In the second year, the weight was similar for 30, 50 and 100 t ha^{-1} biochar but it was significantly higher than 10 t ha^{-1} and control (Figure 4.5). All the biochar rates increased the weight over control.

In the first year, the effect of application rates on shoot fresh weight was significantly greater for 50 and 100 t ha^{-1} than the rest. The height for 30 t ha^{-1} was significantly greater than 10 t ha^{-1} and control. Interestingly, 10 t ha^{-1} and control produced similar shoot fresh weight.

The two-year average data showed that fresh weight increased up to 50 t ha^{-1} thereafter started declining. Here, 50 t ha^{-1} was most effective for shoot fresh weight.

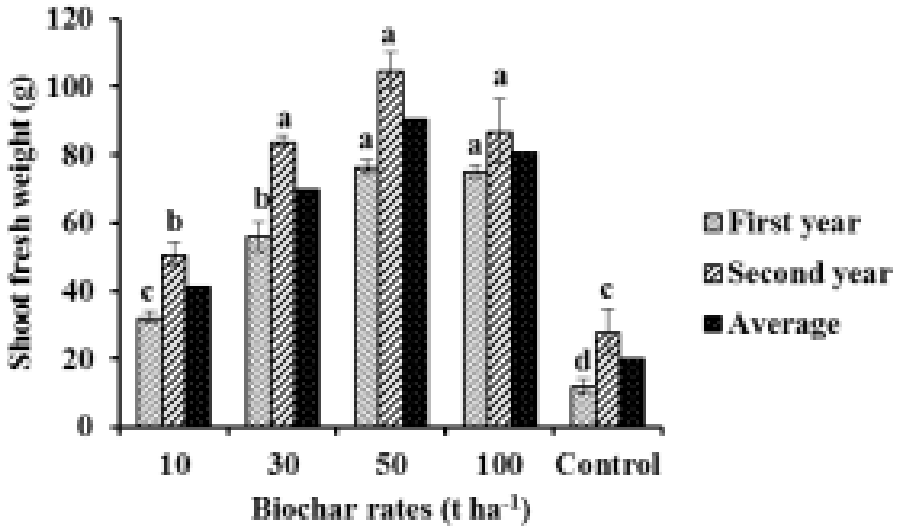


Figure 4.5 Shoot fresh weight of tomato as influenced by biochar rates. The different letters in the same series indicate significant difference between treatments (N = 9 for biochar application rates and 3 for control) at α=0.05 level of significance. The vertical bars represent the standard error of the means.

Application rates were significantly different for shoot dry weight (Figure 4.6). In the second year, weight for 50 t ha^{-1} was greater than for control, 10 and 100 t ha^{-1}. The rate 30 t ha^{-1} was similar for 50 and 100 t ha^{-1}. The rate of 10 t ha^{-1} produced significantly lower weight than higher rates but greater weight than the control.

In the first year, 30, 50 and 100 t ha^{-1} produced similar but significantly greater shoot dry weight than 10 tha^{-1} and control. Control had lower weight than 10 t ha^{-1} and higher rates.

The average weight increased from addition of 10 t to 50 t ha^{-1} biochar and thereafter decreased. The optimum rate was 50 t ha^{-1} biochar for weight but increase in weight was not proportional to increased amount of biochar.

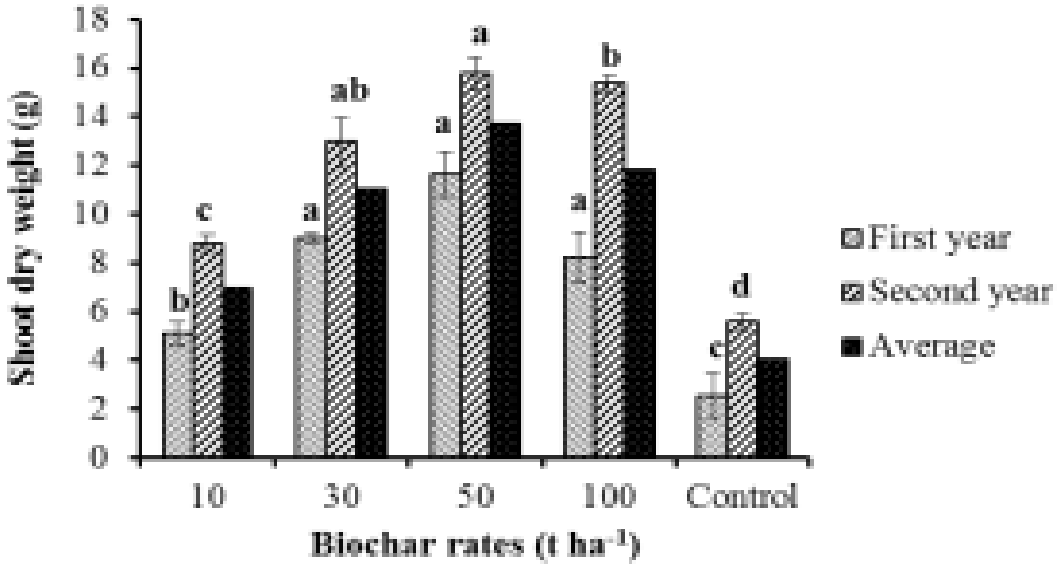


Figure 4.6 Shoot dry weight of tomato as influenced by biochar rates. The different letters in the same series indicate significant difference between treatments (N = 9 for biochar application rates and 3 for control) at α=0.05 level of significance. The vertical bars represent the standard error of the means.

There were significant differences for root fresh weight due to the effect of biochar rates in both years of observation (Table 4.18). In the first year, 10, 30 and 50 t ha^{-1} were similar for weight but these weights were significantly greater than the weight for control. The weights for 10, 30, 50 and 100 t ha^{-1} were respectively 1.9, 2.2, 2.4 and 1.8 times more than for control. In the second year, 50 t ha^{-1} produced the greatest root fresh weight while weights with 30 and 100 t ha^{-1} were similar. All biochar rates produced greater root fresh weight than control. For example, the weight with 50 t ha^{-1} biochar was 3.5 times greater than control. The average weight for 50 t ha^{-1} was 2.9 times the weight of the control.

Soil pH increased as biochar rates increased in both years. All biochar rates increased the pH from neutrality as pH was maintained at neutral by adding dolomite.

Table 4.18 Mean values for root fresh weight of tomato and soil pH as influenced by biochar rates. The different letters in the same column indicate significant difference between treatments (N = 9 for biochar application rates and 3 for control) at α=0.05 level of significance. The ± values represent the standard error of the means.

Application rates (t ha^{-1})	Root fresh weight (g)			Soil pH		
	1st year	2nd year	Mean	1st year	2nd year	Mean
10	6.41 ± 0.49ab	5.36 ± 0.34c	5.89	7.0 ± 0.07c	7.2 ± 0.03b	7.1
30	7.21 ± 0.37ab	8.59 ± 0.32b	7.90	7.2 ± 0.05bc	7.6 ± 0.23b	7.5
50	7.83 ± 0.43a	10.35 ± 0.18a	9.09	7.5 ± 0.13b	7.7 ± 0.03b	7.6
100	6.09 ± 0.38b	9.04 ± 0.18b	7.57	7.9 ± 0.05a	8.6 ± 0.02a	8.3
Control	3.31 ± 1.00c	2.97 ± 0.96d	3.14	7.0 ± 0.06c	6.8 ± 0.02b	6.9

Significant differences between biochar rates were observed for EC and root length in the second year only (Table 4.19). In that year, EC increased as the level of biochar increased. EC increased by 63% and 144% when the biochar rate was raised from 10 to 30 t ha^{-1} and 50 t ha^{-1} respectively. However, EC increased by 50% when the rate was raised from 30 to 50 t ha^{-1}. The increment was 23% when the biochar rate doubled from 50 to 100 t ha^{-1}. It was notable that EC values were not only associated with the salts contained in biochar but they were also related to the salts added from the nutrient solution.

Root length was significantly higher in biochar applied plants than in control plants. Root length increased up to 50 t ha^{-1}; thereafter it decreased. There were 26.5%, 54.4% and 22.1% increments in root length from application of 30, 50 and 100 t ha^{-1} biochar over the rate of 10 t ha^{-1}, respectively. Similarly, there were 67%, 111.3%, 157.9% and 103.9% increments in root length by 10, 30, 50 and 100 t ha^{-1} biochar over control, respectively. However, the increment in root length by increasing from 10 to 30 t ha^{-1} was greater (26.5%) as compared to the same increase from 30 to 50 t ha^{-1} (22%). The results indicated that extremely high rates of biochar could be harmful for root development.

Table 4.19 Mean values for soil EC and root length of tomato as influenced by biochar rates. The different letters in the same column indicate significant difference between treatments (N = 9 for biochar application rates and 3 for control) at α=0.05 level of significance. The ± values represent the standard error of the means.

Application rates (t ha^{-1})	EC (dS m^{-1}) (2^{nd} year)	Root length (cm) (2^{nd} year)
10	0.27 ± 0.02d	1538 ± 32.93c
30	0.44 ± 0.02c	1946 ± 47.77b
50	0.66 ± 0.03b	2375 ± 44.50a
100	0.81 ± 0.02a	1878 ± 26.67b
Control	0.23 ± 0.03e	921 ± 55.08d

Mycorrhizal colonization assessment revealed no clear distinction between the biochar rates in first year but significant difference was observed between 10 t ha^{-1} and higher rates in the second year (Table 4.20). All biochar rates were significantly different from the control. On average, the highest colonized percent of root length was observed for 50 t ha^{-1} which was 5.2 times more than control. The colonized percentage of root length at the lowest biochar rate (10 t ha^{-1}) was 3 times more than control. The colonization observed for highest biochar rate (100 t ha^{-1}) indicated that mycorrhizae could colonize even at these rates but there might be some decrease at extremely high rates. The patterns of colonization are shown in Plate 4.9.

The biochar rate of 10 t ha^{-1} produced significantly greater colonized root length than control but yielded lower colonized root length than the other rates in first year (Table 4.21). The rates 30, 50 and 100 t ha^{-1} showed similar colonized root length. In second year, 50 t ha^{-1} produced significantly higher colonized root length than other rates. Among the rates, 30 and 100 t ha^{-1} were similar for length. However, all biochar rates had greater colonized root length than control. The average colonized root length showed that the length for 10, 30, 50 and 100 t ha^{-1} biochar was 5.1, 9.6, 13.9 and 9.98 times greater than control. The increment in colonized root length was lower (45.2%) with the increase from 30 to 50 t ha^{-1} compared to the same increase from 10 to 30 t ha^{-1} (85.7%).

Table 4.20 Mean values for colonized percent of root length of tomato as influenced by biochar rates. Log values are with the per cent in parenthesis. The different letters in the same column indicate significant difference between treatments (*N* = 9 for biochar application rates and 3 for control) at α=0.05 level of significance. The ± values represent the standard error of the means.

Application rates (t ha^{-1})	Colonized % root length		
	1st year	2nd year	Mean
10	0.71 (4.97) ± 0.10b	0.95 (9.06) ± 0.02b	7.02
30	0.94 (7.90) ± 0.03ab	1.06 (11.53) ± 0.02a	9.72
50	1.11 (11.98) ± 0.02a	1.10 (12.80) ± 0.01a	12.39
100	0.97 (8.54) ± 0.02a	1.10 (12.61) ± 0.02a	10.58
Control	0.24 (1.44) ± 0.24c	0.50 (3.30) ± 0.10c	2.37

Interaction effects

There was no significant difference between the interactions of biochar type and rates for most of the parameters. However, all interactions had greater effect than the control. The difference was observed for plant height in 5th, 6th and 7th week after planting (Table 4.21, Table 4.22) but no conclusive results could be drawn due to the overlapping grouping of treatment means. Mean values for shoot dry weight and soil pH showed that interactions were superior to control for the first year (Table 4.23). In the second year, significant differences for shoot fresh weight and soil EC occurred (Table 4.24) but, again, due to overlapping grouping of the means, no conclusion could be drawn. There was still a great difference between the interactions and control for shoot fresh weight indicating that any biochar could give higher values than the control. As these results were only significant for a single year, they need to be verified in further trials.

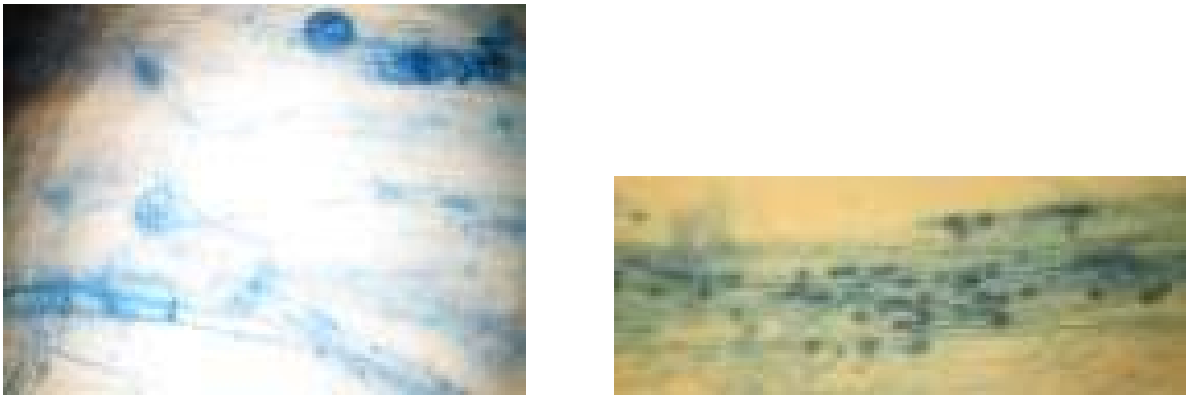


Plate 4.9 Patterns of mycorrhizal colonization of tomato showing network of vesicles and hyphae colonized in roots magnified at 40x left, 20x right.

Table 4.21 Mean values for weekly plant height of tomato as influenced by the interactions of biochar types and application rates in the first year. The different letters in the same column indicate significant difference between treatments (N = 3) at α=0.05 level of significance. The ± values represent the standard error of the means.

Biochar types	Biochar rates (t ha^{-1})	Plant height (cm) in weeks (1^{st} year)		
		Fifth	Sixth	Seventh
Sugarcane trash	10	13.9 ± 1.82bc	21.2 ± 2.74abc	25.5 ± 2.08bcd
	30	23.6 ± 0.64a	31.0 ± 0.39ab	34.5 ± 1.26a
	50	20.0 ± 1.30ab	27.1 ± 2.83abc	31.8 ± 1.80abc
	100	10.6 ± 1.74c	17.2 ± 3.25c	22.3 ± 2.68cd
Green waste A	10	14.8 ± 2.67bc	20.7 ± 2.90abc	23.3 ± 1.20d
	30	18.9 ± 1.47abc	27.5 ± 1.40abc	31.0 ± 1.00abcd
	50	24.1 ± 0.95a	31.9 ± 0.55a	35.6 ± 0.55a
	100	21.2 ± 1.82ab	28.5 ± 1.89abc	32.7 ± 1.33ab
Green waste B	10	14.6 ± 2.21bc	19.5 ± 2.951bc	24.2 ± 2.89bcd
	30	19.5 ± 0.65ab	26.3 ± 2.92abc	32.0 ± 2.02abc
	50	23.8 ± 1.17a	31.2 ± 0.35a	35.5 ± 0.87a
	100	19.7 ± 1.72ab	27.0 ± 1.89abc	31.5 ± 1.76abc
Control		9.4 ± 1.05c	13.1 ± 1.18d	16.5 ± 1.26e

Table 4.22 Mean values for weekly plant height of tomato as influenced by the interactions of biochar types and application rates in the second year. The different letters in the same column indicate significant difference between treatments (N = 3) at α=0.05 level of significance. The ± values represent the standard error of the means.

Biochar types	Biochar rates (t ha^{-1})	Plant height (cm) in weeks (2^{nd} year)		
		Fifth	Sixth	Seventh
Sugarcane trash	10	24.8 ± 0.44c	31.0 ± 2.08e	34.1 ± 0.44e
	30	46.3 ± 2.19ab	54.7 ± 3.33abc	59.8 ± 2.19abc
	50	48.7 ± 2.03ab	60.3 ± 0.88a	65.2 ± 2.03a
	100	44.3 ± 0.67ab	51.0 ± 1.53abcd	54.8 ± 0.66bcd
Green waste A	10	38.0 ± 1.73ab	44.3 ± 0.67d	47.7 ± 1.73d
	30	41.3 ± 0.67ab	49.3 ± 1.33bcd	53.7 ± 0.66bcd
	50	42.3 ± 6.49ab	60.3 ± 0.33a	63.7 ± 6.49ab
	100	41.7 ± 1.67ab	50.7 ± 3.18abcd	53.5 ± 1.67bcd
Green waste B	10	35.7 ± 1.20bc	43.3 ± 2.40d	45.7 ± 1.20d
	30	37.3 ± 4.33abc	45.0 ± 2.89cd	47.6 ± 4.34d
	50	49.0 ± 0.58a	58.3 ± 1.67ab	61.4 ± 0.58abc
	100	41.7 ± 0.88ab	50.7 ± 0.67abcd	53.1 ± 0.88cd
Control		20.2 ± 3.22c	22.8 ± 4.64f	25.1 ± 3.92f

Table 4.23 Mean values for shoot dry weight of tomato and soil pH as influenced by the interactions of biochar types and application rates in the first year. The different letters in the same column indicate significant difference between treatments (N = 3) at α=0.05 level of significance. The ± values represent the standard error of the means.

Biochar types	Biochar rates ($t\ ha^{-1}$)	Shoot dry weight (g)	pH
Sugarcane trash	10	5.05 ± 0.97b	6.83 ± 0.09d
	30	11.27 ± 0.37ab	7.00 ± 0.10cd
	50	9.87 ± 1.37ab	7.10 ± 0.06cd
	100	4.82 ± 1.37b	7.40 ± 0.12bcd
Green waste A	10	5.48 ± 0.72b	7.03 ± 0.09cd
	30	8.15 ± 0.35ab	7.17 ± 0.09cd
	50	14.17 ± 1.10a	7.43 ± 0.18bcd
	100	10.57 ± 2.29ab	7.70 ± 0.12bc
Green waste B	10	4.67 ± 0.86b	7.00 ± 0.17cd
	30	7.67 ± 0.31ab	7.50 ± 0.10bcd
	50	10.88 ± 2.24ab	8.07 ± 0.45ab
	100	9.34 ± 1.81ab	8.63 ± 0.03a
Control		2.52 ± 0.94c	7.00 ± 0.06c

Table 4.24 Mean values for shoot fresh weight of tomato and soil EC as influenced by the interactions of biochar types and application rates in the second year. The different letters in the same column indicate significant difference between treatments (N = 3) at α=0.05 level of significance. The ± values represent the standard error of the means.

Biochar types	Biochar rates (t ha^{-1})	Shoot fresh weight (g)	EC (dS m^{-1})
Sugarcane trash	10	20.50 ± 1.72e	0.27 ± 0.03f
	30	68.37 ± 7.58abc	0.37 ± 0.07ef
	50	86.67 ± 4.12a	0.63 ± 0.03bc
	100	76.73 ± 4.45ab	0.66 ± 0.03bc
Green waste A	10	39.80 ± 4.87de	0.27 ± 0.03f
	30	46.27 ± 6.65cd	0.57 ± 0.03cde
	50	69.17 ± 2.27abc	0.73 ± 0.07bc
	100	75.33 ± 2.21ab	0.80 ± 0.06ab
Green waste B	10	36.17 ± 2.73de	0.27 ± 0.03f
	30	54.93 ± 7.95bcd	0.40 ± 0.00def
	50	73.70 ± 3.75ab	0.60 ± 0.06bcd
	100	73.3 ± 3.19ab	0.97 ± 0.03a
Control		11.93 ± 2.15f	0.23 ± 0.03f

4.5 Discussion

Parameters with non-significant results have not been shown in Tables and Figures but it was notable that biochar type, application rates and their interactions were significantly different from control for both crops. This confirms results of previous experiments on lettuce and cabbage that application of biochar is beneficial for plant growth.

P and Zn were analyzed from plant and soil samples but there were no significant differences. Application of Hoagland's nutrient solution added equal amounts of nutrients but the amount of leachate was unknown. Therefore, the actual amount of the nutrients supplied by biochar and the solution separately could not be detected.

Biochar types were similar for their effect on observed parameters except for a few cases. The main reason could be that nutrient requirement was fulfilled by Hoagland's solution. There was no

interaction of these chars with soil so that no decomposition and release of inherent nutrients could be expected.

There was no difference between biochar types for plant height in the first year. It was associated with less effective growth environment. In the second year, the plants were grown in a controlled glass house with greater light availability for photosynthesis. In the first year, trials were set up in a small bay of a glasshouse where shading effect was prevalent with comparatively low temperature and less light.

The variation in colonization over two years may have also been related to environmental differences. The previous report (Guadarrama & Álvarez-Sánchez 1999) confirmed that the mycorrhizal abundance was greater in the dry season than the rainy season. In the present case the environment in the glasshouse was drier in the second year than that of the glasshouse of the first year because there was shade and more moisture due to less evaporation in the first year.

The soil EC and pH values were not solely dependent upon biochar content. Salts were added by Hoagland's solution. The amount of nutrients and cations leached due to irrigation flush following nutrient application was not measured; therefore, it could not be determined what the contribution of biochars was to EC and pH.

The great difference in biochar application rates for the results over the two years was mainly due to difference in light and temperature. In the first year, the temperature range was 17-33^{0}C in the first week of planting and 12-19^{0}C in the last week of observation. In the second year, the plants were grown under a constant range of temperature (28-30^{0}C).

The trend for plant height was similar to previous research, for example, the increase in height of tomato and pepper due to biochar amendment in soil-less medium was also reported by Graber et al. (2010). To view the real effect of biochars, it may take some years as no significant effect of application rates was observed on plant growth in the first and second years of application but it was significant in the third year when it was applied at 0, 25 and 50 t ha^{-1} (Jones et al. 2012). In the present experiment, similar height due to different rates in initial weeks was found which indicated the slowly decomposing characteristics of biochar. More likely it takes time for differences to be expressed.

The lower plant height at > 30 t ha^{-1} in onion and 50 t ha^{-1} in tomato indicated some negative effect of biochar on plant growth at high rates. This effect could be due to increased stress from accumulation of salts on the surface of biochar applied at higher rates.

Shoot fresh and dry weight of onion were similar for 50 and 100 t ha^{-1} of each biochar indicating tolerance of onion. There was significant increase in crop productivity when biochar was applied at 10, 25, 50 and 100 t ha^{-1} (Jeffery et al. 2011). Significant increase (up to 140%) in maize yield was found for biochar applied at 20 t ha^{-1} two years after application (Major et al. 2010). Yield increase compared to control was 42% at 10 t ha^{-1} and 96% at 50 t ha^{-1} (Chan et al. 2008b). Biochar at 30 t ha^{-1} also had significant effect on increasing grain yield, above ground biomass and dry matter in durum wheat (Vaccari et al. 2011). In the present study, trials were too short for final yield harvest but shoot yield was significantly influenced by application rates. Root fresh weight of onion was highest when 30 t ha^{-1} of biochar was applied. This result indicated optimum development of roots at that rate. Effect of biochar on root biomass and length was significant in the case of rice (Noguera et al. 2010).

The effect of biochars on soil pH was greater in the tomato grown medium than onion. The reason for similarity in pH among biochars in onion grown medium was unclear; however it could be associated with the lower absorption of cations by onion roots than tomato. Sugarcane trash biochar had a lower pH as it had lower (0.66%) acid neutralizing capacity (Apendix 1) than the other chars.

Variation in mycorrhizal colonization might be linked with germinability of spores and ability to colonize in biochar amended sand as well as capacity of plant roots to provide entry for mycorrhizae. Mycorrhizal colonization was denser in onion than in tomato indicating difference in colonization pattern of species. While onion, sweet potato, tomato and cassava are highly dependent on mycorrhizae (Khasa et al. 1992), their percentage of colonization may differ. In the present study, highest colonization of onion was found to be ~21% while it was only ~12% for tomato. This pattern of colonization may also be associated with the feedstock source of biochar and the fertility status of soil. For example, colonization of potato, oat, sunflower, mungbean, wheat, chickpea, berseem, barley, alfalfa, nursery rice and tobacco was less in fertile soils than in marginal soils (Sharif and Moawad 2006). Colonization might well increase if the crop growing period was extended beyond the short period (seven weeks) here.

The nutrient concentrations (especially P) in Sugarcane Trash biochar were higher than other selected biochars which contributed to the deficient sand medium greatly for supplying essential

nutrient for sustaining mycorrhizae. As the sand was irrigated alternately with nutrient solution and water, Sugarcane Trash biochar held enough nutrients for mycorrhizal development in nutrient deficient medium. The lower pH of Sugarcane Trash compared with the other two biochars may have contributed significantly to nutrient availability, plant growth and colonization. As far as the effect of application rates is concerned, colonized per cent of root length of tomato was increased with increasing the dose and the highest effect was observed at 50 t ha^{-1}, while effect of 100 t ha^{-1} decreased and was similar to that for 30 t ha^{-1}. The possible mechanism behind this phenomenon could be that the increased sand pH with increased level of biochar (beyond 50 t ha^{-1}) was less favourable for mycorrhizal development than the other rates. There might also be some negative effects of accumulated salts on mycorrhizae due to the high dose of biochar (100 t ha^{-1}) that elevated the EC level.

Although biochar treatments resulted in increased mycorrhizal colonization, no significant differences in P and Zn uptake were detected perhaps due to effects of applying Hoagland's solution with 25% P. However, it is very important to try to determine the contribution of mycorrhizae to nutrient uptake in the presence of biochar. This experiment was conducted in sand medium necessitating addition of nutrient solution; therefore, further experiments are needed in various soils to discover the optimum rate of a particular biochar for plant growth and mycorrhizal colonization. As the purpose of the present experiments was to detect the influence of biochar on colonization of onion and tomato roots by AM fungi, these were of short duration. However, effects of longer term treatments in field trials are required.

Plant growth and colonization were relatively better at high biochar rates (30 and 50 t ha^{-1}) but economy of these rates may be questioned. These rates were appropriate for soil-less sand medium; however, soils with higher nutritional status may require lower rates.

The interactions showed no significant differences. The most obvious cause was that biochars performed similarly; however, they were derived from different feedstock and processes. To see interaction effects, the trial should be conducted long-term in the same medium and environment.

4.6 Conclusion

Use of biochar as soil amendment was beneficial for growth parameters such as plant height, shoot fresh weight, shoot dry weight and root fresh weight of onion and tomato. Biochars were also beneficial for enhancing soil pH, electrical conductivity and mycorrhizal colonization. All biochars had similar effects on growth parameters when compared at the same rates. Onion roots had more

colonization than tomato. Among the application rates, 30 t ha^{-1} of each biochar had better effect on onion while 50 t ha^{-1} was more effective on tomato in terms of morphological growth and colonization pattern of roots. The experiment was conducted in sand medium and needs to be verified in different soils to address an agro-economically feasible type and application rate of biochar.

Another important consideration is how soil contaminated with heavy metals affects colonization of the crop by the fungi in presence of biochar. The results after the addition of Zn and Cu to biochar amended soil will be discussed in the upcoming chapter.

Chapter 5. Zinc and copper effects on growth and mycorrhizal colonization of onion in biochar amended soil

5.1 Abstract

Three Zn rates, 3 Cu rates and two mycorrhizal inoculation rates were tested along with three controls in a 3x3x2+3 factorial arrangement as a pot trial to find out their effect on growth and colonization of onion in a biochar-added calcareous soil. The experiment consisted of 21 treatments with 4 replications. The pH of alkaline calcareous soil was maintained under neutrality and Sugarcane Trash biochar added at a rate of 30 t ha^{-1} before planting. Growth parameters, uptake of Zn and Cu and mycorrhizal colonization were recorded for analysis. One Way ANOVA for overall effect, General Linear Model for factorial analysis and combined ANOVA for complete effect were applied. The results showed the best positive effect of biochar plus mycorrhizae on all recorded parameters compared to other treatments. Biochar application was more effective than no biochar. Among the Zn and Cu rates, combination of lower rates of 50 mg kg^{-1} of each nutrient had better effect than the other combinations.

5.2 Introduction

Heavy metals are a great concern for their effects on microbial growth and behaviour. Among microbes, arbuscular mycorrhizal fungi are considered as one of the most important symbionts that enhance uptake of some immobile nutrients and still they are under study. Their responses to heavy metals have been studied and many promising results indicating their specific effect on particular AMF species have been found. For example, *Glomus etunicatum* was more sensitive to Cd, Pb and Zn than was *G. intraradices* (Pawlowska & Charvat 2004). Similarly, *Glomus sps* and *Glomus mosseae* were more sensitive than was *Glomus claroideum* (del Val et al. 1999).

Infection of onions with *Glomus mosseae* was reduced when Zn, Cu, Ni or Cd were added to soil medium (Gildon & Tinker 1983). Infection rates of *Glomus caledonium* were the highest but sporulating ability was the poorest among three AMF species when tested for response to heavy metals (Cu and Cd) (Liao et al. 2003). In a separate experiment, *Glomus lamellosum, Glomus intraradices* and *Glmus proliferum* exhibited tolerance to 5 ppm lead (Khade & Adholeya 2008). *Glomus intraradices* showed a heavy metal tolerance in a variety of plants in soils with diverse heavy metals under optimum fertilization (Hildebrandt et al. 1999; Kaldorf et al. 1999).

However, some reports indicated that high concentrations of heavy metals have adverse effect on AMF (Leyval et al. 1997). Similarly, heavy metal tolerance also depends on plant species which can cope with adverse effects of metals; these plants are called metallophytes (Hildebrandt et al. 2007). Protection by AMF that colonize plant roots and reduce the uptake of heavy metals into plant cells could be a mechanism that allows metallophytes to thrive on polluted soils (Weissenhorn et al. 1995; Leyval et al. 1997; Kaldorf et al. 1999; Berreck & Haselwandter 2001; Ouziad et al. 2005; Vogel-Mikus et al. 2005).

Maize grown in heavy metal soils had more essential elements such as K, P, Mg but fewer heavy metals such as Ni, Fe, Zn, or Cu when symbiotically grown with *Glomus intraradices* (Kaldorf et al. 1999) possibly indicating the mycorrhizal species-specific screening of metals.

Heavy metals also have a specific effect on different phases of AMF development. Spores and presymbiotic hyphae are generally sensitive to heavy metals in absence of plants (Göhre & Paszkowski 2006). EC_{50} values (effective concentration reducing germination or hyphal growth by 50%) vary with strain, but overall effect of heavy metals such as Zn, Pb and Cd was negative; however, spores from polluted soils were more tolerant than the spores from non-polluted soils (Shalaby 2003) indicating adaptation of strains in contaminated environments.

The interaction of heavy metals themselves can play a role on the degree of sensitivity of spores and hyphae to heavy metals. For instance, Zn plays an antagonistic role on toxicity of Pb and/or Cd on pre-symbiotic hyphal growth, while Pb and Cd acted synergistically (Shalaby 2003). In soils with 8% Zn and 863 µg g^{-1} Cd, 35% of clover roots were colonized (Gildon & Tinker 1981).

In fact, AMF can decrease Zn toxicity to grasses growing in Zn-polluted soils (Dueck et al. 1986). Similarly, colonization of AMF in *Agrostis capillaris* was significantly higher in Zn and Cd-polluted soils (Griffioen et al. 1994). However, infection was lower in Zn and Pb polluted soil than in less polluted soils (Diaz & Honrubia 1993).

Some authors (Hildebrandt et al. 1999; Audet & Charest 2006) have proposed that mycorrhizal colonization of roots increased with increasing heavy metals in soils, but others (Gildon & Tinker 1981; Graham et al. 1986; McGee 1987; Chao & Wang 1991) indicated some inhibition of AMF colonization by them. In fact, the majority of reports suggest that mycorrhizae have some degree of metal tolerance.

Degree of tolerance may be governed by several physiological mechanisms, for example, expression of genes involved in heavy metal tolerance (Repetto et al. 2003; Rivera-Becerril et al. 2005). A metallothionein (a metal-binding protein) gene of *Gigaspora margarita* (BEG 34) is regulated in symbiotic mycelia by Cu (Lanfranco et al. 2002) and Zn-transporter gene (ZintZnT1) of *Glomus intraradices* is harmonized by short and long term exposure to Zn indicating protection against Zn stress (Gonzalez-Guerrero et al. 2005). Similarly, ABC transporter gene of *Glomus intraradices* depends on Cd and Cu (González-Guerrero et al. 2006). Therefore, symbiotic mycorrhizal cells could cope with heavy metal-induced oxidative stress (Ouziad et al. 2005). However, heavy metal toxicity alleviation by AMF varies with metal types, their concentrations, symbiotic partners and plant growth conditions (Weissenhorn et al. 1995; Leyval et al. 1997; Hildebrandt et al. 1999; Turnau & Mesjasz-Przybylowicz 2003).

A metal binding mechanism has been illustrated in two processes: fungi release glomalin in soil that binds metals outside the rhizosphere (Gonzalez-Chavez et al. 2004; Göhre & Paszkowski 2006) or metals are bound to chitin of hyphal cell walls that reduces their local concentrations in the soil (Zhou 1999; Göhre & Paszkowski 2006). On average, 28 mg Cu per gram of glomalin is sequestered by *Gigaspora rosea* (Joner et al. 2000) while up to 0.5 mg Cd is bound per mg biomass of fungal hyphae (Joner et al. 2000). Gonzalez-Chavez et al. (2004) found that up to 4.3 mg Cu, 0.08 mg Cd and 1.12 mg Pb can be extracted from a gram of glomalin.

Several heavy metals have been found in biochar but concentrations vary. For example, biochars used in this study contained aluminium, arsenic, cadmium, cobalt, chromium, copper, nickel, lead, selenium, zinc and others (Kochanek et al. 2014) but zinc and copper were selected for this study.

The effect of heavy metals and biochar amended soil on colonization of plant roots by AMF has not been adequately studied. To discover the extent of colonization, this study describes the influence of biochar on the colonization of onion roots by AM fungi in Zn and Cu amended low fertility soil.

5.3 Materials and methods

5.3.1 Experimental Site and Environment

The trials were conducted in winter season in 2012 and 2013 in a glasshouse at the University of Queensland, Gatton Campus, Australia. The first year trials were conducted in a bay of a small glasshouse with fluctuating temperatures and light. There was shading effect from shade cloth on the roof and other adjacent bays. The daily temperature range was 17-33^{0}C during the first week of planting and 11-19^{0}C during the last week of observation. Later trials were conducted in a large

glasshouse where the plants had full access to sunlight and temperature was controlled within a fixed range.

5.3.2 Biochar type

Sugarcane Trash biochar was used as a soil amendment at a rate of 30 t ha^{-1}.

5.3.3 Mycorrhizal inoculum and species

The inoculum described in Chapter 4 was also used in this study.

Seed source and seedlings

Onion (variety: Rio Red Rock) seeds were prepared as described in Chapter 4.

To ensure optimum mycorrhizal colonization, plants were allowed to grow for six weeks and then cut at ground level and seedlings of the same onion variety produced by the same procedure were planted. This was done in both years. Observations were recorded on the second crop.

5.3.4 Soil source and characteristics

Soil with low P and Zn was collected from Felton, Queensland from the top 10 cm surface layer and autoclaved at 121^0C with a pressure of 105 kPa for an hour to sterilize it. This autoclaving system was available at preparation room of soil science laboratory at the University of Queensland, Gatton Campus. EC, P, Zn and Cu content of this soil reported by Phosyn Analytical and Yara Megalab were 0.18 dS m^{-1}, 4 ppm (Olsen), 0.4 ppm (DTPA) and 1.8 ppm (DTPA), respectively. As the soil was calcareous alkaline (pH 9), elemental sulphur was added at 100 g m^{-3} to reduce pH to nearly neutral. Soil was kept moist for two weeks to enhance the reaction of sulphur. Soil of 1 kg per pot was used.

5.3.5 Biochar, Zn and Cu calculation and mixing

In the exploratory experiment in Chapter 3.1, Green Waste biochar at a rate of 30 t ha^{-1} was the best for plant growth. In cabbage and tomato, the higher rates were effective. Consideration was given to the fact that the greater amount of phosphorus contained in Sugarcane Trash biochar would reduce the mycorrhizal colonization at higher rates. High rates also increased soil pH in previous experiments which might be unfavourable for colonization of AMF and also would be expensive from an economic point of view, therefore the rate of 30 t ha^{-1} was selected for this trial. Sugarcane Trash biochar at 30 t ha^{-1} (25 g kg^{-1} of soil which is equivalent to 2.5% w/w) was mixed in the soil. Characteristics of this biochar are discussed in Chapter 4. Amounts of Zn sulphate heptahydrate ($ZnSO_4.7H_2O$) and Cu sulphate pentahydrate ($CuSO_4.5H_2O$) were calculated on the basis of Zn (22.7%) and Cu (32%) requirement for treatments of 50, 500 and 1000 mg kg^{-1}. Amounts of these

metals in soil and biochar were considered as negligible. The calculated amounts of biochar, Zn and Cu were added to the soil using a mixer as described in Chapter 4.

5.3.6 Pots and nutrients

Plastic pots of 1.6 L volume (12 cm diameter) were used. Nutrients were supplied through modified Hoagland's solution (Hoagland and Arnon 1950; Mattson and Lieth 2008; Epstein and Bloom 2004). This solution was modified with 25% phosphorus to minimize the negative effect on mycorrhizal infection and was applied at 50 mL per pot only once after planting of the first and second crops.

5.3.7 Experimental design and treatments

A factorial arrangement (3 x 3 x 2 + 3; Zn, Cu and mycorrhizae, respectively + 3 extra treatments) (Table 5.1) was applied for growth parameters and nutrients while 3 x 3 +1, Zn (3 levels), Cu (3 levels) and a control was used for colonization data (Table 5.2). Thus there were 21 treatments for growth and nutrient data and 10 treatments for colonization study.

This trial design for growth and nutrients can be described as a 3 x 3 x 2 factorial + 3. The data were analyzed as a simple treatment structure. Assuming the design as completely randomized (not blocked), with no missing data, the ANOVA was like Analysis 1 as given in Table 5.3.

Means and standard errors from this were used for further illustrations. This gave an overall test of treatment, but did not give information about the specific effects of the factors (Zn, Cu and mycorrhiza and interactions). Therefore, the factorial subset was analysed by General Linear Model of Minitab 16, version 4.0 (Minitab 2005) to get results as Analysis 2 as given in Table 5.4.

Table 5.1 Trial design for growth parameters and nutrients

Treatment combinations	Factor A (Zn Levels, mg kg^{-1})	Factor B (Cu levels, mg kg^{-1})	Factor C (Mycorrhiza, g kg^{-1})
1	50	50	10
2	50	50	0
3	50	500	10
4	50	500	0
5	50	1000	10
6	50	1000	0
7	500	50	10
8	500	50	0
9	500	500	10
10	500	500	0
11	500	1000	10
12	500	1000	0
13	1000	50	10
14	1000	50	0
15	1000	500	10
16	1000	500	0
17	1000	1000	10
18	1000	1000	0
19	Soil only		
20	Soil + Biochar		
21	Soil + Biochar		10

Table 5.2 Trial design for mycorrhizal colonization data

Treatment combinations	Factor A (Zn, mg kg^{-1})	Factor B (Cu, mg kg^{-1})
1	50	50
2	50	500
3	50	1000
4	500	50
5	500	500
6	500	1000
7	1000	50
8	1000	500
9	1000	1000
10	Soil + Biochar + mycorrhiza	

Table 5.3 Analysis of variance for Analysis 1

Source	df
Treatment	20
Error	42
Total	62

Table 5.4 Analysis of variance obtained from General Linear Model for Analysis 2

Source	df
Zn	2
Cu	2
Zn*Cu	4
Mycorr	1
Zn*Mycorr	2
Cu*Mycorr	2
Zn*Cu*Myc	4
(total factorial)	17
Error	36
Total	53

Table 5.5 Analysis of variance for analysis 3 (Combined for growth parameters and nutrients)

Source	df	ss	ms
Overall treatment	20	from (1)	
Zn	2	from (2)	
Cu	2	from (2)	
Zn*Cu	4	from (2)	
Mycorr	1	from (2)	
Zn*Mycorr	2	from (2)	
Cu*Mycorr	2	from (2)	
Zn*Cu*Myc	4	from (2)	
(total factorial)	17		
'Extra treatments'	3	(by subtraction)	SS/df
Error	42	from (1)	
Total	62	from (1)	

Table 5.6 Combined ANOVA for colonization data (Process of (1) and (2) is similar to Analysis 1 and 2 above after excluding factor 3, Mycorrhiza)

Source	df	ss	ms
Overall treatment	9	from (1)	
Zn	2	from (2)	
Cu	2	from (2)	
Zn*Cu	4	from (2)	
(total factorial)	8		
'Extra treatment'	1	(by subtraction)	SS/df
Error	20	from (1)	
Total	29	from (1)	

Finally, these two analyses were combined because the second analysis gave correct degrees of freedom, sums of squares and mean squares for the factorial part, but an incorrect error term and total term (it did not use all the data). The first analysis had the correct error and total terms, but had not split the variation up fully. It was obtained by manipulating the sum of squares from both analysis as Treatment SS from (1) – Total factorial SS from (2) = SS for extra treatments (with 20-17 = 3 degrees of freedom). Then factorial SS from (2) and the error and total from (1) were taken

to get the following combined ANOVA (Table 5.5). For colonization data, ANOVA was as described in Table 5.6.

5.3.8 Mycorrhizal care and Analysis

The procedure for mycorrhizal care and analysis was the same as given in Chapter 4.

5.3.9 Nutrient analysis

Nutrients were analysed in the Chemical Analysis Laboratory, St Lucia Campus of the University of Queensland. Methods were taken from different references mentioned in Appendix 5-9.

5.3.10 Statistical analysis

Observations were recorded on weekly plant height for six weeks, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, Zn, Cu and P content of plant tissue, and electrical conductivity, pH, Zn and Cu content of soil. Root length, percent of root length colonized and colonized root length were also observed.

Plant height was recorded between the soil level and tip of the longest upright leaf. Shoot fresh weight was recorded immediately after harvest while shoot dry weight was determined by drying at 65^0C for two weeks.

The bulk of roots was washed with tap water and cleaned by removing all traces of soil and biochar. The roots were then air dried on a bed of thick tissue paper to absorb the external moisture. Then the fresh weight of roots was taken on an electronic balance that measured to three decimal places.

Root length was determined by WinRhizo software and Epson 1680 modified flatbed scanner (Régent Instruments Inc., Québec, CA). Mycorrhizal colonization was recorded as recommended by Brundrett et al. (1996) and described in Chapter 4.

Statistical analyses were undertaken in Minitab 16, version 4.0 (Minitab 2005). Comparisons of means were made by Tukey's Pair comparison test in MiniTab.

5.4 Results

5.4.1 Effect of Zn rates

Zn rates were significantly different for plant height up to six weeks of growth in both years of observation (Table 5.7). Plant height was greater in the second year than the first year of observation due to the effect of Zn. In every week, plant height decreased as the rate of Zn was increased indicating the negative effect of high Zn rates on plant growth. The height of onion plants

in the sixth week showed that height was 3.6, 4 and 3.8 cm less in first year, second year and average of two years respectively when Zn rates were increased from 50 to 1000 mg kg^{-1} of soil.

Shoot fresh weights in both years of observation were significantly different for Zn rates (Figure 5.1). The weight increased by 308.8%, 313.6% and 351.8% in the second year compared to the first year from Zn rates of 50, 500 and 1000 mg kg^{-1}, respectively. Shoot fresh weight decreased as rates of Zn increased. Shoot fresh weight decreased by 33.4%, 26.4% and 28.0% in the first year, second year and average of both years when the Zn rates increased from 50 to 1000 mg kg^{-1} of soil.

Statistically, there were significant differences for shoot dry weight for 50 mg and other rates of Zn but values for 500 and 1000 mg kg^{-1} remained similar in both years (Figure 5.2). Shoot dry weight increased by 138.9%, 12.3% and 0.9% due to 50, 500 and 1000 mg kg^{-1} respectively in the second year of observation. Weight decreased by 10.5% in the first year and 62.2% in the second year when rates increased from 50 to 1000 mg kg^{-1}. On average, weight decreased by 46.9% when Zn rate increased from 50 to 1000 mg kg^{-1}.

Significant differences among Zn rates were observed for root length of onion in both years (Figure 5.3A). Root length increased by 3%, 8.9% and 12.9% due to 50, 500 and 1000 mg kg^{-1} respectively in the 2^{nd} year compared to the 1^{st} year. Root length decreased by 39.3%, 33.4% and 36.3% in the 1^{st} year, the 2^{nd} year and on an average respectively when rates increased from 50 to 1000 mg kg^{-1}. Similarly, root fresh weight increased by 47.6%, 36.3% and 27.9% in the 2^{nd} year compared to the 1^{st} year due to rates of 50, 500 and 1000 mg kg^{-1} (Figure 5.3B). Weight decreased by 28.8%, 38.4% and 34.6% in the 1^{st} year, 2^{nd} year and on an average when rates increased from 50 to 1000 mg kg^{-1}.

Soil electrical conductivity was less in the second year than the first year (Table 5.8). In the second year, it decreased by 4.9%, 13.1% and 14.4% in the soil amended with Zn at 50, 500 and 1000 mg kg^{-1}. Increasing rates from 50 to 1000 mg kg^{-1} increased conductivity by 78.5%, 60.6% and 69.5% in the first year, the second year and on an average of two years respectively. However, available soil phosphorus content was significantly increased by Zn rates in the second year only, indicating 17.6% more phosphorus in soil amended with Zn at 1000 mg kg^{-1} than 50 mg kg^{-1} (Table 5.8). Soil Zn content also increased significantly in both years as Zn rates increased. The increment in Zn content was greater in the second year which could be due to less leaching of salts from the pots after irrigation.

Table 5.7 Mean values for plant height of onion as influenced by Zn rates in 1st year, 2nd year and mean of the two years. The different letters in the same column indicate significant difference between treatments (N = 18) at α=0.05 level of significance. The ± values represent the standard error of the means.

Zn rates ($mg\ kg^{-1}$)	Plant height (cm) in weeks														
	2nd week			3rd week			4th week			5th week			6th week		
	1st year	2nd year	Mean	1st year	2nd year	Mean	1st year	2nd year	Mean	1st year	2nd year	Mean	1st year	2nd year	Mean
50	9.7 ± 0.05a	10.8 ± 0.05a	10.3	13.9 ± 0.11a	15.0 ± 0.11a	14.5	19.1 ± 0.29a	20.8 ±. 0.32a	19.9	23.4 ± 0.34a	26.9 ± 0.50a	25.1	27.6 ± 0.39a	29.3 ± 0.49a	28.5
500	8.1 ± 0.06b	9.2 ± 0.11b	8.7	11.6 ± 0.24b	12.6 ± 0.26b	12.1	16.9 ± 0.42b	18.5 ± 0.46b	17.7	21.1 ± 0.43b	24.6 ± 0.60b	22.9	25.2 ± 0.56b	26.8 ± 0.63b	26.0
1000	7.3 ± 0.05c	8.5 ± 0.10c	7.9	10.5 ± 0.11c	11.6 ± 0.11c	11.1	15.6 ± 0.28c	17.1 ± 0.29c	16.3	19.7 ± 0.32c	22.7 ± 0.40c	21.2	24.0 ± 0.49c	25.3 ± 0.56b	24.7

The values within the column that do not follow the same letter are significantly different ($p < 0.05$). The ± values represent the standard error of the means (N = 18).

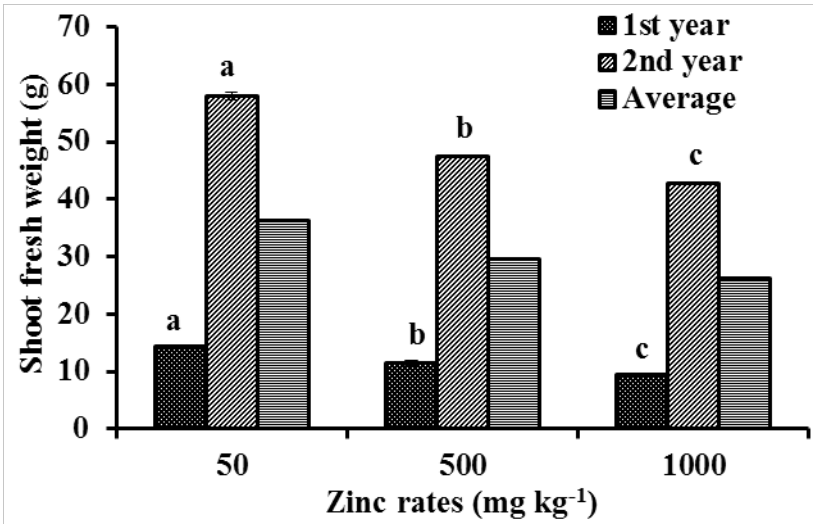


Figure 5.1 Shoot fresh weight of onion as influenced by Zn rates in 1st year, 2nd year and the average of the two years. The different letters in the same series indicate significant difference between treatments (N = 18) at α=0.05 level of significance. The vertical bars represent the standard error of the means.

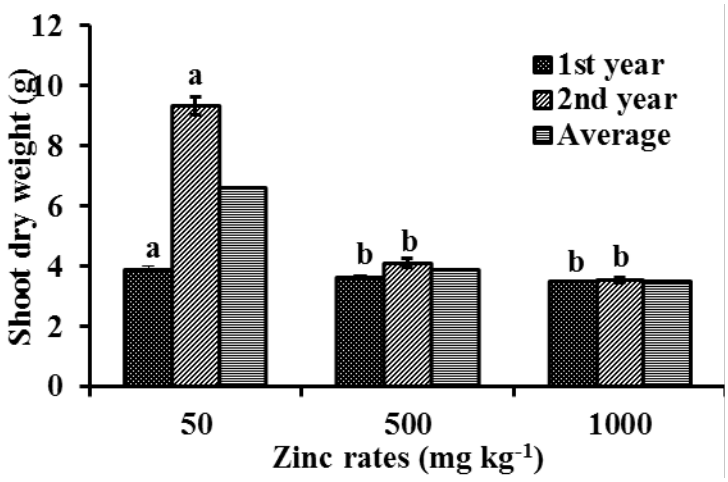
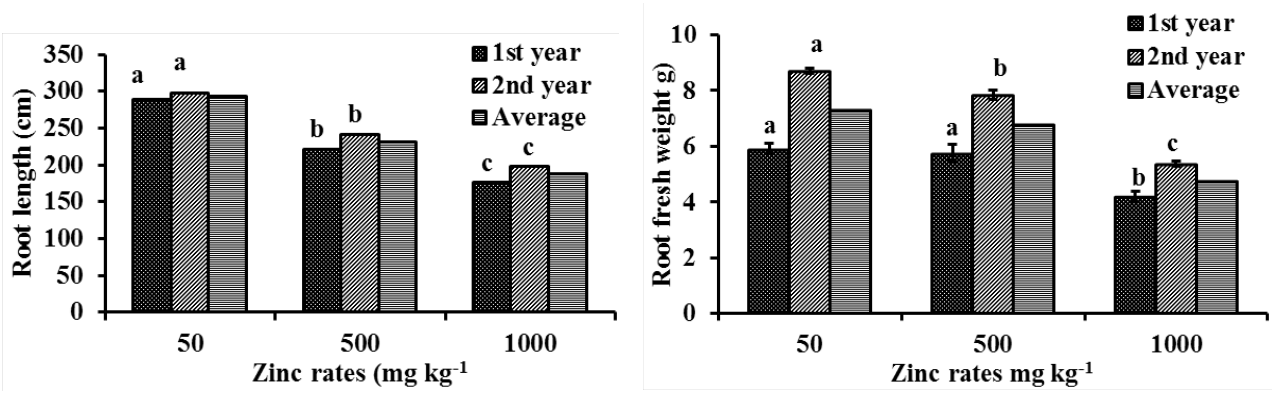


Figure 5.2 Shoot dry weight of onion as influenced by Zn rates in 1st year, 2nd year and the average of the two years. The different letters in the same series indicate significant difference between treatments (N = 18) at α=0.05 level of significance. The vertical bars represent the standard error of the means.



A B

Figure 5.3 A. Root length; B. root fresh weight of onion as influenced by Zn rates in 1st year, 2nd year and mean of two years. The different letters in the same series indicate significant difference between treatments (*N* = 18) at α=0.05 level of significance. The vertical bars represent the standard error of the means.

Table 5.8 Mean values for electrical conductivity, soil P and soil Zn as influenced by Zn rates in 1st year, 2nd year and mean of the two years. The different letters in the same column indicate significant difference between treatments (*N* = 18) at α=0.05 level of significance. The ± values represent the standard error of the means.

Zn rates (mg kg^{-1})	Electrical conductivity (dS m^{-1})			Soil P (mg kg^{-1})	Soil Zn (mg kg^{-1})		
	1st year	2nd year	Mean	2nd year	1st year	2nd year	Mean
50	1.44 ± 0.00c	1.37 ± 0.02c	1.41	3.6 ± 0.04c	14.7 ± 0.19c	15.0 ± 0.21c	14.9
500	2.06 ± 0.00b	1.79 ± 0.01b	1.93	4.1 ± 0.01b	148.7 ± 0.92b	406.6 ± 0.97b	277.7
1000	2.57 ± 0.00a	2.20 ± 0.01a	2.39	4.3 ± 0.02a	284.3 ± 1.19a	804.7 ± 0.60a	544.5

Phosphorus and Zn content in plants were significantly different due to the effect of Zn rates (Table 5.9). Phosphorus content was higher in the soil amended with low rate (50 mg kg^{-1}) of Zn compared to the rates of 500 and 1000 mg kg^{-1} in both years of observation. There was 0.8, 0.6 and 0.7 units less phosphorus content in soil amended with 1000 mg kg^{-1} than with 50 mg kg^{-1} in the first year, the second year and on average, respectively. Decrease in P with higher Zn may be due to inhibition of AM and is supported by reduced colonization. Zn content of plants was also higher in the second

year than in the first year at all levels of Zn (Table 5.6). The Zn content of plants due to 1000 mg kg^{-1} was 5.9, 5.6 and 5.8 times the Zn content due to 50mg kg^{-1} in the first year, the second year and on average, respectively.

Table 5.9 Mean values for plant P and plant Zn as influenced by Zn rates in 1st year, 2nd year and the mean of the two years. The different letters in the same column indicate significant difference between treatments (N = 18) at α=0.05 level of significance. The ± values represent the standard error of the means.

Zn rates (mg kg^{-1})	Plant P (mg kg^{-1})			Plant Zn (mg kg^{-1})		
	1st year	2nd year	Mean	1st year	2nd year	Mean
50	1.6 ± 0.12a	1.4 ± 0.03a	1.5	37.9 ± 0.53c	35.3 ± 0.17c	36.6
500	0.9 ± 0.01b	0.9 ± 0.00b	0.9	88.2 ± 1.20b	92.8 ± 1.16b	90.5
1000	0.8 ± 0.02b	0.8 ± 0.00c	0.8	225.3 ± 0.96a	196.9 ± 0.51a	211.1

Colonized percent of roots by mycorrhizae was greater in the first year than in the second year for all application rates of Zn (Table 5.10). Colonization decreased as the level of Zn increased from 50 to 1000 mg kg^{-1} in both years. The colonized percent of root length in the soil amended with Zn at 50 mg kg^{-1} was 80%, 120% and 92.6% greater than the Zn rate of 1000 mg kg^{-1} in the first year, the second year and on average, respectively.

Table 5.10 Mean values for colonized percent of root length as influenced by Zn rates in 1st year, 2nd year and the mean of the two years. The different letters in the same column indicate significant difference between treatments (N = 18) at α=0.05 level of significance. The ± values represent the standard error of the means.

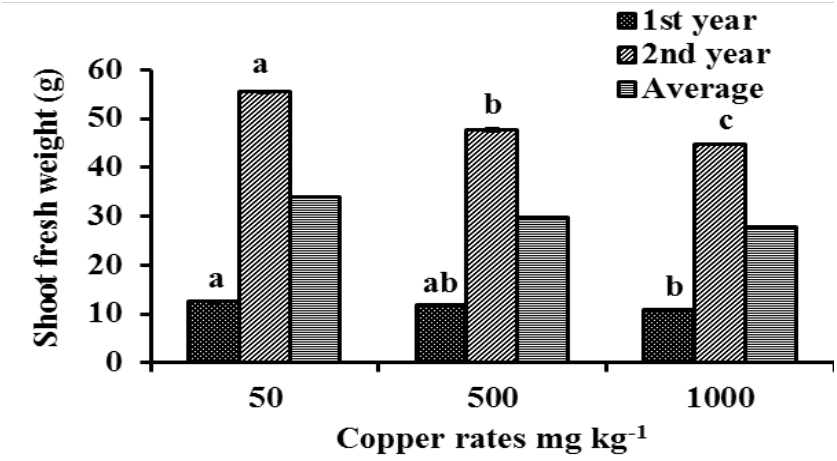
Zn rates (mg kg^{-1})	Colonized % of root length		
	1st year	2nd year	Mean
50	55.6 ± 3.60a	30.8 ± 0.75b	43.2
500	47.8 ± 3.51a	18.1 ± 0.66c	32.9
1000	30.8 ± 3.27b	14.0 ± 0.45c	22.4

5.4.2 Effect of Cu rates

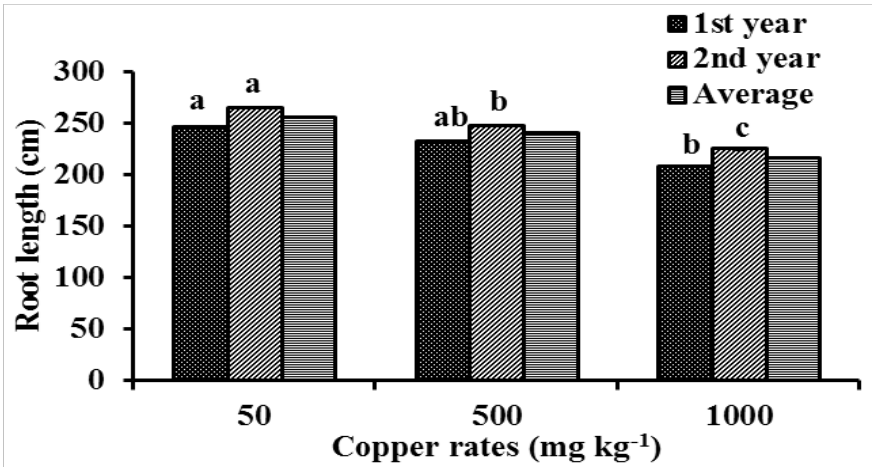
Significant differences ($P < 0.05$) were observed for shoot fresh weight, root length, root fresh weight, electrical conductivity, plant content of P, Zn and Cu, soil content of Zn and Cu, percent of colonized root length and colonized root length for application rates of Cu in both years of

observation. Significant differences were also observed for shoot dry weight and soil phosphorus in the second year.

Shoot fresh weight was greater in the second year compared to the first year (Figure 5.4A). Weight significantly decreased as the rate of Cu increased in both years. Shoot fresh weight decreased by 13.5%, 19.2% and 18.2% in the first year, the second year and on the average respectively when Cu rates were raised from 50 to 1000 mg kg^{-1}. Shoot dry weight reduced by 63.4% as Cu rates rose by 20 times (Table 5.11). Root length decreased by 15.7%, 15.0% and 15.4% in the first year, the second year and on average respectively as Cu rates increased from 50 to 1000 mg kg^{-1} (Figure 5.4B). Similarly, root fresh weight decreased by 17.9% in the first year, 13.3% in the second year and 15.2% on average respectively when the Cu rates increased by 20 times (Figure 5.5).



A



B

Figure 5.4 A. Shoot fresh weight and B. root length of onion as influenced by Cu rates in 1st year, 2nd year and the average of the two years. The different letters in the same series indicate significant difference between treatments (N = 18) at α=0.05 level of significance. The vertical bars represent the standard error of the means.

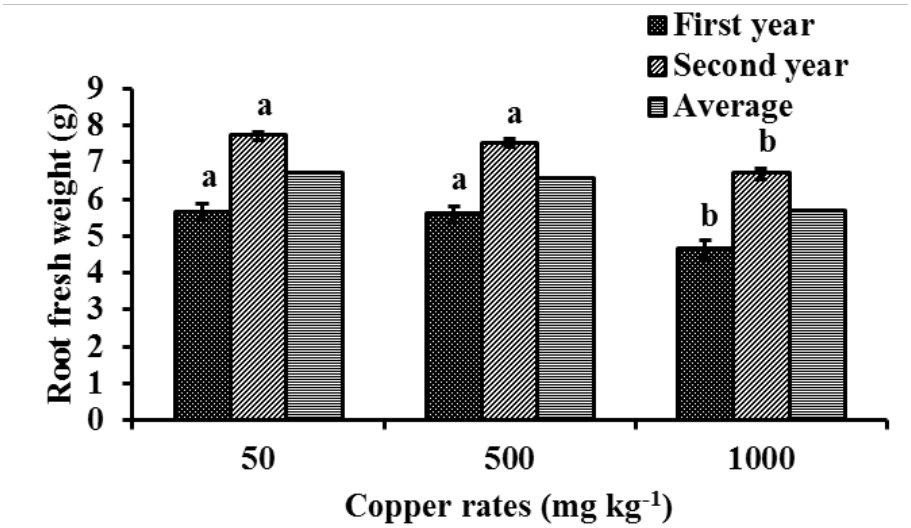


Figure 5.5 Root fresh weight of onion as influenced by Cu rates in 1st year, 2nd year and the average of the two years. The different letters in the same series indicate significant difference between treatments (N = 18) at α=0.05 level of significance. The vertical bars represent the standard error of the means.

Table 5.11 Mean values for shoot dry weight and EC as influenced by Cu rates in 1st year, 2nd year and the mean of the two years. The different letters in the same column indicate significant difference between treatments (N = 18) at α=0.05 level of significance. The ± values represent the standard error of the means.

Cu rates (mg kg^{-1})	Shoot dry weight (g)	EC (dS m^{-1})		
	2nd year	1st year	2nd year	Mean
50	8.94 ± 0.30a	1.42 ± 0.00c	1.35 ± 0.00c	1.39
500	4.75 ± 0.07b	2.07 ± 0.00b	1.85 ± 0.02b	1.96
1000	3.28 ± 0.14c	2.57 ± 0.00a	2.17 ± 0.01a	2.37

Electrical conductivity increased as Cu rates increased (Table 5.11) while P content decreased by 0.5, 0.42 and 0.46 units in the first year, the second year and on average respectively as Cu rates increased from 50 to 1000 mg kg^{-1} (Table 5.12). Zn content of plant increased by 69.1, 7.5 and 30.8 units in first, second years and on average respectively when Cu rates increased by 20 times, indicating a difference between years. High rates of Cu may have inhibited mycorrhizal colonization, thereby reducing uptake of phosphorus. The plant Zn was stimulated by Cu rates in the first year but not in the second year. The reason behind this was unclear. Cu content of plant due to Cu at 1000 mg kg^{-1} was 64.7, 113.3 and 89.0 units greater than the rate of 50 mg kg^{-1} in the first year, second year and on average respectively.

Table 5.12 Mean values for plant P, Zn and Cu of onion as influenced by Cu rates in 1st year, 2nd year and mean of the two years. The different letters in the same column indicate significant difference between treatments (N = 18) at α=0.05 level of significance. The ± values represent the standard error of the means.

Cu rates (mg kg^{-1})	Plant P (mg kg^{-1})			Plant Zn (mg kg^{-1})			Plant Cu (mg kg^{-1})		
	1st year	2nd year	Mean	1st year	2nd year	Mean	1st year	2nd year	Mean
50	1.4 ± 0.10a	1.3 ± 0.03a	1.3	87.0 ± 0.76b	111.5 ± 0.47a	99.3	10.3 ± 0.71c	12.2 ± 0.52b	11.3
500	1.1 ± 0.06b	1.0 ± 0.01b	1.0	108.4 ± 0.79b	109.0 ± 0.43a	108.7	26.7 ± 1.33b	115.7 ± 0.72a	71.6
1000	0.9 ± 0.03b	0.8 ± 0.01c	0.9	156.1 ± 1.20a	104.0 ± 1.11b	130.1	75.0 ± 0.60a	125.5 ± 0.86a	100.2

Soil Zn and Cu increased as Cu rates increased in both years (Table 5.13). Soil Zn was 33.5, 6.0 and 19.7 units more in soil amended with 1000 mg kg^{-1} than with 50 mg kg^{-1} in the first year, the second year and on average, respectively. However, soil Cu was 278.3, 808.8 and 543.6 units greater due to effect of 1000 mg kg^{-1} than 50 mg kg^{-1} in the first year, the second year and average. Soil P in the first year increased by 0.4 units from Cu rate of 1000 mg kg^{-1} compared to 50 mg kg^{-1}. Colonized percent of root length of onion decreased as Cu rates increased (Table 5.14). Colonized percent of root length was 21.4, 10.5, and 16.0 units greater due to 50 mg kg^{-1} compared to 1000 mg kg^{-1} in the first year, the second year and on average respectively.

5.4.3 Interaction effect of Zn and Cu

There were significant differences for shoot fresh weight, shoot dry weight, soil Zn and plant P for interactions of Zn and Cu rates in both years of observation (Figure 5.6, Figure 5.7 and Table 5.15). The differences were also significant for plant Cu in first year, soil P, plant Zn, EC, root length and colonized per cent of root length in the second year. Shoot fresh weight was significantly higher due to the interaction of low rates of Zn and Cu compared to other interactions (Table 5.16). The interactions of Cu rates beyond 50 mg kg^{-1} with higher rates of Zn were similar in the first year while the interactions of 50 mg kg^{-1} of Zn with 50 and 500 mg kg^{-1} Cu had greater fresh weight than the other rates. Fresh weight was greater in the second year than in the first year in all interactions. In the second year, the interaction of 50 and 50 mg of each element and 50 and 500 mg of each element were similar in that they had higher shoot dry weight than the remaining interactions. On average, interactions of lowest rates (50 and 50 mg kg^{-1}) were 3.8 times higher than highest rates (100 and 100 mg kg^{-1}) of both elements.

Table 5.13 Mean values for soil Zn, soil Cu and soil P as influenced by Cu rates in 1st year, 2nd year and mean of the two years. The different letters in the same column indicate significant difference between treatments (N = 18) at α=0.05 level of significance. The ± values represent the standard error of the means.

Cu rates (mg kg^{-1})	Soil Zn (mg kg^{-1})			Soil Cu (mg kg^{-1})			Soil P (mg kg^{-1})
	1st year	2nd year	Mean	1st year	2nd year	Mean	2nd year
50	131.7 ± 0.95c	405.8 ± 0.64c	268.8	22.1 ± 0.78c	38.9 ± 0.66c	30.5	3.8 ± 0.04c
500	150.9 ± 1.13b	408.8 ± 0.50b	279.9	165.7 ± 1.41b	389.6 ± 0.85b	277.7	4.0 ± 0.02b
1000	165.2 ± 0.35a	411.8 ± 0.82a	288.5	300.4 ± 0.72c	847.8 ± 1.01a	574.1	4.2 ± 0.01a

Table 5.14 Mean values for colonized percent of root length of onion as influenced by Cu rates in 1st year, 2nd year and mean of the two years. The different letters in the same column indicate significant difference between treatments (*N* = 9) at α=0.05 level of significance. The ± values represent the standard error of the means.

Cu rates (mg kg^{-1})	Colonized % of root length		
	1st year	2nd year	Mean
50	55.3 ± 2.64a	26.0 ± 0.77a	40.7
500	45.0 ± 3.18ab	21.4 ± 0.42b	33.2
1000	33.9 ± 3.70b	15.5 ± 0.68c	24.7

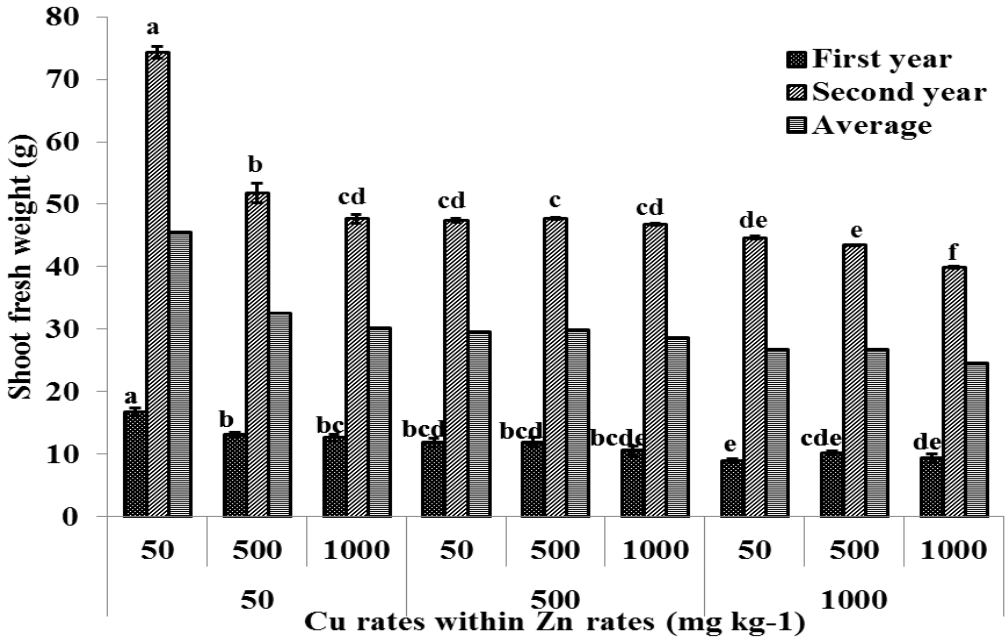


Figure 5.6 Shoot fresh weight of onion as influenced by the interaction of Zn and Cu rates in 1st year, 2nd year and the average of the two years. The inner Cu rates are split under the outer Zn rates. The different letters in the same series indicate significant difference between treatments (N = 6) at α=0.05 level of significance. The vertical bars represent the standard error of the means.

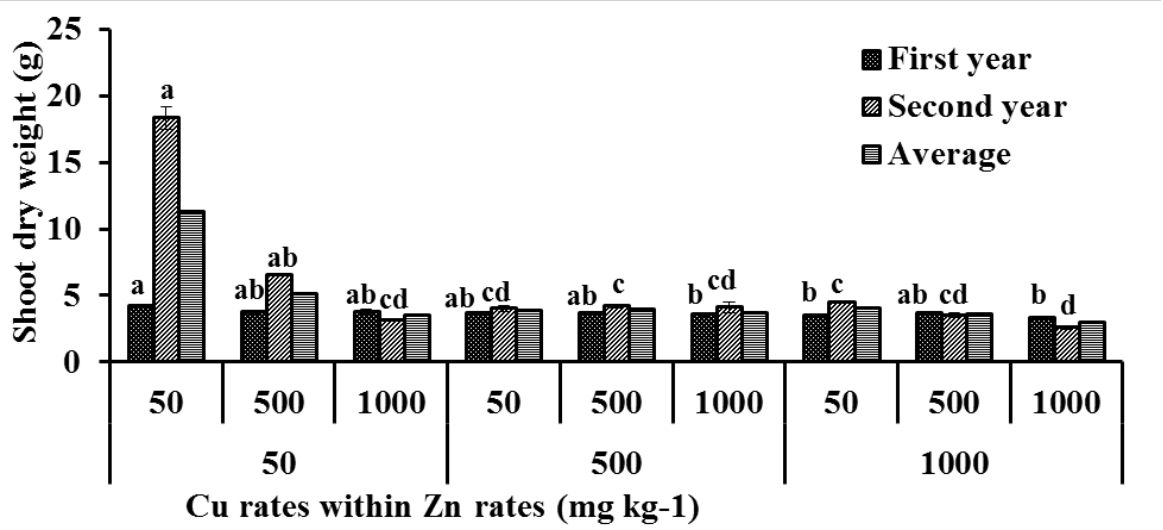


Figure 5.7 Shoot dry weight of onion as influenced by the interaction of Zn and Cu rates in 1st year, 2nd year and the average of the two years. The inner Cu rates are split under the outer Zn rates. The different letters in the same series indicate significant difference between treatments (N = 6) at α=0.05 level of significance. The ± values represent the standard error of the means.

Soil P, plant Zn and soil EC content were higher in soil amended with the higher rates of Zn in the second year; however, the interaction effects overlapped for treatment means. Soil Zn was also higher in soils amended with high rates of Zn and Cu. Plant P was greater for the interaction of low rates of each element. Plant Cu was higher for the interaction of higher rates of each element in the first year. In fact, most of the interaction means of the above-mentioned parameters overlapped statistically; no clear recommendations could be made.

Root length and colonized percent of root length were significantly higher due to the effect of the interactions of low rates of each element in the second year (Table 5.17). Root length was 34% less for the highest rates of each element as compared to their lowest rates. Colonized percent of root length in the interactions of lowest rates (50 mg kg^{-1} of each element) was 3.8 times the length of the highest rates (1000 mg kg^{-1}).

5.4.4 Effect of mycorrhizae

Plant height (Table 5.18), shoot fresh weight (Figure 5.8A), shoot dry weight (Figure 5.8B), and root length, root fresh weight and plant P were significantly different for mycorrhizal rates in both years (Table 5.18). Treatments were also significantly different for soil EC, soil P, soil Zn and plant Zn in the second year (Table 5.19). Mycorrhizal inoculum at 10 g kg^{-1} was found beneficial over no inoculation. In the sixth week of growth, plant height was 5.9, 6.8 and 6.4 units greater in mycorrhizae inoculated plants. Shoot fresh weight of inoculated plants was 65.9% greater than the non-inoculated plants in the first year while it was 9.5% greater in the second year. Average shoot fresh weight increased by 18.4% from mycorrhizae. Shoot dry weight was increased by 16.1%, 15.8% and 15.9%.

Table 5.15 Mean values for soil P, soil Zn and plant P as influenced by the interaction of Zn and Cu rates (mg kg^{-1}) in 1st year, 2nd year and the mean of the two years. The different letters in the same column indicate significant difference between treatments (N = 6) at α=0.05 level of significance. The ± values represent the standard error of the means.

Zn rates (mg kg^{-1})	Cu rates (mg kg^{-1})	Soil P (mg kg^{-1})	Soil Zn (mg kg^{-1})			Plant P (mg kg^{-1})		
		2nd year	1st year	2nd year	Mean	1st year	2nd year	Mean
50	50	3.1 ± 0.10e	13.3 ± 0.30g	13.3 ± 0.19d	13.3	2.3 ± 0.30a	1.9 ± 0.10a	2.1
	500	3.8 ± 0.02d	14.3 ± 0.45g	14.9 ± 0.32d	14.6	1.4 ± 0.17b	1.2 ± 0.02b	1.3
	1000	4.0 ± 0.02cd	16.6 ± 0.17g	16.9 ± 0.50d	16.8	1.09 ± 0.07bc	1.0 ± 0.03c	1.1
500	50	4.0 ± 0.01c	136.7 ± 1.87f	402.1 ± 1.57c	269.4	1.0 ± 0.02bc	1.0 ± 0.00c	1.0
	500	4.1 ± 0.01c	145.8 ± 1.90e	405.6 ± 0.93c	275.7	1.00 ± 0.02bc	0.9 ± 0.01cd	1.0
	1000	4.1 ± 0.02bc	163.7 ± 0.74d	412.3 ± 2.26b	288.0	0.9 ± 0.01bc	0.9 ± 0.01cd	0.9
1000	50	4.2 ± 0.03bc	245.2 ± 2.12c	801.9 ± 1.10a	523.6	0.9 ± 0.01bc	0.9 ± 0.00cd	0.9
	500	4.3 ± 0.04ab	292.5 ± 2.78b	805.9 ± 1.12a	549.2	0.8 ± 0.03c	0.8 ± 0.01d	0.8
	1000	4.4 ± 0.01a	315.2 ± 0.74a	806.2 ± 0.87a	560.7	0.7 ± 0.06c	0.6 ± 0.01e	0.6

Table 5.16 Mean values for plant Zn, plant Cu and soil electrical conductivity as influenced by the interaction of Zn and Cu rates. The different letters in the same column indicate significant difference between treatments (N = 6) at α=0.05 level of significance. The ± values represent the standard error of the means.

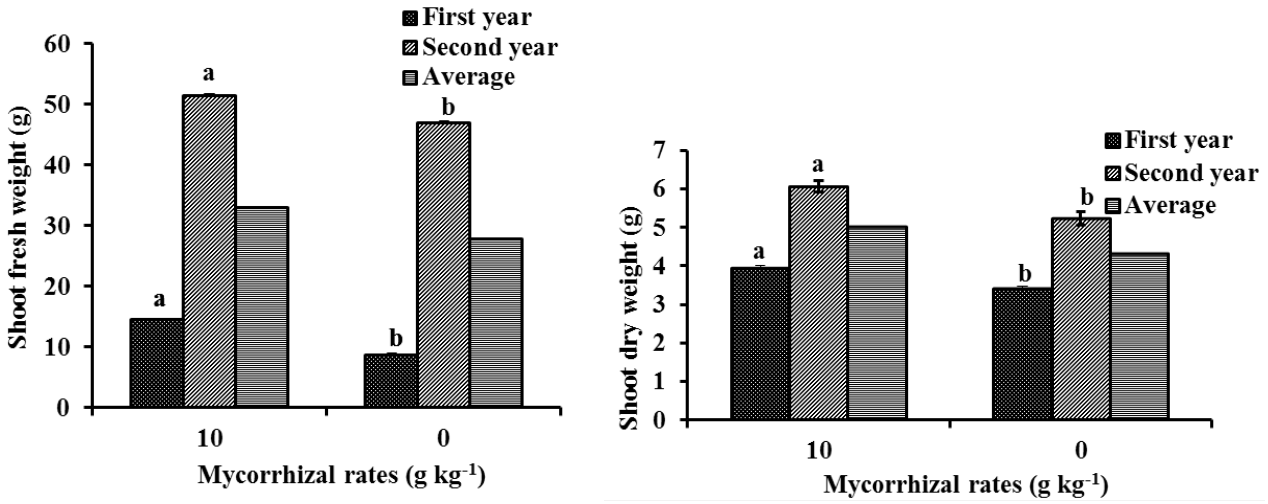
Zn rates (mg kg^{-1})	Cu rates (mg kg^{-1})	Plant Zn (mg kg^{-1})	Plant Cu (mg kg^{-1})	EC (dS m^{-1})
		2^{nd} year	1^{st} year	2^{nd} year
50	50	37.21 ± 0.13d	7.81 ± 0.42d	0.88 ± 0.01h
	500	35.49 ± 0.35d	19.39 ± 3.09cd	1.46 ± 0.06g
	1000	33.07 ± 0.35d	46.47 ± 0.84bc	1.78 ± 0.00e
500	50	97.38 ± 0.93b	9.46 ± 0.79d	1.45 ± 0.00g
	500	94.99 ± 0.92b	25.32 ± 2.31cd	1.89 ± 0.01d
	1000	85.92 ± 3.24c	65.73 ± 0.93b	2.04 ± 0.02c
1000	50	199.90 ± 1.07a	13.72 ± 1.94cd	1.71 ± 0.01f
	500	196.53 ± 0.84a	35.23 ± 0.96bcd	2.19 ± 0.02b
	1000	194.37 ± 0.72a	112.83 ± 1.31a	2.69 ± 0.03a

Table 5.17 Mean values for root length, colonized per cent of root length as influenced by the interaction of Zn and Cu rates in 2^{nd} year. The different letters in the same column indicate significant difference between treatments (N = 6) at α=0.05 level of significance. The ± values represent the standard error of the means.

Zn rates (mg kg^{-1})	Cu rates (mg kg^{-1})	2^{nd} year	
		Root length	Colonized % of root length
50	50	382.5 ± 1.78a	39.5 ± 1.53a
	500	348.6 ± 2.41b	32.2 ± 1.17b
	1000	342.4 ± 0.82b	20.6 ± 1.13cd
500	50	315.1 ± 1.65c	20.9 ± 1.57c
	500	311.9 ± 1.70c	18.1 ± 0.25de
	1000	303.8 ± 1.97cd	15.4 ± 1.22def
1000	50	292.9 ± 2.13d	17.6 ± 0.52cde
	500	259.7 ± 0.59e	13.9 ± 0.52ef
	1000	251.3 ± 1.40e	10.4 ± 0.35f

Table 5.18 Mean values for plant height as influenced by mycorrhizal rates over weeks in 1st year, 2nd year and the mean of the two years. The different letters in the same column indicate significant difference between treatments (N = 27) at α=0.05 level of significance. The ± values represent the standard error of the means.

Mycorrhizal rates (mg kg^{-1})	Plant height (cm) in weeks														
	2nd week			3rd week			4th week			5th week			6th week		
	1st year	2nd year	Mean	1st year	2nd year	Mean	1st year	2nd year	Mean	1st year	2nd year	Mean	1st year	2nd year	Mean
10	9.8 ± 0.04a	10.8 ± 0.08a	10.3	14.2 ± 0.06a	15.3 ± 0.05a	14.7	20.0 ± 0.06a	22.1 ± 0.09a	21.0	24.2 ± 0.14a	28.2 ± 0.19a	26.2	28.6 ± 0.18a	30.5± 0.22a	29.6
0	7.0 ± 0.04b	8.3 ± 0.07b	7.7	9.9 ± 0.18b	10.9 ± 0.20b	10.4	14.4 ± 0.38b	15.5 ± 0.41b	15.0	18.6 ± 0.41b	21.3 ± 0.55b	20.0	22.7 ± 0.53b	23.7 ± 0.61b	23.2



A B

Figure 5.8 A. Shoot fresh weight and B. shoot dry weight of onion as influenced by the mycorrhizal rates in 1st year, 2nd year and the average of the two years. The different letters in the same series indicate significant difference between treatments (N = 27) at α=0.05 level of significance. The vertical bars represent the standard error of the means.

Mycorrhizal inoculation increased root lengths in the first year, the second year and on average they were 1.9, 1.7 and 1.8 times the lengths produced in no inoculation, respectively. Root fresh weight also increased by 1.9, 1.6 and 1.8 times by mycorrhizae over no mycorrhizae in the first year, the second year and on the average, respectively. Plant P increased by 0.2, 0.12 and 0.16 units by the mycorrhizae in the first year, the second year and on an average, respectively. Soil EC, soil P and soil Zn was higher in non-mycorrhizal plants in the second year. However, plant Zn content was enhanced by mycorrhizae by 7.8 units over no mycorrhizae in the same year (Table 5.20).

Table 5.19 Mean values for root length, root fresh weight and plant P as influenced by mycorrhizal rates in 1st year, 2nd year and the mean of the two years. The different letters in the same column indicate significant difference between treatments (N = 27) at α=0.05 level of significance. The ± values represent the standard error of the means.

Mycorrhizal rates (g kg^{-1})	Root length (cm)			Root fresh weight (g)			Plant P (mg kg^{-1})		
	1st year	2nd year	Mean	1st year	2nd year	Mean	1st year	2nd year	Mean
10	298.0 ± 0.96a	312.0 ± 0.78a	305.0	6.98 ± 0.22a	9.10 ± 0.12a	8.04	1.2 ± 0.07a	1.1 ± 0.02a	1.2
0	159.4 ± 0.71b	179.3 ± 0.83b	169.3	3.60 ± 0.16b	5.52 ± 0.07b	4.56	1.0 ± 0.04b	1.0 ± 0.01b	1.0

Table 5.20 Mean values for soil electrical conductivity, soil P, soil Zn, and plant Zn as influenced by mycorrhizal rates in the 2nd year. The different letters in the same column indicate significant difference between treatments (N = 27) at α=0.05 level of significance. The ± values represent the standard error of the means.

Mycorrhizal rates ($g\ kg^{-1}$)	Mean values in 2nd year			
	Soil EC ($dS\ m^{-1}$)	Soil P ($mg\ kg^{-1}$)	Soil Zn ($mg\ kg^{-1}$)	Plant Zn ($mg\ kg^{-1}$)
5	1.71 ± 0.01b	3.9 ± 0.02b	405.3 ± 0.54b	112.2 ± 0.63a
0	1.87 ± 0.01a	4.1 ± 0.01a	412.2 ± 0.55a	104.4 ± 0.58b

5.4.5 Interaction effect of Zn and Mycorrhizae

The interactions of Zn and mycorrhizae were significantly different for plant height and soil Zn in both years of observation (Table 5.21). They were also significantly different for shoot fresh weight in the first year (Table 5.22) and shoot dry weight, root fresh weight, soil EC, soil P and plant P in the second year (Table 5.23). Interaction of Zn rates with mycorrhizal inoculation had a positive effect on plant height over no inoculation. Plant height was 8.8, 4.4, 4.5 units greater in the interaction of Zn rates 50, 500 and 1000 $mg\ kg^{-1}$ with mycorrhizae over no mycorrhizae in the 1st year while it was 9.3, 5.5, 5.7 units greater in the 2nd year.

Table 5.21 Mean values for plant height over weeks and soil Zn as influenced by the interaction of Zn and mycorrhizal rates in 1st year, 2nd year and mean of the two years; a. 2-4 weeks; b.5-6 weeks and soil Zn. The different letters in the same column indicate significant difference between treatments (*N* = 9) at α=0.05 level of significance. The ± values represent the standard error of the means.

a.

		Plant height (cm) in weeks								
Zn rates (mg kg^{-1})	Mycorrhizal rates (g kg^{-1})	2nd week			3rd week			4th week		
		1st year	2nd year	Mean	1st year	2nd year	Mean	1st year	2nd year	Mean
50	10	12.18 ± 0.06a	13.11 ± 0.08a	12.65	17.58 ± 0.07a	18.62 ± 0.08a	18.10	23.29 ± 0.06a	25.53 ± 0.09a	24.41
	0	7.27 ± 0.08d	8.51 ± 0.05d	7.89	10.32 ± 0.20c	11.42 ± 0.22c	10.87	14.96 ± 0.58c	15.97 ± 0.63c	15.47
500	10	9.09 ± 0.11b	10.03 ± 0.20b	9.56	12.81 ± 0.13b	13.85 ± 0.12b	13.33	18.81 ± 0.16b	20.74 ± 0.20b	19.78
	0	7.10 ± 0.03d	8.43 ± 0.06de	7.77	10.29 ± 0.46c	11.21 ± 0.51c	10.75	15.06 ± 0.82c	16.31 ± 0.90c	15.69
1000	10	7.99 ± 0.05c	9.14 ± 0.07c	8.57	12.11 ± 0.08b	13.20 ± 0.07b	12.66	17.99 ± 0.08c	19.88 ± 0.18b	18.94
	0	6.67 ± 0.09e	7.91 ± 0.18e	7.29	8.97 ± 0.20d	10.07 ± 0.20d	9.52	13.17 ± 0.56c	14.30 ± 0.55c	13.74

b.

Zn rates (mg kg^{-1})	Mycorrhizal rates (g kg^{-1})	Plant height (cm) in weeks								
		5^{th} week			6^{th} week			Soil Zn (mg kg^{-1})		
		1^{st} year	2^{nd} year	Mean	1^{st} year	2^{nd} year	Mean	1^{st} year	2^{nd} year	Mean
50	10	27.58 ± 0.18a	31.64 ± 0.21a	29.61	32.06 ± 0.12a	33.91 ± 0.23 a	32.99	13.12 ± 0.25e	13.10 ± 0.28e	13.11
	0	19.13 ± 0.66c	22.09 ± 0.97c	20.61	23.28 ± 0.76c	24.64 ± 0.94c	23.96	16.33 ± 0.28e	16.96 ± 0.31e	16.65
500	10	22.99 ± 0.22b	27.17 ± 0.21b	25.08	27.37 ± 0.30b	29.53 ± 0.34b	28.45	130.53 ± 1.59d	401.60 ± 1.28d	266.07
	0	19.23 ± 0.84c	22.10 ± 1.18c	20.67	22.96 ± 1.08c	24.01 ± 1.21c	23.49	166.94 ± 0.93c	411.69 ± 1.45c	289.32
1000	10	21.91 ± 0.28b	25.74 ± 0.50b	23.83	26.22 ± 0.43b	28.17 ± 0.51b	27.20	232.41 ± 1.89b	801.31 ± 0.94b	516.86
	0	17.41 ± 0.58c	19.69 ± 0.62c	18.55	21.73 ± 0.88c	22.47 ± 1.00c	22.1	336.12 ± 1.44a	808.01 ± 0.74a	572.07

Shoot fresh weight from interactions of Zn rates 50, 500 and 1000 mg kg^{-1} with mycorrhizae in the first year increased by 44.2%, 19.6% and 25.8% respectively over no mycorrhizae. In the second year, shoot dry weight increased by 20.5% in the interaction of Zn at 50 mg kg^{-1} with mycorrhizae compared to without mycorrhizae. Other interactions were similar for shoot dry weight. Root fresh weight was two times greater in the interaction of Zn at a rate of 50 mg kg^{-1} with mycorrhizae compared to the interaction without mycorrhizae. Root fresh weight was 1.2 and 1.98 times greater for the interaction of Zn rate 50 mg kg^{-1} with mycorrhizae compared to interaction of Zn rate 500 and 1000 mg kg^{-1} with mycorrhizae, respectively.

Soil EC was greater in the interactions in which there was an absence of mycorrhizae compared to the respective interactions with mycorrhizae. For soil P, there was no difference between the respective Zn rates with and without mycorrhiza but difference was observed between Zn rates with mycorrhiza. For instance, interaction of Zn rate 50 mg kg^{-1} with mycorrhizae resulted in 13.2% and 18.6% less soil P compared to the interaction of Zn rate 500 and 1000 mg kg^{-1} with mycorrhizae, respectively. However, plant P was 57.9%, 92.3% greater in the interaction of Zn rates 50 mg kg^{-1} with mycorrhizae compared to 500 and 1000 mg kg^{-1} with mycorrhizae, respectively.

Table 5.22 Mean values for shoot fresh weight as influenced by the interaction of Zn and mycorrhizal rates in the 1st year. The different letters in the same column indicate significant difference between treatments (N = 9) at α=0.05 level of significance. The ± values represent the standard error of the means.

Zn rates (mg kg^{-1})	Mycorrhizal rates (g kg^{-1})	Mean values for the first year
		Shoot fresh weight (g)
50	10	27.58 ± 0.14a
	0	19.13 ± 0.56c
500	10	22.99 ± 0.48b
	0	19.23 ± 0.69c
1000	10	21.91 ± 0.44b
	0	17.41 ± 0.31c

Table 5.23 Mean values for shoot dry weight, root fresh weight, soil electrical conductivity, plant P and soil P as influenced by the interaction of Zn and mycorrhizal rates in 2nd year. The different letters in the same column indicate significant difference between treatments (N = 6) at α=0.05 level of significance. The ± values represent the standard error of the means.

Zn rates (mg kg^{-1})	Mycorrhizal rates (g kg^{-1})	Mean Values for the second year				
		Shoot dry weight (g)	Root fresh weight (g)	Soil electrical conductivity (dS m^{-1})	Plant P (mg kg^{-1})	Soil P (mg kg^{-1})
50	10	10.21 ± 0.38a	11.66 ± 0.17a	1.30 ± 0.00f	1.50 ± 0.07a	3.50 ± 0.07c
	0	8.47 ± 0.46b	5.77 ± 0.04c	1.44 ± 0.04e	1.23 ± 0.02b	3.77 ± 0.02c
500	10	4.42 ± 0.22c	9.73 ± 0.27b	1.74 ± 0.01d	0.95 ± 0.01c	4.03 ± 0.01b
	0	3.78 ± 0.20c	5.94 ± 0.21c	1.84 ± 0.01c	0.92 ± 0.00c	4.08 ± 0.01b
1000	10	3.58 ± 0.12c	5.89 ± 0.19c	2.08 ± 0.02b	0.78 ± 0.00d	4.25 ± 0.03a
	0	3.48 ± 0.11c	4.84 ± 0.07d	2.31 ± 0.01a	0.72 ± 0.01d	4.30 ± 0.02a

5.4.6 Interaction effect of Cu and Mycorrhizae

Interactions of Cu rates and mycorrhizal rates were significantly different for shoot fresh weight, shoot dry weight and soil Zn in the first year while differences were observed for soil P and plant P in the second year (Table 5.24).

In the first year, Cu rates with mycorrhizae produced similar shoot fresh weight while they were different from interaction without mycorrhizae, indicating positive effect of interaction of Cu and mycorrhizae. Shoot dry weight had overlapping rankings for most treatments but a clear difference between lowest and highest Cu rates with mycorrhizae was found. Soil Zn increased with increased rate of Cu for interactions lacking mycorrhizal inoculation.

In the second year, the interactions of lowest Cu rates with and without mycorrhizae gave less soil P compared to the highest rates with or without mycorrhizae. However, plant P was greater in the interaction of the lower rates of Cu with or without mycorrhizae. Soil P was greater in the interactions with the absence of mycorrhizae.

Table 5.24 Mean values for shoot fresh weight, shoot dry weight and soil Zn in first year and soil P and plant in 2nd year as influenced by the interaction of Cu and mycorrhizal rates. The different letters in the same column indicate significant difference between treatments (N = 9) at α=0.05 level of significance. The ± values represent the standard error of the means.

Cu rates (mg kg^{-1})	Mycorrhizal rates (g kg^{-1})	Mean values for the 1st year			Mean values for the 2nd year	
		Shoot fresh weight (g)	Shoot dry weight (g)	Soil Zn (mg kg^{-1})	Soil P (mg kg^{-1})	Plant P (mg kg^{-1})
50	10	16.26 ± 0.32a	4.18 ± 0.04a	111.5 ± 1.58f	3.6 ± 0.07d	1.4 ± 0.07a
	0	8.72 ± 0.54c	3.37 ± 0.10c	152.0 ± 1.04c	3.9 ± 0.01c	1.1 ± 0.00b
500	10	14.28 ± 0.38ab	3.95 ± 0.03ab	126.5 ± 1.79e	4.0 ± 0.02bc	1.0 ± 0.01bc
	0	9.22 ± 0.45c	3.47 ± 0.09c	175.2 ± 1.39b	4.1 ± 0.02ab	0.9 ± 0.01cd
1000	10	13.20 ± 0.45b	3.74 ± 0.15bc	138.1 ± 0.69d	4.2 ± 0.01a	0.8 ± 0.01d
	0	8.42 ± 0.63c	3.41 ± 0.08c	192.2 ± 0.15a	4.2 ± 0.02a	0.8 ± 0.02d

5.4.7 Interaction effect of Zn, Cu and Mycorrhizae

The results revealed that there were some differences between interaction of Zn, Cu and mycorrhizae for shoot fresh weight, shoot dry weight and soil Zn in the first year (Table 5.25), and for EC, root fresh weight, plant P and soil P in second year (Table 5.26). Treatment groupings overlapped for all parameters but the lowest rates of Zn and Cu with mycorrhizae had more positive effect for the described parameters than the rest of the interactions.

The general morphological differences and mycorrhizal associations between plants grown in the treatment structures are shown in Plate 5.1 and Plate 5.2 below.

Table 5.25 Mean values for shoot fresh weight, shoot dry weight and soil Zn for the first year as influenced by the interaction of Zn, Cu and mycorrhizal rates. The different letters in the same column indicate significant difference between treatments (N = 3) at α=0.05 level of significance. The ± values represent the standard error of the means.

Zn rates (mg kg^{-1})	Cu rates (mg kg^{-1})	Mycorrhizal rates (g kg^{-1})	Mean values for the first year		
			Shoot fresh weight (g)	Shoot dry weight (g)	Soil Zn (mg kg^{-1})
50	50	10	24.47 ± 0.25a	5.04 ± 0.10a	11.61 ± 0.13k
		0	9.03 ± 1.12fgh	3.32 ± 0.10bc	15.04 ± 0.58k
	500	10	17.10 ± 0.26b	3.85 ± 0.04bc	13.17 ± 0.67k
		0	9.27 ± 0.57efgh	3.61 ± 0.25bc	15.35 ± 0.61k
	1000	10	16.63 ± 0.22bc	3.98 ± 0.32b	14.59 ± 0.32k
		0	8.53 ± 1.03fgh	3.63 ± 0.22bc	18.60 ± 0.12k
500	50	10	14.60 ± 0.87bcd	3.80 ± 0.05bc	116.66 ± 3.37j
		0	9.03 ± 0.96fgh	3.53 ± 0.26bc	156.69 ± 1.62hi
	500	10	13.43 ± 1.04bcde	3.85 ± 0.08bc	128.00 ± 3.05j
		0	10.47 ± 1.09defgh	3.58 ± 0.08bc	163.64 ± 2.27h
	1000	10	12.57 ± 0.50cdef	3.82 ± 0.31bc	146.93 ± 1.48i
		0	8.53 ± 1.49fgh	3.30 ± 0.09c	180.50 ± 0.14g
1000	50	10	9.70 ± 0.26efgh	3.71 ± 0.02bc	206.17 ± 3.34f
		0	8.10 ± 0.56gh	3.25 ± 0.08c	284.20 ± 2.60c
	500	10	12.30 ± 0.35defg	3.15 ± 0.02c	238.37 ± 4.37e
		0	7.93 ± 0.52h	3.20 ± 0.07c	346.57 ± 3.44b
	1000	10	10.40 ± 1.25defgh	3.42 ± 0.12bc	252.70 ± 1.42d
		0	8.20 ± 0.50gh	3.29 ± 0.01c	377.60 ± 0.40a

Table 5.26 Mean values for soil electrical conductivity, root fresh weight, plant P, and soil P for the second year as influenced by the interaction of Zn, Cu and mycorrhizal rates. The different letters in the same column indicate significant difference between treatments (N = 3) at α=0.05 level of significance. The ± values represent the standard error of the means.

Zn rates (mg kg^{-1})	Cu rates (mg kg^{-1})	Mycorrhizal rates (g kg^{-1})	Mean values for the 2nd year			
			Electrical conductivity (dS m^{-1})	Root fresh weight (g)	Plant P (mg kg^{-1})	Soil P (mg kg^{-1})
50	50	10	0.78 ± 0.01n	11.64 ± 0.42ab	2.32 ± 0.20a	2.69 ± 0.20h
		0	0.99 ± 0.00m	6.30 ± 0.05d	1.47 ± 0.01b	3.52 ± 0.01g
	500	10	1.37 ± 0.01l	12.67 ± 0.12a	1.26 ± 0.02bc	3.76 ± 0.02fg
		0	1.54 ± 0.12ij	5.58 ± 0.07d	1.14 ± 0.03cd	3.86 ± 0.03ef
	1000	10	1.76 ± 0.01h	10.68 ± 0.23bc	0.94 ± 0.02def	4.06 ± 0.03cdef
		0	1.81 ± 0.00gh	5.43 ± 0.11d	1.07 ± 0.04cde	3.92 ± 0.04def
500	50	10	1.42 ± 0.00jk	10.39 ± 0.04bc	098 ± 0.00def	4.01 ± 0.01 cdef
		0	1.48 ± 0.00ij	5.87 ± 0.45d	0.96 ± 0.00def	4.02 ± 0.01cdef
	500	10	1.87 ± 0.01fg	9.46 ± 0.20c	0.94 ± 0.02def	4.04 ± 0.03 cdef
		0	1.92 ± 0.01f	6.11 ± 0.25d	0.94 ± 0.00def	4.07 ± 0.01cde
	1000	10	1.94 ± 0.01ef	9.34 ± 0.79c	0.94 ± 0.01def	4.04 ± 0.02 cdef
		0	2.14 ± 0.03d	5.85 ± 0.34d	0.88 ± 0.00ef	4.15 ± 0.03bcde
1000	50	10	1.63 ± 0.01h	6.53 ± 0.16d	0.87 ± 0.00efg	4.19 ± 0.06abcd
		0	1.78 ± 0.00gh	5.57 ± 0.03d	0.86 ± 0.00efg	4.16 ± 0.02bcde
	500	10	2.04 ± 0.04e	5.93 ± 0.55d	0.85 ± 0.00efg	4.19 ± 0.05abcd
		0	2.34 ± 0.01c	5.33 ± 0.07d	0.75 ± 0.01fgh	4.29 ± 0.05abc
	1000	10	2.59 ± 0.06b	5.22 ± 0.06de	0.62 ± 0.01gh	4.39 ± 0.01ab
		0	2.80 ± 0.01a	3.63 ± 0.20e	0.55 ± 0.01h	4.46 ± 0.02a

5.4.8 Effect of extra treatments

The three extra (control) treatments were evaluated in the experiments to compare their effect on the given parameters within them and with rates of Zn, Cu, mycorrhizae and their interactions (Table 5.27 to Table 5.33). Results for controls revealed that application of biochar was more beneficial than none confirming results from previous experiments (Chapters 2, 3 and 4). Here, application of mycorrhizae, in addition to biochar, had greater effect than none of each.

When these controls were compared to sole effects and interaction effects of the above-mentioned results, application of biochar plus mycorrhizae was superior to all treatments for growth of onion and mycorrhizal colonization. Soil was low in P and Zn, so application of biochar, Zn, Cu, mycorrhizae or interaction of Zn, Cu and mycorrhizae was positive compared to no amendment.

Table 5.27 Effect of extra treatments on plant height over weeks in the 1st year, 2nd year and mean of the two years. The different letters in the same column indicate significant difference between treatments (N = 3) at α=0.05 level of significance. The ± values represent the standard error of the means.

Treatments	Plant height (cm) in weeks														
	2nd week			3rd week			4th week			5th week			6th week		
	1st year	2nd year	Mean	1st year	2nd year	Mean	1st year	2nd year	Mean	1st year	2nd year	Mean	1st year	2nd year	Mean
soil	6.5 ± 0.15c	7.0 ± 0.32c	6.8	7.4 ± 0.09c	9.0 ± 0.21c	8.2	9.6 ± 0.22b	13.7 ± 0.15c	11.7	12.5 ± 0.18b	18.9 ± 0.50c	15.7	16.1 ± 0.38b	20.8 ± 0.36c	18.4
Soil + Biochar	6.9 ± 0.37b	11.0 ± 0.72b	9.0	9.6 ± 1.10b	13.9 ± 0.35b	11.8	11.5 ± 1.17b	18.0 ± 0.27b	14.8	14.2 ± 0.70b	21.9 ± 0.31b	18.0	18.3 ± 0.79b	26.6 ± 0.15b	22.5
Soil + Biochar + Mycorrhiza	11.3 ± 0.13a	14.9 ± 0.61a	13.1	20.1 ± 0.24a	21.8 ± 0.53a	21.0	28.1 ± 0.30a	28.9 ± 0.50a	28.5	32.3 ± 0.66a	32.5 ± 5.14a	32.4	35.6 ± 0.10a	39.4 ± 0.74a	37.6

Table 5.28 Effect of extra treatments on shoot fresh weight and shoot dry weight in the 1st year, 2nd year and mean of the two years. The different letters in the same column indicate significant difference between treatments (N = 3) at α=0.05 level of significance. The ± values represent the standard error of the means.

Treatments	Shoot fresh weight (g)			Shoot dry weight (g)		
	1st year	2nd year	Mean	1st year	2nd year	Mean
soil	11.30 ± 0.17c	45.21 ± 7.92b	28.26	4.50 ± 0.03a	5.39 ± 0.82b	4.95
Soil + Biochar	14.70 ± 1.55b	43.91 ± 11.00b	29.31	4.57 ± 0.06a	7.44 ± 1.83ab	6.01
Soil + Biochar + Mycorrhiza	36.33 ± 0.46a	69.63 ± 16.86a	52.98	4.84 ± 0.14a	7.47 ± 1.17a	6.16

Table 5.29 Effect of extra treatments on root length and root fresh weight in the first year, second year and the mean of the two years. Values within the same column that do not follow the same letter are significantly different ($p < 0.05$). The ± values represent the standard error of the mean ($N = 3$).

Treatments	Root length (cm)			Root fresh weight (g)		
	1st year	2nd year	Mean	1st year	2nd year	Mean
soil	359.4 ± 6.21b	240.4 ± 13.17b	299.9	3.53 ± 0.22b	3.60 ± 0.53c	3.57
Soil + Biochar	350.9 ± 3.86b	255.1 ± 3.37b	303.0	4.96 ± 0.10b	6.77 ± 0.35b	5.87
Soil + Biochar + Mycorrhiza	478.8 ± 2.79a	311.2 ± 12.62a	395.0	11.56 ± 0.07a	10.80 ± 1.00a	11.18

Table 5.30 Effect of extra treatments on soil electrical conductivity, plant P and plant Zn in the first year, second year and the mean of the two years. Values within the same column that do not follow the same letter are significantly different ($p < 0.05$). The ± values represent the standard error of the mean ($N = 3$).

Treatments	Electrical conductivity (dS m^{-1})			Plant P (mg kg^{-1})			Plant Zn (mg kg^{-1})		
	1st year	2nd year	Mean	1st year	2nd year	Mean	1st year	2nd year	Mean
soil	1.02 ± 0.18a	0.11 ± 0.21b	0.57	0.9 ± 0.3a	0.8 ± 0.51b	0.8	0.8 ± 0.41c	0.5 ± 0.10c	0.6
Soil + Biochar	1.05 ± 0.36a	0.80 ± 0.44a	0.93	1.1 ± 0.25a	1.4 ± 0.20b	1.2	1.4 ± 0.69a	0.8 ± 0.06b	1.1
Soil + Biochar + Mycorrhiza	0.98 ± 0.19a	0.80 ± 0.75a	0.89	1.5 ± 0.33a	2.4 ± 0.21a	2.0	1.6 ± 0.31b	1.3 ± 0.15a	1.4

Table 5.31 Effect of extra treatments on soil P in second year and soil Zn in the first year, second year and the mean of the two years. The different letters in the same column indicate significant difference between treatments ($N = 3$) at α=0.05 level of significance. The ± values represent the standard error of the means.

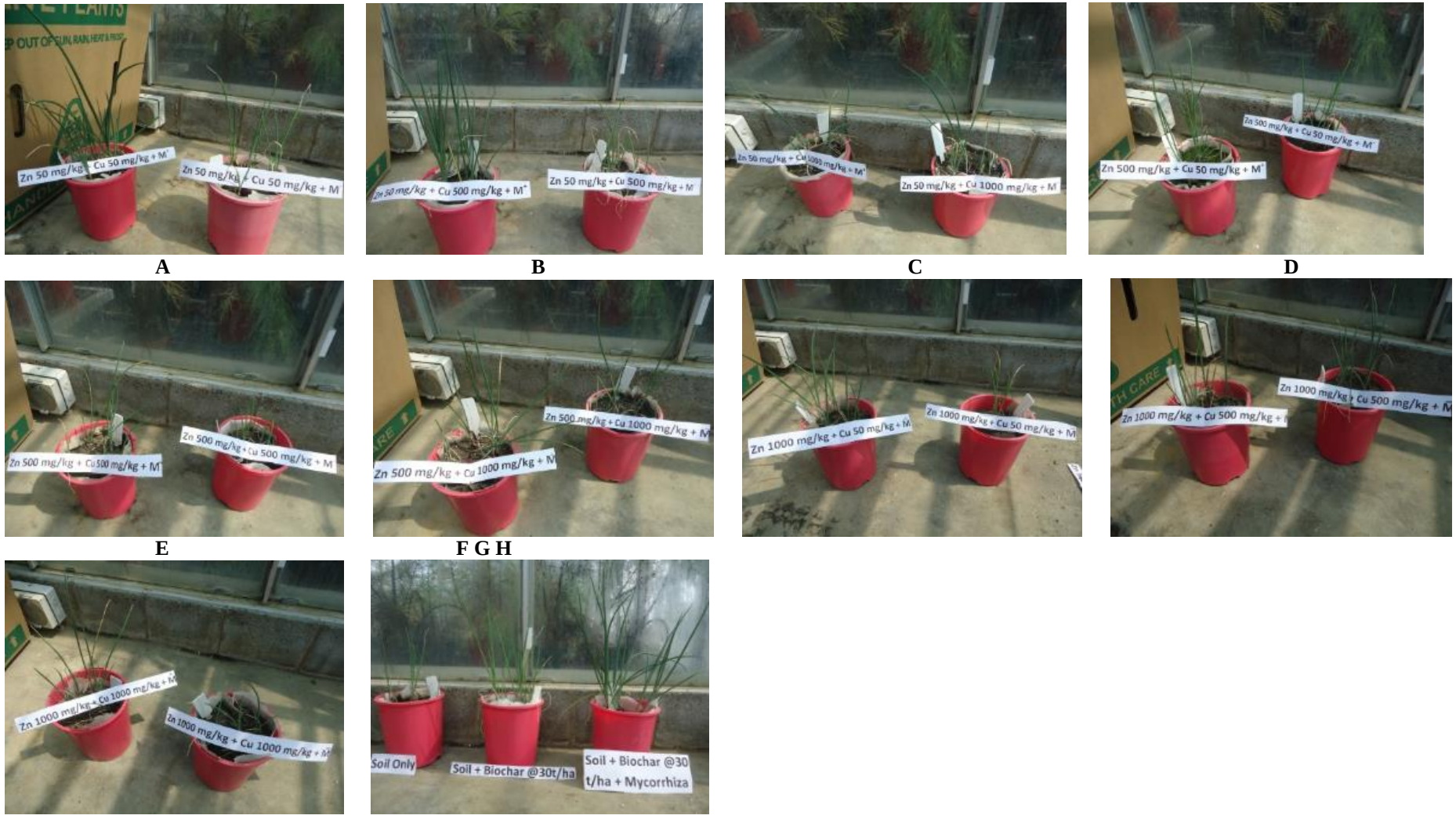
Treatments	Soil P (mg kg^{-1})	Soil Zn (mg kg^{-1})		
	2nd year	1st year	2nd year	Mean
soil	3.00 ± 0.40a	0.78 ± 0.23a	1.28 ± 1.39d	1.03
Soil + Biochar	3.60 ± 0.20a	1.68 ± 0.60a	0.87 ± 0.31d	1.28
Soil + Biochar + Mycorrhiza	2.57 ± 0.21b	0.89 ± 0.27a	1.20 ± 0.12d	1.05

Table 5.32 Effect of extra treatments on soil Cu and plant Cu in the first year, second year and the mean of the two years. The different letters in the same column indicate significant difference between treatments (N = 3) at α=0.05 level of significance. The ± values represent the standard error of the means.

Treatment	Soil Cu (mg kg^{-1})			Plant Cu (mg kg^{-1})		
	1^{st} year	2^{nd} year	Mean	1^{st} year	2^{nd} year	Mean
Soil	1.06 ± 0.16	1.04 ± 0.15d	1.05	0.42 ± 0.24	0.80 ± 0.10b	0.61
Soil + Biochar	1.90 ± 0.76	1.60 ± 0.41d	1.75	0.93 ± 0.27	1.17 ± 0.06a	2.1
Soil + Biochar + Mycorrhiza	1.46 ± 0.84	1.18 ± 0.07d	1.32	0.66 ± 0.24	1.00 ± 0.10a	0.83

Table 5.33 Effect of control on colonized per cent of root length in the 1^{st} year, 2^{nd} year and the mean of the two years. The ± values represent the standard error of the mean (N = 3).

Treatment	Colonized % of root length		
	1^{st} year	2^{nd} year	Mean
Biochar + Mycorrhizae	78.3 ± 4.64	61.9 ± 3.8	70.12



A **B** **C** **D**

E **F G H**

I **J**

Plate 5.1 Effect of mycorrhizal inoculation on growth of onion plants grown in various combinations of Zn and Cu rates added to a medium of soil and biochar at a rate of 30 t ha^{-1}, A-C = Cu 50, 500 and 1000 mg kg^{-1} with Zn 50mg kg^{-1} of soil; D-F = Cu 50, 500 and 1000 mg kg^{-1} with Zn 500mg kg^{-1} of soil; G-I = Cu 50, 500 and 1000 mg kg^{-1} with Zn 1000mg kg^{-1} of soil; J = control pots with soil, soil + biochar, soil + biochar + mycorrhiza. In each plate, mycorrhizal inoculation shows better growth of plants than no mycorrhiza.

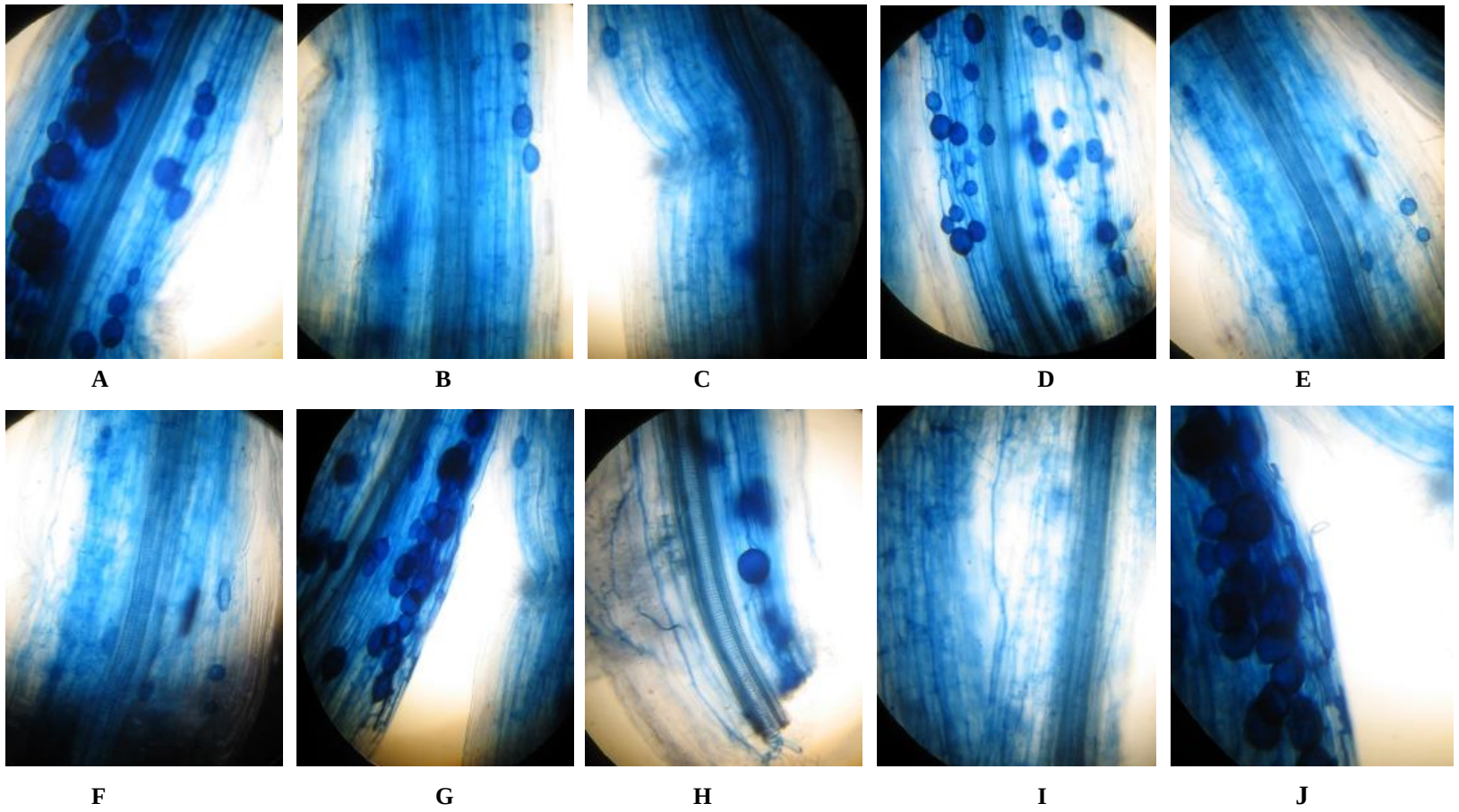


Plate 5.2 Effect of various combinations of Zn and Cu rates added to a medium of soil and biochar at a rate of 30 t ha^{-1} on mycorrhizal colonization, A-C = Cu 50, 500 and 1000 mg kg^{-1} with Zn 50mg kg^{-1} of soil; D-F = Cu 50, 500 and 1000 mg kg^{-1} with Zn 500mg kg^{-1} of soil; G-I = Cu 50, 500 and 1000 mg kg^{-1} with Zn 1000mg kg^{-1} of soil; J = control plants with soil + biochar + mycorrhiza. Plates showing the lower rates of Cu should have more mycorrhizal colonization and increasing rates of both metals reduce the colonization. Magnified by 40x.

5.5 Discussion

Soil was amended with biochar for all treatments except a control (soil only) at a rate of 30 t ha^{-1}. This rate was experimentally verified for other horticultural crops such as lettuce and tomato in sand medium. Influence of all other treatments was more positive on the recorded parameters than soil without any amendment; however it also showed some degree of colonization. From this experiment, it can be emphasized that biochar had positive effects on plant traits, mycorrhizal development and soil and plant nutrient contents. EC was quite high which would be associated with acid neutralizing value of biochar and added nutrients. Biochar has been reported by Brandstaka et al. (2010) and others for its positive influence on improving soil quality and plant growth (Chan et al. 2007; Chan et al. 2008a), liming values (Van Zwieten et al. 2010a) and contaminant destruction (Glover 2009).

Some reports emphasize that biochar amendments can increase AMF % root colonization in plant roots (Elmer & Pignatello 2011) grown in acidic soils (Ezawa et al. 2002; Matsubara et al. 2002; Yamato et al. 2006), while others show decreased abundance (Warnock et al. 2010). However, the colonization was detected in the present study even at the higher rates of Zn, Cu and biochar amended soil. In some cases, inhibited colonization after char amendment might be due to improved availability of P (Warnock et al. 2007) if soil had adequate P.

Comparing the results on mycorrhizal colonization in biochar and mycorrhizae added sand in Chapter 4 and biochar and mycorrhizae added calcareous soil in Chapter 5, the findings were interesting. In Table 4.5 of Chapter 4, mycorrhizal colonization was around 4% in control while it was about 17% in biochar treatments. Even though they were statistically different, the colonization was very low in both conditions. It indicated that the commercial inoculum was less effective in sand medium. But, in Table 5.33 of chapter 5, mycorrhizal colonization in biochar and mycorrhizae added soil was about 70%. This difference might come from different structure of the two soil media in which rate of mycorrhizal establishment, association and development differed. The other possible reason for this difference may be less leaching of mycorrhizal spores from clay soil than from sand medium due to their difference in porosity. There was still some chance of leaching of nutrients and mycorrhizal spores from the sand medium, however, fabric cloth placed on the bottom of the pots in both experiments was used to reduce leaching of nutrients and spores.

Plant Zn and Cu were observed in higher amounts where higher rates of Zn or Cu were applied. However, these results did not exceed the data range given in previous literature. For example in this study, the highest content of Zn 225.32 mg kg^{-1} was found in plants treated with Zn at a rate of

1000 mg kg^{-1} of soil. It was reported that the Zn content of leafy vegetables and herbs grown in heavy metal contaminated soil were 738mg and 414 mg kg^{-1} dry weight, respectively (Kachenko & Singh 2004). Similarly, the Zn content of vegetables grown in soils contaminated by base metal mining was 39-710 mg kg^{-1} (Davies & White 1981). The Zn content in plants of heavy metal contaminated soil ranged from 98 to 244 mg kg^{-1} (Kashem & Singh 1999), though the crops were not vegetables. Similarly Cu content of plant was 125 mg kg^{-1} dry weight in the present study which was also comparable with the previous reports; for example, it was 9.6 – 245 mg kg^{-1} in vegetables (Kachenko & Singh 2004).

Zn increased plant height up to 50 mg kg^{-1}. It might increase beyond this level but that requires further study. As onion has been classified as sensitive to Zn deficiency (Chapman 1966), the rate needs to be verified. As a recommendation, 10 kg ha-^{1} was optimum for onion crop (Khan et al. 2007) while in the present study, Zn rate of 50 mg per pot was equivalent to about 11 kg ha^{-1} considering the pot diameter (12 cm) and the soil had very low Zn content (0.4 mg kg^{-1} of soil). Thus, a basal rate of 50 mg kg^{-1} of soil was used. Reduced plant height due to increased Zn rates could be due to Zn toxicity. Similar results were found for Cu. Plants showed Cu toxicity at higher rates, however, Cu is less available if soil pH is above 4 (Mathur & Levesque 1983). In the experimental soil, pH was around 7. High rates of Cu could change the soil nutrient composition so that plant height would be reduced. Similar effects of Cu on plant height in later weeks would be associated with increased plant tolerance after vigorous growth. Greater plant height in mycorrhizal plants from the second week to the sixth week of planting suggested that networks of mycorrhiza were established from the earlier planting.

High rate of Zn reduced Cu content in plants and created stress that ceased growth (Bonnet et al. 2000). This fact was also associated with the present study as higher rates of Zn had negative effect on plant growth. Interaction of Zn and mycorrhiza increased growth during the recording period indicating that mycorrhizal inoculation had positive effect on Zn uptake. Mycorrhiza can detoxify high rates of Zn (Dueck et al. 1986). The effect could also be associated with other factors such as P, as elevated Zn levels interfere with P uptake (Shetty et al. 1995). Interaction of Cu and mycorrhiza on plant height was visible only in the second week of planting but the reason behind this was unclear. However, it could be associated with supply of Cu from mycorrhizal activity.

Lower shoot fresh weight due to elevated Zn levels would be linked to Zn toxicity and low P as the soil was designated as low P soil. Nutrient solution with 25% P was added to sustain plants but it might not have been sufficient for optimum shoot growth.

Minimum temperature of about 10^0C at night was maintained for onion by air cooling in the glasshouse during the morning and evening but higher temperatures were still a problem during the day (beyond 26^0C) in the first year. In the second year, the experiment was conducted in a larger glasshouse with a fixed range of temperature. This could be a major factor for better plant growth in the second year.

The shoot fresh and dry weights were very high in second year in part because plants were at bulbing stage. Day length is not very important in bulb formation but high temperature plays a major role (Abdalla 1967). The temperature and light conditions were also improved in the second year of the study.

Root mass could be increased if the soil were loamy or more porous but clayey calcareous soil in this study could impair porosity and root aeration. Increased level of Zn also decreases root length (Denny & Wilkins 1987) but the toxic concentration of a particular nutrient may vary depending upon crop type (Dang et al. 1990), soil composition, biological activity in soil and other factors (Påhlsson 1989). Zn with Cu and some other nutrients in excess amount can reduce rooting capacity as observed in white poplar (Castiglione et al. 2007).

Mycorrhizal colonization can increase plant dry weight (Chen et al. 2007). Higher shoot fresh weight and dry weight due to interaction of mycorrhiza and Cu have also been reported by Malekzadeh & Ordubadi (2012) indicating that mycorrhizae prevented Cu toxicity in plants by acting as an agent to filter its flow from roots to plant tissue (Malekzadeh et al. 2007).

The interaction effect of Zn, Cu and mycorrhiza were not consistent over the term of the present study. However, there could be positive influence on plant and mycorrhizal parameters because mycorrhizae develop protective mechanisms against heavy metals (Gildon & Tinker 1983). To determine the interaction effects more consistently, long-term experiments should be carried out maintaining the soil pH around or below neutral.

Elemental sulphur was used to reduce pH of soil to near 7; pots were planted after two weeks which would not be long enough duration for soil reactions. The pH level should be in the acidic range for optimum uptake of Zn, but to make a balance for all nutrients it was maintained at 7. Soil pH was tested after addition of biochar due to the limitation of planting but soil was alkaline after plant

harvest. The results may have been clearer if the pH were maintained at 7 or below after the addition of biochar.

Salt concentration increased due to the effect of Zn and Cu, however, mycorrhiza was observed to increase salt concentration in soil as EC was higher in mycorrhizal plants. A previous study also explains that the mycorrhiza restrict salt absorption by plants (Huang et al. 2005), thus they can accumulate in the rhizosphere increasing EC; however, there was no significant difference between mycorrhizal and non-mycorrhizal soil for EC content. Yet there was some difference in Zn content of soil. Interaction of Zn with Cu, Cu with mycorrhiza and Zn with Cu and mycorrhiza had the similar trend that explains the role of mycorrhiza in restricting the absorption of excess Zn.

Cu content of plants was lower in mycorrhizal plants in extra treatments which supports the theory on the role of mycorrhiza in restriction of excess intake of heavy metals explained above. Plant P content was higher for low rate of Zn in this experiment, however, Zn supply had little effect on tissue P in wheat (Zhu et al. 2001) but excess phosphorus can induce Zn deficiency (Marschner & Cakmak 1986). Soil was low P and plant P content was probably added by biochar and nutrient solution by the activity of mycorrhiza. Therefore, the higher concentration of P at higher rates of Zn could be linked with mycorrhiza and other factors. Similar trend of P content was observed for Cu supply; however the role of Cu on P uptake has been inadequately explained by previous researchers. Mycorrhizae increase the uptake of Zn and Cu, but mycorrhizal activity is suppressed by P fertilization (Lambert et al. 1979). Interaction effect of Zn and Cu on plant P content is not adequately studied but some authors mentioned that Cu in excess interferes with the mechanism to absorb or translocate other nutrients (Struckmeyer et al. 1969) and inhibits root elongation (Woolhouse & Walker 1981; Fageria 2001). Plant P increase by mycorrhizal inoculum is well-established and the mechanism for enhanced absorption of P by hyphae and solubilization of soil P by releasing organic acids from mycorrhizal network has been explained by Bolan (1991).

Mycorrhizal tolerance to heavy metals has been described by Hildebrandt et al. (2007). Mycorrhizal development on soybean plants grown in the greenhouse in soil was enhanced by adding 18 mg Zn per kg soil on a dry weight basis while higher rates of 45 and 135 mg kg^{-1} Zn resulted in decreased infection (McIlveen & Cole Jr 1979). In the present study, the lowest rate was 50 mg kg^{-1} and better colonization occurred at this rate than higher rates. Thus this study supports the previous one that lower rates of Zn stimulate but higher rates reduce development. It could not be concluded that the highest rate was inhibitory because some colonization was also detected at those rates, however, 50 mg Zn and 50 mg Cu per kilogram of soil could be recommended from this experiment. Yet, a

rigorous study is needed to identify the deleterious rates of heavy metals for mycorrhiza. *Glomus mosseae* was able to colonize pepper in Cu contaminated soils (Latef & Hamed 2011). Present results also supported the view of this author. Adequate literature is lacking on the interaction effects of Zn and Cu on mycorrhizal development, however there is a potential of mycorrhiza for remediation in Cu and Zn contaminated soils. In this study, increasing rates of Zn and Cu in combination reduced mycorrhizal development which could be associated with the rooting ability of the plants in contaminated soil.

A low P calcareous soil was used for the experiment. Other types of soil as well as other biochars need to be trialled. Similarly, the rates of Zn covered a wide range but even the highest concentration did not prevent mycorrhizal colonization. Therefore, rates more than 1000 mg kg^{-1} of each metal should be included to determine the lethal doses. However, the aim was to find whether the AMF could tolerate the range of Zn and Cu levels in biochar amended soil. It was noteworthy that AM application increased nutrient uptake but application of higher rates of Zn and Cu had inhibitory effect on colonization of crops by arbuscular mycorrhizal fungi.

5.6 Conclusion

Effects of Zn, Cu and mycorrhizae were tested to determine the effective rates and interactions on plant growth and mycorrhizal colonization. The study confirmed that the addition of biochar and mycorrhizae was beneficial over no use. This study also confirmed that the mycorrhizal colonization could be found in a calcareous alkaline soil. Biochar amended soil increased overall growth and mycorrhizal colonization. Individual effects of Zn and Cu were more distinct than their interactions. Mycorrhizal effect was greater in all recorded parameters than where there was no application of mycorrhizae. Limited mycorrhizal colonization was found for high rates of Zn and Cu thus verifying the tolerance of mycorrhizae to these metals. This experiment adds information on the soil management system and resolving environmental pollution caused by Zn and Cu contamination in agricultural soils.

In these studies, effects of biochar with nutrient solution or biochar with Zn and Cu were studied. However, actual contribution of biochar could not be detected, though it was found beneficial over no biochar addition. To determine the influence of nutrients present in biochar on growth of a crop, further study was conducted to compare the effect of biochar alone and nutrients supplied equivalent to biochar in two types of soils viz. ferrosol and podsol. The details of this study will be described in the upcoming chapter.

Chapter 6. Performance of tomato in pH adjusted ferrosol and podsol soils amended with biochar, nitrogen and equivalent amounts of P and K

6.1 Abstract

Two pot experiments were conducted in a ferrosol and a podsol soils to determine the effect of biochar, nitrogen and equivalent amount of P and K on growth of tomato crop. The soils were balanced for pH with the use of biochar and lime to determine the difference between lime + biochar, lime + biochar + N and lime + N + PK equivalent to biochar consisting of nine treatments with 4 replications. Sugarcane Trash, Green Waste A and Green Waste B biochars were used for the experiments. Nitrogen was applied at a rate of 110 kg ha^{-1} and P and K were calculated based on their amount in respective biochar. Pots were irrigated up to field capacity preventing leaching of water and nutrients. Observations were recorded on growth parameters, NPK content in plant and soil. The treatments had positive effect on shoot fresh weight and shoot dry weight of tomato. The treatment lime + biochar + nitrogen (L + B + N) was beneficial over lime + biochar (L + B) application. The application of lime + nitrogen + phosphorus and potassium equivalent to biochar (L + N + PK) had the significantly greatest positive effect on performance of tomato in both soils.

6.2 Introduction

There are a number of papers which report positive effects of biochar addition on crop growth and development (Asai et al. 2009; Van Zwieten et al. 2010a; Coomer et al. 2012; Zhang et al. 2012; Carter et al. 2013; Saxena et al. 2013; Vinh et al. 2014). Some reports have also illustrated negative (Lehmann et al. 2003; Chan et al. 2008a) or no (Brandstaka et al. 2010; Borsari 2011; Lai et al. 2013) response of crops to biochar. Some reports emphasized that the effect was positive when biochar was added in combination with N fertilization (Hottle 2013). Others have noted that biochar in combination with mineral fertilizers had greater positive effect than mineral fertilizer alone (Alburquerque et al. 2014). However, more studies are required to understand the difference in the performance of plants when different biochar preparations are used.

Biochar preparations contain different amounts of ash depending upon the method and conditions of preparations as well as biochar itself. Ash and biochar particles are different fractions of biochar. For example, biochars (n = 94) can have ash contents from 0.4% to 88.2% (Enders et al. 2012). These variations were mainly due to different feedstocks and pyrolysis methods. Ash contains all

the P and K originally present in the pyrolised material (Kuligowski & Poulsen 2009). Biochar ash contains nutrients including K and P (Amonette & Joseph 2009). Biochar also contains minerals including K chloride, amorphous silica (SiO_2), calcite ($CaCO_3$) hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), Ca phosphates, anhydrite ($CaSO_4$), nitrates, oxides and hydroxides of Ca, Mg, Aluminium, Titanium, Mn, Zn or Fe (Amonette & Joseph 2009).

Generally, biochar raises soil pH (Chan et al. 2008b) which is mostly dependent on ash content. The acidity or alkalinity of a biochar is a function of both the ash content as well as the pyrolysis temperature, for example, pH ranged from 4.48 for Oak biochar pyrolysed at 300 °C to 11.62 for Paper at 600 °C; and from 8.6 for the biochar with less than 8% ash to below 7.5 for less than 1.5% ash content (Enders et al. 2012). Biochar pH also varied significantly among feedstocks, ranging from 6.93 to 10.26, which resulted from the presence of greater quantities of salts of alkali and alkaline elements (Na, K, Ca, Mg) and calcite (Singh et al. 2010). Alkalinity of ash is explained as K_2O and/or Na_2O formed during the combustion of plant material and these dissolve in water during extraction to form hydroxides (Onyegbado et al. 2004).

The ash of biochar made from plant parts generally contains very small amounts of N (Major no date). During the pyrolysis process, significant proportions of biomass N are lost by volatilization (Chan & Xu 2009) and the rest may be converted into more resistant forms that may not be readily plant available. The N remaining in the biochar is poorly available to plants (Gaskin et al. 2010), since a fraction of it is found inside aromatic C structures and heterocyclic compounds (Chan & Xu 2009). One exception may be N in biochars derived from animal manures (Chan et al. 2008b; Tagoe et al. 2008). Limiting soil N content by biochar application in N deficient soils could also be due to the high C/N ratio, hence it might reduce crop productivity temporarily (Lehmann et al. 2003). Thus, it is essential to compare the effect of biochar applied alone and in combination with other nutrient sources.

More studies are required to understand the difference in the performance of plants when different biochar preparations are used. There is a need to identify the differences between the effects of biochars and the equivalent NPK contents amended to balance the nutrient concentrations at the same soil pH in different type of soils.

The present study is concerned with whether the effect of biochar (with associated ash and nutrients) on plant growth is greater than that caused by the equivalent amount of nutrients alone.

The other issue this study tries to resolve is whether the N contained in the carbon matrix of biochar is available in soils for plant growth.

This study aims to compare the performance of tomato plants influenced by the nutrients contained in biochar preparation (mixture of biochar and ash, the product of charring after pyrolysis) with their equivalent amounts added to a podsol and a ferrosol soil each adjusted to a given pH value. The experiment also verifies if N present in biochar particles of the biochar preparations is available in soil to improve plant growth.

This chapter addresses the effect of nutrients present in biochar preparations and their equivalent amounts added to low fertility podsol and ferrosol soils on tomato crop when they are adjusted to a similar level of pH and balanced for P and K.

6.3 Materials and Methods

As mentioned above, biochar preparations are mixture of ash and char particles, these two components were not separated in the study. This experiment was designed to test two hypotheses. Firstly, the P and K contents of biochar preparations (supplied mostly from ash) had similar effects to their equal amounts supplied from chemical fertilizers. Secondly, the N content of biochar preparations (contained in char particles) and N supplied from chemical fertilizer had similar effects on plant growth. To determine availability of N (from char particles) and P and K (from ash), plant performance was compared in two types of soil: ferrosol and podsol soils. To determine the availability of N separately, N treated pots were included as treatments. For N treatments, a recommended dose of 110 kg N per hectare was applied as described in online information on the nutrient management of tomato section published by Ikisan (Annonymous 2000). The details of the methodology are given below.

6.3.1 Experimental site

The experiments were conducted in a controlled environment of a glasshouse that was used for the trials in Chapter 4 and 5 at the Gatton Campus, The University of Queensland, Australia.

6.3.2 Cultivar and source of seeds

Tomato seeds of a determinate variety Rebel F-1 were obtained from South Pacific Seeds, New South Wales. These seeds were used for other previous experiments of the present study (thesis).

6.3.3 Pot preparation, seed germination and seedling preparation

Plastic pots of 2.5 litre capacity and 16 cm diameter were selected for the experiments. A fine fabric cloth was placed on the bottom of the pots and pots were placed on the trays in which the drained water and nutrient solutions could be collected and reused to prevent loss of nutrients and water. The pots were then filled with the soils treated with biochar or nutrients. Seeds were germinated in 4.5 x 3 x 2.5 cm (height-length-breadth) dimensioned, rectangular (100 cells, 10 rows and 10 cells per row) germination tray filled with propagation mix. Two seeds were sown at a depth of 2 cm and covered with propagation mix. The trays were watered gently through a wash bottle immediately after seeding. Just after maximum germination, the seedlings were thinned to one seedling per cell. The tray was kept in an air-conditioned bay of a glasshouse to protect the seedlings from high temperature stress. Seedlings were watered every morning until they germinated.

After germination, the watering was reduced to once every alternate day to harden seedlings so that they could tolerate transplanting shock after transfer from fertile potting mix to less fertile podsol and ferrosol soils. The seedlings were watered two hours before transplanting and then uprooted gently to minimize damage and stress to the seedlings. Roots of uprooted seedlings were cleaned by dipping and gentle shaking into water in a tray as shown in the Plate 6.1. After removing all clods or particles of propagation mix, seedlings were transplanted into the pots filled with treated podsol and ferrosol soils.

Plate 6.1 Cleaning of roots to remove nutrients and propagation mix

6.3.4 Sources and characteristics of soils

A low-fertility podsol soil, as used by Spark and Swift (2008a), was collected from the Poona region close to the Queensland Coast (~200km north of Brisbane) for use for these experiments. The podsol soil sample was taken from the A Horizon consisting of an ash-grey layer made up of largely quartz (sand). The soil had bulk density 1.45 g cm-^{3}, water holding capacity 0.28 cm^{3}, cation

exchange capacity 0.96 and lime requirement 1.2 t ha^{-1}. The pH and EC were 4.0 and 30 µS cm^{-1} (Spark & Swift no date).

A low-fertility ferrosol soil as described by Spark and Swift (Spark & Swift 2008b) was collected from a cleared forested site in the Kingaroy region of Queensland (~300 km west of Brisbane) and was also used for the experiment. These soils are formed from rocks of volcanic origins and are nearly always red coloured (Spark & Swift 2008a). They generally have good soil structure, lack strong texture contrast between A and B horizons and have a high free iron content in the B horizon (subsoil). These soils are well drained and are often deep and highly fertile, and are typically found on the crests, upper, mid- and lower slopes of plateau remnants. The soil had bulk density 0.93 g cm-^{3}), water holding capacity 0.39 cm^3, cation exchange capacity 14 and lime requirement 6.2 tha^{-1}. The pH and EC were 5.38 and 198 µS cm^{-1} (Spark & Swift 2008a).

The bulk density was determined to select appropriate size of the pots for two soils as they had different volume. However, the same size pots were used for this experiment to maintain uniformity between the trials.

6.3.5 Source and characteristics of biochar

Three biochars from different feedstock (Sugarcane Trash, Green Waste A and Green Waste B) described in previous chapters was used for the experiment. The details of N, P, K content of these biochar is given in Appendix 1. The pH and electrical conductivity (EC) of the biochars have been determined by Kochanek et al. (2014). The pH of Sugarcane Trash, Green Waste A and Green Waste B was 8, 9.4 and 8.9 respectively. The EC of these biochars were 1.4, 3.4 and 2.3 µS cm^{-1} respectively.

6.3.6 Soil preparation

A bulk of 24 kg soil (2 kg x 3 treatments without biochar x 4 replications) was separated from the whole lot and mixed with the recommended amount of lime. This soil was used in the pots for N, P, K additions equal to the nutrients contained in each of the three biochars plus supplementary micronutrient application. The remaining 48 kg of soil was divided into three for three types of biochars and each biochar was mixed at a rate of 20 t ha^{-1} in case of podsol soil and 30 t ha^{-1} for the ferrosol soil. The rate was different because their liming value was different as described below. These separate bulks of soil-biochar mixtures were used to fill the pots. After filling into the pots, the nutrients were calculated, weighed and applied as specified in the treatments illustrated in Table 6.4 and Table 6.6.

6.3.7 Calculation of lime requirement

To verify the previous results of lime requirement of podsol and ferrosol soils to maintain pH 6.5 as recommended by Spark & Swift (2008a), the previous requirement of 1.2 t ha^{-1} for podsol soil was tested with 1.0, 1.2 and 1.4 t ha^{-1} and the requirement of 6.2 t ha^{-1} for ferrosol soil was tested with 6.0, 6.2 and 6.4 t ha^{-1} to confirm the requirement experimentally.

The initial pH of podsol and ferrosol soils was analysed by the methods 1:5 water and 1:1 water described by Rayment and Higginson (1992). Recording of podsol soil was taken on the fifth day and of ferrosol soil on the sixth day after adding water. The amount of lime that was required to increase pH up to 6.5 was selected for the experiments. For podsol soil, the required amounts of lime at a rate of 1.0, 1.2 and 1.4 t ha^{-1} were 100.4 mg, 120.6 mg and 140.4 mg per 100 g of soil, respectively. Similarly, the rates of 6.0, 6.2 and 6.4 t ha^{-1} of lime for ferrosol soil were equal to 612.9, 623.0 and 643.1 mg per 100 g of soil. Each of the biochars had different lime equivalence. From the calculations, Sugarcane Trash 19.9 mg, Green Waste A 181.2 mg and Green Waste B 42.2 mg were required for 100 g of soils at a rate of 30 t ha^{-1}. The results given in Table 6.1 revealed that the rate of Sugarcane Trash should be increased to 40 t ha^{-1} while the rate of Green Waste A should be reduced to 20 t ha^{-1} to meet the requirement according to their liming values.

The results revealed that the application of biochars at 30 t ha^{-1} was not sufficient to raise the pH of ferrosol soil above pH 6 because mean ferrosol soil pH values following the addition of 30 t ha^{-1} biochar are 5.5, 6.0 and 5.7 for Sugarcane Trash, Green Waste A and Green Waste B, respectively. This requires certain amounts of lime to increase the values above 6. In podsol soil, Green Waste B had the pH within the range of 6-7; while the Green Waste A raised the pH beyond 7 and Sugarcane Trash had a value below 6. The results indicated that 30 t ha^{-1} of Green Waste A raised the soil pH too high so that it was decided to apply a lower amount (20 t ha^{-1}) of all three biochars in podsol soil and to add lime to Sugarcane Trash and Green Waste B to balance the amount of $CaCO_3$ required. This was considered to be the effective way to retain the podsol soil pH within the range of 6-7 which is the optimum requirement for growth of tomato.

The recommended doses of lime for podsol and ferrosol soil indicated by Spark and Swift (no date) were 1.2 and 6.2 t ha^{-1}, respectively to raise the pH to around 6. These values were verified experimentally (Table 6.2). The rate (1.2 t ha^{-1}) recommended for podsol soil was still the most effective among 1, 1.2 and 1.4 t ha^{-1} as pH was around 6.6 in the soil. Thus the lime rates of 1.2 t ha^{-1} for podsol soil and 6.0 t ha^{-1} for ferrosol soil were applied for the main experiment.

Thus, it can be calculated as 10,000m^2= 1.2 t ha^{-1}, for a pot of 0.020096 m^2 = 2411.52 mg lime for podsol soil. Similarly for ferrosol soil, 10,000m^2 = 6 t ha^{-1}, for a pot of 0.020096 m^2 = 12057.6 mg lime.

6.3.8 Nutrient solutions

Solutions of N, P, and K, Ca and Mg were prepared separately as mentioned at Appendix 2 (Hoagland recipes). They were applied to each pot just after transplanting. A supplementary solution of micronutrients was applied as calculated on the basis of Hoagland's solution in Appendix 2. After adding the solutions, water was added to the soil to maintain it near field capacity. Depending upon the plant performance, an additional dose of micronutrients was added during the growth phase of tomato.

Table 6.1 Soil pH for ferrosol and podsol soils amended with same amounts of $CaCO_3$ from different biochars and lime.

Soil type	Source of lime	Amount of lime/lime equivalent (mg 100 g^{-1} of soil)	Soil pH (1:1 soil-water)			
			Rep 1	Rep 2	Rep 3	'Mean'
Podsol	Sugarcane Trash	19.94	5.6	5.6	5.8	5.7
Podsol	Green Waste A	181.2	7.07	6.9	7.13	7.0
Podsol	Green Waste B	42.2	6.5	6.2	6.4	6.4
Podsol	lime	19.94	5.91	5.95	5.2	5.7
Podsol	lime	181.2	7.3	7.02	7.02	7.1
Podsol	lime	42.2	6.32	6.18	6.23	6.2
Podsol			3.85		3.70*	3.8
Ferrosol	Sugarcane Trash	19.94	5.46	5.45	5.45	5.5
Ferrosol	Green Waste A	181.2	6.04	5.97	5.97	6.0
Ferrosol	Green Waste B	42.2	5.80	5.63	5.74	5.7
Ferrosol	lime	19.94	5.38	5.38	5.37	5.4
Ferrosol	lime	181.2	6.00	6.00	6.00	6.0
Ferrosol	lime	42.2	5.40	5.48	5.55	5.5
Ferrosol			5.15*	5.25		5.2

*=1:5water method

Table 6.2 Verification of lime requirement for podsol and ferrosol soils

Treatments	Lime rate (t ha^{-1})	soil type	pH of soils				
			Stirring method (1:1 water)		Shaking method (1:5 water)		Mean
			Rep 1	Rep 2	Rep 3	Rep 4	
1	1	Podsol	6.5	6.5	6.57	6.5	6.5
2	1.2	Podsol	6.6	6.6	6.6	6.5	6.6
3	1.4	Podsol	7.7	6.9	6.97	6.1	6.9
4	6	Ferrosol	6.62	6.64	6.72	6.7	6.7
5	6.2	Ferrosol	6.85	6.88	6.82	6.87	6.9
6	6.4	Ferrosol	6.99	7.00	6.98	7.01	7.0

6.3.9 Determination of field capacity

To determine the water content in the pots near field capacity, three pots were weighed after placing fabric cloth in the pots and filling with soil in each (dry weight) (Table 8.3). Then water was supplied through a shower applied gently with lowest force so that the water spread into soil as much as possible and drainage could be seen. As the podsol soil was fine and sandy, the water was applied to all inner areas of the soil surface. When it started draining from the bottom holes, then watering was stopped. When drainage came to an end, the moist pot was weighed and the dry weight was subtracted from the wet weight to give the water holding capacity. Experimentally, the water requirement was 776 ml per pot for ferrosol soil and 564 ml per pot for podsol soil. Water movement in ferrosol soil was very slow while it was fast in podsol soil. Considering this fact, about 80% of required water (620~600 ml) was supplied to minimize the drainage and water logging. Similarly, 70% (394~400 ml) of required water was supplied for podsol soil. During plant growth, roots were dense and reduced the space for water; less water was required to discourage drainage and water logging. Thus, the amount of water supplied did not exceed the amounts indicated and there was no drainage from the pots during the experiment.

6.3.10 Experimental design

Pot experiments were conducted as a multi-factorial arrangement with three treatments under each biochar for each soil. Thus nine treatment combinations were arranged for a soil type (Table 6.3 and 6.5) in four replications. These treatment combinations consisted of limed soil with three biochars

(three treatments), limed soil with biochars plus N at a rate of 110 kg ha^{-1} (three treatments), and limed soil plus N 110 kg ha^{-1} plus P and K equal to biochars (three treatments).

These treatments were designed to test whether the difference is within and between the soils of similar pH amended with the same amounts of nutrients (P and K) supplied by biochar preparations (mixture of char and ash) and by chemical fertilizers. The treatments were also designed to compare the N effect of that contained in biochar versus the same amount supplied through chemical fertilizer. Treatments 1, 4 and 7 were lime and biochar which is the amount of lime required to balance the pH supplied by Sugarcane Trash, Green Waste A and Green Waste B biochar preparations, respectively. Treatment 2, 5 and 8 were designed by adding N at the rate of 110 kg ha^{-1} to the treatments 1, 4 and 7 to see the difference of N application. Treatments 3, 6 and 9 were designed by adding N as previous treatments and P and K equivalent to respective biochars to compare the effect of P and K supplied by chemical fertilizer and that contained in biochar preparation (char and ash).

Treated pots of ~ 2.5 l volume were arranged randomly on the benches of the glasshouse. A standard amount of 2 kg soil was weighed and all pots were filled with the same amount of soil. Biochars were added at a rate of 20 t ha^{-1} for podsol soil and 30 t ha^{-1} for ferrosol soil. Seedlings were germinated and transplanted as discussed in Seed Germination and Seedling Preparation section of this Chapter. The experiments were continued until flowering at which stage roots were developed and plants were harvested.

6.3.11 Observations

Observations were recorded on weekly plant height, number of branches, fresh and dry weights of roots and shoots, electrical conductivity, and soil pH, NPK of soil and plant tissues. Plant height was measured from the ground level to the base of the uppermost leaf. Fresh weights of shoots and roots were recorded separately. Fresh plant materials were kept in a dryer at $65^{O}C$ for two weeks after being put into thin paper envelopes in an upright position and leaving the envelope open to ventilate. Dry weight was recorded after two weeks in the drying room. Electrical conductivity, soil pH and nutrients were determined by the methods given in Appendix 3-9.

Table 6.3 Treatment combinations for ferrosol soil trial calculated on a hectare basis

Treatment code	Description
L + B1	Lime @ 6 t ha^{-1} (5.8 t ha^{-1} $CaCO_3$ from lime + 0.2 t ha^{-1} CaCO3 from Sugarcane Trash biochar @ 30 t ha^{-1})
L + B1 + N	Lime @ 6 t ha^{-1} (5.8 t ha^{-1} $CaCO_3$ from lime + 0.2 t ha^{-1} $CaCO_3$ from Sugarcane Trash biochar @ 30 t ha^{-1}) + N (110kg ha^{-1})
L +N + PK1	Lime @ 6 t ha^{-1} (lime only) + N (110kg ha^{-1}) + P & K~ Sugarcane Trash biochar @ 30 t ha^{-1}
L + B2	Lime @ 6 t ha^{-1} (4.2 t ha^{-1} $CaCO_3$ from lime + 1.8 t ha^{-1} $CaCO_3$ from Green Waste A @ 30 t $ha^{-1)}$
L + B2 + N	Lime @ 6 t ha^{-1} (4.2 t ha^{-1} $CaCO_3$ from lime + 1.8 t ha^{-1} $CaCO_3$ from Green Waste A @ 30 t ha^{-1}) + N (110 kg ha^{-1})
L + N + PK2	Lime @ 6 t ha^{-1} (lime only) + N (110kg ha^{-1}) + P & K~ Green Waste A @ 30 t ha^{-1})
L + B3	Lime @ 6 t ha^{-1} (5.58 t ha^{-1} $CaCO_3$ from lime + 0.42 t ha^{-1} CaCO3 from Green Waste B @ 30 t ha^{-1})
L + B3 + N	Lime @ 6 t ha^{-1} (5.58 t ha^{-1} $CaCO_3$ from lime + 0.42 t ha^{-1} CaCO3 from Green Waste B @ 30 t ha^{-1}) + N (110 kg ha^{-1})
L + N + PK3	Lime 6 t ha^{-1} (lime only) + N (110kg ha^{-1}) + P & K~ Green Waste B @ 30 t ha^{-1}

Table 6.4 Amount of NPK (mg pot^{-1}), lime (mg pot^{-1}) and biochar (g pot^{-1}) added to the ferrosol soil

Treatment code	N (as NH_4NO_3)	P (as TSP)	K (as KCl)	Lime ($CaCO_3$)	Biochar
L + B1				11661.6	60.3
L + B1 + N	632			11661.6	60.3
L +N + PK1	632	330.5	376.6	12057.6	
L + B2				8439.6	60.3
L + B2 + N	632			8439.6	60.3
L + N + PK2	632	222.2	852.0	12057.6	
L + B3				11212.8	60.3
L + B3 + N	632			11212.8	60.3
L + N + PK3	632	174.5	672.6	12057.6	

Table 6.5 Treatment combinations for podsol soil trial calculated on a hectare basis

Treatment code	Description
L + B1	Lime @ 1.2 t ha^{-1} (1.068 t ha^{-1} $CaCO_3$ from lime + 0.132 t ha^{-1} CaCO3 from Sugarcane Trash @ 20 t ha^{-1})
L + B1 + N	Lime @ 1.2 t ha^{-1} (1.068 t ha^{-1} $CaCO_3$ from lime + 0.132 t ha^{-1} CaCO3 from Sugarcane Trash @ 20 t ha^{-1}) + N (110 kg ha^{-1})
L + N + PK1	Lime @ 1.2 t ha^{-1} (lime only) + N (110 kg ha^{-1}) + P and K equivalent to Sugarcane Trash @ 20 t ha^{-1}
L + B2	Lime @ 1.2 t ha^{-1} (only from Green Waste A @ 20 t ha^{-1})
L + B2 + N	Lime @ 1.2 t ha^{-1} (only from Green Waste A @ 20 t ha^{-1}) + N (110 kg ha^{-1})
L + N + PK2	Lime @ 1.2 t ha^{-1} (lime only) + N (110 kg ha^{-1}) + P and K equivalent Green Waste A @ 20 t ha^{-1}
L + B3	Lime @ 1.2 t ha^{-1} (0.92 t ha^{-1} CaCO3 from lime + 0.28 t ha^{-1} $CaCO_3$ from Green Waste B @ 20 t ha^{-1})
L + B3 + N	Lime @ 1.2 t ha^{-1} (0.92 t ha^{-1} $CaCO_3$ from lime + 0.28 t ha^{-1} $CaCO_3$ from Green Waste B @ 20 t ha^{-1}) + N (110 kg ha^{-1})
L + N + PK3	Lime @ 1.2 t ha^{-1} (lime only) + N (110 kg ha^{-1}) + P and K equivalent to Green Waste B @ 20 t ha^{-1}

Table 6.6 Amount of NPK (mg pot^{-1}), lime (mg pot^{-1}) and biochar (g pot^{-1}) added to the podsol soil

Treatment code	N (as NH_4NO_3)	P (as TSP)	K (as KCl)	Lime ($CaCO_3$)	Biochar
L + B1				2147	40.2
L + B1 + N	632			2147	40.2
L + N + PK1	632	220.3	251.1	2411	
L + B2				0	40.2
L + B2 + N	632			0	40.2
L + N + PK2	632	148.23	568.1	2411	
L + B3				1849	40.2
L + B3 + N	632			1849	40.2
L + N + PK3	632	98.16	448.5	2411	

6.3.12 Soil and plant sample analysis

Soil and plant samples were analyzed by adapting the methods explained in Appendix 4-9.

6.3.13 Statistical analysis

ANOVA were undertaken using Minitab version 16 and graphs were plotted by Excel 8 package. The ANOVA were applied as the General Linear Model of Minitab. Paired grouping was organised by Tukey's family error test. Standard errors of the means were derived by standard deviations divided by square root of number of observations (*N*). Correlation coefficients were derived by Excel.

6.4 Results

6.4.1 Plant height

Treatments were significantly different for plant height from the fifth to the eighth week of transplanting (Figure 6.1). Morphological differences are shown in Plate 6.2. Initially, there was less difference between soils; however the difference was more at the eighth week. In the eighth week, plants were taller in podsol soil than in ferrosol soil treated with L + B1 and L + B1 + N, L + B2 and L + B2 + N and L + B3 and L + B3 + N. Interestingly, the plants were taller in ferrosol soil than in podsol soil when treated with L + N + PK1, L + N + PK2 and L + N + PK3. Among the treatments, L + N + PK1, 2 and 3 were superior within their SCT, GWA and GWB categories of biochar, respectively. Comparing these three treatments, there was difference in plant height but the trend was decreasing from L + N + PK1 through L + N + PK2 to L + N + PK3.

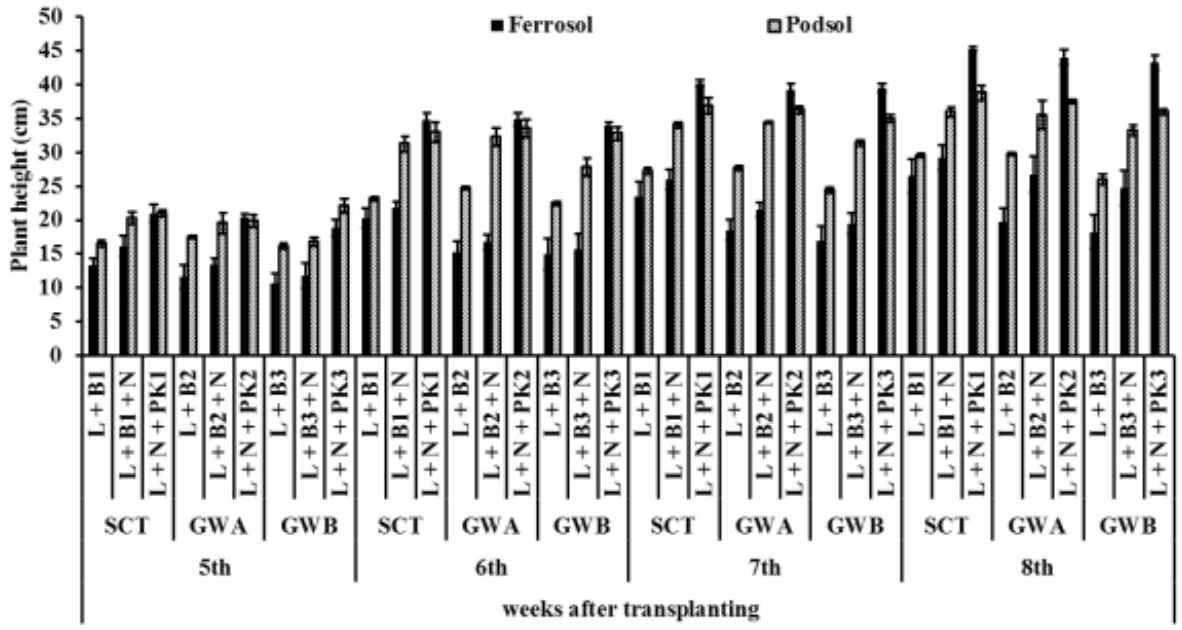


Figure 6.1 Tomato plant height from 5th to 8th week of transplanting in ferrosol and podsol soils. The treatments were significantly different at α=0.05 level of significance. The vertical bars represent standard error of the mean (*N* = 4). SCT = Sugarcane Trash, GWA = Green Waste A and GWB = Green Waste B. Description of treatment combination is given for ferrosol in Table 6.4 and for podsol in Table 6.5.

6.4.2 Shoot fresh weight and shoot dry weight

Shoot fresh weight and shoot dry weight were greater in ferrosol soil than in podsol soil (Figures 6.2 and 6.3). For all categories of biochar, the treatments L + B1, L + B2 and L + B3 produced less fresh weight than the treatments with + N and + N + PK. Among the sole biochar treatments with lime L + B1, L + B2 and L + B3, the L + B1 had greater weight than the remaining treatments. The treatments L + B1 + N and L + B2 + N had greater weight than L + B3 + N. The treatments with N + PK of all categories of biochar were superior to other treatments within the biochar category, however, the treatments with N + PK were similar in the case of podsol soil while the weights were slightly decreasing in ferrosol soil from SCT through GWA to GWB.

The category of all biochar had greater shoot dry weight in ferrosol soil than in podsol soil, however the difference was higher in SCT and GWA categories. In all categories, L + N + PK treatments were superior to others. Among the L + N + PK treatments, L + N + PK1 had the highest shoot dry weight in podsol soil while L + N + PK3 had the highest weight in

podsol soil. Overall results revealed that addition of N at a rate of 110 kg ha^{-1} plus P and K from chemical fertilizers with their equivalent amounts contained in respective biochar to the soils had greater positive effect than their absence. Addition of N including biochar had lesser positive effect than the addition of P and K. Among the biochar sole treatments, there was less difference in shoot dry weight in podsol soil but Sugarcane Trash produced greater weight in ferrosol soil.

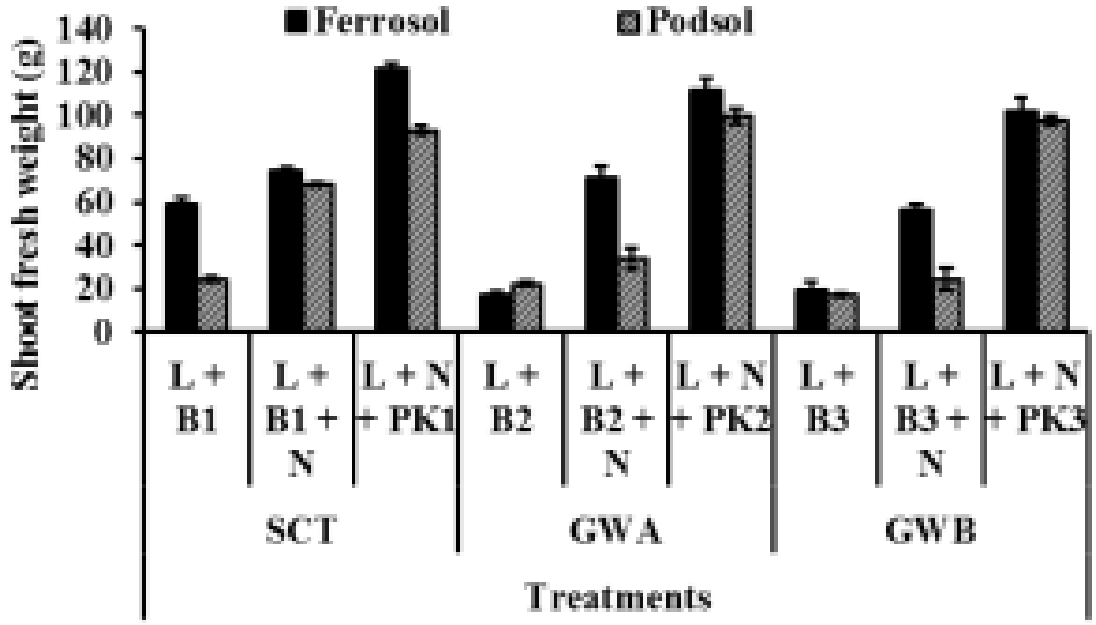


Figure 6.2 Tomato shoot fresh weight in ferrosol soil and podsol soils. The bars represent standard error of the mean (N = 4). All treatments were significantly different from each other at α=0.05 level of significance. SCT = Sugarcane Trash, GWA = Green Waste A and GWB = Green Waste B. Description of treatment combination is given for ferrosol in Table 6.4 and for podsol in Table 6.5.

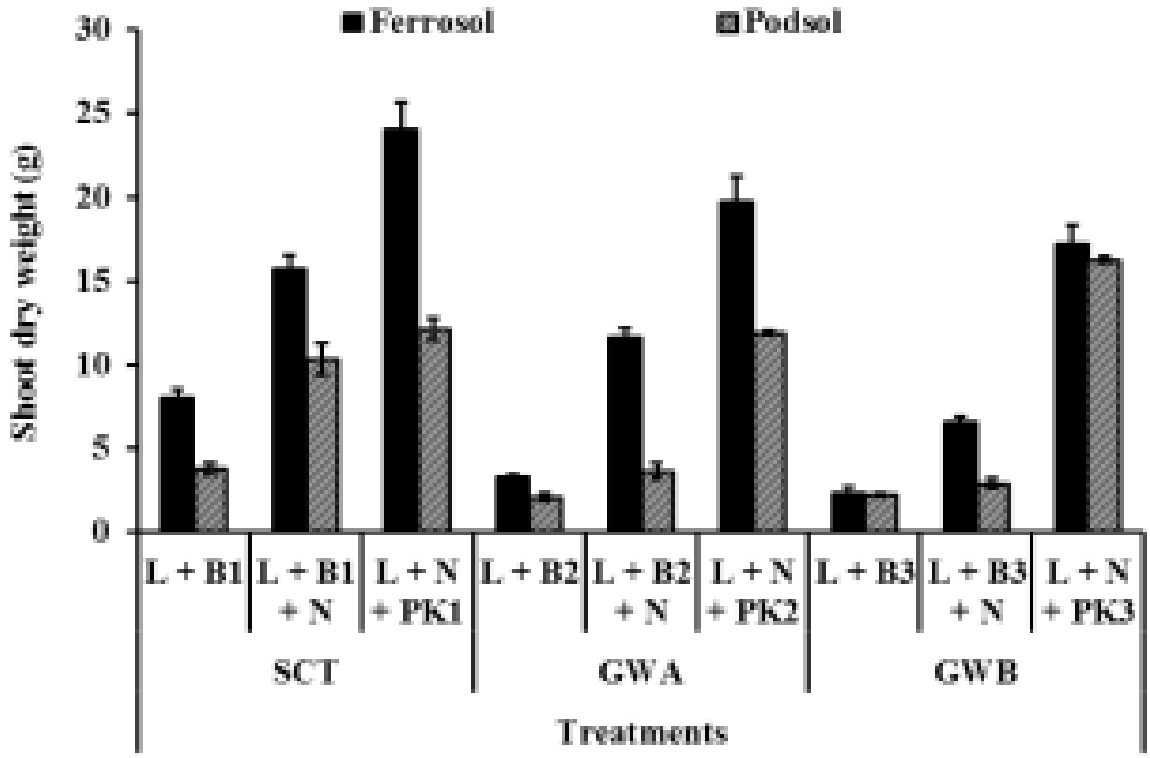


Figure 6.3 Tomato shoot dry weight in ferrosol and podsol soils. The bars represent standard error of the mean (N = 4). All treatments were significantly different from each other at α=0.05 level of significance. SCT = Sugarcane Trash, GWA = Green Waste A and GWB = Green Waste B. Description of treatment combination is given for ferrosol in Table 6.4 and for podsol in Table 6.5.

6.4.3 Root fresh weight and root dry weight

Root fresh weight was greater in podsol soil than in ferrosol soil; however, the results for root dry weight were opposite which would be associated with higher moisture content of the roots at harvesting in podsol soil (Table 6.7). Greater root dry weight in ferrosol soil should be linked with appropriate soil environment for root development. The root dry weight was greater when the soil was treated with L + B + N or L + N + PK in each category of biochar. As mentioned above, the treatments with L + N + PK were superior to others within the category. Among the biochar sole, L + B1 had the greatest positive effect followed by L + B2 and L + B3 in ferrosol soil but the results were inconsistent in podsol soil.

Table 6.7 Mean values for root fresh weight and root dry weight as influenced by the treatments in ferrosol and podsol soils. The ± values represent the standard error of the means (N = 4). The column values within each subset of means followed by the same letter are significantly different at α=0.05 level of significance. Description of treatment combination is given for ferrosol in Table 6.4 and for podsol in Table 6.5. ($p < 0.05$).

Treatments	Root fresh weight (g)		Root dry weight (g)	
	Ferrosol	Podsol	Ferrosol	Podsol
L + B1	8.42 ± 0.73b	6.30 ± 0.18b	1.87 ± 0.06b	0.55 ± 0.07b
L + B1 + N	7.69 ± 0.31b	21.59 ± 0.51a	2.12 ± 0.09b	1.90 ± 0.29a
L + N + PK1	18.90 ±1.50 a	24.72 ± 0.51a	3.20 ± 0.34a	2.20 ± 0.11a
L + B2	6.69 ± 0.36b	8.29 ± 0.77c	1.25 ± 0.25b	0.59 ± 0.12c
L + B2 + N	8.76 ± 1.07b	14.33 ± 1.79b	1.32 ± 0.11b	1.45 ± 0.08b
L + N + PK2	24.57 ±1.42a	25.23 ± 1.02a	3.50 ± 0.12a	2.15 ± 0.15a
L + B3	5.97 ± 0.54b	8.74 ± 0.67c	1.05 ± 0.48b	0.58 ± 0.33c
L + B3 + N	6.87 ± 0.49b	14.72 ± 188b	1.22 ± 0.09b	1.39 ± 0.24b
L + N + PK3	12.53 ± 2.33a	21.91 ± 1.81a	3.10 ± 0.15a	2.27 ± 0.31a

6.4.4 Soil EC and N P K levels after plant harvest

Soil electrical conductivity represents the concentration of salts in soil. N content of soil was total N, P was Colwell P (available P) and K values were exchangeable K. The cmol(+) kg^{-1} values of K were multiplied by 390 to convert into mg kg^{-1} and were further divided by 1000 to express in g kg^{-1} of soil. Total N was determined by combustion method, P by 0.5 M sodium bicarbonate at pH 8.5 and analyzed by Inductively Coupled Plasma Atomic Emission Spectrometry (ICPAES) and exchangeable K was derived from 1 M ammonium choride at pH 7 followed by ICPAES analysis as given in Appendices 5, 6 and 7, respectively.

Table 6.8 Mean values for soil electrical conductivity (dS m^{-1}), total amounts (g kg^{-1}) of total N, extractable P and extractable K in soil as influenced by the treatments in ferrosol and podsol soils. Description of treatment combination is given for ferrosol in Table 6.4 and for podsol in Table 6.5.

Treatments	Soil EC		N		P		K	
	Ferrosol	Podsol	Ferrosol	Podsol	Ferrosol	Podsol	Ferrosol	Podsol
L + B1	1.45	1.53	2.72	0.67	0.031	0.019	1.08	0.06
L + B1 + N	1.55	1.70	2.75	0.72	0.020	0.010	0.94	0.02
L + N + PK1	1.46	1.50	2.40	0.58	0.003	0.005	0.76	0.01
L + B2	2.30	2.53	2.75	0.63	0.026	0.014	1.29	0.15
L + B2 + N	2.50	2.80	2.77	0.73	0.015	0.012	1.13	0.13
L + N + PK2	2.33	2.62	2.46	0.59	0.002	0.004	0.94	0.03
L + B3	1.93	2.03	2.79	0.66	0.020	0.010	1.26	0.12
L + B3 + N	2.18	2.33	2.85	0.77	0.016	0.009	1.18	0.11
L + N + PK3	2.07	2.30	2.52	0.53	0.002	0.001	0.96	0.01

Soil electrical conductivity was greater in podsol soil than in ferrosol soil. The treatments L + B2, L + B2 + N and L + N + PK2 had greater EC the than the rest of the treatments (Table 6.8). Within the categories, L + B + N had the highest EC followed by L + N + PK and L + B. Soil N was greater in L + B + N treatments as N was added by both biochar and ammonium nitrate. Extractable P and K content were higher in L + B treatments followed by L + B + N and L + N + PK. The reason behind this could be due to the fact that P and K supplied by TSP and KCl should be more readily available than that supplied by biochar. Another reason could be the amount of P and K attached to or absorbed by the dense roots of L + N + PK treatments as the root fresh and dry weights were higher in these treatments.

6.4.5 Plant N, P and K

Plant N was derived by the combustion method; P and K were derived by acid digestion methods as given in Appendices 5 and 9. Plant N was greater in treatments for ferrosol soil than podsol soil. The L + N + PK treatments of all of the three categories showed greater amounts of N indicating that most of the N provided by biochar and soil was unavailable (Table 6.9). Plant N was less in L + B compared to the other treatments. The treatments L + B3, L + B3 + N and L + N + PK3 had greater amounts of N compared to their corresponding

treatments. However, some differences were observed in podsol soil. In podsol soil, the effect of L + N + PK1 had greater effect than L + B1 while the effect of L + B2 + N and L + N + PK2 was similar but greater than L + B2. The effect of L + N + PK3 was greatest followed by L + B3 + N and L + B3 within the category. Sufficiency ranges of N for tomato showed that the plant N was deficient for optimum plant growth in all the treatments.

Table 6.9 Mean values for plant N, P and K (mg g^{-1} dry weight) as influenced by the treatments in ferrosol and podsol soils. Sufficiency ranges of NPK for tomato was adapted from Rosen and Eliason (1996). Description of treatment combination is given for ferrosol in Table 6.4 and for podsol in Table 6.5.

Treatments	N		P		K		Sufficiency range
	Ferrosol	Podsol	Ferrosol	Podsol	Ferrosol	Podsol	
L + B1	17.4	9.1	2.4	2.52	35.0	12.42	N = 40-60
L + B1 + N	19.6	9.9	2.7	2.65	35.9	12.88	P = 2.5-8.0
L + N + PK1	28.6	13.6	3.1	3.15	38.1	13.44	K = 29-50
L + B2	15.6	8.4	2.3	2.42	36.6	15.88	
L + B2 + N	19.6	9.8	2.6	2.49	36.9	19.77	
L + N + PK2	28.8	12.4	2.8	3.01	40.8	22.56	
L + B3	18.4	10.2	2.0	1.70	36.2	14.44	
L + B3 + N	20.9	11.3	2.2	1.79	36.8	15.44	
L + N + PK3	25.5	16.7	2.4	1.89	38.7	17.46	

Plant P in L + B1, L + N + PK1, L + B2 and L + N + PK3 showed greater plant P in podsol soil than ferrosol soil while the rest of the treatments gave higher plant P in ferrosol soil. The plant P ranged from 2.2 mg kg^{-1} dry weight (L + B3 + N) to 2.8 mg kg^{-1} dry weight (L + N + PK2) indicating the plants of the treatments L + B1, L + B2, L + B3 and L + B3 + N had P deficiency. Similar effects were observed in podsol soil, that the plant P was greater in L + N + PK treatments followed by L + B + N and L + B treatments of the same category. It was noticed that the P ranged from 1.70 (L + B3) to 3.15 (L + N + PK1) indicating all treatments of the first category and L + N + PK2 of the second category had sufficient P content in the plant for optimum growth.

Interestingly, the plant K content was significantly greater and sufficient for plant growth in the treatments for ferrosol soil while it was deficient in the treatments for podsol soil. In both

soils, the K content was in order of L + N + PK > L + B + N > L + B in all categories. It indicated that the added amount of K could not meet the requirement of plant growth. Overall results demonstrated that the nutrients supplied by chemical fertilizers were more effective than that contributed by biochar.

6.4.6 Comparison between supplied and harvested amount of NPK

The amounts of NPK were determined from soil, biochar and chemical fertilizers added to the respective treatments and their amounts harvested from the dry matter of the plants (shoot and root) (Table 10, 11 and 12). The little amount of N taken up by the plants ranged from 0.04 g to 0.69 g per pot as compared to supplied N from 5.48 to 5.84 g per pot in ferrosol soil. A significantly higher amount of N was absorbed by the plants in the treatments of L + N + PK treatments of all categories in both soils. The treatments L + B1, L + B1 + N and L + N + PK1 showed the greater amounts of harvested N compared to their corresponding treatments of the other categories in both soils. The treatments with L + B had the least amount of harvested N. Similarly, the plant N ranged from 0.02 to 0.27 g per pot as compared to the supplied N range from 1.28 to 1.56 g per pot in podsol soil; however the per cent of amounts harvested was greater in ferrosol soil. In the trial, the same amount of N was supplied to all the treatments except to L + B treatments. Plants could not take up the entire readily available N supplied by chemical fertilizer because the growth was continuing and plants were harvested during their optimum growth (8 weeks) when they had just started flowering.

The amount of P absorbed by the plants ranged from 0.005 to 0.075 g per pot as compared to supplied P from 0.045 to 0.081 g per pot in ferrosol soil. There was significantly higher amount of P absorbed by the plants in the L + N + PK treatments of all categories in both soils. The treatments L + B1, L + B1 + N and L + N + PK1 showed the greater amounts of harvested P compared to their corresponding treatments of the other categories in both soils. The treatments with L + B had the least amount of harvested P in both soils. Similarly, the plant P ranged from 0.004 to 0.038 g per pot as compared to the supplied P range from 0.023 to 0.047 g per pot in podsol soil; however the per cent of amounts harvested was greater in ferrosol soil.

The amount of K harvested by the plants ranged from 0.08 to 0.92 g per pot as compared to supplied K from 2.44 to 2.69 g per pot in ferrosol soil. There was significantly higher amount of K absorbed by the plants in the treatments of L + N + PK treatments of all categories in

both soils. The treatments L + B1, L + B1 + N and L + N + PK1 showed the greater amounts of harvested K compared to their corresponding treatments of the other categories in ferrosol soil while L + B1, L + B1 + N and L + N + PK2 had greater amounts of harvested K in podsol soil. The treatments with L + B had the least amount of harvested K in ferrosol soil. Similarly, the plant K ranged from 0.03 to 0.27 g per pot as compared to the supplied N range from 0.17 to 0.33 g per pot in podsol soil; however the per cent of amounts harvested was greater in ferrosol soil.

In conclusion, the N, P and K were higher in the plants treated with chemical fertilizers. The same amounts of P and K supplied by biochar had less effect than those supplied from chemical fertilizer. This fact indicates further verification of this research is needed by means of a longer-term study.

6.4.7 Correlation between NPK and shoot dry weight

Correlation coefficients for shoot dry weight against N, P and potash contained in soil and shoot dry matter were determined by their values of four replications (N = 4) (Table 6.13 and Table 6.14). In both soils, the relationship between shoot dry weight and N, P, or K of plant and soil was strong enough in all treatments to contribute to increase the shoot dry weight (r = >0.55) except for soil K and shoot dry weight in ferrosol soil in L + N + PK3 treatment (r = 0.45). The main reason behind this lower correlation coefficient was that the K values were similar in three replications, yet the shoot dry weight was increasing. The similar values might be reliant on the sampling of soils. These results confirmed that the increasing level of NPK in soil or plant of each treatment will contribute to increase the shoot dry weight.

6.5 Discussion

These experiments were conducted to determine if there was any difference in the performance of tomato plants when biochars and the equivalent NPK contents were amended to balance the nutrient concentrations at the same soil pH in a podsol soil and a ferrosol soil. The overall results revealed that tomato plants performed better in the soils treated with the N at a rate of 110 kg ha^{-1} plus P and K equivalent to the amount in biochar. As P level was greater in Sugarcane Trash biochar, and K was higher in Green Waste A, the treatments with the equivalent level of nutrients contained in the biochars but supplied instead by fertilizers had better performance of tomato plants.

Table 6.10 Amounts of N (g pot^{-1}) content of soil before planting and plant uptake after harvest in the treatments for ferrosol and podsol soils. Description of treatment combination is given for ferrosol in Table 6.4 and for podsol in Table 6.5.

Treatments	N content									
	Ferrosol					Podsol				
	Soil				Plant	Soil				Plant
	Total	Soil	Char	Chemical		Total	Soil	Char	Chemical	
L + B1	5.58	5.26	0.32		0.14	1.36	1.10	0.22		0.03
L + B1 + N	5.80	5.26	0.32	0.22	0.31	1.54	1.10	0.22	0.22	0.10
L + N + PK1	5.48	5.26		0.22	0.69	1.32	1.10		0.22	0.17
L + B2	5.54	5.26	0.28		0.05	1.28	1.10	0.18		0.02
L + B2 + N	5.76	5.26	0.28	0.22	0.23	1.50	1.10	0.18	0.22	0.04
L + N + PK2	5.48	5.26		0.28	0.57	1.32	1.10		0.22	0.15
L + B3	5.62	5.26	0.36		0.04	1.34	1.10	0.24		0.02
L + B3 + N	5.84	5.26	0.36	0.22	0.14	1.56	1.10	0.24	0.22	0.03
L + N + PK3	5.48	5.26		0.22	0.44	1.32	1.10		0.22	0.27

Table 6.11 Amounts of P (g pot^{-1}) content of soil before planting and plant uptake after harvest in the treatments for ferrosol and podsol soils. The soil P values are extractable and plant P values are total. Description of treatment combination is given for ferrosol in Table 6.4 and for podsol in Table 6.5.

Treatments	P content							
	Ferrosol			Plant	Podsol			Plant
	Total	Soil	Char/Chemical		Total	Soil	Char/Chemical	
L + B1	0.081	0.015	0.066	0.019	0.047	0.003	0.044	0.010
L + B1 + N	0.081	0.015	0.066	0.042	0.047	0.003	0.044	0.027
L + N + PK1	0.081	0.015	0.066	0.075	0.047	0.003	0.044	0.038
L + B2	0.059	0.015	0.044	0.008	0.033	0.003	0.030	0.005
L + B2 + N	0.059	0.015	0.044	0.030	0.033	0.003	0.030	0.009
L + N + PK2	0.059	0.015	0.044	0.055	0.033	0.003	0.030	0.026
L + B3	0.045	0.015	0.030	0.005	0.023	0.003	0.020	0.004
L + B3 + N	0.045	0.015	0.030	0.014	0.023	0.003	0.020	0.005
L + N + PK3	0.045	0.015	0.030	0.041	0.023	0.003	0.020	0.021

Table 6.12 Amounts of K (g pot^{-1}) content of soil before planting and plant uptake after harvest in the treatments for ferrosol and podsol soils. The soil K values are extractable and plant K values are total. Description of treatment combination is given for ferrosol in Table 6.4 and for podsol in Table 6.5.

Treatments	K content							
	Ferrosol			Plant	Podsol			Plant
	Total	Soil	Char/Chemical		Total	Soil	Char/Chemical	
L + B1	2.44	2.24	0.198	0.28	0.17	0.038	0.132	0.05
L + B1 + N	2.44	2.24	0.198	0.56	0.17	0.038	0.132	0.13
L + N + PK1	2.44	2.24	0.198	0.92	0.17	0.038	0.132	0.16
L + B2	2.69	2.24	0.447	0.12	0.33	0.038	0.292	0.03
L + B2 + N	2.69	2.24	0.447	0.43	0.33	0.038	0.292	0.07
L + N + PK2	2.69	2.24	0.447	0.81	0.33	0.038	0.292	0.27
L + B3	2.59	2.24	0.353	0.08	0.27	0.038	0.235	0.03
L + B3 + N	2.59	2.24	0.353	0.24	0.27	0.038	0.235	0.05
L + N + PK3	2.59	2.24	0.353	0.67	0.27	0.038	0.235	0.26

Table 6.13 Correlation coefficient (*r*) between N and P and K content of soil and shoot dry weight. Description of treatment combination is given for ferrosol in Table 6.4 and for podsol in Table 6.5.

Treatments	Correlation coefficient between (r =)					
	N and Shoot dry weight		P and shoot dry weight		K and shoot dry weight	
	Ferrosol	Podsol	Ferrosol	Podsol	Ferrosol	Podsol
L + B1	0.96	0.94	0.81	0.97	0.97	0.94
L + B1 + N	0.89	0.88	0.87	0.89	0.88	0.77
L + N + PK1	0.98	0.93	0.93	0.77	0.83	0.66
L + B2	0.90	0.80	0.91	0.82	0.94	0.97
L + B2 + N	0.82	0.56	0.80	0.94	0.94	0.80
L + N + PK2	0.83	0.78	0.74	0.96	0.87	0.74
L + B3	0.98	0.41	0.64	0.59	0.71	0.82
L + B3 + N	0.98	0.96	0.90	0.99	0.92	0.92
L + N + PK3	0.89	0.59	0.72	0.79	0.45	0.94

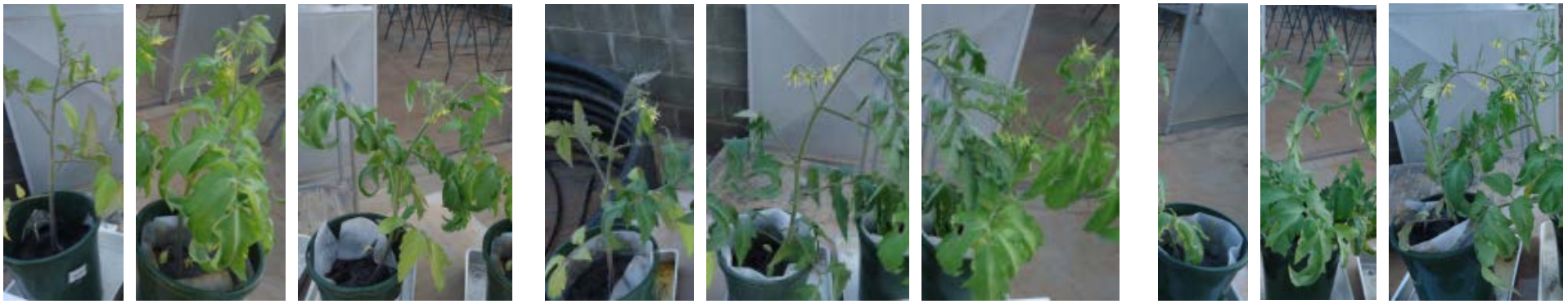
Table 6.14 Correlation coefficient (*r*) between N, P and K content of plants and shoot dry weight. Description of treatment combination is given for ferrosol in Table 6.4 and for podsol in Table 6.5.

Treatments	Correlation coefficient between (*r*=)					
	N and shoot dry weight		P and shoot dry weight		K and shoot dry weight	
	Ferrosol	Podsol	Ferrosol	Podsol	Ferrosol	Podsol
L + B1	0.98	0.98	0.75	0.87	0.95	0.98
L + B1 + N	0.97	0.84	0.98	0.65	0.95	0.97
L + N + PK1	0.82	0.96	0.90	1.00	0.87	0.72
L + B2	0.98	0.99	0.99	0.54	0.99	1.00
L + B2 + N	0.93	0.62	0.97	0.90	0.98	0.95
L + N + PK2	0.67	0.57	0.93	0.93	0.97	0.97
L + B3	0.99	0.64	1.0	0.80	1.00	0.87
L + B3 + N	0.97	0.99	0.98	0.93	0.83	0.96
L + N + PK3	0.95	0.99	0.69	0.89	0.93	0.74

L + B1 **L + B1 +N** **L + N + PK1** **L + B2** **L + B2 + N** **L + N + PK2** **L + B3** **L + B3 + N** **L + N + PK3**

Plate 6.2 Morphological performance of tomato in ferrosol soil.

L + B1= Lime @ 6 t ha^{-1} (5.8 t ha^{-1} CaCO3 from lime + 0.2 t ha^{-1} CaCO3 from Sugarcane Trash biochar @ 30 t ha^{-1}); L + B1 + N = Lime @ 6 t ha^{-1} (5.8 t ha^{-1} CaCO3 from lime + 0.2 t ha^{-1} CaCO3 from Sugarcane Trash biochar @ 30 t ha^{-1}) + N (110kg ha^{-1}); L + N + PK1 = Lime @ 6 t ha^{-1} (lime only) + N (110kg ha^{-1}) + P & K~ Sugarcane Trash biochar @ 30 t ha^{-1}; L + B2 = Lime @ 6 t ha^{-1} (4.2 t ha^{-1} CaCO3 from lime + 1.8 t ha^{-1} CaCO3 from Green Waste A @ 30 t ha^{-1}); L + B2 + N = Lime @ 6 t ha^{-1} (4.2 t ha^{-1} CaCO3 from lime + 1.8 t ha^{-1} CaCO3 from Green Waste A @ 30 t ha^{-1}) + N (110 kg ha^{-1}); L + N + PK2 = Lime @ 6 t ha^{-1} (lime only) + N (110kg ha^{-1}) + P & K~ Green Waste A @ 30 t ha^{-1}); L + B3 = Lime @ 6 t ha-1 (5.58 t ha^{-1} CaCO3 from lime + 0.42 t ha^{-1} CaCO3 from Green Waste B @ 30 t ha^{-1}); L + B3 + N = Lime @ 6 t ha^{-1} (5.58 t ha^{-1} CaCO3 from lime + 0.42 t ha^{-1} CaCO3 from Green Waste B @ 30 t ha^{-1}) + N (110 kg ha^{-1}); L + N + PK3 = Lime 6 t ha^{-1} (lime only) + N (110kg ha^{-1}) + P & K~ Green Waste B @ 30 t ha^{-1}. In each row, well growing plants (right) treated with lime plus N at a rate of 110 kg ha^{-1} plus P and K equivalent to biochar, some growth but less branching plants (middle) were treated with biochar plus N at a rate of 110 kg ha^{-1}. Nutrient deficient plants (left) were treated with biochar sole (left). The plants supplied nutrients from chemical fertilizer had better growth and early flowering.



L + B1 **L + B1 +N** **L + N + PK1** **L + B2** **L + B2 +N** **L + N + PK2** **L + B3** **L + B3 +N** **L + N + PK3**

Plate 6.3 Morphological performance of tomato in podsol soil.

L + B1 = Lime @ 1.2 t ha^{-1} (1.068 t ha^{-1} CaCO3 from lime + 0.132 t ha^{-1} CaCO3 from Sugarcane Trash @ 20 t ha^{-1}); L + B1 + N = Lime @ 1.2 t ha^{-1} (1.068 t ha^{-1} CaCO3 from lime + 0.132 t ha^{-1} CaCO3 from Sugarcane Trash @ 20 t ha^{-1}) + N (110 kg ha^{-1}); L + N + PK1 = Lime @ 1.2 t ha^{-1} (lime only) + N (110 kg ha^{-1}) + P and K equivalent to Sugarcane Trash @ 20 t ha^{-1}; L + B2 = Lime @ 1.2 t ha^{-1} (only from Green Waste A @ 20 t ha^{-1}); L + B2 + N = Lime @ 1.2 t ha^{-1} (only from Green Waste A @ 20 t ha^{-1}) + N (110 kg ha^{-1}); L + N + PK2 = Lime @ 1.2 t ha^{-1} (lime only) + N (110 kg ha^{-1}) + P and K equivalent Green Waste A @ 20 t ha^{-1}; L + B3 = Lime @ 1.2 t ha^{-1} (0.92 t ha^{-1} CaCO3 from lime + 0.28 t ha^{-1} CaCO3 from Green Waste B @ 20 t ha^{-1}); L + B3 + N = Lime @ 1.2 t ha^{-1} (0.92 t ha^{-1} CaCO3 from lime + 0.28 t ha^{-1} CaCO3 from Green Waste B @ 20 t ha^{-1}) + N (110 kg ha^{-1}); L + N + PK3 = Lime @ 1.2 t ha^{-1} (lime only) + N (110 kg ha^{-1}) + P and K equivalent to Green Waste B @ 20 t ha^{-1}. In each row, well growing plants (right) treated with lime plus N at a rate of 110 kg ha^{-1} plus P and K equivalent to biochar, some growth but less branching plants (middle) were treated with biochar plus N at a rate of 110 kg ha^{-1}. Nutrient deficient plants (left) were treated with biochar sole (left). The plants supplied nutrients from chemical fertilizer had better growth and early flowering.

These experiments were developed to test the hypothesis that the effect of biochar (with associated nutrients) on plant growth was greater than that caused by the equivalent amount of nutrients alone. This hypothesis was not substantiated because the nutrients alone had greater effect on plant growth than the biochars with their associated nutrients. Another hypothesis tested in this study was that N contained in the carbon matrix of biochar was not available in soils for plant growth. This hypothesis was supported because the biochar without added N had less effect on plant growth.

The growth pattern of the plants was similar between the treatments equivalent to each of the biochars (L + N + PK). There was no difference in plant growth between the treatments during the early stage which was mainly due to the low fertility soils that have fewer nutrients to supply. The added nutrients had little effect on the growth in that period because the seedlings had less developed root systems which were inadequate to absorb the soil nutrients and they largely used their own stored nutrients for growth. Morphologically, the ferrosol soil had better growth of plants than the podsol soil; however the effect of nutrients supplied by the chemical fertilizer was more than that of the biochar. By visual observation, plants appeared to be nutrient deficient in L + B treatments. There was also some difference between the L + B and L + B + N treatments. The great difference for plant growth between ferrosol and podsol was mainly associated with their nutrient contents. The NPK contents were higher in ferrosol (5.26, 0.015 and 2.24 g pot-1, respectively) than in podsol (1.10, 0.003 and 0.038 g pot^{-1}); these are given in Table 6.10, 6.11 and 6.12, respectively.

The literature on comparison of the effect of biochar in different soils and comparing the effect of different biochars and their interactions is inadequate. However, some previous reports have summarized the benefits of biochar as sequestration of carbon, improvement of cation exchange capacity, durability of soil aggregates, microbial activity, bioenergy production and water retention capacity; reduction of nitrous oxide and methane emissions from soils, leaching, soil erosion and need of fertilization and thereby enhancement of soil fertility and crop yields (Brandstaka et al. 2010). Other reports emphasised the adsorption of anions and cations by biochar to prevent leaching of applied nutrients (Major et al. 2009). In this study leaching of applied nutrients was prevented by maintaining the water content by applying the calculated amount of water so as not to exceed field capacity. However there could also be a role for biochar to reduce leaching of nutrients which could not be determined from this experiment.

Van Zwieten et al. (2010a) tested two biochars produced from the slow pyrolysis of paper mill waste, in two agricultural soils in a glasshouse and found that the biochars differed slightly in their liming values (33% and 29%). The lime requirement of podsol and ferrosol soils in the present

study also confirmed that the ferrosol soil needed more biochar than the podsol; soil and Green Waste A had the higher liming value than Sugarcane Trash and Green Waste B (Appendix 1).

No immediate effect on plant growth of adding biochar to these two soils was observed. Hardie et al. (2014) incorporated biochar in soil and found after thirty months observation that it had no significant effect on soil moisture content, drainable porosity between −1.0 and −10 kPa, field capacity, plant available water capacity, aggregate stability and the permanent wilting point but had significantly higher near-saturated hydraulic conductivity, soil water content at −0.1 kPa, and significantly lower bulk density than the unamended control. Tammeorg et al. (2014) reported that biochar improved nitrate N content, water retention capacity, soil organic carbon and K content, while the present study had less effect on the availability of NPK which could be associated with the shorter duration of the study. In another study biochar derived from wheat straw decreased available P (Alburquerque et al. 2014) which was not comparable to the present results because the control soils (without biochar and nutrients) were not included in the study.

A biochar produced from corn cobs was found to increase nitrate N in the first ten days and thereafter it decreased; while it decreased P content when applied alone and increased after addition of nitrogenous or phosphate fertilizer (Nelson et al. 2011). In the present study, nitrate N was not studied but total N and extractable P and K were increased after addition of chemical fertilizers (Table 6.10).

Mineralization of N could be enhanced by application of biochar produced from slow pyrolysis rather than the fast pyrolysis (Bruun et al. 2012). The biochar tested in this study were medium type as described in the Materials and Methods Section of this Chapter.

In a three-year field experiment, there was no difference between biochar added and not-added soil but reapplication of biochar after three years significantly increased available P, exchangeable K and calcium, dissolved organic carbon, soil moisture and electrical conductivity (Quilliam et al. 2012). These results recommend continuing the experiment to see the long-term effect; however, the present study had limitations of time so it could not be extended for years.

Van Zwieten et al. (2010a) tested two biochars produced from the slow pyrolysis of paper mill waste, in two agricultural soils in a glasshouse and found that they significantly increased N uptake in wheat and biomass in wheat, soybean and radish in ferrosol soil. In this study, soils with and without biochar were not compared but N uptake differed in both soils.

In separate studies presented in previous chapters (Chapter 5), a positive effect of biochar addition on plant growth and mycorrhizal colonization was observed in a calcareous soil. The use of biochar was beneficial over no use for growth of cabbage, lettuce, onion and tomato, although equal amounts of nutrients were supplied through Hoagland's nutrient solution. Comparison of biochar and no biochar could also be observed in ferrosol and podsol soils in upcoming chapters.

Asai et al. (2009) showed that biochar increased rice grain yields at sites with low P availability; but in this study, biochar addition alone produced less dry matter than the addition of P and K equivalent to the biochar plus 110 kg ha^{-1} N. The N content in L + B treatment and L + B + N treatment had similar effect. Biochar at the rates of 20 and 40 t ha^{-1} without N fertilization in a carbon poor calcareous soil in China increased maize yield by 15.8% and 7.3% while the rates with 300 kg ha^{-1} N fertilization enhanced the yield by 8.8% and 12.1%, respectively (Zhang et al. 2012). This result was contradictory to our results for ferrosol and podsol soils which found that shoot fresh weight and dry weight were higher in biochar with N fertilization. However, it might not be comparable between different crops and different soil types.

In addition, biochar application in a nutrient-poor, slightly acidic loamy sand soil had little effect on wheat yield in the absence of mineral fertilization but when applied with the highest rate of mineral fertilization, it increased yield by 20–30% more than mineral fertilizer alone (Alburquerque et al. 2014) which is also similar to the present results on dry matter yield, however, the crop was harvested before fruiting. Yet, the present study showed that the chemically fertilized plants started flowering six weeks after planting while the plants with biochar sole remained un-flowered until the time of harvest (eight weeks after planting).

The soil test after plant harvest showed that the application of biochar and lime balanced the pH within the range of plant requirement (~7). The dry matter yield was strongly correlated with the application of biochar, biochar plus N or N plus PK showing the increased correlation by increased level of nutrients in soil samples (within a replication). The overall treatment effect on plant morphology was outstanding, which can be seen in the Plate 6.3.

Biochars had different concentrations of NPK (Appendix 1) and also had different bulk densities. Therefore, they need to be balanced for their comparison. A brief illustration of general information is presented in Appendix 10.

6.6 Conclusion

The soils balanced for pH with the use of biochar and lime were prepared to determine the difference between lime + biochar, lime + biochar + N and lime + N + PK equivalent to biochar in ferrosol and podsol soils. The overall results showed the treatments had positive effect on shoot fresh weight and shoot dry weight of tomato. The treatment L + B + N was beneficial over L + B application. The application of L + N + PK had the significantly greatest positive effect on performance of tomato.

The present study was conducted for only eight weeks which was insufficient to address many plant and nutrient related issues. It is considered that the experiment should be extended for at least three years to see the biochar effect on soil and plant growth. It would also be worthwhile to compare different types of crops and soils during the same period.

As the main purpose of the PhD research was developed for mycorrhizal study, an experiment was further established to determine the mycorrhizal colonization in similar treatments. The details of the study on the influence of the similar treatments on mycorrhizal colonization of onion roots was carried out in both the podsol and ferrosol soil, which will be discussed in the next chapter.

Chapter 7. Comparative effects of soil types, biochar types and mycorrhizae on growth and colonization of onion in soils with adjusted pH.

7.1 Abstract

Ferrosol and podsol soils, Sugarcane Trash and Green Waste A biochars, and inoculum of AM fungi at a rate of 0 and 5 g kg^{-1} of soil were tested to determine their sole and interaction effects on growth of onion plants and colonization of their roots by AM fungi. Experiment was conducted in a 2 x 2 x 2 factorial arrangement consisting of 8 treatment combinations in 4 replications. Biochars were applied at a rate of 30 t ha^{-1}. Observations were recorded on growth parameters, mycorrhizal colonization and nutrient contents. The sole effect of soil type, biochar type and mycorrhizal rates was prominent but there were little effects of their interaction. Ferrosol soils, Sugarcane Trash biochar and mycorrhizal rates of 5 g kg^{-1} were positively effective over their corresponding treatments. The interactions were not enough to affect the plant performance and colonization of onion roots by mycorrhizae.

7.2 Introduction

There are many references on the study of biochars, their effects on crops and mycorrhizae, which were cited in previous chapters. From the conclusions drawn in the previous chapter, it was recommended that a comparative study on soil types and biochar types be conducted. Therefore, the research question was to determine the difference in mycorrhizal colonization and performance of onion when biochars were applied at the same rate to a podsol and a ferrosol soil balanced to the same pH. The hypothesis was that biochar types have different influences on mycorrhizal colonization of onion when added to ferrosol and podsol. The specific aim of the study was to compare the plant performance and mycorrhizal colonization as influenced by the types of soil and biochars when each soil is adjusted to the same pH value and thus nutrient availability of biochars should be similar. The experiment also emphasized the examination of mycorrhizal colonization in two soil types and two biochars.

7.3 Materials and Methods

7.3.1 Biochar types

The biochars were made from sugarcane trash and green wastes and had different compositions. These two biochars were relatively better for plant growth and mycorrhizal colonization from my

previous experiments in sand medium (Chapter 4). The general information about biochar and their NPK concentration is given in Appendix 1.

Biochar amounts were calculated based on the area of pots. The pot area based on the average diameter of 12 cm was 0.011304 cm^2. The biochar rate of 30 t ha^{-1} was ~ 34.0 g pot^{-1}.

7.3.2 Cultivar, source of seeds and pot preparation

Onion seeds of variety Rio Red Rock were obtained from South Pacific Seeds, New South Wales. These seeds were also used for previous experiments of the present study.

Plastic pots of 1.5 litre volume with the average diameter of ~12cm were selected for the experiments. A fine fabric cloth was placed on the bottom of the pots and pots were placed on the trays in which the drained water and nutrient solutions could be collected and reused to prevent loss of nutrients and water. The pots were then filled with the soils treated with biochar with or without mycorrhizal inoculum.

7.3.3 Seed germination and seedling preparation

Seeds were germinated in 4.5 x 3 x 2.5 cm (height-length-breadth) dimensioned, rectangular (100 cells, 10 rows and 10 cells per row) germination trays filled with propagation mix. Three seeds were sown at a depth of 1.5 cm and covered with the potting mix. The tray was watered gently through a wash bottle immediately after seeding. After maximum germination, the seedlings were thinned to one seedling per hole. Seedlings were watered every morning until they germinated. After germination, the watering was reduced to once every alternate day to harden them so that they could tolerate transplanting shock after transfer from fertile propagation mix to less fertile podsol and ferrosol soils.

The seedlings were watered 2 hours before transplanting and then uprooted gently without any damage and stress to the seedlings. Roots of uprooted seedlings were cleaned by dipping and gentle shaking into water in a tray. After removing all particles of propagation mix, seedlings were transplanted into pots filled with treated podsol and ferrosol soils.

7.3.4 Source of mycorrhizae

The MycoApply product of mycorrhizae was used as soil inoculum of mycorrhizae. The inoculum was the same as described in previous chapters. Five grams were placed in treatment pots.

7.3.5 Source and characteristics of soil

The low fertility and acidic podsol soil was collected from the Tent Hill area near Lockyer Valley, Queensland (~120 km from Brisbane) while low-fertility ferrosol was collected from fallow grassy land in the Kingaroy region of Queensland (~300 km west of Brisbane) in 2014. The composite podsol soil was taken from both the A and B Horizon (up to 30 cm depth from the surface) consisting of a light-red layer. The soil had bulk density 1.41 g cm-^{3}), water holding capacity 0.26 cm^3, cation exchange capacity 0.92 and lime requirement 1.5 t ha^{-1}. The pH and EC were 4.5 and 0.04 dS m^{-1}.

Ferrosol soils are generally formed from rocks with volcanic origins and are nearly always red coloured (Spark & Swift no date). They generally have good soil structure, lack strong texture contrast between A and B horizons and have a high free iron content in the B horizon (subsoil). These soils are well drained and are often deep and highly fertile, and are typically found on the crests, upper, mid- and lower slopes of plateau remnants. The soil had bulk density 0.95 g cm-^{3}), water holding capacity 0.40 cm^3, cation exchange capacity 1.2 and lime requirement 6.0 t ha^{-1}. The pH and EC were 5.2 and 0.08 dS m^{-1} (Spark & Swift no date).

7.3.6 Calculation of lime requirement

The current pH of podsol soil was analyzed by the methods 1:5 water and 1:1 water described in Appendix 4. The amount of lime required to increase pH up to 6.5 was selected for the experiment and the procedure was the same as described in Chapter 6. The pH of the podsol and ferrosol soils was 4.6 and 5.2 respectively. The rates of lime for podsol and ferrosol to raise the pH up to 6.5 were 1.89 and 6.2 t ha^{-1}, respectively. The acid neutralizing capacity (% $CaCO_3$) of Sugarcane Trash and Green Waste A biochar was 6% and 0.66%, respectively. When a rate of 30 t ha^{-1} of each biochar was applied, the amount of lime equivalent supplied through Sugarcane Trash was 0.2 t ha^{-1} while it was 1.8 t ha^{-1} through Green Waste A. For podsol, 1.69 and 0.09 t ha^{-1} of pure lime were added with the addition of 30 t ha^{-1} Sugarcane Trash and Green Waste A biochar, respectively. Similarly, for ferrosol, 6 and 4.2 t ha^{-1} of pure lime were added with the addition of 30 t ha^{-1} Sugarcane Trash and Green Waste biochar, respectively. Thus, the same amount of biochar was added to both soils. Lime was mixed in the soils two weeks before planting. Then the pots were filled with soils and water maintained at field capacity until planting to allow sufficient reaction time between soil and lime.

7.3.7 Nutrient solution

A low-P (25% P) Hoagland's nutrient solution (Appendix 2) prepared for other previous experiments was applied at a rate of 50 mL per pot just after transplanting. After adding the solutions, water was added to maintain soil at field capacity.

7.3.8 Determination of field capacity

Field capacity was determined by the same procedure followed in Chapter 6.

7.3.9 Experimental design

Pot experiments were conducted in a glasshouse as a 2 x 2 x 2 factorial arrangement with soil types, biochar types and mycorrhizal inoculum rates as factors in four replications. The treatment combinations consisted of two soils (ferrosol and podsol), two biochar types (Sugarcane Trash and Green Waste A) and two inoculum rates of mycorrhizae (5 g and 0 g kg^{-1} of soil) (Table 7.1). The pots of ~ 1.5L volume were arranged randomly on the bench of the glasshouse. A standard amount of 1 kg soil was weighed and all pots were filled with the same amount of soil. Biochars were added at a rate of 30 t ha^{-1} to both soils.

7.3.10 Observations

Observations were recorded on plant height at harvest (eight weeks after planting), fresh and dry weight of shoots, fresh weight of roots, soil EC, soil pH, soil N, P, K and plant N, P, K. Plant height was measured from the ground level to the tip of the longest leaf. Fresh above ground parts were weighed and kept in a dryer at $65^{O}C$ for two weeks after putting them into thin paper envelopes in an upright position and leaving the envelopes open to ventilate. Dry weight was taken after two weeks in the drying room. Electrical conductivity, soil pH and nutrients were determined by the methods given in Appendix 3-9. Nutrients and mycorrhizal colonization were analyzed by the procedures adopted in previous chapters.

7.3.11 Statistical analysis

ANOVA was undertaken in Minitab 16, version 4.0 (Minitab 2005) and graphs were plotted by Sigma Plot programme, version 12.0 (Systat Software 2007). The standard error was derived from the standard deviation of the mean divided by the number of observations. The number of observations for soil types, biochar types and mycorrhizal rates was 16 while it was 8 for the interactions of soil and biochar, soil and mycorrhizae, and biochar and mycorrhizae. The number for the interaction of soil types, biochar types and mycorrhizal rate was 4. The standard errors of the factors and their dual interactions were calculated in Microsoft Excel 2010, version 14.0 (Microsoft 2010) by using the following formula:

$$Standard\ error = \left(\frac{SQRT\left(\frac{SUMSQ(a:b)}{n}\right)}{\sqrt{N}}\right)$$

Where, SQRT = Square root

SUMSQ = Sum of squares

a:b = the standard deviations to be averaged after squared

n = number of standard deviations to be averaged

N = Number of observations for the factors and interactions.

7.3.12 Nutrients and lime content (%$CaCO_3$) of biochars

Biochars had different concentrations of NPK and also had different bulk densities. Therefore, they need to be balanced for their comparison. A brief illustration of general information is presented in Appendix 1.

Table 7.1 Treatment structures in factorial arrangement.

Soil Type	Biochar type	Mycorrhizal rates (g kg^{-1})
Ferrosol	Sugarcane Trash	5
		0
	Green Waste A	5
		0
Podsol	Sugarcane Trash	5
		0
	Green Waste A	5
		0

7.4 Results

7.4.1 Effect of soil type

Ferrosol and podsol soils were significantly different for plant height at harvest, shoot fresh and dry weight, root fresh weight, soil P, soil K, plant P (Table 7.2), root length, percent of colonized root length and colonized root length of onion. Overall plant performance was better in ferrosol than podsol. Plant height in ferrosol was 13.5% greater than in podsol. Shoot fresh weight was 25.2% higher in ferrosol while shoot dry weight was 32.5% higher in the same soil. Comparing the root fresh weight, ferrosol had 18.9% greater root fresh weight than the podsol. It would be noteworthy that the nutrient availability of the soils was different given that pHs of the soils were balanced.

Soil N and P but not K were higher in ferrosol before planting (Table 7.3). However, the soils had similar N content after harvest. Soil P after plant harvest was 2.11 units higher in ferrosol than in the podsol. In addition, the K content was 1.5 times higher in podsol than the ferrosol. Plant P was also higher in ferrosol than in podsol. Root length, percent of colonized root length and colonized root length were higher by 28.2%, 3.58 units and 26.5 units in ferrosol over podsol (Table 7.4).

7.4.2 Effect of biochar type

Biochar types were different for root fresh weight, soil EC, plant N, plant P, root length and % colonized root length (Table 7.5). Remaining parameters had similar mean values for both biochars. Root fresh weight, plant P, root length and % colonized root length were 0.63, 0.02, 53.7 and 1.7 units higher, respectively, due to Sugarcane Trash biochar compared to Green Waste A. Conversely, soil EC and plant N were higher in Green Waste by 0.33 and 0.07 units over Sugarcane Trash.

Table 7.2 Mean values for plant and soil parameters influenced by the soil types. The means within the column that do not share the same letter are significantly different at α=0.05 level of significance. The ± values indicate the standard error of the mean (N = 16).

Soil type	Plant height at harvest (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Soil P (mg kg^{-1})	Soil K (mg kg^{-1})	Plant P (%)
Ferrosol	38.61 ± 0.68a	15.74 ± 0.35a	1.586 ± 0.051a	4.24 ± 0.10a	7.03 ± 0.23a	115.3 ± 8.2b	0.20 ± 0.002a
Podsol	34.02 ± 1.06b	12.57 ± 0.31b	1.197 ± 0.052b	3.55 ± 0.06b	4.92 ± 0.24b	281.9 ± 10.2a	0.14 ± 0.002b

Table 7.3 N, P and K content of ferrosol and podsol before adding biochar, lime and mycorrhizae

Soil type	N (%)	P (mg kg^{-1})	K (mg kg^{-1})
Ferrosol	0.36	10	132.6
Podsol	0.22	7	302.3

Table 7.4 Mean values for root length, percentage colonized root length and colonized root length influenced by the soil types. The means within the column that do not share the same letter are significantly different at α=0.05 level of significance. The ± values indicate the standard error of the mean (*N* = 16).

Soil type	Root length (cm)	% colonized root length
Ferrosol	364.6 ± 13.8a	20.06 ± 0.34a
Podsol	284.5 ± 19.4b	16.48 ± 0.23b

Table 7.5 Mean values for plant and soil parameters influenced by the biochar types. The means within the column that do not share the same letter are significantly different at α=0.05 level of significance. The ± values indicate the standard error of the mean (*N* = 16). The means without ± values had negligible standard error.

Biochar type	Root fresh weight (g)	Soil electrical conductivity (dS m^{-1})	Plant N (%)	Plant P (%)	Root length (cm)	colonized root length (%)
Sugarcane Trash	4.21 ± 0.05a	0.44 ± 0.02b	1.17b	0.181a	351.4 ± 19.5a	19.1 ± 0.35a
Green Waste A	3.58 ± 0.04b	0.78 ± 0.03a	1.24a	0.161b	297.7 ± 13.8b	17.4 ± 0.21b

7.4.3 Effect of mycorrhizal rates

Application of mycorrhizae had significant effect on shoot fresh weight, shoot dry weight and root fresh weight over no application of mycorrhizae (Figure 7.1). Shoot fresh weight, shoot dry weight and root fresh weight were increased by 8.1%, 15.4% and 12.8%, respectively by mycorrhizal inoculum at a rate of 5 g kg^{-1} of soil over no application. Mycorrhizal effects were also significant on soil EC, soil P and plant P (Table 7.6). Soil EC was reduced by 10.3% by application of mycorrhizae over no application while available soil P and plant P increased by 26.4%, and 26.7% for mycorrhizae over no application.

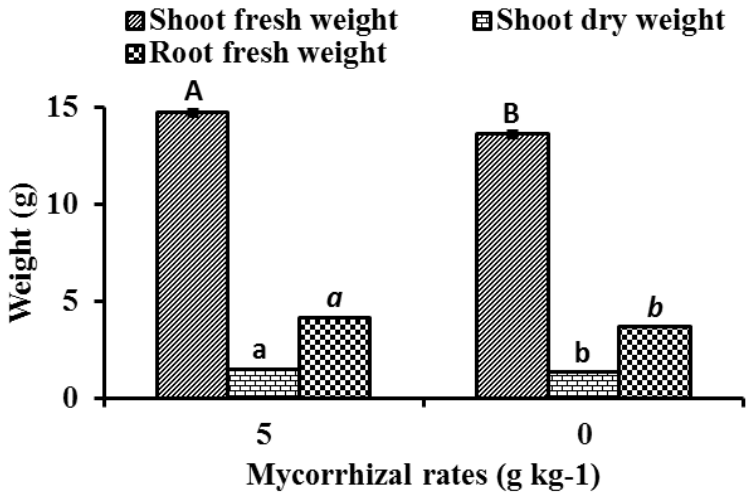


Figure 7.1 Shoot fresh weight, shoot dry weight, and root fresh weight influenced by the mycorrhizal rates. The blocks within the series that do not share the same letter are significantly different at α=0.05 level of significance. The bars on the blocks indicate the standard error of the mean (*N* = 16).

Table 7.6 Mean values for soil electrical conductivity, soil P and plant P content influenced by the soil types. The means within the column that do not share the same letter are significantly different at α=0.05 level of significcance.The ± values indicate the standard error of the mean (*N* = 16).

Mycorrhizal rates ($g\ kg^{-1}$)	Soil electrical conductivity ($dS\ m^{-1}$)	Soil P ($mg\ kg^{-1}$)	Plant P (%)
5	0.58 ± 0.02b	5.2 ± 0.10b	0.187a
0	0.64 ± 0.03a	6.7 ± 0.13a	0.154b

7.4.4 Interaction effects

The interactions were similar for many parameters. Interaction of soil type and biochar types were significantly different for soil P (Figure 7.2) and plant P (Figure 7.3) only. Soil P and plant P were in the order of Ferrosol + Sugarcane Trash biochar> Ferrosol + Green Waste A biochar> Podsol + Sugarcane Trash biochar> Podsol + Green Waste A biochar. Soil P and plant P in both biochars within podsol were similar but the trend was decreasing from Sugarcane Trash to Green Waste A. Interactions of soil type and mycorrhizal inoculation were similar for all parameters (data not shown) indicating mycorrhiza could be similarly active with both soils. Interaction of biochar type and mycorrhizal rates had significant effect on soil EC (Figure 7.4). The EC was reduced by 46.7% by the interaction of Sugarcane Trash biochar with 5g of mycorrhiza per kg of soil over the interaction of Green Waste A with the same rate of mycorrhizae. The reduction was 66.3% by the

interaction of Sugarcane Trash with the mycorrhizal inoculum over the interaction of Green Waste and no mycorrhiza. In both the biochars, interaction of biochar with mycorrhizal inoculum had better effect on soil EC than their interaction with no mycorrhizal inoculum.

The interactions of soil type, biochar type and mycorrhizal rates were significantly different for plant uptake of P only (Figure 7.5). The P content was greater in the interactions with ferrosol than the podsol. Interactions of Sugarcane Trash biochar and mycorrhizal rate of 5 g per kg were more effective than the other interactions. With both biochars, application of mycorrhiza was beneficial over no application.

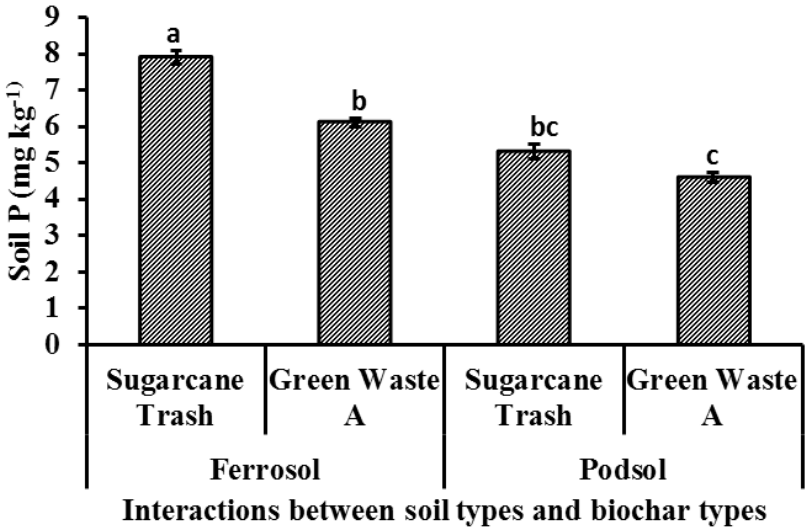


Figure 7.2 Soil P content after harvesting the crop influenced by the interaction of soil types and biochar types. The blocks that do not share the same letter are significantly different at α=0.05 level of significance. The bars on the blocks indicate the standard error of the mean (N = 8).

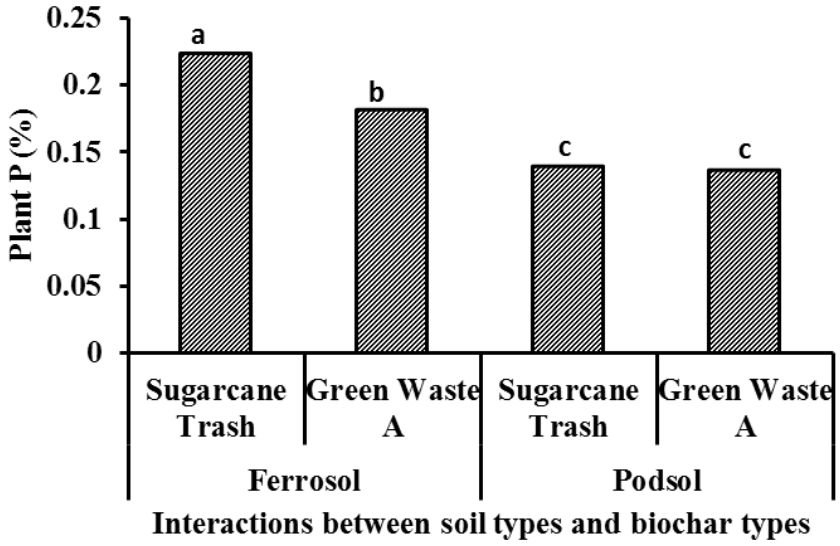


Figure 7.3 Plant P content after harvesting the crop influenced by the interaction of soil types and biochars. The blocks that do not share the same letter are significantly different at α=0.05 level of significance. The bars on the blocks indicate the standard error of the mean (*N* = 8).

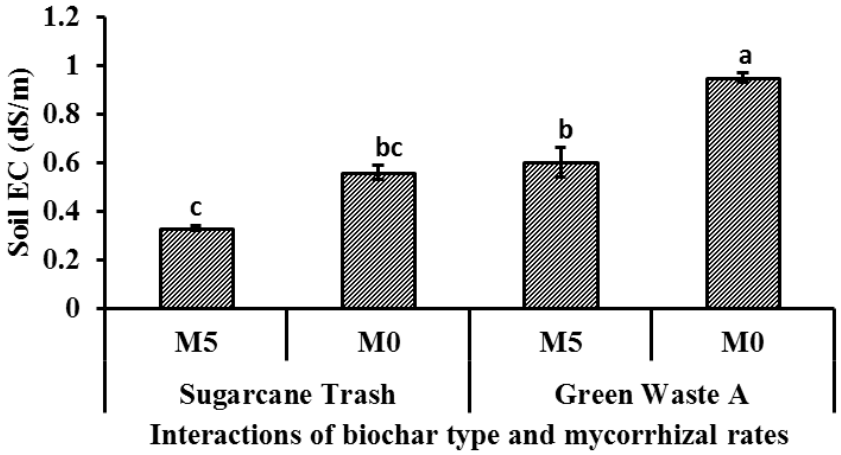


Figure 7.4 Soil electrical conductivity influenced by the interactions of biochar types and mycorrhizal rates (M5= 5g per kg which is 10g per pot; M0 = No mycorrhizal inoculation). The bars on the blocks indicate the standard error of the mean (*N* = 8). The blocks that do not share the same letter are significantly different. Presumably the mycorrhizal treatments took up more nutrients thereby reducing soil levels.

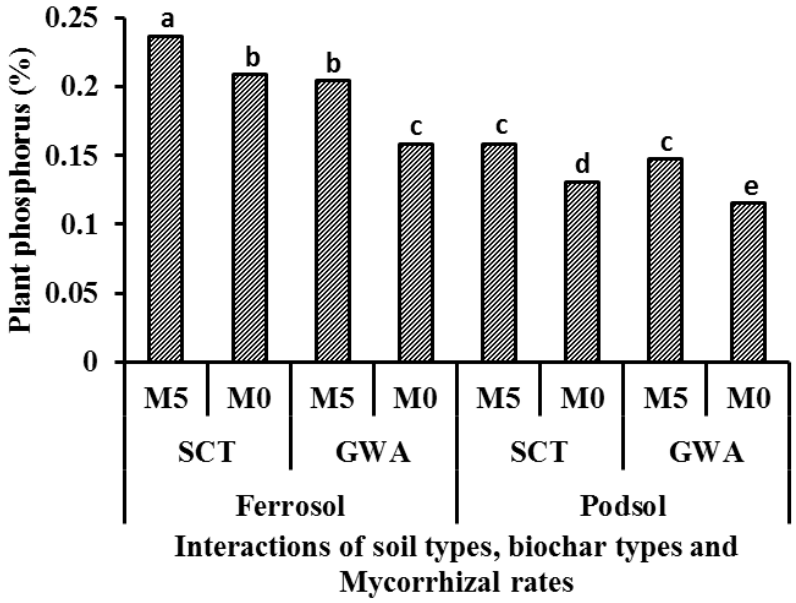


Figure 7.5 Plant P content influenced by the interaction of soil types, biochar types and mycorrhizal rates. (SCT = Sugarcane Trash; GWA = Green Waste A; M5= 5g per kg which is 10g per pot; M0 = No mycorrhizal inoculation). The blocks that do not share the same letter are significantly different at α=0.05 level of significance.

7.5 Discussion

The differences in the effects of soil types, biochar types and mycorrhizal rate were highly promising for some parameters of onion. The interactions were similar for most of the parameters indicating that there is no need to integrate these factors as they can work solely. However, the effects of biochar and mycorrhizae were encouraging for some parameters even in this short duration trial which has significance for further studies.

The performance of onion plants was better in ferrosol than the podsol. The main reason could be the comparatively higher content of N and P in ferrosol than the podsol (Table 7.3). Mycorrhizal colonization was better in ferrosol which could be due to the finer soil structure and higher water holding capacity. The AM dependency may be dependent upon several soil and plant factors. For example, generally, plants perform better when soil nutrients are adequately available. However, the adequate concentration of P in the soil (and root) is not compatible with mycorrhizal colonization and infection (Menge et al. 1978). In the present study, the P content in both soils was low (<10 mg kg^{-1}); that is most likely the main reason for colonization in both soils.

Among the biochars, Sugarcane Trash had better effect on plant parameters. This could be due to its fine structure that provided favourable conditions to hold moisture and nutrients. However the P

content of Sugar Cane Trash is 1100 mg kg^{-1} of biochar, the amount of P added to the soil from 30 t ha^{-1} biochar (34 g per pot) was 37.4 mg. When this amount is added to the amount of P in ferrosol and podsol, the figure becomes 47.5 mg for ferrosol and 44.4 mg per pot for podsol. These levels of P are favourable for mycorrhizal development which is discussed below.

There are several studies on critical levels of P for mycorrhizal colonization. A list of previous works on the P levels critical for mycorrhizal colonization has been reported (Swift 1999). When the soil level of bicarbonate-soluble P exceeded 140 mg kg^{-1}, the rate of infection decreased (Amijee et al. 1989). Abbott and Robson (1977) reported that the mycorrhizae *Glomus fasciculatum* ceased to be effective when the soil P level reached 133 mg kg^{-1}. Schubert and Hayman (1986) indicated that mycorrhizae became ineffective when 100 mg or more of P was added per kilogram of soil (100 ppm). No infection was observed when 1.5 grams or more of monocalcium phosphate was added to each kilogram of soil (Mosse 1973). Other researchers have also emphasized that soils containing or given more P are detrimental for mycorrhizal infections (Baylis 1967). The development of mycorrhizal relationships was greatest when soil P levels were 50 mg kg^{-1} (Schubert & Hayman 1986).

The soil EC on mycorrhizal soils was less due to the mobilization of salts especially P and zinc by mycorrhizae for plant uptake and their own use. Thus less nutrient was left in soil to affect EC readings. In a study, mycorrhizal colonization was observed at different salt concentrations (Neera and Machanda 2008). The role of AM fungi in alleviating salt stress has been reviewed by Evelin et al. (2009) concluding that the fungi enhanced salt tolerance of plants by enhanced nutrient acquisition (P, Na, Mg and Ca), maintaining K^+ : Na^+ ratio and other biochemical changes. Inoculation with AM fungi reduced soil EC readings (Sheng et al. 2012), for example, Glomus fasciculatum reduced EC under saline conditions (Suhail and Mahdi 2013).

Many of the interactions were ineffective for plant growth and mycorrhizal colonization. The most important consideration is the duration of the crop and soil. The trial length was eight weeks which was possibly not enough for soil reactions between biochar and lime. Greater effectiveness of mycorrhizal inoculation could be observed by planting a second crop with minimum disturbance of soil allowing the first crop roots to act as an inoculum. Limitation of time and season for the trial as well as the nature of the trial were not possible here. The duration was possibly also not long enough for the reaction of soil and biochar as it was reported in the previous chapters that the carbon sequestered in biochar could be utilized in the long-term.

The study was organized in a glasshouse for a single season, but the need to recommend a multi-season trial was noted.

7.6 Conclusion

The sole effect of soil type, biochar type and mycorrhizal rates was prominent but there were little effects of their interaction. Ferrosol soils, Sugarcane Trash biochar and mycorrhizal rates of 5 g kg^{-1} were positively effective over their corresponding treatments. The interactions were not enough to affect the plant performance and colonization of onion roots by mycorrhizae. Considering the results of this experiment, the effects of washed and pure biochars in addition to their equivalent P and K will be evaluated in the next chapter.

Chapter 8. Effect of washed biochar, extracted nutrient of biochar and P and K equivalent to biochar on onion growth with or without mycorrhizae

8.1 Abstract

Two pot trials were conducted to compare the effects of washed biochar and extracted nutrients with and without mycorrhizae on growth and colonization of onion in pH adjusted ferrosol and podsol soil. The experiment was carried out in a randomized complete block design consisting of 7 treatments (lime, lime + washed biochar, lime + washed biochar + mycorrhizae, lime + extracted nutrients, lime + extracted nutrients + mycorrhizae, lime + P and K equivalent to biochar, lime + P and K equivalent to biochar + mycorrhizae) in 4 replications. Observations were recorded on plant growth, mycorrhizal colonization and soil and plant nutrient contents. The results showed that the effect of ferrosol was greater than podsol on most of the parameters. Application of mycorrhizae was beneficial over soil with lime only. Extracted nutrients had beneficial effect over washed biochar indicating the importance of the soluble component in ash. Extracted nutrients had slightly greater effect than washed biochar; however, they were statistically similar in many cases. Lime plus P and K equivalent to biochar plus mycorrhizae had greater effect on plant growth and nutrient content. The P and K levels were increased when biochar was added with or without mycorrhizae.

8.2 Introduction

This chapter describes an experiment to determine whether there is any difference in the performance and colonization of onion plants by mycorrhizae when washed biochar, their extracted water soluble nutrients and equivalent amount of P and K are amended to podsol and ferrosol soils balanced for the same pH. This study compares the effect of washed biochar, extracted nutrients, P and K equivalent to the biochar. The study is also seeking to compare the application of mycorrhizae and no mycorrhiza within each amendment. The comparison is also carried out between the amendments and no amendment on plant growth and mycorrhizal colonization. The emphasis of the research is to determine differences between the washed biochar and the P and K equivalent to it.

When water is applied to biochar, a large amount of ash is washed out and nutrients may be lost through this process. The effect of washing biochar on nutrients availability is also a matter for study. Therefore, the present study aims to determine the effect of washed biochar and extracted

solution of nutrients from biochar. This study was developed to confirm if the amount of nutrients in ash content are adequate to make a positive influence on plant performance and mycorrhizal colonization.

8.3 Materials and methods

8.3.1 Preparation of Biochar

The biochar was made of sugarcane trash with composition given in Appendix 1. It had acid neutralizing capacity of 0.66%, P content 1100 mg kg-1 and K 8.4 cmol(+) (8.4 x 390 = 3276 mg kg^{-1}) compositions. This biochar was relatively better for plant growth and mycorrhizal colonization than other biochars (previous chapters). This biochar was washed, dried and applied to the soils.

Biochar amount was calculated based on the area of pots. The pot area based on the average diameter of 12 cm was 0.011304 cm^2. The biochar rate of 30 t ha^{-1} was equivalent to ~ 34.0 g pot^{-1}.

Thirty t ha^{-1} of biochar (34 g pot^{-1}) was weighed for the experiment because this amount had relatively greater positive effects on plant performance in previous experiments. The fine ground dry biochar was poured into the water in a beaker and was stirred for 5 minutes. The solution was sieved through a 2-mm sieve. The filtered solution was used as water extracted nutrients and the screened fine particles were considered as washed biochar. Washing of this biochar was carried out by applying deionized water at a proportion of 1:5 (biochar: water, w: v). Beakers were rewashed with de-ionized water and rewashed solution was also applied to the pot. Beakers were thoroughly cleaned with household bleach and deionized water and wiped dry with tissue paper after each lot to maintain the nutrient content of each dose. The washed biochar and extracted solution were applied to pots filled with the soils separately.

8.3.2 Cultivar, and source of seeds and pot preparation

Onion seeds of variety Rio Red Rock were obtained from South Pacific Seeds, New South Wales. These seeds were also used for other previous experiments of the present study.Plastic pots of 1.5 litre volume with average diameter of ~12cm were selected for the experiments. A fine fabric cloth was placed on the bottom of the pots and pots were placed on trays in which the drained water and nutrient solutions could be collected and reused to prevent their loss. Pots were then filled with the soils treated with washed biochar, extracted nutrient solution and equivalent P and K of whole biochar with or without mycorrhizal inoculum. Seeds were germinated as described in Chapter 7.

8.3.3 Source of mycorrhizae, soil and lime calculation

The MycoApply product of mycorrhizae was used as soil inoculum of mycorrhizae. The inoculum was the same as described in previous chapters. Soils were same as described in Chapter 7.

The current pH of podsol and ferrosol soils was analyzed by the methods 1:5 water and 1:1 water described in Appendix 4. The amount of lime required to increase pH up to 6.5 was selected for the experiment and the procedure was the same as described in Chapter 6. The pH of podsol and ferrosol soils was 4.6 and 5.2 respectively.

The doses of lime for podsol and ferrosol to raise the pH to 6.5 were 1.89 and 6.2 t ha^{-1}, respectively. The acid neutralizing capacity (% $CaCO_3$) of Sugarcane Trash was 0.66%. When a rate of 30 t ha^{-1} of biochar was applied, the amount of lime supplied through Sugarcane Trash was 0.2 t ha^{-1}.

However, the pH of podsol raised by the fractions of washed biochar and extracted solutions of 30 t ha^{-1} biochar was only 4.7 and 4.8. Similarly, the pH of ferrosol raised by the fractions of washed biochar and extracted solutions of 30 t ha^{-1} biochar was 5.25 and 5.3. Thus, the lime requirement to raise the pH after adding biochar fractions was 1.84 and 6.12 t ha^{-1} for podsol and ferrosol. Lime was mixed in the soils two weeks before planting. Then the pots were filled with soils and biochar mix and nutrients and water maintained at field capacity until planting to allow sufficient reaction between soil and lime.

Nutrient solutions: No nutrients were applied except for ammonium nitrate at a rate of 125 mg pot^{-1}. For treatments 6 and 7, i.e. P and K equivalent to biochar, the amounts of P and K added to ferrosol and podsol were 37.8 mg and 111.4 mg pot^{-1}.

8.3.4 Determination of field capacity

Field capacity was determined by the same procedure followed in Chapter 6.

8.3.5 Experimental design

Pot experiments were conducted in a glasshouse as a randomized complete block design with four replications with seven treatments. The treatment details are given in Table 8.1. All pots were filled with 1 kg of soil. Biochar was added at a rate of 30 t ha^{-1} to both soils.

Table 8.1 Treatment compositions for ferrosol and podsol.

Treatments for ferrosol	Treatments for podsol
Lime	Lime
Lime + Washed biochar	Lime + Washed biochar
Lime + Washed biochar + Mycorrhizae	Lime + Washed biochar + Mycorrhizae
Lime + extracted nutrients	Lime + extracted nutrients
Lime + extracted nutrients + mycorrhizae	Lime + extracted nutrients + mycorrhizae
Lime + P and K equivalent to biochar	Lime + P and K equivalent to biochar
Lime + P and K equivalent to biochar + mycorrhizae	Lime + P and K equivalent to biochar + mycorrhizae

Amount of lime, biochar and P and K are discussed above in lime calculation, biochar and nutrient sections of materials and methods.

8.3.6 Observations

Observations were recorded on plant height at harvest (eight weeks after planting), fresh and dry weights of shoots, fresh weight of roots, soil EC, soil pH, soil N, P, K and plant N, P, K. Plant height was measured from the ground level to the tip of the longest leaf. Fresh above ground parts were weighed and kept in a dryer at $65^{O}C$ for two weeks, after putting them into thin paper envelopes in an upright position and leaving the envelopes open to ventilate. Dry weight was taken after two weeks in the drying room. Electrical conductivity, soil pH and nutrients were determined by the methods given in Appendix 3-9. Mycorrhizal colonization was analyzed by the procedures adopted in previous chapters.

8.3.7 Statistical analysis

ANOVA was undertaken in Minitab 16, version 4.0 (Minitab 2005) and graphs were plotted by Microsoft Excel 2010, version 14.0 (Microsoft 2010). The standard error was derived from the standard deviation of the mean divided by the number of observations. The grouping of treatments was organized by the Tukey's family error test.

8.4 Results

Plant height (Figure 8.1), shoot fresh weight (Figure 8.2) and shoot dry weight (Figure 8.3) of onion are illustrated below. The overall results showed that there was a greater positive influence of ferrosol on these parameters than the podsol. For plant height, application of lime plus mycorrhizae

with biochar or nutrients equivalent to biochar was beneficial over lime plus no treatments in ferrosol, but in podsol the lime treatment was similar to lime plus washed biochar. Mycorrhizae did not cause differences for plant height between treatments with and without mycorrhizae in podsol. In ferrosol, the treatments lime plus extracted nutrients plus mycorrhizae and lime plus extracted nutrient and no mycorrhizae were significantly different indicating mycorrhizal inoculation was beneficial in promoting plant height. Similarly, effect of P and K equivalent to biochar was superior to washed biochar in both soils. Among the mycorrhizal treatments, the mycorrhizae applied with lime plus P and K equivalent to biochar was superior to mycorrhizae with lime plus extracted nutrients and lime plus washed biochar. The treatment mycorrhizae with lime plus extracted nutrients produced taller plants than mycorrhizae with lime plus washed biochar.

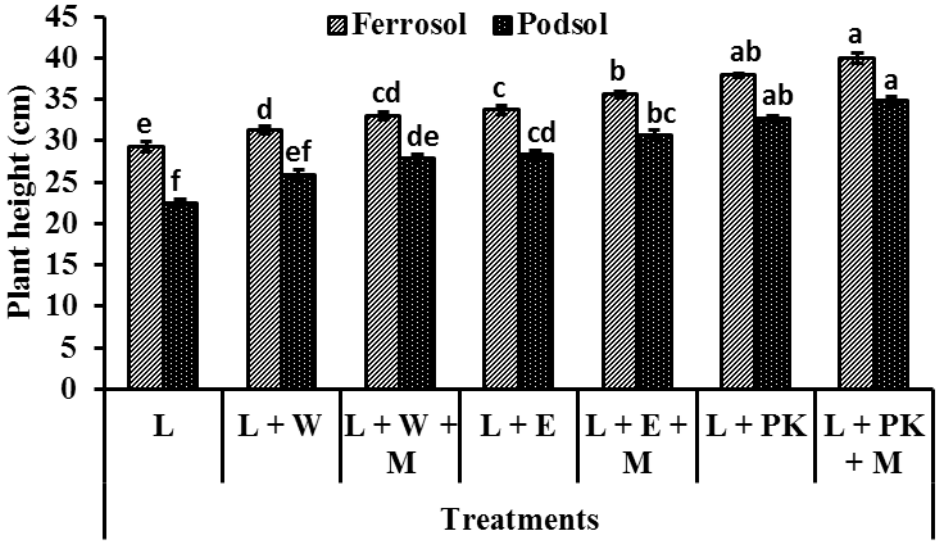


Figure 8.1 Plant height of onion in ferrosol and podsol. Different letters in the same series indicate significant differences between the treatments at α=0.05 level of significance. The bars on each block represent the standard error (SE) of the mean. L= Lime, L + W = Lime + Washed biochar, L + W + M = Lime + washed biochar + Mycorrhizae @ 5 g kg^{-1}, L + E = Lime + water extracted nutrients, L + E + M = Lime + water extracted nutrients + Mycorrhizae @ 5 g kg^{-1}, L + PK = Lime + P and K equivalent to biochar, L + PK + M = Lime + P and K equivalent to biochar + Mycorrhizae @ 5 g kg^{-1}.

There was a trend for increasing shoot fresh weight from treatments L to L + PK + M from left to right in Figure 8.2. Ferrosol soil was more effective for producing shoot fresh weight than podsol. In both soils, there was no difference between lime, lime plus washed biochar and lime plus washed biochar plus mycorrhizae. In podsol, the treatments with or without mycorrhizae within washed biochar, extracted nutrients and P and K equivalent to biochar were not different but the differences

were significant between washed biochar plus mycorrhizae, extracted nutrients plus mycorrhizae and P and K equivalent to biochar plus mycorrhizae. Similar trends were found in ferrosol. The highest shoot fresh was observed in L + PK + M followed by L + PK.

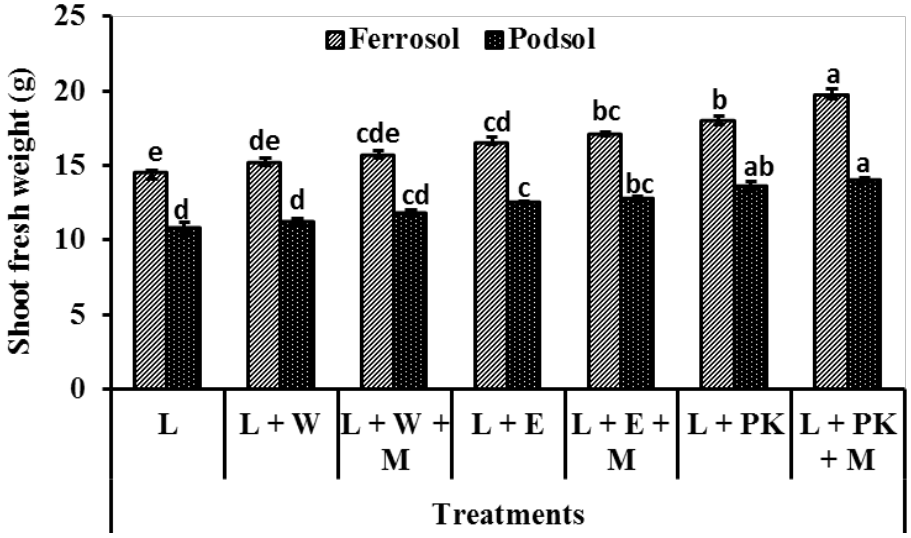


Figure 8.2 Effect of treatments on shoot fresh weight of onion in ferrosol and podsol. Different letters in the same series indicate significant differences between the treatments (N = 4) at α=0.05 level of significance. The bars on each block represent the standard error (SE) of the mean. L= Lime, L + W = Lime + Washed biochar, L + W + M = Lime + washed biochar + Mycorrhizae @ 5 g kg^{-1}, L + E = Lime + water extracted nutrients, L + E + M = Lime + water extracted nutrients + Mycorrhizae @ 5 g kg^{-1}, L + PK = Lime + P and K equivalent to biochar, L + PK + M = Lime + P and K equivalent to biochar + Mycorrhizae @ 5 g kg^{-1}.

Root fresh weight and root length were higher in ferrosol than in the podsol (Table 8.2). All amendments with biochar and with or without mycorrhizae had higher root fresh weight and root length than absence of these amendments in both soils. There was no difference between the treatments with and without mycorrhizae within each pair of treatments but the treatments with mycorrhizae were significantly different from lime alone.

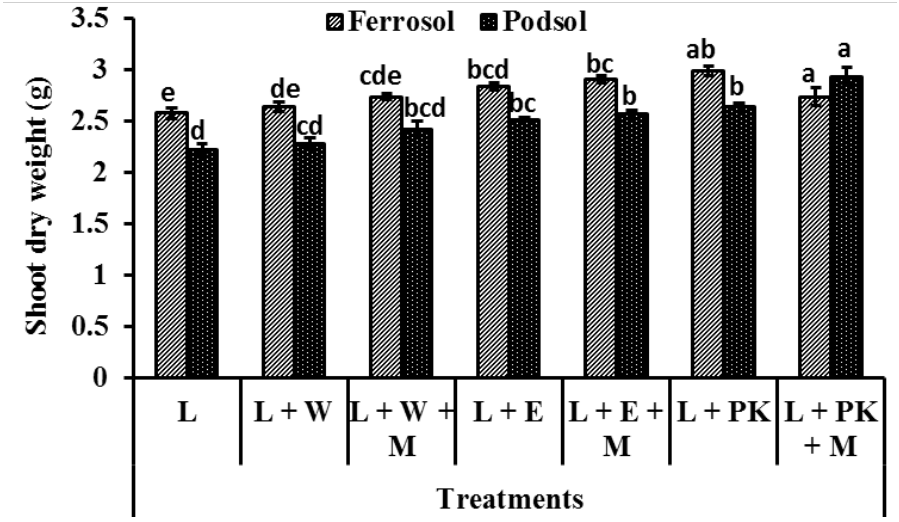


Figure 8.3 Effect of treatments on shoot dry weight of onion in ferrosol and podsol. Different letters in the same series indicate significant differences between the treatments (*N* = 4) at 0.05 level of significance. The bars on each block represent the standard error (SE) of the mean. L= Lime, L + W = Lime + Washed biochar, L + W + M = Lime + washed biochar + Mycorrhizae @ 5 g kg^{-1}, L + E = Lime + water extracted nutrients, L + E + M = Lime + water extracted nutrients + Mycorrhizae @ 5 g kg^{-1}, L + PK = Lime + P and K equivalent to biochar, L + PK + M = Lime + P and K equivalent to biochar + Mycorrhizae @ 5 g kg^{-1}.

The grouping was overlapped among treatments for soil EC (Table 8.3). Soil pH was similar (6.5-6.6) in all treatments. The ECs of all biochar, mycorrhizae or P and K added treatments were similar but their ECs were significantly higher than for no addition (lime only). Soil N was similar in all treatments of both soils (data not shown). Lime plus extracted nutrients had higher soil P and soil K content than lime plus washed biochar. Lime plus P and K equivalent biochar had the greatest amount of P and K among the treatments.

Plant N was similar in all treatments of both soils (data not shown). Plant P was the highest in lime plus P and K equivalent to biochar plus mycorrhizae. Due to the overlapping groupings of treatments, no recommendations could be made but the applications of P and K equivalent to biochar showed greater positive effect on plant P and K content than the other treatments. Mycorrhizae colonized between 20-24% root lengths in all mycorrhizae applied treatments but there were no significant differences.

Table 8.2 Root fresh weight and root length of onion in ferrosol and podsol. Different letters in the same column indicate significant differences ($P < 0.05$) between the treatments ($N = 4$) α=0.05 level of significance. The ± values indicate the standard error (SE) of the mean.

Treatments	Root fresh weight		Root length	
	Ferrosol	Podsol	Ferrosol	Podsol
Lime	3.03 ± 0.09e	2.30 ± 0.20e	178.3 ± 4.0d	153.0 ± 2.7e
Lime + Washed biochar	3.63 ± 0.06d	3.23 ± 0.10d	219.3 ± 13.5c	195.3 ± 11.9d
Lime + Washed biochar + Mycorrhizae @ 5 g kg^{-1}	3.98 ± 0.09cd	3.68 ± 0.11cd	246.3 ± 6.3bc	222.0 ± 5.7cd
Lime + Water extracted nutrients	4.20 ± 0.15bc	3.85 ± 0.13bc	259.0 ± 6.3ab	236.3 ± 7.0bc
Lime + Water extracted nutrients + Mycorrhizae @ 5 g kg^{-1}	4.50 ± 0.12ab	4.20 ± 0.82abc	273.8 ± 4.3ab	250.3 ± 6.4abc
Lime + P and K equivalent to biochar	4.65 ± 0.13ab	4.35 ± 0.13ab	279.3 ± 3.8a	259.8 ± 5.4ab
Lime + P and K equivalent to biochar + Mycorrhizae @ 5 g kg^{-1}	4.88 ± 0.15a	4.55 ± 0.10a	286.2 ± 3.9a	269.0 ± 6.6a

Table 8.3 Soil electrical conductivity, P and K content in ferrosol and podsol soil. Different letters in the same column indicate significant differences between the treatments (N = 4) at α=0.05 level of significance. The ± values indicate standard error (SE) of the mean.

Treatments	Electrical conductivity (dS m^{-1})		P mg kg^{-1})		K (mg kg^{-1})	
	Ferrosol	Podsol	Ferrosol	Podsol	Ferrosol	Podsol
Lime	0.21 ± 0.01b	0.16 ± 0.01d	3.15 ± 0.30c	5.2 ± 0.1b	412.4 ± 5.1c	122.0 ± 0.0d
Lime + Washed biochar	0.46 ± 0.01a	0.37 ± 0.01c	3.30 ±0.40c	6.0 ± 0.3b	434.5 ± 3.2bc	130.5 ± 0.9c
Lime + Washed biochar + Mycorrhizae @ 5 g kg^{-1}	0.44 ± 0.01a	0.41 ± 0.01b	4.05 ± 0.30c	6.5 ± 0.3b	440.3 ± 4.3bc	132.5 ± 0.9c
Lime + Water extracted nutrients	0.48 ± 0.01a	0.45 ± 0.01a	7.45 ± 0.53b	8.5 ± 0.7b	447.3 ± 3.6b	147.0 ± 1.2b
Lime + Water extracted nutrients + Mycorrhizae @ 5 g kg^{-1}	0.47 ± 0.01a	0.47 ± 0.01a	7.83 ± 0.54b	9.2 ± 0.4b	448.0 ± 3.2b	150.5 ± 2.0b
Lime + P and K equivalent to biochar	0.48 ± 0.01a	0.47 ± 0.00a	8.73 ± 0.08b	14.6 ± 1.4a	487.28 ± 11.7a	175.5 ± 3.2a
Lime + P and K equivalent to biochar + Mycorrhizae @ 5 g kg^{-1}	0.47 ± 0.01a	0.48 ± 0.01a	12.90 ± 0.37a	15.9 ± 1.9a	488.27 ± 11.7a	175.0 ± 1.7a

Table 8.4 Plant P and K content of onion in ferrosol and podsol. Different letters in the same column indicate significant differences between the treatments (N = 4) at α=0.05 level of significance. The ± values indicate the standard error (SE) of the mean.

Treatments	Plant P (%)		Plant K (%)	
	Ferrosol	Podsol	Ferrosol	Podsol
Lime	0.20 ± 0.00d	0.11 ± 0.01d	2.50 ± 0.12d	1.98 ± 0.07c
Lime + Washed biochar	0.22 ± 0.01cd	0.12 ± 0.00cd	2.68 ± 0.04cd	2.58 ± 0.10b
Lime + Washed biochar + Mycorrhizae @ 5 g kg^{-1}	0.23 ± 0.01bc	0.14 ± 0.00bc	3.14 ± 0.15bc	2.60 ± 0.09b
Lime + Water extracted nutrients	0.24 ± 0.01bc	0.15 ± 0.01b	3.18 ± 0.15bc	2.84 ± 0.05b
Lime + Water extracted nutrients + Mycorrhizae @ 5 g kg^{-1}	0.25 ± 0.01abc	0.16 ± 0.01b	3.29 ± 0.14b	2.88 ± 0.04b
Lime + P and K equivalent to biochar	0.26 ± 0.00ab	0.22 ± 0.01a	4.46 ± 0.11a	4.52 ± 0.13a
Lime + P and K equivalent to biochar + Mycorrhizae @ 5 g kg^{-1}	0.28 ± 0.01a	0.22 ± 0.01a	4.47 ± 0.10a	4.46 ± 0.16a

8.5 Discussion

The superior performance of ferrosol to podsol for most of the parameters was possibly associated with their texture and nutrient content. N and K of the soils before the trial were not analysed but results for P content showed a greater value (10 mg kg^{-1}) for ferrosol than the podsol.

The beneficial effect of application of biochar with extracted nutrients confirms that the majority of nutrients may be available in the ash content. The washed biochar had little effect on all parameters indicating that most of the nutrients responsible for plant growth were leached. This could also be compared with the treatments of lime plus extracted nutrients plus or minus mycorrhizae which produced similar effects as the treatment lime plus P and K equivalent to biochar plus or minus mycorrhizae. This indicates that the major part of nutrients were in extracted nutrient solution.

Values for plant height, shoot fresh weight and shoot dry weight of the treatment P and K equivalent to biochar were expected because the amount was calculated from the whole biochar (unwashed). No differences for soil and plant N were obvious because the same amount of N was applied to each plot and the N contained in biochar was sequestered. During the pyrolysis process, significant proportions of biomass N are lost by volatilization (Chan & Xu 2009) and the rest may be converted into more resistant forms that may not be readily available to the plant. The N remaining in the biochar is poorly available to plants (Gaskin et al. 2010), since a fraction of it is found inside aromatic C structures and heterocyclic compounds (Chan & Xu 2009). Limiting soil N content by biochar application in N deficient soils could also be due to the high C/N ratio, hence it might reduce crop productivity temporarily (Lehmann et al. 2003). The retention and immobilization of N from biochar have been explained by several mechanisms, for example, adsorption of NH_3 or organic-N onto biochar, cation or anion exchange reactions, and enhanced immobilisation of N as a consequence of labile C addition in the biochar (Clough et al. 2013). The N contained in all biochars used for the experiments as explained throughout the thesis was in the form of total N.

Mycorrhizal colonization was similar in all treatments and the colonization percentage was quite low in both soils. The most possible cause may be that mycorrhizae started to colonize but did not develop associations or network in the whole root system. The nutrient content of soil was very low so that might not have been enough for sustaining mycorrhizae and plants at the same time.

The addition of biochar to soils increased above ground productivity, crop yield, soil microbial biomass, rhizobia nodulation, plant K tissue concentration, soil P , soil K , total soil N , and total soil carbon (C) compared to control (Biederman & Harpole 2013). In the present study, biochar added treatments produced greater plant and soil P and K content but soil and plant N remained similar. The reason behind this may be associated with the effect of applied N rather than the N of soil and biochar.

Previous research showed that application of biochar increased mycorrhizal colonization in wheat roots where biochar was applied with inoculated mineral fertilizer (Solaiman et al. 2010). In the present study, mycorrhizal colonization was similar for biochar applied and nutrient added plants. Soil P and soil pH ranges were considered adequate for mycorrhizal colonization.

8.6 Conclusion

The effect of ferrosol was greater than podsol on most of the parameters. Application of mycorrhizae was beneficial over soil with lime only. Extracted nutrients had beneficial effect over washed biochar indicating the importance of the soluble component in ash. Extracted nutrients had slightly greater effect than washed biochar, however, they were statistically similar in many cases. Lime plus P and K equivalent to biochar plus mycorrhizae had greater effect on plant growth nutrient content. The P and K levels were increased when biochar was added with or without mycorrhizae.

From the results of all experiments so far, the need for verification of the application rates of biochar was expected and a field trial was conducted. The results of the field trial will be discussed in the next chapter.

Chapter 9. Verification of biochar effects under field conditions

9.1 Abstract

Field experiment was conducted in a less fertile ferrosolic field to determine the growth of shallot and its root colonization by AM fungi in response to biochars. The experiment was conducted in a 2 x 3 + 2 factorial arrangement with two types of biochar (Sugarcane Trash and Green Waste A) at three application rates (10, 20 and 30 t ha^{-1}) plus two extra treatments (control with no amendment and N at a rate of 110 kg ha^{-1}). As a result, the rate of 10 t ha^{-1} was considered as the best rate as all rates of biochar had similar and more positive effects on most of the parameters than the control. The pH and electrical conductivity increased as the biochar rates increased. These results provide information for soil management strategy by using biochars as an amendment in onion in less fertile ferrosolic soil in a particular season, however, multi-season, multi-soil and multi-crop experiments are important for specific recommendations.

9.2 Introduction

The effects of biochar on plant growth may vary depending upon soil type and fertilizer application. For instance, Van Zwieten et al. (2010a) reported that biochars increased wheat and radish biomass in ferrosol but reduced it in calcaresol. Asai et al. (2009) showed that biochar increased rice grain yields in low P soils. In addition, biochar had little effect on wheat yield in the absence of mineral fertilization but with the highest rate of mineral fertilization, yield increased by 20–30% more than mineral fertilizer alone (Alburquerque et al. 2014). These results suggest that soil properties, including pH and nutrient levels, influence biochar effect on crop response.

Tomato fruit yield increased with application of charcoal compared to without charcoal (Yilangai et al. 2014). Biochar also increased vegetable yield by 4.7-25.5% as compared to farmers' practice (Vinh et al. 2014). Biochar positively influenced growth and yield of French bean as compared to no biochar (Saxena et al. 2013). A rice-husk biochar tested in lettuce-cabbage-lettuce rotation increased final biomass, root biomass, plant height and number of leaves in all cropping cycles in comparison to no biochar treatments (Carter et al. 2013). On the other hand, biochar did not increase annual yield of winter wheat and summer maize but the cumulative yield over four growing seasons significantly increased in a

calcareous soil (Liang et al. 2014). Biochar of maple showed no significant effect on root elongation of pea (Borsari 2011). Wood chip biochars produced at 290^0C and 700^0C had no effect on growth and yield of either rice or leaf beet (Lai et al. 2013).

Application rates may have varied effects on crop growth. Dry matter of radish significantly decreased when biochar was applied at 10 ton ha^{-1} (Chan et al. 2008a); however, there was no significant effect of biochar rates (0, 7 and 15 tons ha^{-1}) on turnip, wheat, rape and faba bean yields (Brandstaka et al. 2010). Biochar at rates of 20 and 40 t ha^{-1} without N fertilization increased maize yield by 15.8% and 7.3% while addition of 300 kg ha^{-1} N with biochar enhanced yield by 8.8% and 12.1%, respectively (Zhang et al. 2012).

An oak biochar derived from a slow pyrolysis process was tested for four years at 0, 5 and 25 t ha^{-1} with 100% and 50% of the normal N fertilizer rate on a maize -soybean rotation in an alfisol soil; this resulted in an overall positive trend in total above-ground biomass and grain yield (Hottle 2013). A higher rate (3 t ha^{-1}) with urea of a poultry-litter biochar derived from slow pyrolysis produced better cotton growth than the lower rate (1.5 t ha^{-1}) (Coomer et al. 2012).

Biochar amendments can increase AMF % root colonization in plant roots (Elmer & Pignatello 2011) grown in acidic soils (Ezawa et al. 2002; Matsubara et al. 2002; Yamato et al. 2006), while others showed decrease in AMF abundance (Warnock et al. 2010).

In trials described earlier in this thesis, biochar had encouraging effects on plant growth and colonization in the controlled environment of the glasshouse. For example, biochar was beneficial for growth of lettuce, cabbage, tomato and onion while it was ineffective for potato. An application rate of 30 t ha^{-1} was found to be most effective for plant growth. The mycorrhizal colonization of tomato and onion roots was found for up to 100 t ha^{-1} of biochar application. This chapter describes the results of a field trial on the effects of biochar on shallots for comparison with the glasshouse studies.

9.3 Materials and methods

9.3.1 Biochar

Biochars from two different sources *viz.* Sugarcane Trash and woody Green Waste (Green Waste A) were used for the experiment. The nutritional and other properties of these biochars

are given in Appendix 1. These biochars were repeatedly tested in previous experiments as described in earlier chapters. Biochar amount was calculated based on the area of land. As the application rates were 10, 20 and 30 t ha^{-1}, a 2 m^2 area required 2, 4 and 6 kg of each biochar.

9.3.2 Cultivar and source of seedlings

A popular shallot variety recommended for the Lockyer Valley was obtained from Jackwitz Seedlings, Tenthill. The seedlings were grown in plastic trays filled with propagation mix. Three week old seedlings were planted directly into the field.

9.3.3 Experimental site and soil

The experiment was conducted in the research field of the University of Southern Queensland, Toowoomba. , the site was situated at around 51 km west from the University of Queensland, Gatton Campus. The climate during the study was warm summer. The site was a previously grassed, uncultivated fallow with acidic, red ferrosol soil. The baseline soil test results showed that the soil pH was 5.2 and the soil contained 0.19% total N, 14.6 mg kg^{-1} Colwell P and 347.7 mg kg^{-1} exchangeable K, respectively.

9.3.4 Land preparation

Land was prepared by deep ploughing with a tractor and three cultivations with a rotary hoe. Weeds were removed from the experimental plot. The biochars and fertilizer were applied manually to each plot as calculated and mixed by rotary hoe to a depth of 10 cm. The plots were separated by a distance of 50 cm and made into flat beds.

No additional inoculum was applied as it was assumed that sufficient mycorrhizal spores and hyphae were prevalent in the soil.

9.3.5 Experimental design

The experiment was conducted in a 2 x 3 + 2 factorial arrangement with two types of biochar at three application rates plus two extra treatments (control with no amendment and N at a rate of 110 kg ha^{-1}). Thus there were eight treatments with four replications. Treatment details are given in Table 9.1.

Four blocks of eight plots in each block with 2 x 1 m plot size were maintained as replications. The inter-block and inter-plot spacing was maintained at 50 cm. The row to row and plant to plant distance was 25 and 13.3 cm respectively. Thus the plot area was 2 m^2.

9.3.6 Transplanting of seedlings

Seedlings were transplanted on 9th September 2014 by covering the whole root system with soil. Seedlings with five tillers were maintained in each hill after establishment. As the plot size was 2 m^2, there were 15 plants in a row and 60 plants in a plot. After transplanting, irrigation was supplied to enhance establishment and later, for plant growth.

9.3.7 Care of the crop

No disease or insect pests were observed during the experiment. No chemical pesticides were applied. Weeds were a major problem during the period but they were uprooted manually. To protect the experiment from hares, a wire net was erected 1 m around the perimeter of the site (Plate 9.1). For irrigation, two sprinklers were set to ensure irrigation one hour every day for a week until the seedlings were established and thereafter two hours every alternate day (three days a week).

Plate 9.1 Part of experimental area showing wired fences to protect crop from hares and sprinkler to irrigate the crop.

9.3.8 Harvesting of the plants and roots

Plants were harvested manually on 14 November 2014. Plants and roots were harvested from the two middle rows after discarding all border plants. Thus the harvested net plot area was 0.87 m^2. The total number of harvested plants from the net plot was 26. The shoots were harvested by uprooting nine weeks after transplanting. The shoots were cut at ground level and put into paper envelopes. The roots were uprooted, washed and stored in 50% ethanol in Falcon tubes for mycorrhizal analysis.

9.3.9 Soil sampling

Four random samples of soil were collected from 10 cm depth from each plot and mixed. The composite sample was divided into four quarters. The two opposite quarters were discarded and then the process was repeated with the remaining soil accepted for the sample for analysis. The samples were kept in plastic bags and air-dried for a week in a glasshouse with a temperature range of 30-33^{0}C.

Table 9.1 Treatments and application rates for field trial.

Biochar	Application rates	Amount per plot
	No amendment	-
	N @ 110 kg ha^{-1}	64.7 g (Ammonium nitrate)
	10 t ha^{-1}	2 kg
SCT	20 t ha^{-1}	4 kg
	30 t ha^{-1}	6 kg
	10 t ha^{-1}	2 kg
GWA	20 t ha^{-1}	4 kg
	30 t ha^{-1}	6 kg

9.3.10 Observations

Observations were recorded on plant height at harvest (eight weeks after planting), fresh and dry weights of shoots, fresh weight of roots, soil EC, soil pH as well as soil and plant N, P, K. Plant height was measured from the ground level to the tip of the longest leaf. Fresh above ground parts were weighed and kept in a dryer at 65^{o}C for two weeks, after putting them into thin paper envelopes in an upright position and leaving the envelopes open for ventilation. Dry weight was taken after two weeks in the drying room. Electrical conductivity, soil pH

and nutrients were determined by the methods given in Appendix 3-9. Mycorrhizal colonization was analyzed by the procedures adopted in the materials and methods section of Chapter 4. Benefit cost ratio was derived from the total gross income divided by total costs for production.

9.3.11 Statistical analysis

A General Linear Model of biochar, application rate, biochar x application rate was applied for the factorial subset in Minitab (Minitab 2005). One way ANOVA was organized by Minitab for whole treatment effect, means and standard deviation of the mean. A combined ANOVA was also assessed by combining one way ANOVA and factorial subset to draw a perfect ANOVA with extra treatments by applying similar methodology from Chapter 5. Graphs were plotted in Microsoft Excel 2010, version 14.0 (Microsoft 2010). The standard error of the mean was derived from the standard deviation of the mean divided by the number of observations (replications). The grouping of treatments was organized by Tukey's family error test in Minitab 16, version 4.0 (Minitab 2005).

9.4 Results

9.4.1 Effect of biochar types

Significant differences between biochars were observed for root length, soil pH, soil N and plant N, P, and K. Root length (931.8 cm), soil N (Total N, 0.25%), plant N (3.6%) and P (0.29%) content were greater due to the effect of Sugarcane Trash biochar while the K (3.8%) was greater due to Green Waste A biochar. Soil pH of Green Waste A biochar treated plots was higher (6.2) than that of Sugarcane Trash. These differences in pH and nutrients reflected the composition of biochars.

9.4.2 Effect of biochar application rates

Plant height, shoot fresh and dry weight, root length, soil NPK and plant NPK showed significant differences for biochar application rates. Soil pH, EC and N, P, K were higher in the soils amended at the higher rates (30 t ha^{-1}) of biochar while plant height, shoot fresh weight, shoot dry weight, root length and plant NPK were greater at the lowest rate (10 t ha^{-1}).

9.4.3 Interaction effect

All interactions were non-significant for all parameters except for soil P. Soil P was the greatest due to the interaction of Sugarcane Trash biochar and 30 t ha^{-1} of application rate followed by the interaction of Green Waste A biochar and 30 t ha^{-1}. The rest of the interactions were not different from each other.

9.4.4 Combined effect of factors and extra treatments

Combined ANOVA (One - way ANOVA for all treatments) for factors and extra treatments revealed that the application rates were significantly different for plant height (Figure 9.1), shoot fresh weight (Figure 9.2), shoot dry weight (Figure 9.3), soil pH (Figure 9.4), soil EC (Figure 9.5), root fresh weight and root length and % of colonized root length (Table 9.2) and soil NPK and plant NPK (Table 9.3).

The results showed most of the biochar treatments were similar or overlapped each other for the parameters. It was noteworthy that the application of biochar rates had greater positive effect than no amendment (control). Application of N at a rate of 110 kg ha^{-1} had a large positive effect on plant height, shoot fresh weight and shoot dry weight and it was at a par with biochar rates. Soil pH and EC increased as the biochar levels were raised. Root fresh weight and root length and colonized root length were higher in biochar and N added soil than in no amendment. When comparing the effect of N and biochar application rates, they were similar for plant height, shoot fresh and dry weight, root length and colonized root length. N had less effect on soil pH and EC, yet greater effect on root fresh weight.

Soil N, P and K contents were higher in higher application rates of biochar (30 t ha^{-1}). On the other hand, plant NPK contents increased as application rates of biochar decreased. Among the rates, many were overlapping so that no distinct conclusions could be made.

9.4.5 Benefit cost ratio (BCR) analysis

Total costs for production of the crop and gross income were recorded in a square meter basis. The results showed that all of the treatments were beneficial. The fertilizer and biochar treatments were more beneficial that the control. Among the treatments, application of N from ammonium nitrate @ 110 kg ha^{-1} had the highest BCR value followed by Sugarcane Trash biochar @ 10 t ha^{-1}. Comparing the two biochars, Sugarcane Trash had greater BCR values than Green Waste A.

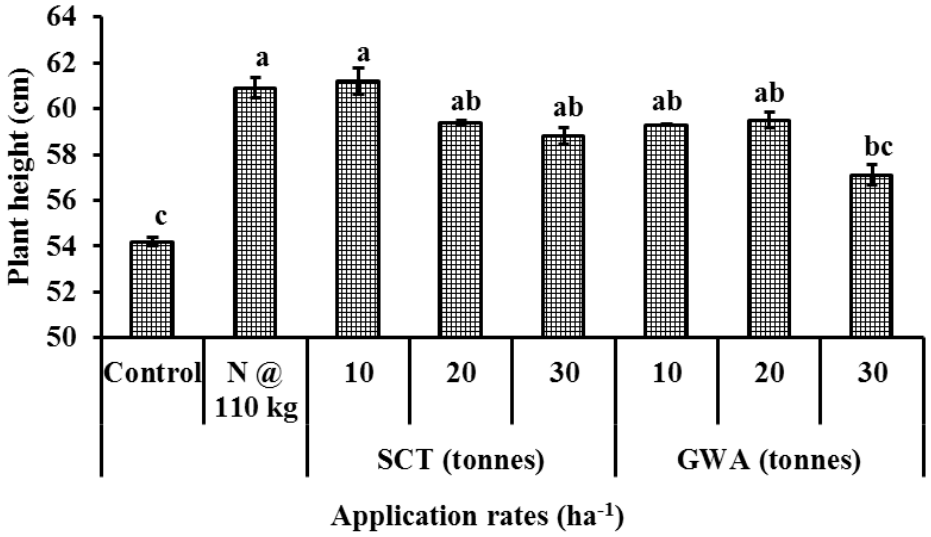


Figure 9.1 Plant height of shallot in response to treatments. Different letters indicate significant differences between the means (N = 4) at α=0.05 level of significance.The vertical bars represent the standard error of the mean (SE). Means were average of 4 replications; randomly sampled 5 recordings were averaged in each replication. N = N; SCT = Sugarcane Trash; GWA = Green Waste A.

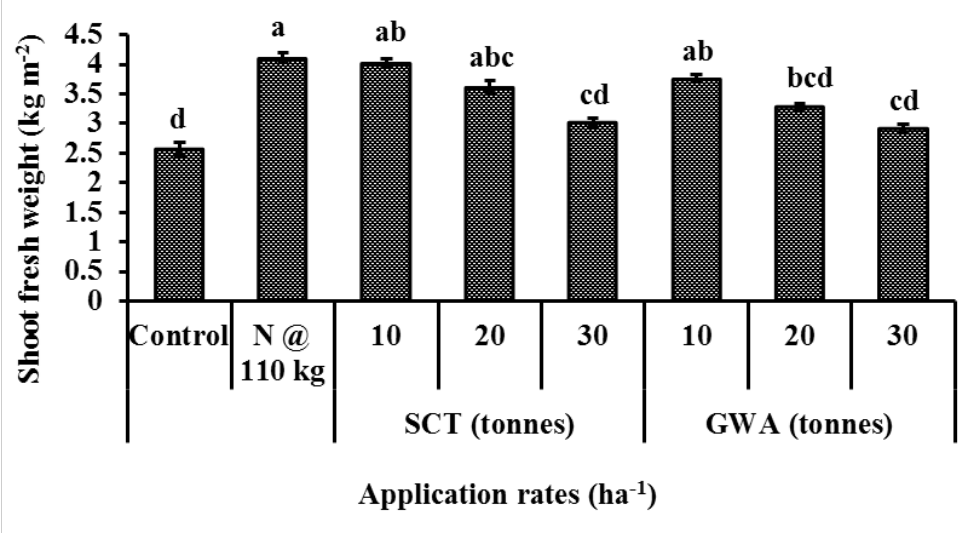


Figure 9.2 Shoot fresh weight of shallot in response to the application rates of biochar, N and control. Different letters indicate significant differences between the means (N = 4) at α=0.05 level of significance.The vertical bars represent the standard error of the mean (SE). Means were average of 4 replications; randomly sampled 5 recordings were averaged in each replication. N = N; SCT = Sugarcane Trash; GWA = Green Waste A.

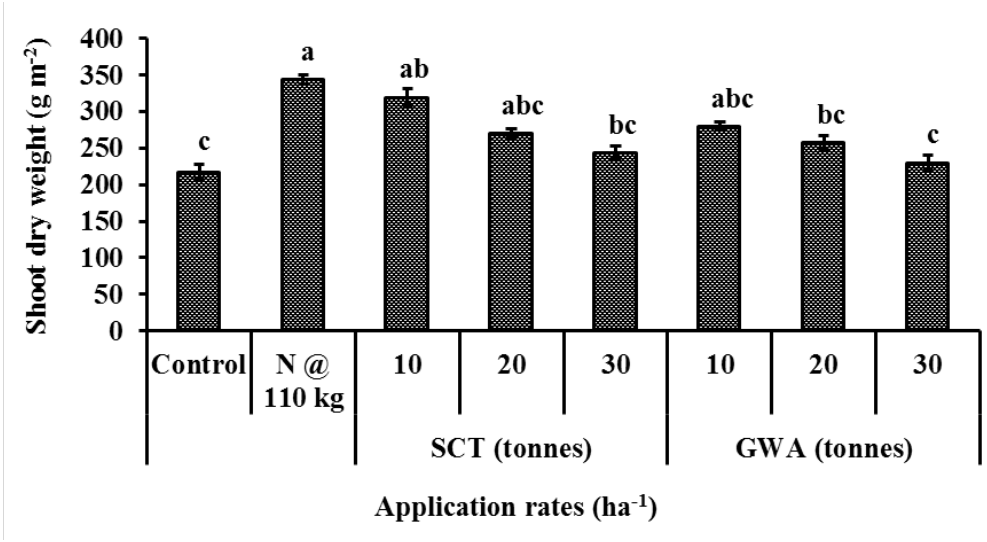


Figure 9.3 Shoot dry weight of shallot in response to the application rates of biochar, N and control. Different letters indicate significant differences between the means (N = 4) at α=0.05 level of significance.The vertical bars represent the standard error of the mean (SE). Means were average of 4 replications; randomly sampled 5 recordings were averaged in each replication. N = N; SCT = Sugarcane Trash; GWA = Green Waste A.

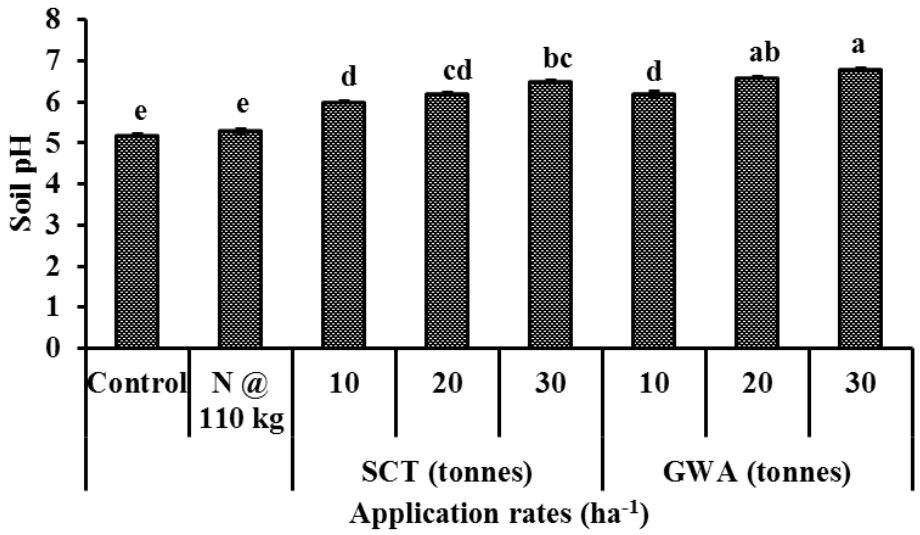


Figure 9.4 Soil pH of shallot in response to the application rates of biochar, N and control. Different letters indicate significant differences (N = 4) between the means at α=0.05 level of significance. The vertical bars represent the standard error of the mean (SE). Recordings of three subsamples were averaged in each replication. N= N; SCT = Sugarcane Trash; GWA = Green Waste A.

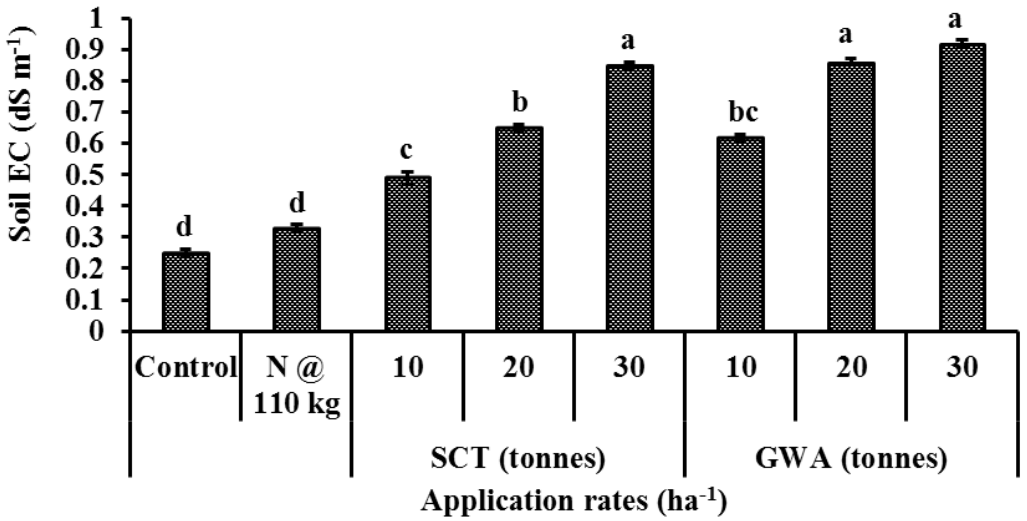


Figure 9.5 Soil EC of shallot in response to the application rates of biochar, N and control. Different letters indicate significant differences (N = 4) between the means. The vertical bars represent the standard error of the mean (SE). Recordings of three subsamples were averaged in each replication. N= N; SCT = Sugarcane Trash; GWA = Green Waste A.

Table 9.2 Mean values for root fresh weight, root length and colonized root length in response to the application rates of biochar, N and control. Different letters of the same column indicate significant differences between the means (N = 4) at α=0.05 level of significance. The ± values represent the standard error of the mean (SE).

Application rates	Root fresh weight (g)	Root length (cm)	Colonized root length (cm)
Control	2.40 ± 0.04c	629.3 ± 23.9d	92.6 ± 6.6b
N @ 110 kg ha^{-1}	4.53 ± 0.08a	1108.0 ± 18.2a	228.0 ± 18.9ab
Sugarcane Trash @ 10 t ha^{-1}	3.58 ± 0.02b	1056.5 ± 20.4ab	255.3 ± 17.9a
Sugarcane Trash @ 20 t ha^{-1}	3.55 ± 0.01b	954.0 ± 10.3ab	219.2 ± 18.9ab
Sugarcane Trash @ 30 t ha^{-1}	3.30 ± 0.05b	784.9 ± 23.2cd	182.2 ± 15.5ab
Green Waste A @ 10 t ha^{-1}	3.56 ± 0.08b	1008.8 ± 14.1ab	215.8 ± 17.4ab
Green Waste A @ 20 t ha^{-1}	3.48 ± 0.01b	902.7 ± 13.4bc	198.8 ± 19.2ab
Green Waste A @ 30 t ha^{-1}	3.50 ± 0.05b	704.4 ± 9.6d	149.1 ± 12.6ab

Table 9.3 Mean values for N, P and K content of soil and plant in response to application rates of biochar, N and control. Different letters of the same column indicate significant differences between the means (N = 4) at α=0.05 level of significance. The ± values represent the standard error of the mean (SE).

Application rates	Soil content			Dry matter content		
	N (%)	P (mg kg^{-1})	K (mg kg^{-1})	N (%)	K (%)	K (%)
Control	0.17 ± 0.00d	10.1 ± 0.15f	334.2 ± 2.35b	2.93 ± 0.02f	0.22 ± 0.01g	3.32 ± 0.01e
N @ 110 kg ha^{-1}	0.24 ± 0.00bc	10.9 ± 0.07ef	334.9 ± 1.08b	4.40 ± 0.06a	0.25 ± 0.00f	3.44 ± 0.01e
Sugarcane Trash @ 10 t ha^{-1}	0.21 ± 0.00cd	13.0 ± 0.31de	366.7 ± 0.88b	3.86 ± 0.03b	0.28 ± 0.00de	3.76 ± 0.01cd
Sugarcane Trash @ 20 t ha^{-1}	0.23 ± 0.01bc	17.0 ± 0.14bc	394.0 ± 1.28b	3.58 ± 0.00c	0.31 ± 0.00bc	3.98 ± 0.01bc
Sugarcane Trash @ 30 t ha^{-1}	0.32 ± 0.00a	21.4 ± 0.64a	452.38 ± 3.95ab	3.24 ± 0.01de	0.34 ± 0.00a	4.62 ± 0.07a
Green Waste A @ 10 t ha^{-1}	0.19 ± 0.00cd	14.7 ± 0.09cd	379.0 ± 1.50b	3.65 ± 0.01bc	0.27 ± 0.00ef	3.58 ± 0.02de
Gren Waste A @ 20 t ha^{-1}	0.21 ± 0.00bcd	15.7 ± 0.15c	423.3 ± 1.59b	3.39 ± 0.02cd	0.29 ± 0.00cd	3.84 ± 0.01bcd
Green Waste A @ 30 t ha^{-1}	0.27 ± 0.00b	18.3 ± 0.11b	591.7 ± 4.30a	3.09 ± 0.01ef	0.32 ± 0.00ab	4.07 ± 0.02b

A

B

C

D

E

F

G

Plate 9.2 Effect of treatments on performance of shallot; A. Control plot showing poor growth with less tillers, B. N @ 110 kg ha^{-1} with intensive tillers, C. Sugarcane Trash biochar @ 10 t ha^{-1}, D and E. Views of whole experimental area, F. Experimental plots one week after transplanting, G. Manual weeding of the plots with a hoe. Colours of images are due to the effect of light and shade at the time of taking the photos.

Table 9.4 Benefit cost ratio analysis for the treatments. Calculations are presented for a square metre land area. Currencies are in Australian dollar.

Costs items	Treatments							
	Control	N (110 kg ha^{-1})	Sugarcane Trash (10 t ha^{-1})	Sugarcane Trash (20 t ha^{-1})	Sugarcane Trash (30 t ha^{-1})	Green Waste A (10 t ha^{-1})	Green Waste A (20 t ha^{-1})	Green Waste A (30 t ha^{-1})
Biochar/fertilizer ($)	0	0.4	0.3	0.6	0.9	0.3	0.6	0.9
Seedlings ($)	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3
Labourer ($)	2.7	5.1	5.1	4	2.8	5.1	4	2.8
Machinery tools ($)	2	2	2	2	2	2	2	2
Irrigation($)	4	4	4	4	4	4	4	4
Total cost ($)	11	13.8	13.7	12.9	12	13.7	12.9	12
Production (kg)	2.57	4.11	4.02	3.62	3.02	3.76	3.28	2.91
Price $ kg^{-1}	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Gross Income ($)	11.57	18.50	18.09	16.29	13.59	16.92	14.76	13.10
Benefit: cost ratio	1.05	1.34	1.32	1.26	1.13	1.24	1.14	1.09

9.5 Discussion

This experiment confirmed some previous issues of this thesis research. For example, the glass house experiments showed that application of biochar was beneficial for crop growth and colonization of roots by arbuscular mycorrhizal fungi (Chapters 3, 4, and 5). This experiment also confirmed that both tested biochars were beneficial for the growth and colonization of shallots. The previous chapters showed Sugarcane Trash was comparatively better for some parameters which were also observed here. As before, there was little effect of interaction between biochar and application rates in this experiment.

The effects of biochars and their application rates were similar for many of the growth parameters, soil and plant nutrients perhaps because the growth period was very short (nine weeks) which might not be enough to allow decomposition of biochars to increase availability of nutrients. The duration of glasshouse experiments was also similar (six to eight weeks) but as nutrients were supplied through Hoagland's nutrient solution, (Appendix 2) and grown in sand, most were leached out. The initial nutrient content of sand was zero while higher nutrient content was available in soil so that high rates performed similar to the low rate in the field trial. However, all application rates were competitive with N applied at a rate of 110 kg ha^{-1}. N applied (22 g) for this treatment was greater than that supplied per plot from biochars at the rate of 10 t ha^{-1} (12 g) but less than from 20 and 30 t $ha^{-1}$of both biochars (24 g and 36 g).

There should be three possible reasons behind the similar effects of N application and biochar at a rate of 10 t ha^{-1} on plant parameters. Firstly, there should be a greater amount of leaching of N from chemical fertilizer than that from biochar because the field was irrigated for two hours every day for the first week. The mobility of nutrients in soil should be greater from chemical fertilizers than from biochar as biochar was mixed within the top 10 cm but nitrogenous fertilizer may move below that depth due to leaching. Secondly, the contribution of P, K and other nutrients of biochar to plant parameters should enhance plant performance; however, the N content in biochar at 10 t ha^{-1} was low. Thirdly, the elevated EC (Figure 9.5) reduced the growth.

Varied responses of crops to biochar were reported by other authors also (Chan et al. 2008a). Van Zwieten et al. (2010a) reported two biochars increased crop biomass. However, application of biochar at a rate of 10 t ha^{-1} significantly decreased dry matter content of radish (Chan et al. 2008a). No differences were observed between the application rates of biochar (0, 7 and 15 tons ha^{-1}) on turnip, wheat, rape and faba bean yields (Brandstaka et al. 2010). Biochar rates of 20 and 40 t ha^{-1} without N fertilization increased maize yield by 15.8 and 7.3% (Zhang et al. 2012).

A previous experiment conducted in sand medium (this thesis) confirmed that application rate of 30 t ha^{-1} was the most beneficial for plant growth (Upadhyay et al. 2014). The recommended rate for this specific soil might be even lower. From the present experiment, it was confirmed that application of 10, 20 and 30 t ha-1 of biochar had similar effects on most of the parameters. Economically, the positive effect of 20 t ha^{-1} and 30 t ha^{-1} was not two and three times greater than that of 10 t ha^{-1}, so the application of 10 t ha^{-1} may be the recommendation from this work. In the glasshouse experiments (Chapter 3 and 4), biochars were applied in sand medium which was not sufficient to prevent leaching of nutrients due to a flush watering. One of the major roles of biochar in sand medium was to conserve moisture, so increased rates of biochar conserved more moisture and became more effective.

Application of biochar derived from maple tended to be beneficial for root elongation of pea and wheat but no significant difference was observed (Borsari 2011), possibly due to little effect of biochar in the short-term. The wood chip biochars had no effect on growth and yield of rice and leaf beet (Lai et al. 2013) but other biochars had positive impact on growth and yield of French bean (Saxena et al. 2013). An oak biochar also had positive effect on biomass and grain yield at 5 and 25 t ha^{-1} (Hottle 2013). A poultry-litter biochar at 3 t ha^{-1} with urea produced better cotton growth than the lower rate (1.5 t ha^{-1}) (Coomer et al. 2012).

In the soil, there was increase in NPK with increased rate of biochar as expected. For SCT there was more N from 30 t ha^{-1} in soil than the 110 kg N treatment as SCT contains more N. However, the plant N % does not reflect this may be because the amount of available N in 110 kg N treatment was higher than in biochar, as biochar N is total N and all N may not be available. The P and K contents in plants were greater in biochar treated plants than N treated, as there was no additional P and K in N treated plants.

Colonization of roots was observed in all treatments but N and biochar treated plants had significantly greater colonization than control. This result was similar to previous information that biochar amendments could increase colonization in plant roots (Elmer & Pignatello 2011) grown in acidic soils (Ezawa et al. 2002; Matsubara et al. 2002; Yamato et al. 2006). In some reports, addition of char inhibited colonization probably due to improved availability of P (Warnock et al. 2007). In the present study, colonization was improved because the soil P level was within acceptable limit (<25 mg kg^{-1}, Table 9.3) for mycorrhizal growth. After addition of all levels of biochar, the soil pH remained between 6 and 7, which was suitable for availability of most of the

nutrients. This could be a cause of similar growth and dry weight in all rates of biochars. The similar effect of 10 t ha^{-1} and the higher rates could be associated with the elevated EC (Figure 9.5) that reduced the growth. Another presumption is the addition of more heavy metals by rates of biochar higher than 10 t ha^{-1} may have affected growth; however their actual amounts in the soil were unknown. Interestingly, N alone stimulated colonisation equal to that for low rates of biochar and resulted in a slight but significant increase in plant P.

Benefit cost ratio showed higher value for N @ 110 kg ha^{-1}. It proved that biochar was less profitable than chemical nitrogenous fertilizer for immediate effect. However, the biochar rates had greater BCR values than control which indicates possibility of enhancing profitability by using biochar as compared to no fertilizer application. The values for sequestered carbon and leached N were not done under this single season study but they were considered important for future long-term research.

9.6 Conclusion

This experiment compared two types of biochar (Sugarcane Trash and Green Waste A) and their application rates (10, 20 and 30 t ha^{-1}) including a control and N at a rate of 110 kg ha^{-1}. The results confirmed that biochar application was beneficial for plant growth and colonization. Lower rates of biochar were equally beneficial as the higher rates and both biochars were effective for specific traits. As a result, the rate of 10 t ha^{-1} was considered as the best rate as all rates of biochar had similar and more positive effects on most of the parameters than the control. However, these results were based on a short-term experiment. To observe complete effects, a series of experiments should be conducted in different soils for a longer period.

Chapter 10. General discussion

Experiments confirmed that there were crop-specific responses to biochar. For example, lettuce was more responsive than potato, true potato seedlings and single node cuttings of true potato seedlings. Addition of biochar increased pH of the medium in all experiments; this was also confirmed by previous researchers (Chan et al. 2008a), however the liming value of different biochars may vary (Van Zwieten et al. (2010a).

The beneficial effect on crops like cabbage in the present study was also reported by previous researchers (Tayxayngavong 2008; Fujiia et al. 2011). The significant effect of biochar on leaf length at the later stages of growth showed that biochar may influence growth in the long-term, because cabbage leaf size and number significantly increased 4-12 weeks after sowing (Olaniyi & Ojetayo 2011). It may take a long period to see the influence of biochar (Graber et al. 2010), even years (Jones et al. 2012). Onion and tomato are highly dependent on mycorrhizae (Khasa et al. 1992), however, colonization was less in fertile soils than in marginal soils (Sharif and Moawad 2006).

It was argued that biochar amendments could increase AMF % root colonization in plant roots (Elmer & Pignatello 2011) grown in acidic soils (Ezawa et al. 2002; Matsubara et al. 2002; Yamato et al. 2006), or decrease AMF abundance in some cases (Warnock et al. 2010). It depends on availability of P in soil (Warnock et al. 2007) as low P soils in the experiments showed good colonization (Chapter 5).

Zn and Cu effects on plant growth and colonization increased up to 50 mg kg^{-1}. As onion has been classified as sensitive to zinc deficiency (Chapman 1966), a recommendation of 10 kg $ha-^{1}$ was made (Khan et al. 2007) while in the present study, the Zn rate of 50 mg per pot was equivalent to about 11 kg ha^{-1} as the experimental soil had very low zinc content (0.4 mg kg^{-1} of soil) (Chapter 5). However, Cu is less available for soil pH above 4 (Mathur & Levesque 1983). Mycorrhizal colonization increased plant dry weight (Chen et al. 2007), the reason could be that mycorrhizae prevented Cu toxicity in plants (Malekzadeh & Ordubadi 2012) and acted to filter its flow from roots to plant tissue (Malekzadeh et al. 2007).

Mycorrhizae restrict salt absorption by plants (Huang et al. 2005) and thus they can accumulate in the rhizosphere increasing EC. Zn supply had little effect on tissue P (Zhu et al. 2001) but excess P

can induce Zn deficiency (Marschner & Cakmak 1986). Mycorrhizae increase the uptake of Zn and Cu, but mycorrhizal activity is suppressed by P fertilization (Lambert et al. 1979).

Mycorrhizal tolerance to heavy metals has been described by Hildebrandt et al. (2007). Mycorrhizal development was enhanced by levels of 18 mg zinc kg^{-1} soil basis while higher rates of 45 and 135 mg kg^{-1} Zn resulted in decreased colonization (McIlveen & Cole Jr 1979). In the present study, it could not be concluded that the highest rate was inhibitory because some degree of colonization was also detected at those rates.

A pot experiment (Chapter 7) confirmed that soil types, biochar types and mycorrhizal rate can work independently. The rate of mycorrhizal infection decreased when soil P (bicarbonate-soluble) exceeded 140 mg kg^{-1} (Amijee et al. 1989), 133 mg kg^{-1} (Abbott and Robson (1977) or 100 mg kg^{-1} (Schubert and Hayman (1986). Colonization was greatest when soil P was 50 mg kg^{-1} (Schubert & Hayman 1986).

The experiment (Chapter 8) confirmed that washed biochar and extracted nutrients had similar effects on plant growth and colonization in both soils and that most of the available nutrient was contained in ash. The effects of biochar on crop, soil and microbes are subject to climate and soil variation (Biederman & Harpole 2013). Colonization can be enhanced by mineral fertilizer (Solaiman et al. 2010) in less fertile soil.

The field experiment confirmed that both biochars tested (Sugarcane Trash and Green Waste A) were beneficial for growth and colonization of onion. The previous chapters showed Sugarcane Trash was comparatively better for some parameters as was also observed in this experiment. All application rates were competitive with N applied at a rate of 110 kg ha^{-1}. The result was different from a previous report in that application of biochar at a rate of 10 t ha^{-1} significantly decreased dry matter content of radish (Chan et al. 2008a).

Green Waste A biochar rates were beneficial for growth of lettuce (Upadhyay et al. 2014). Biochar increased colonization in plant roots (Elmer & Pignatello 2011) grown in acidic soils (Ezawa et al. 2002; Matsubara et al. 2002; Yamato et al. 2006). In some reports, addition of char inhibited colonization probably due to improved availability of P (Warnock et al. 2007). In the present study, colonization was improved because soil P level was within acceptable limits (<25 mg kg^{-1}, Table 9.3) for mycorrhizal growth.

10.1 Research issues arisen from the thesis

There are some research issues that should be considered in future research work. The experiments of the present study were short-term (six to nine weeks); however, the effects of biochar and mycorrhizae may be greater if trials were conducted for a longer time period. The pot trials of this study had limitations for observation of growth and colonization, however, the crop yield after full growth and development is equally important. Soils in these experiments were less fertile, therefore information was lacking for effects of biochar on more fertile soils. All biochars were amended without other organic sources of nutrients such as manure and composts; these should be considered in future research. The limitation of this study was that the field trial was conducted for only a single season; results would be more useful if the trial could be continued for a longer period, perhaps up to three years. There would be benefits in maintaining AM levels for longer periods and examining their interactions with biochar which is likely to result in more sustained release of nutrients over the extended time.

10.2 Relevance of findings

The findings of this thesis are relevant to long-term soil fertility management. Soil fertility management has been a great challenge to developing countries like Nepal because of continued soil degradation due to several natural factors and inappropriate management practices. Natural factors are inevitable so they cannot be altered. However, their adverse effects can be reduced by applying sustainable management practices. For example, in Nepalese soils, N and P are the most limiting factors for crop yields which are lower than for other developing countries. Poor soil management practices play a major role in reducing crop yields. Research findings of this thesis indicate that the application of biochar is beneficial for crop growth and mycorrhizal colonization. Biochar application under field conditions has also proven to be beneficial over no use of biochar. As a carbon sequestering material, biochar can also be used to manage carbon in degraded soils of those countries. The colonization of mycorrhizae for all rates of zinc and copper were useful, even for Zn and Cu contaminated soils under certain conditions.

10.3 General conclusion

Experiments were conducted to determine effects of biochar on crop growth and mycorrhizal colonization. From the results, biochar was beneficial for growth of lettuce and cabbage but not effective for potato indicating crop-specific responses to biochar. The rate of 30 t ha^{-1} was optimum for lettuce growth. Biochar effects on growth of onion and tomato as well as mycorrhizal colonization of these crops confirmed that 30 t ha^{-1} for onion and 50 t ha^{-1} for tomato were effective. Sugarcane Trash biochar was comparatively better than Green Waste. Lower rates (50

mg kg^{-1}) of Zn and Cu were best for growth of onion and mycorrhizal colonization. Combination of lime, N at a rate of 110 kg ha^{-1} and P and K equivalent of biochar was the best for plant growth. The combination of ferrosol soil, sugarcane trash biochar and mycorrhizal inoculum was more effective than other treatments. Lime and extracted nutrients was more effective than lime and washed biochar. The field trial confirmed the results of glasshouse experiments that application of biochar was beneficial over no application. It was concluded that verification of treatments for longer periods in different types of soils and crops would be more effective in making specific recommendations. Externally added other major nutrients such as N P and K influenced the outcomes more than similar amounts of these nutrients in biochar alone. From the final experiment in the field, glasshouse results were verified and observed that the biochars were beneficial with greater positive effect on plant growth and colonization than no biochar. A long-term study is important for general recommendation in different types of soils.

Appendices

Appendix 1 Biochar properties (Kochanek et al. 2014)

Properties	Unit	Sugarcane Trash	Green Waste A	Green Waste B
EC	dS m^{-1}	1.4	3.4	2.3
pH	($CaCl_2$ 0.01M)	8	9.4	8.9
Total Nitrogen	%	0.53	0.47	0.61
Colwell Phosphorus	mg kg^{-1}	1100	740	490
Acid Neutralising capacity	% $CaCO_3$	0.66	6	1.4
Exchangeable Potassium	cmol(+) kg^{-1}	8.4	19	15
Copper (DTPA)	mg kg^{-1}	1.1	13	5.9
Zinc (DTPA)	mg kg^{-1}	5.7	44	16

Appendix 2 Hoagland recipes for supplementary nutrient solution (Hoagland & Arnon 1950; Epstein & Bloom 2005; Mattson & Lieth 2008)

Macronutrient STOCK A. Added first to the solution: 5 mL L^{-1} used.

Salts	Mass of each salt (g) per L stock
	atomic mass*moles = mass
$Ca(NO_3)_2.4H_2O$	94.4656
KNO_3	40.444
NH_4NO_3	24.0156

Macronutrient STOCK B CONTROL. Added third to the solution: 5 mL L^{-1} used.

Salts	Mass of each salt (g) per L of stock
	atomic mass*moles = mass
KH_2PO_4	27.2172
$MgSO_4.7H_2O$	24.6492
K_2SO_4	17.427

Micronutrient stock solution. Added second to the solution: 1 mL L^{-1} used.

Salts	Mass of each salt (g) per L in stock
	atomic mass*moles = mass
KCl	1.864
H_3BO_3	0.773
$MnSO_4.H_2O$	0.169
$ZnSO_4.7H_2O$	0.288
$CuSO_4.5H_2O$	0.062
$Na_2MoO_4.2H_2O$	0.0597
$NiSO_4.6H_2O$	0.066

Iron chelate stock solution. Added last to the solution: 1 mL L^{-1} used.

Fe EDTA	Fe is 10.5% of Fe EDTA (100/10.5 = 9.524) Mass Fe EDTA (g) = 9.524*1.0053 9.574285714 :. We need 9.574 g Fe EDTA per L of stock

Half-strength Hoagland solution with 25% P

Salts	Mass of each salt (g) per L of stock
	atomic mass*moles = mass
KH_2PO_4	6.8043
$MgSO_4.7H_2O$	24.6492
$Ca(NO_3)_2.4H_2O$	94.4656
K_2SO_4	30.49725
KNO_3	40.444
NH_4NO_3	24.0156

Appendix 3: Electrical conductivity (EC) of 1:5 soil/water extract (Rayment & Higginson 1992; Rayment & Lyons 2011)

Procedure: Prepare 1:5 w/v soil/water suspension. For example, weigh 20.0 g air-dry soil into a suitable bottle or jar and add 100 ml deionised water. Mechanically shake (end-over-end preferred), at 25^0C in a closed system for 1 h to dissolve soluble salts. Allow around 20-30 min minimum for the soil to settle.

Calibrate the conductivity cell and meter in accordance with manufacturer's instructions, using the KCl reference solution at the temperature of the suspensions.

Dip the conductivity cell into the settled supernatant, moving it up and down slightly without disturbing the settled soil. Take the reading with the cell stationary when the system has stabilized (see notes 2 and 3). Rinse the EC cell with deionised water between samples and remove excess water. Complete EC measurements within 3-4 h of obtaining the aqueous supernatant. Reference soil should be included in each batch of unknown samples.

Report EC (ds m^{-1}) at 25^0C on an air-dry (40^0C basis).

Appendix 4: pH of 1:5 soil/water suspension (Rayment & Higginson 1992; Rayment & Lyons 2011)

Procedure: Prepare a 1:5 soil/water suspension. For example, weigh 20.0 g air-dry soil (<2mm) into a suitable bottle or jar and add 100 ml deionised water. Mechanically shake, end-over-end, at 25^0C in a closed system for 1 h. Allow around 20-30 min for the soil to settle and make all measurements on the day of extraction, ideally within 4 h.

Standardize the pH meter according to manufacturer's instructions using the buffer at pH 6.86 or pH 7.0, and either the 4.0 or 9.183 buffer depending on the expected values for the soils. The use of three buffers during calibration provides a check on the linearity of electrode response. When soil pH values >10.0 are expected, use a glass electrode designed for highly alkaline conditions.

Stir these buffer solutions with a mechanical stirrer during measurements. Occasionally confirm there is adequate leakage of KCl from the calomel electrode, otherwise inaccurate readings may be obtained. This is achieved by placing the calomel electrode in 10.0 ml of deionised water for 1 min before testing for presence of Cl^- with $AgNO_3$. Thoroughly wash electrodes between the measurements of buffer solutions and between buffer solutions and soil solutions/extracts.

When measuring pH of soil suspension, ensure electrodes are well immersed. Record the pH value obtained when the meter appears steady while the suspension is being mechanically stirred. Replicate determinations should give results within 0.1 pH unit.

Report pH (1:5 soil/water) on an air-dry basis.

Appendix 5: Total Soil N – Dumas high temperature combustion (Method 7A5), (Rayment & Higginson 1992; Rayment & Lyons 2011)

Apparatus: LECO^{TM} CNS-2000 analyser or equivalent, plus essential gases and other accessories.

Reagents: Ethylenediaminetetraacetic Acid Reference Standard (EDTA): Use dry (105^0C for 2 h), high-grade EDTA ($C_{10}H_6N_2O_8$), calibrated against EDTA certified by the instrument manufacturer. When fully dry, this contains 9.586% N.

Procedure: Set up and maintain the high-temperature combustion analyser in accord with the Manufacturer's Operation and Procedures' Manual. This includes performing door maintenance and a combustion-leak check.

Run three separate ceramic 'boats' of EDTA reference standard (9.586% N) to stabilise the detectors, noting that irritating, toxic NO_x is released when EDTA is heated to its decomposition temperature of 240^0C. Next combust three empty ceramic 'boats' as blanks, using 0.200 g as the weight, to set the instrument blank from these results. Follow this by weighing into ceramic 'boats' analysing three separate replications of EDTA reference standard. Use the two closest results to perform a drift correction. Confirm the instrument setup and calibration by analysing at least one internal LCS (Laboratory Control Sample) for quality assurance purposes, using a weight between 0.3 and 0.75 g depending on the expected concentration.

If the LCS sample/s test within its/their accepted concentration/s, proceed to analyse unknown samples. Should the analyser be 'out of range', analyse another EDTA reference standard portion of known weight. Again perform a drift correction. Follow this with the reanalysis of another portion of the LCS sample/s. If the expected result/s is/are still out of specification, the instrument, gas lines and detectors should be double checked before proceeding any further.

When optimum analytical performance specifications are confirmed, prepare a known weight (e.g. 0.5-0.75 g of finely ground (<0.5 mm) air dry soil) and proceed to analyze all samples. If results are 'out-of-range', adjust sample weights as necessary. Include an LCS, followed by an EDTA reference standard portion of known weight to check instrumental drift about every 25 samples. Finally include a further LCS and an EDTA reference standard portion of known weight at the end of the samples' 'run', then finish with 2 blanks to enable the gas blank to be reset if necessary.

At the end of the run, go back and check that the LCS values are within their accepted range. If not, use the next measured EDTA reference standard value to 'drift correct', then recalculate the results. In general, recalculate the results half-way back to the last in-range LCS.

Calculation: Total Soil N (%N) = [a x MF]

Where, a = N concentration in air-dry sample (%N)

MF = air-dry moisture to oven-dry moisture ratio

Report TSN (%N) on an oven-dry basis.

Appendix 6: Bicarbonate extractable P (Colwell-P) – manual color (Method 9B1) (Rayment & Higginson 1992; Rayment & Lyons 2011)

Reagents

Extracting solution – 0.5 M Sodium Bicarbonate at pH 8.5: Dissolve 42.0 g sodium bicarbonate ($NaHCO_3$) in deionised water, dilute to almost 1.0 L, adjust pH to 8.5 (usually requires 0.8 g NaOH), and make volume to 1.0 L. Take care not to exceed pH 8.55 (refer to note 1). This extracting solution is best prepared on day of use. If storage is necessary, keep under nitrogen or mineral oil or utilise a CO_2 trap to prevent entry of this atmospheric contaminant (refer to note 2).

2.25 M sulphuric acid

1.0 M sulphuric acid

Reagent A (Ammonium molybdite – sulphuric acid – Sb solution

Dissolve 12.0 g of ammonium molybdite $(NH_4)_6Mo_7O_{24}.4H_2O$) in 400 ml of warmed (not above 50^0C) distilled (preferred) or deionised water then cool. Add 140 ml sulphuric acid (H_2SO_4; 18 M) slowly and with stirring to another 400 ml distilled or deionised water and cool. To a further 100 ml of water dissolve 0.267 g potassium antimony tartarate ($KSbO.C_4H_4O_6$). Combine by adding with stirring the ammonium molybdite solution to the diluted H_2SO_4. Re-cool, add the potassium antimony tartarate solution and make 1.0 L with water. Store in borosilicate glass in a cool place to achieve a shelf life of several months. This solution contains 1.2% ammonium molybdate and 0.1 mg Sb ml^{-1} in ≈2.5 M H_2SO_4.

Mixed colour reagent: For each 100 ml required, dissolve 1.056 g *l*-ascorbic acid ($C_6H_8O_6$) in 100 ml of Reagent A. Prepare only as required as the shelf life does not exceed 24 h.

Phosphorus primary standard: 1 L contains 50.0 mg of P. Dissolve 0.1098 g potassium dihydrogen phosphate (KH_2PO_4; previously dried at 130^0C for 2 h) in deionised water. After making volume to 500 ml with deionised water, add 2 drops of chloroform ($CHCl_3$) to supress biological activity. When stored in a sealed, chemically inert container at ≈4^0C, this solution should remain stable for at least 2-3 months.

Phosphorus secondary standard: 1 L contains 10.0 mg of P. Take 100 ml P primary standard and dilute accurately to 500 ml in a volumetric flask with extracting solution (0.5 M $NaHCO_3$ at pH 8.5). This solution should be freshly prepared each time working standards are made.

Phosphorus working standards: Add 0, 1.0, 2.5, 5.0, 10.0, 15.0, 20.0, 30.0, 40.0, 50.0 ml of P secondary standard to separate 500 ml volumetric flasks. Dilute to 500 ml with 0.5 M $NaHCO_3$ at pH 8.5 extracting solution. These working standards cover the range 0-1 mg P L-1 and for a 1:100 soil/solution ratio are equivalent to soil concentrations of 0, 2.0, 5.0, 10.0, 20.0, 30.0, 40.0, 60.0, 80.0, 100.0, mg P kg^{-1}.

Procedure: Weigh 1.00 g of air-dry soil (<2 mm) into a 250 ml extracting bottle and add 100 ml extracting solution (0.5 M $NaHCO_3$ at pH 8.5), stopper and mechanically shake end-over-end for 16 h at 25^0C. Centrifuge or filter (Whatman No. 42 – tested P free) soil extracts then pipette duplicate 25 ml aliquots into 100 ml volumetric flasks. Add 50 ml deionised water and mix thoroughly. Add 2 ml 1M H_2SO_4, mix and, after effervescence has ceased, add a further 5 ml of 1 M H_2SO_4. Mix well and allow to stand overnight to complete the removal of CO_2.

To one set of volumetric flasks add 8 ml mixed colour reagent, then make to 100 ml and mix well. To the duplicate flasks (the reagent blanks) add 8 ml of Reagent A, make to 100 ml and mix well. Concurrently with the sample, take 25 ml of each P working standard and treat in a similar manner to the samples.

After 30 minutes, measure the absorbance at 882 nm of samples, standards, and soil-reagent blanks against deionised water as a reference. The absorbance values remain stable for up to 24 h. If necessary, dilute over-range extracts with $NaHCO_3$ extracting solution. Same-day measurement following acidification is preferred to limit the possibility of chemical and/or biological change.

Calculation: Bicarbonate extractable P = [Sample Value – Reagent blank] mg P kg^{-1}.

Appendix 7: Exchangeable bases (K^+ for the experiments mentioned above) by 1 M ammonium chloride at pH 7.0, pre-treatment for soluble salts (Method 15A1 and 15A2) (Rayment & Higginson 1992; Rayment & Lyons 2011)

There is pre-treatment with aqueous ethanol and auqueous glycerol to remove soluble salts. This pre-treatment is desirable when the soil EC (1:5 soil/water) exceeds ≈ 0.3 dS m^{-1}.

Reagents

Extracting solution: 1 M Ammonium Chloride at pH 7.0: Dissolve 535 g ammonium chloride (NH_4Cl – low in Ca, Mg, Na and K impurities) in deionised water and dilute to 9 L. Adjust to pH 7.0 by adding ammonium hydroxide (NH_4OH). Wash the electrodes of the pH meter thoroughly before placing them in the extracting solution; otherwise K+ salts from the calomel electrode may cause contamination.

Make the volume to 10 L with deionised water and store in sealed containers. Plastic containers are preferred, however borosilicate glassware may be substituted; soda glass should not be used.

5 M Ammonium Chloride at pH 7.0: Dissolve 267.5 g NH_4Cl (identical to that used for the extracting solution) and dilute to 900 ml. Adjust to pH 7.0 as described for the extracting solution and make to 1.0 L.

Wetting agent Brij 35: Shake 30 g of polyoxyethylene 23 lauryl ether (Brij 35) with 20 ml iso-propyl alcohol [propane-2-ol, $(CH_3)_2$-CH-OH] until dissolved; several hours may be required. Make to 100 ml with deionised water.

Lithium chloride for automated Na+ and K+: Dissolve 0.11 g lithium chloride (LiCl), add 1 ml Briz 35 wetting agent and make to 1 L with deionised water.

60% aqueous ethanol (w/w): Mix 665 ml of 96% ethanol (C_2H_5OH; e.g. special grade serina – SMF3; S.G. 0.803) and make to 1 L with deionised water. Deionise if EC > 10^{-3} dS m^{-1}) or if pH is not within the range 5.5-7.0. Pass through a column of fresh, mixed bed ion exchanger in the H+/OH- form, such as Zero-Karb 225/De Acidite FF or equivalents. Remove dissolved air by boiling or by drawing the prepared reagent through a fine jet under vacuum into a Buchner filtration flask connected through a trap to a vacuum pump.

20% aqueous glycerol: Combine 200 ml 87-88% technical glycerol ($CH_2OH.CHOH.CH_2OH$; wt/ml about 1.23 g) with 800 ml deionised water. Deionised if necessary as described for 60% aqueous ethanol. Boil to sterilize and add 0.5g of thymol crystals ($C_{10}H_{14}O$) as a preservative.

Mixed Ca and Mg primary standard: 1 L contains 100 $mmol_C$ of Ca and Mg. Use certified commercial concentrates or dry calcium carbonate ($CaCO_3$, primary standard grade) by heating at 110^0C to constant weight. Also dry magnesium oxide (MgO, heavy) by heating in an electric muffle furnace at 600-700^0C for 2 h. Cool and store the chemicals in a desiccator without desiccant.

Weigh 2.0152 g MgO and 5.0045 g $CaCO_3$ and wash into a 1 L conical flask with about 50 ml deionised water. Add 240 ml 1 M HCL and boil until all CO_2 is expelled. Cover and allow cooling, and then transfer quantitatively to a 1 L volumetric flask. Dilute to volume with CO2-free (boiled) deionised water and mix well. Transfer to a clean plastic bottle. Should MgO not assay at 100% purity, adjust the weight according to the assay obtained.

Mixed Na and K primary standard: 1 L contains 50 $mmol_C$ of Na and 12.5 $mmol_C$ of K. Use certified commercial standard concentrates or dry sodium chloride (NaCl) at 105^0C for 2 h and potassium chloride (KCl) for 2 days at 115-120^0C. When dry, cool and store in a desiccator without desiccant. Weigh 2.9221 g NaCl and 0.9319 g KCl and dissolve separately with deionised water. Transfer quantitatively to 1 L volumetric flask and make to 1 L with deionised water. Store in a clean plastic bottle.

Mixed Ca and Mg secondary method: 1 l contains 10 $mmol_C$ of ca and Mg. Take 50 ml of mixed Ca and Mg primary standard and dilute to 500 ml in a volumetric flask with CO2-free (boiled) deionised water. This solution should be freshly prepared each time working standards are required.

Mixed Na and k secondary standard: 1 L contains 5 $mmol_C$ of Na and 1.25 $mmol_C$ of K. Take 50 ml of mixed Na and K primary standard and dilute 500 ml in a volumetric flask with CO2-free deionised water. This solution should be freshly prepared each time working standards are required.

Mixed working standards for exchangeable bases: Dispense mixed Ca and Mg primary or secondary standards as indicated in Table A and mixed Na and K primary standards as indicated in Table B below, into 500 ml volumetric flasks. Add 100 ml 5 M NH4Cl to each and dilute to 500 ml with CO2-free deionised water.

Table A. Examples of dilutions and concentrations for Ca and Mg working standards – 1M NH4Cl at pH 7.0.

ml of mixed primary or secondary standard in 500 ml	Initial solution concentration ($mmol_C$ Ca and Mg L^{-1})	Equivalent soil content ($cmol_C$ kg^{-1}) of Ca and Mg, respectively, for 1:20 soil/extract ratio (final) following	
		1 + 9 dilution of samples and standards*	1 + 49 dilution* of samples 1 + 9 dilution of standards
Mixed Ca and Mg secondary standard (10 $mmol_C$ Ca and Mg L^{-1})			
2.5	0.05	0.1	0.5
5.0	0.10	0.2	1.0
7.5	0.15	0.3	1.5
12.5	0.25	0.5	2.5
25.0	0.50	1.0	5.0
50.0	1.00	2.0	10.0
Mixed Ca and Mg primary standard (100 $mmol_C$ Ca and Mg L^{-1})			
7.5	1.5	3.0	15.0
10.0	2.0	4.0	20.0
12.5	2.5	5.0	25.0
15.0	3.0	6.0	30.0
20.0	4.0	8.0	40.0
25.0	5.0	10.0	50.0

*NH4Cl as working solution for ICPAES

Table B. Examples of dilutions and concentrations for Na and K working standards – 1M NH4Cl at pH 7.0.

ml of mixed primary or secondary standard in 500 ml	Initial solution concentration ($mmol_C\ L^{-1}$)		Equivalent soil content ($cmol_C\ kg^{-1}$) for 1:20 soil/extract ratio*	
	Na	K	Na	K
Mixed Na and K secondary standard (5 $mmol_C$ Na L^{-1} and 1.25 $mmol_C$ K L^{-1})				
2.5	0.025	0.006	0.05	0.0125
7.5	0.075	0.019	0.15	0.038
12.5	0.125	0.031	0.2	0.063
25.0	0.25	0.063	0.5	0.125
50.0	0.50	0.125	1.0	0.25
Mixed Na and K primary standard (50 $mmol_C$ Na L^{-1} and 12.5 $mmol_C$ K L^{-1})				
7.5	0.75	0.188	1.5	0.375
10.0	1.00	0.250	2.0	0.500
12.5	1.25	0.313	2.5	0.625
15.0	1.50	0.375	3.0	0.75
20.0	2.0	0.500	4.0	1.00
25.0	2.5	0.625	5.0	1.25

If necessary, dilute extracts of high concentration with 1 M NH_4Cl extracting solution to bring these within the optimum range of the instrument, and to maintain the same concentrations of NH_4Cl in standards and sample extracts.

Procedure: Weigh 5.00 g air-dry soil (<2mm) into a pre-weighed 50 ml centrifuge tube and add 25 ml 60% aqueous ethanol. Seal and shake for 30 min. Within 30 min of that action, centrifuge and remove the supernatant solution by suction.

Drain the tube upside down on a piece of absorbent paper to remove excess solvent. Disperse the soil mechanically and add a second 25 ml of aqueous ethanol, centrifuge and decant and drain as before. Repeat the process a third time using 20% aqueous glycerol in place of aqueous ethanol. Weigh the centrifuge tube to determine the approximate volume of entrained aqueous solvents. Transfer the pre-treated soil to a 250 ml plastic extracting bottle using 100 ml 1 M NH_4Cl at pH 7.0 extracting solution. Stopper securely and mechanically shake end-over-end at $\approx 25^0$C for 1 h. Centrifuge or filter soil extracts. If filtering, prepare Whatman No. 40 filter papers in 75 mm plastic

funnels and place suitable clean, dry, receiving containers in position. Condition the filter paper by discarding the first 10-20 ml of filtered extract then collect sufficient extract (30-50 ml) to determine all basic cations. If centrifuging, ensure centrifuge tubes are clean and dry. Retain the clarified extracts for Ca^{2+}, Mg^{2+}, Na^{+}, K^{+} analyses. The batch should be sized to allow filtration and/or centrifugation to occur within 30 min of completion of mechanical shaking.

Determination of Ca^{2+}, Mg^{2+}, Na^{+}, K^{+} by ICPAES (Inductively coupled plasma atomic emission spectrometry)

Set up and operate the ICPAES instrument as advised by the manufacturer. Suitable wavelengths are: Ca = 430.25 nm; Mg = 285.21 nm; Na = 588.96 nm; and K = 766.49 nm. Calibrate the instrument using an appropriate range of working standard solution, guided by examples in Table A and B above. The 1:20 soil/extraction ratio can be factored into the calibration on the ICPAES. A reagent blank should also be measured and adjustments made as necessary.

Calculation and reporting: When 5.0 g of soil are extracted with 100 ml of NH_4Cl, increase the determined values by the ratio [100 + mass (g) of entrained aqueous solvent]/100 to obtain the concentration of exchangeable bases on an air-dry basis.

Report exchangeable Ca^{2+}, Mg^{2+}, Na^{+}, K^{+} ($cmol_C\ kg^{-1}$), expressed on an oven-dry soil basis. Use the air dry moisture to oven-dry moisture ratio to make the oven dry conversion.

Appendix 8: DTPA-extractable Cu, Zn, Mn and Fe (Method 12A1) (Rayment & Higginson 1992; Rayment & Lyons 2011)

Reagents

DTPA Extracting Solution: This solution is 0.005 M with respect to DTPA, 0.01 M to CaCl2 and 0.10 M triethanolamine (TEA). For 1 l of extracting solution, dissolve 1.97 g diethylenetriamine penta aceticacid (DTPA), 1.47 g calcium chloride dehydrate ($CaCl_2.2H_2O$) and 14.92 g triethanolamine [$N(CH_2CH_2OH)_3$] separately in deionised water and combine. Add ≈6.8 g of 35% w/w HCl and dilute to ≈990 ml with deionised water. Check pH and adjust to 7.3±0.05 with either dilute HCL or triethanolamine, then make volume to 1.0 L. Store in a Teflon or low density polyethylene container not previously used to store any of the four metals under test; the solution remains stable for at least three months if kept cool (≈4^0C) and away from direct sunlight.

Copper primary standard: 1 ml contains 1 mg of Cu. Clean a piece of Cu foils then accurately weigh 1.000 g of the cleaned metal and place in a 1 l volumetric flask. Dissolve in 20 ml of 1+1 HNO_3 and dilute to volume with deionised water.

Zinc primary standard: 1 ml contains 1 mg of Zn. Clean a piece of Zn rods then accurately weigh 1.000 g of the cleaned metal and place in a 1 l volumetric flask. Dissolve in 20 ml of 1+1 HCl and dilute to volume with deionised water.

Manganese primary standard: 1 ml contains 5 mg of Mn. Weigh 6.8712 g anhydrous manganous sulphate (prepared by dehydrating manganese sulphate monohydrate ($MnSO_4.H_2O$) at 200^0C for 4 h) into a 500 ml volumetric flask. Dissolve in a mixture of 200 ml water and 1 ml 18 M H_2SO_4 and make to volume with deionised water.

Iron primary standard: 1 ml contains 5 mg Fe. Weigh 17.5538 g ammonium ferrous sulphate [$(NH_4)_2SO_4FeSO_{4.6}H_2O$] and transfer to a 500 ml volumetric flask. Dissolve in deionised water containing 1 ml 18 M H_2SO_4 and make to volume with deionised water.

Mixed ‘low strength’ secondary standard: Take 10 ml Cu primary standard, 10.0 ml Zn primary standard, 20 ml Mn primary standard and 20 ml Fe primary standard and dilute with deionised water to 1.0 L. This solution contains 10 mg L^{-1} of both Cu and Zn and 100 mg L^{-1} of both Mn and Fe.

Mixed ‘high strength’ secondary standard: Take 40 ml Cu primary standard, 40 ml Zn primary standard, 200 ml Mn primary standard and 200 ml Fe primary standard and dilute with deionised

water to 1.0 L. This solution contains 40 mg L^{-1} of both Cu and Zn and 1000 mg L^{-1} of both Mn and Fe.

Mixed working standards: Take aliquots of freshly prepared low and high strength secondary standard solutions as given in Table A and B below. Add 83 ml triple strength DTPA extracting solution (45 g triethanolamine, 5.91 g DTPA, 4.41 g $CaCl_2.2H_2O$ and 20.65 g HCl to 1 L with deionised water) and make volume of each working standard to 250 ml with deionised water. Store in a black polyethylene bottles or in the dark in standard polyethylene or teflon bottles. Actual solution concentrations and equivalent soil contents for a 1:2 soil/extract ratio are given in Tables A and B below.

Procedure: A reagent blank with no soil should be included with each batch of samples. Weigh 25.0 g of air-dry soil (<2mm) into a 100 or 250 ml polyethylene bottle. Add 50 ml DTPA extracting solution, stopper, and mechanically shake end-over-end continuously for 2 h at 25^0C. Filter (No. 2 Whatman paper) or centrifuge the extracts without delay, discarding the first portion, and retain the particle-free extracts for analysis. Measure metal concentrations in these filtrates by ICPAES as soon as possible to avoid microbial growth and/or chemical changes.

Use an appropriate selection of working standards and determine concentrations of each element (mg kg^{-1}) from the appropriate calibration curve, after adjusting for any significant reagent blank. It is important to follow manufacturer's recommendation with respect to instrument parameters and wavelengths selections (Preferred spectral lines for ICPAES are typically 324.754, 213.856, 257.610 and 259.940 nm for Cu, Zn, Mn and Fe, respectively. No background corrections are necessary when these wavelengths are used over concentrations ranges of 0-10 mg L^{-1} for Cu and Zn and 0-240 mg L^{-1} for Mn and Fe).

Report each element (Cu, Zn, Mn, Fe; mg kg^{-1}) on air-dry basis.

Table A. Volumes of 'Low Strength' secondary standard and consequential concentrations of 'Low Range' Mixed Working Standards for DTPA-extractable Cu, Zn, Mn and Fe.

ml of low strength secondary standard in 250 ml	Actual solution concentration (mg L^{-1})		Equivalent soil context (mg kg^{-1}) for a 1:2 soil/extract ratio	
	Cu and Zn	Mn and Fe	Cu and Zn	Mn and Fe
2.5	0.1	1	0.2	2
5.0	0.2	2	0.4	4
7.5	0.3	3	0.6	6
10.0	0.4	4	0.8	8
12.5	0.5	5	1.0	10
15.0	0.6	6	1.2	12
20.0	0.8	8	1.6	16
25.0	1.0	10	2.0	20
37.5	1.5	15	3.0	30
50.0	2.0	20	4.0	40

Table B. Volumes of 'High Strength' secondary standard and consequential concentrations of 'High Range' Mixed Working Standards for DTPA-extractable Cu, Zn, Mn and Fe.

ml of high strength secondary standard in 250 ml	Actual solution concentration (mg L^{-1})		Equivalent soil context (mg kg^{-1}) for a 1:2 soil/extract	
	Cu and Zn	Mn and Fe	Cu and Zn	Mn and Fe
2.5	0.4	10	0.8	20
5.0	0.8	20	1.6	40
10.0	1.6	40	3.2	80
15.0	2.4	60	4.8	120
20.0	3.2	80	6.4	160
25.0	4.0	100	8.0	200
30.0	4.8	120	9.6	240
35.0	5.6	140	11.2	280
40.0	6.4	160	12.8	320
45.0	7.2	180	14.4	360
50.0	8.0	200	16.0	400
60.0	9.6	240	19.2	480

Appendix 9: Procedures for plant nitrogen analysis by acid digestion and ICPAES method (Mills & Jones Jr 1996)

1. Weigh 0.5 g dried (80^0C) and ground (20- or 40- mesh) plant tissue into a digestion tube. Place a glass funnel in the mouth of the digestion tube.
2. Add 1.5 ml concentrated nitric acid (HNO_3). Let it stand overnight.
3. Place the digestion tube into a port of the digestion block and heat at 120^0C for 1 h. Remove the digestion tube from the digestion block and let cool.
4. Add 1.5 ml perchloric acid ($HClO_4$) with precautions.
5. Place the tube back into the digestion block and heat at 200^0C for 1 h, until the digest is clear (colourless).
6. Remove the funnel from the digestion tube and set the temperature of the digestion block at 100^0C. Keep the digestion tube in the block until the fumes of perchloric acid have dissipated.
7. Remove the digestion tube from the digestion block and allow it to cool.
8. Add pure water to dilute it to 10 ml, or to another appropriate volume.
9. The digest is ready for elementary assay. The may be further diluted as necessary to achieve an element concentration that is within the analysis range of the analyser.
10. Analyse N by LECO CNS 2000 Carbon-Nitrogen-Sulfur Analyser and other elements (important for this thesis) by ICPAES (ICP polychromator).

Appendix 10

Nutrients and lime content (%CaCO3) of biochars (Chapter 6)

Calculation of biochar

For podsol, recommended dose: 20 t ha^{-1}

Pot diameter: 16cm

Pot area = πr^2 = 0.020096 m^2

= 40.2 g biochar per pot

For Ferrosol, recommended dose: 30 t ha^{-1} = 60.3 g biochar per pot

Calculation of lime equivalent to biochars

For podsol, lime requirement = 1.2 t ha^{-1} = 2412 mg

Green Waste A = 40.2g x 0.06 =2412 mg

Green Waste B = 40.2 g x 0.014=562.8 mg

Sugarcane Trash = 40.2 g x 0.0066=265.3 mg

To equalize these three values, Green Waste B needs 2412-562.8=1849.2 mg lime and Sugarcane Trash requires 2412-265.3=2146.7 mg lime to be added.

For ferrosol,

Lime requirement = 6 t ha^{-1} = 12057.6 mg pot^{-1}

Green waste A = 60.3 g x 0.06 =3618 mg of lime

Green waste B = 60.3 g x 0.014=844.2 mg of lime

Sugrcane Trash = 60.3 g x 0.0066=396 mg of lime

To equalize these three values, Green Waste A 12057.6-3618=8439.6 mg, Green Waste B 12057.6-844.2 =11212.8 and Sugarcane Trash 12057.6-396=11661.6 mg lime to be added per pot.

Nitrogen calculation for podsol and ferrosol

Recommended dose of nitrogen for tomato hybrids = 110 kg N in a hectare

= (100/35) x 110 = 314.3 kg of Ammonium nitrate per hectare

= (314300/10000) x 0.020096 = 632 mg Ammonium nitrate per pot.

Phosphorus and Potassium calculation for podsol

Phosphorus equivalent to Sugarcane Trash biochar

1 kg biochar contains 1100 mg of P,

= (1100/1000) x 40.2 = 44.22 mg of P,

= (100/20.07) x 44.22 mg = 220.3 mg TSP per pot

Phosphorus calculation equivalent to Green Waste A

1 kg biochar contains 740 mg of P,

= (740/1000) x 40.2 = 29.75 mg of P

= (100/20.07) x 29.75 mg = 148.23 mg TSP per pot

Phosphorus calculation equivalent to Green Waste B

1 kg biochar contains 490 mg of P,

= (490/1000) x 40.2 = 19.7 mg of P

= (100/20.07) x 19.7 mg = 98.16 mg TSP per pot

Potassium calculation equivalent to Sugarcane Trash biochar

1 kg biochar contains 8.4 cmol(+) which is equal to 8.4 x 390 = 3276 mg of K,

= (3276/1000) x 40.2 = 131.7 mg of K

= (100/52.44) x 131.7 mg = 251.1 mg KCl per pot

Potassium calculation equivalent to Green Waste A

1 kg biochar contains 19 cmol (+) which is equal to 19 x 390 = 7410 mg of K,

= (7410/1000) x 40.2 = 297.9 mg of K

= (100/52.44) x 297.9 mg = 568.1 mg KCl per pot

Potassium calculation equivalent to Green Waste B

1 kg biochar contains 15 cmol (+) which is equal to 15 x 390 = 5850 mg of K,

= (5850/1000) x 40.2 = 235.17 mg of K

= (100/52.44) x 235.17 mg = 448.5 mg KCl per pot

Phosphorus and Potassium calculation for ferrosol

Phosphorus equivalent to Sugarcane Trash biochar

1 kg biochar contains 1100 mg of P,

= (1100/1000) x 60.3 = 66.33 mg of P,

= (100/20.07) x 66.33 mg = 330.5 mg TSP per pot

Phosphorus calculation equivalent to Green Waste A

1 kg biochar contains 740 mg of P,

= (740/1000) x 60.3 = 44.6 mg of P

= (100/20.07) x 44.6 mg = 222.2 mg TSP per pot

Phosphorus calculation equivalent to Green Waste B

1 kg biochar contains 490 mg of P,

= (490/1000) x 60.3 = 29.6 mg of P

= (100/20.07) x 29.6 mg = 147.5 mg TSP per pot

Potassium calculation equivalent to Sugarcane Trash biochar

1 kg biochar = 8.4 cmol(+) = 8.4 x 390 = 3276 mg of K,

= (3276/1000) x 60.3 = 197.5 mg of K

= (100/52.44) x 197.5 mg = 376.6 mg KCl per pot

Potassium calculation equivalent to Green Waste A

1 kg biochar = 19 cmol (+) = 19 x 390 = 7410 mg of K,

= (7410/1000) x 60.3 = 446.8 mg of K

= (100/52.44) x 446.8 mg = 852.0 mg KCl per pot

Potassium calculation equivalent to Green Waste B

1 kg biochar = 15 cmol (+) = 15 x 390 = 5850 mg of K,

= (5850/1000) x 60.3 = 352.7 mg of K

= (100/52.44) x 352.7 mg = 672.6 mg KCl per pot

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