EFFECTS OF BIOCHAR AND COMPOSTS ON SUBSTRATES PROPERTIES AND CONTAINER-GROWN BASIL (*Ocimum basilicum*) AND TOMATO (*Solanum lycopersicum*) PLANTS

A Thesis

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ABSTRACT

Biochar (BC) has the potential to be used as container substrates. However, effects of BC on container-grown plants depend on various factors including container substrate components mixed with BC, BC percentage and plant type. The purpose of this project is to test the potential of BC and composts mixes to be used as replacements for the commercial container substrates.

In the first experiment, mixes of 20%, 40%, 60% or 80% (by vol.) BC with 5%, 10%, 15% or 20% (by vol.) vermicompost (VC) were evaluated as container substrate on basil (*Ocimum basilicum*) and tomato (*Solanum lycopersicum*) plant growth compared to the commercial peat-based substrate (control). The commercial substrate made up the remaining volume when the BC and VC did not add up to 100%. Growth index (GI) and the total dry weight (DW) of basil and tomato in BC:VC mixes were similar to or higher than the control at 9 weeks after transplanting (WAT). Therefore, the BC (20%, 40%, 60% or 80%, by vol.) and VC (5%, 10%, 15% or 20%, by vol.) mixes could be used as the alternative container substrates.

Because of the high price of VC, the lowest VC percentage (5%) from the first experiment was selected for the second experiment. Chicken manure compost (CM) has similar fine texture to VC and is cheaper than VC. In the second experiment, mixes with either CM or VC (5%, by vol.) and BC (60%, 70%, 80% or 90%, by vol.) with the rest being the commercial substrate were evaluated to grow tomato and basil and compared to the commercial substrate. At 8 WAT, the GI, shoot DW and fresh weight (FW), and root and total DW of basil in BC-compost mixes (except 80BC:5CM, 90BC:5VC and 90BC:5CM) were similar to the control, respectively. The GI, stem, root, total DW, and red and total fruit FW and DW of tomato plants in BC-compost mixes (except 90BC:5CM) were similar to or higher than the control. Therefore, 60% and 70% BC mixed with as low as 5% (by vol.) CM and VC can be used to grow basil and tomato plants in containers.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

Container plant production needs significant amount of potting mixes or substrates, and the primary substrate components include peat moss, vermiculite, perlite, bark, and compost (Landis and Morgan, 2009; Wright and Browder, 2005). Sphagnum peat moss is an excellent and also major substrate component, which has suitable characteristics, such as low pH and bulk density, high cation exchange capacity (CEC), appropriate aeration and good water holding capacity (Bohlin and Holmberg, 2001; Fascella, 2015; Landis, 1990). However, excess harvest of peat moss led to damage to peatlands to some extent, which has caused increasing ecological concern (Verhagen and Blok, 2007). There is also increasing cost of the major substrate components (Carlile et al., 2015; Landis and Morgan, 2009), limited supply of commonly used substrates, such as bark, which became scarcer or less available for increased competition as energy sources (Bilderback et al., 2013; Lu et al., 2006), and rising interest in using environmentally friendly and local substrates components (Landis and Morgan, 2009). A lot of research has concluded that it would be beneficial and necessary to search for alternative substrate components for many years (Carlile et al., 2015; Landis and Morgan, 2009; Li et al., 2017b; Wright and Browder, 2005).

Biochar, attracting a lot of interest in recent years for its use in agriculture, can be a potential alternative to commonly used substrates. Biochar refers to the carbon-rich material derived from biomass (Lehmann, 2007; Nartey and Zhao, 2014). Biochar is renewable and fast to generate (Yu et al., 2012), compared to peat moss. Research has shown that addition of biochar to soil or container substrate could increase water and nutrient holding capacity, sequestrate carbon, ameliorate soil acidity and provide a suitable environment for microbial activity, which could improve plant growth and make it an excellent alternative to the current substrates (Dumroese et al., 2011; Laird, 2008; Vaughn et al., 2013; Woolf et al., 2010).

Although effects of biochar on agriculture production was generally positive, the effects of biochar on soil or soilless substrates depend on a lot of factors including biochar feedstock sources, production conditions, percentage of biochar applied, other substrate components mixed with biochar, soil type, plant type and fertility. There is no universal standard for biochar addition for all plants. All biochars are not equal. Therefore, it would be of interest to examine the characteristics of biochars, different combinations of biochars with other substrate components and their effects on diverse types of container-grown plants.

Biochar Production

There are many variables prior to, during and after production of biochar. These factors will eventually affect biochar properties and how biochar performs when being incorporated in soil or soilless substrates.

Biochars could be made from different feedstocks, such as green waste (Tian et al., 2012), wheat straw (Vaughn et al., 2013; Xu et al., 2016), marginal biomass (Buss et al., 2016), wood (Hansen et al., 2016; Spokas et al., 2009; Vaughn et al., 2013), and rice hull (Locke et al., 2013). Biochar made from woodchips has higher C/N ratios and specific surface area than animal manure biochar produced under the same condition (Lei and Zhang, 2013). Straw biochar has higher pH, exchangeable cations, and K content compared to wood biochar (Vaughn et al., 2013). Biochar made from rice hulls has a high content of P and K, which should be considered when making P and K fertilization plans (Locke et al., 2013). Therefore, the physical and chemical properties of biochars depend on the basic properties of the original feedstock and should be taken into account.

There are mainly three processes to produce biochar: pyrolysis, gasification and hydrothermal carbonization. Pyrolysis refers to the thermal decomposition of biomass by heating it (around 400-600°C) without oxygen (Gvero et al., 2016; Hansen et al., 2015; Lehmann et al., 2011). The pyrolysis temperature influenced the characteristics of biochars significantly. Biochar produced at low temperatures (below 400°C) from pyrolysis retains more phosphorus, which could then enhance plant growth. As pyrolysis temperature increases, more water-soluble and organic phosphorus are converted to unavailable phosphorus (Xu et al., 2016). Raising pyrolysis temperature can contribute to decreased biochar yield (Li et al., 2014; Rahman et al., 2016) and increased pH (Butnan et al., 2015). Compared to pyrolysis, gasification is conducted under small amounts of oxygen at relatively higher temperatures (around 700-1200°C) (Hansen et al., 2016). Gasification produces smaller quantities of biochar than pyrolysis and with lower C content (Bruun et al., 2011; Hansen et al., 2016; Hansen et al., 2015). Hydrothermal carbonization (HTC) uses water and catalysts at lower temperatures (180-300°C) under high pressure to convert biomass to different biochar products, hydrochars (Kalderis et al., 2014; Libra et al., 2011). Hydrochars are acidic, and have low surface area, less aromatic compounds and higher CEC than those produced by pyrolysis and gasification (Kalderis et al., 2014; Wiedner et al., 2013).

The main purpose of fast pyrolysis is to produce syngas and bio-oil (Fuchs et al., 2014; Gvero et al., 2016; Laird, 2008) and gasification syngas (Zheng et al., 2010) with biochar being a byproduct. Syngas could be used to provide energy for another pyrolysis process. Bio-oil could be burned to produce heat or further processed to be used as fuels (Bridgwater et al., 1999). Using biochar in agriculture adds values to biomass pyrolysis or gasification. Specific process and its heating rate or temperature could be modified to produce desirable products. For example, gasification is preferred over pyrolysis when more energy product (i.e. syngas) is desirable (Ahmed and Gupta, 2009). Slow pyrolysis produces more biochar and syngas and fast pyrolysis more bio-oil (Fuchs et al., 2014). The residence time of slow pyrolysis is from 5 to 30 minutes, while that of fast pyrolysis is from seconds to less than a second and the temperature is higher (Gvero et al., 2016; Panwar et al., 2012).

Pretreatment of feedstocks has been reported to have significant influences on biochar properties. Pre-treatment of biomass, such as washing with water or acid, could help to remove some ash culprits in feedstock to reduce fouling, and improve the quality of biomass feedstock and final biochar product (Jenkins et al., 1996; Rahman et al., 2016). Rahman et al. (2016) tested the effectiveness of different pre-treatments by comparing the electrical conductivity (EC) of the initial washing medium and leachate collected after treatment. The result showed that EC from the leachates of palm kernel shell (PKS) pre-treated with dilute acid, dilute alkali and distilled water were observed to increase and the highest increase in EC was found in the dilute acid leachate, which could be the result of removal of soil and alkaline metal by the acid solution and degradation of the biomass chemical composition. It was also shown that the ash content of PKS was reduced if pretreated with distilled water or diluted acid. And the ash concentration increased with alkaline pre-treatment since abundant sodium ions in alkaline medium prevented ions from leaching into the medium and ions were bound and tied up to the biomass particles, which resulted in high amount of ash content (Rahman et al., 2016). Pre-treatment of biochar feedstock bark with tannery slurry as an alkaline treatment also resulted in increased ash content, as well as surface functional groups and greater NH_4^+ absorption capacity than untreated ones (Hina et al., 2010). Another research showed that biochar feedstock paper mill sludge pre-treated with phosphoric acid and torrefaction followed by pyrolysis resulted in reduced volatile matter content, increased inorganic matter and increased biochar yield (Reckamp et al., 2014). Torrefaction pre-treatment could also increase the yield of biochar during pyrolysis (Boateng and Mullen, 2013).

In addition to pre-treatments of feedstocks, post-treatments could also change biochar properties. Biochar could contain toxic compounds polycyclic aromatic hydrocarbons (PAHs) during production. Drying biochars at temperature of 100, 200 and 300°C significantly decreased the amount of PAHs in biochars (Kołtowski and Oleszczuk, 2015). Biochar could be treated and mixed with other substances. Dumroese et al. (2011) dry-blended biochar with wood flour, polylactic acid and starch to pelletize biochar, which is a more preferred form than the fine-textured and dusty biochar for its handling convenience and evenly incorporation. McCabe et al. (2016) evenly blended soybean-based bioplastics with biochar in a pelletized form as a source of nutrients in soilless substrate.

Effects on Physical and Chemical Properties of Soil and Soilless Substrates

The addition of biochar to the soil or soilless substrates could have an effect on the physical properties. In most situations, adding biochar reduces the soil bulk density (Ashworth et al., 2014; Bruun et al., 2014; Laird et al., 2010b; Lei and Zhang, 2013; Zhang et al., 2016). However, adding biochar from wood and straw gasification did not affect the bulk density of sandy loam and sandy soils in the research conducted by Hansen et al. (2016). For soilless substrates, biochar has higher bulk density than some commonly used substrate components, such as peat moss and vermiculite. Using biochar to replace certain percentage of peat could increase the bulk density of the substrates (Bilderback et al., 2005; Dumroese et al., 2011; Tian et al., 2012; Vaughn et al., 2015a; Vaughn et al., 2013).

Adding biochar may affect the total porosity, air space and container capacity of soil or soilless substrates, but the effects are variable. Total porosity of the substrates could be increased with increasing biochar rate (Méndez et al., 2015; Ruqin et al., 2015; Zhang et al., 2014). However, total porosity decreased with increasing addition of pelleted biochar (Dumroese et al., 2011). Substituting peat with 50% biochar (by vol.) made from green waste had no effect on total porosity and container capacity, and significantly decreased air space, however, the air space was still in the ideal range for container substrates, which is 15%-30% (Tian et al., 2012). Méndez et al. (2015) concluded that the addition of biochar produced from deinking sludge increased the total porosity and air space. Ruqin et al.

(2015) found there was no significant effect of biochar addition on air space. And Vaughn et al. (2013) also showed that the effects of biochar on air space, container capacity and total porosity were mixed and there was no specific trend, when mixing biochar with peat.

Biochar addition could increase soil or soilless substrate EC. Vaughn et al. (2013) showed that mixing 5, 10 and 15% (by vol.) pelletized wheat straw and hardwood biochars with soilless substrates containing peat moss and vermiculite increased the EC. Tian et al. (2012) also found that adding 50% (by vol.) biochar made from green waste to peat moss media significantly increased EC. And Ruqin et al. (2015) concluded that substrate EC increased with increased biochar rates. Aridisol and Alfisol amended with 10% (approximately equivalent to 100 t ha^{-1}) biochar showed increased EC too (Kelly et al., 2015). And sandy soil with 5 or 10 t ha^{-1} biochar corn stalk biochar also showed increased EC (Wang et al., 2016).

Biochar addition could change hydraulic properties of soil or soilless substrates, and this impact varies in individual conditions. Laird et al. (2010a) found that soil saturated hydraulic conductivity was not affected by biochar addition. However, Lei and Zhang (2013) and Asai et al. (2009) found that saturated hydraulic conductivities increased with biochar addition. Dumroese et al. (2011) indicated that application of 25% pelleted biochar with 75% peat (by vol.) had improved hydraulic conductivity. Liu et al. (2016b) observed that as the amount of biochar added to sandy soil increased, hydraulic conductivity decreased, and this depends on the biochar particle size. Biochar with finer particle size than sand had a more significant effect on decreasing hydraulic conductivity than the ones coarser than sand, because of increased tortuosity and reduced pore size. And when mixing biochar with similar particle size as sand, the results showed no effect on hydraulic conductivity (Liu et al., 2016b).

Biochar addition can also influence water holding capacity (WHC) of soil or soilless substrates, and the effects are variable. It was shown that biochar addition to soil could improve water holding capacity of soil (Asai et al., 2009), especially with biochars produced at higher pyrolysis temperatures (Lei and Zhang, 2013). Bruun et al. (2014) found that the amount of plant available water increased after the addition of straw biochar made from gasification to subsoil. Laird et al. (2010b) also concluded that Clarion soil (fine-loamy soil) with biochar produced from mixed hardwood [primarily oak (*Quercus spp.*) and hickory (*Carya spp.*)] held more water at gravity-drained equilibrium. Hansen et al. (2016) found that both biochar made from straw and wood gasification addition to sandy loam or coarse sandy soil could increase the plant available water content by 17-42%. Dumroese et al. (2011) found that a proper mixture of 25% pelleted biochar and 75% peat (by vol.) reserved more water than 100% peat substrates at low matric potentials. However, adding 47 t ha^{-1} acacia green waste biochar had no impact on available water content in sandy loam soil (Hardie et al., 2014). Wang et al. (2016) also confirmed that there was no difference between the water holding capacity of sandy soil with or without 5 or 10 t ha^{-1} corn stalk biochar, although 10 t ha^{-1} biochar and 20 t ha^{-1} compost applied together into sandy soil increased the water holding capacity. Streubel et al. (2011) showed that although switchgrass straw and softwood bark biochar amendment at the rate of 8 t ha^{-1}, 19.5 t ha^{-1}, and 39.0 t ha^{-1} increased WHC in silt loam soils, there was no significant difference in the Naff or Palouse silt loams amended with wood pellets biochar.

In general, biochar is effective in increasing the pH of soil or soilless substrates since pH of biochars used in most research is neutral to basic (Chan and Xu, 2009; O'Toole et al., 2013; Van Zwieten et al., 2010; Verheijen et al., 2010). A lot of research used biochar with high pH and showed that biochar can ameliorate soil acidity because of its alkaline nature (Chan et al., 2008; Ducey et al., 2015; Dumroese et al., 2011; Laird et al., 2010b; Streubel et al., 2011), and of its acidity buffering due to the negative charge on the surface of biochar (Initiative, 2010). Many of the positive plant responses after biochar addition are found in those studies conducted in acidic soils. Adding alkaline biochar raises the pH of the soils and leads to a more suitable soil environment for plants growth. However, pH of biochar depends on the nature of the feedstock and the temperatures during biochar production, and could also be acidic. The lower the temperature of production condition is, the lower the pH of biochar is. The pH of oak wood biochar is 3.16 when produced at 60°C and 5.18 at 350°C (Lehmann et al., 2011). Khodadad et al. (2011) also showed pH of biochar made from pyrolysis of oak and grass at 250°C was 3.5. Lima et al. (2009) showed that the pH was around 5.9 for biochar made from pecan shell at 350 °C and switchgrass at 250 °C.

Biochar addition could significantly increase CEC (Headlee et al., 2014; Van Zwieten et al., 2010) and affect nutrient availability (Zhang et al., 2016). Biochar-amended soil had higher CEC up to 20% compared to the control soil (Laird et al., 2010a). As the temperature of pyrolysis increase, CEC of biochar increases (Ashworth et al., 2014). Some forms of biochars can serve as a source of P and K, which lead to increased P and K availability in soil and improved soil fertility (Altland and Locke, 2012; Altland and Locke, 2013; Locke et al., 2013; Xu et al., 2016). Biochar addition in soil increased total nitrogen (up to 7%) and organic carbon (up to 69%), while reducing nutrient leaching, thus resulting in decreased need for fertilizer (Laird et al., 2010a). Altland and Lockel (2012) indicated that N could be bound to biochar and released more slowly. However, another study has found that available N and K were decreased after addition of green waste biochar to peat substrates (50% each) (Tian et al., 2012). When amending pelleted biochar to peat to compare five different growing media, Dumroese et al. (2011) found that as increasing percentage of pellets in the growing media, Fe, K, Na, P and B increased, while Al, Ca, Mg, Mn and S decreased. Xu et al. (2016) found that biochar may precipitate P and P availability in soil decreased when biochar and P fertilizer were applied together.

So, available nutrients in soils would depend on different kinds of biochars application and soil type.

Effects on the Microbial Communities

Ducey et al. (2015) demonstrated changes in microbial community composition along with a significant increase in Mehlich-1 extractable nutrient in response to biochar amendment. Other studies also found microbial activity increased after adding biochar to the soil (Biederman and Harpole, 2013; Rutigliano et al., 2014). Adding biochar causes increased pH, available water content, carbon availability and influx of nutrients as discussed above, thus stimulating microbial communities and increasing microbial biomass (Lehmann et al., 2011; Lei and Zhang, 2013; Steiner et al., 2007). Also, porous biochar with high surface area provides favorable shelter for microorganism's activity and growth (Khodadad et al., 2011).

Carbon Sequestration and Greenhouse Gas Reduction

Adding biochar mitigates greenhouse gas emission and has a beneficial effect for climate change. Agriculture produces large amounts of greenhouse gasses into the atmosphere, such as nitrous oxide and carbon dioxide (Change, 2007). Biochar addition in agriculture was found to reduce N_2O emissions (Jia et al., 2012; Spokas et al., 2009; Wang et al., 2012; Woolf et al., 2010). But the reason why biochar leads to N_2O reduction is still not well understood (Spokas et al., 2009). One possible explanation is that biochar addition may promote or provide a more favorable environment for abiotic reactions of biological species with nitrous oxide and oxygen (Avdeev et al., 2005). Biochar amendment in soil could also suppress net CH_4 oxidation (Spokas et al., 2009). Biochar can also be used to sequester the applied carbon in agricultural soil and mitigate CO_2 emissions (Woolf et al., 2010). Biochar addition in the soil helps to stabilize organic carbon in it, which is completed by decreasing the mineralization rates (Spokas et al., 2009).

Effects of Biochar on Plant Growth in Soil

Biochar has a lot of positive effects on physical, chemical and biological properties of soil, such as ameliorating soil acidity, increasing water and nutrient holding capacity and increasing microbial activity, which could have an indirect beneficial impact on plant growth. A meta-analysis conducted to quantify the effect of biochar on crop productivity showed that biochar amendments in soil have a statistically positive effect on plant yield (Jeffery et al., 2011). It was shown that adding 10 t ha^{-1} miscanthus biochar in soil increased the shoot and root biomass and rhizosphere zones of spring barley (Prendergast-Miller et al., 2014). Also, adding biochar from wood residue and rosewood at the rate of 8 t ha^{-1} in soil had the potential to improve grain yield (Asai et al., 2009). And a lot of the positive effects of biochar on plant growth were seen in acidic and neutral soils (Asai et al., 2009; Hansen et al., 2016; Liu et al., 2016a; O' Toole et al., 2013; Park et al., 2011;

Prendergast-Miller et al., 2014; Van Zwieten et al., 2010), where addition of biochar increased the soil pH. It was shown in the meta-analysis made by Jeffery et al. (2011) that there is a significant increase in crop yield after addition of biochar to acid and neutral soils and the more soil pH increases after biochar amendment, the more crop productivity increases.

Biochar has positive effects on plant growth in soil under stress conditions. It was reported that addition of biochar to compost promoted plant growth including root and shoot growth, stem diameter, biomass and yield of mung bean in a semi-arid area in China (Wang et al., 2016). And using 30% biochar (by vol.) to blend with sandy loam or quartz sand increased tomato plants drought resistance (Mulcahy et al., 2013). Also, biochar can decrease salinity stress. It can adsorb salt, and thus alleviates osmotic stress and promotes plant growth in saline soil (Drake et al., 2016; Xu et al., 2016). The absorbable capacity of biochar could reduce the activity of heavy metals and immobilize polycyclic aromatic hydrocarbons (PAHs) in soil, thus increasing agricultural production. This resulted in reduced phytotoxicity of heavy metals to plant growth (Park et al., 2011). Liu et al. (2016a) showed that plant biomass increased when adding biochar to the Cd-polluted soil. Cd content in rhizomes, petiole and leaves all decreased with 32% biochar addition (Liu et al., 2016a). Adding sewage sludge biochar into soil contaminated with PAHs reduced the bioaccumulation of PAHs from contaminated soil in lettuce and increased the lettuce biomass (Khan et al., 2013).

The impacts of biochar on plant growth in soil vary in relation to the biochar addition rate, soil condition, plant type and fertilizer application. The aboveground growth of tobacco was promoted by addition of 0.2%-1.0% biochar, while decreased by 5% biochar (Zhang et al., 2016). Mixing up to 2% biochar to coarse sandy subsoil increased the biomass of *Hordeum vulgare cv. Anakin*, while plant biomass decreased when using 4% biochar (Bruun et al., 2014). Kelly et al. (2015) found that as the amount of biochar applied increased from 25 to 100 t ha^{-1} to Aridisol from Colorado, the wheat shoot biomass decreased, but there is not significant difference found in the dry weight of wheat grown in Alfisol in Virginia. Hansen et al. (2016) showed that adding straw gasified biochar to coarse sand increased *Hordeum vulgare L.* growth under both water regimes (70% and 30% of the water-holding capacity), whereas there was no effect on plant growth in sandy loam. Asai et al. (2009) found that adding 8 t ha^{-1} biochar in two sites in Houay-Khot and Long-Or increased grain yield, while no significant difference was found in other sites (Seng-Oudom, Somusa-Nuck and Long-Sang), which all have different soil chemical and physical properties. Van Zwieten et al. (2010) demonstrated that application of 10 t ha^{-1} biochar with fertilizer resulted in increased soybean and radish biomass in the Ferrosol and increased soybean and decreased wheat and radish biomass in the loamy Calcarosol. The biomass yield of perennial rye grass (*Lolium perenne L.*) was not affected by kiln-produced wheat-straw biochar addition at a rate of 17 t ha^{-1} or 54 t ha^{-1} in sandy loam with 192, 240 and 288 kg N ha^{-1} but was slightly decreased at 144 kg N ha^{-1} (O'Toole et al., 2013). Asai et al. (2009) showed that grain yield increased with nitrogen fertilizer and 4 and 8 t ha^{-1} biochar addition, while grain yield was not affected by nitrogen fertilizer and 16t ha^{-1} biochar addition.

Effects of Biochar on Plant Growth in Soilless Substrates

Recently, there is an increasing amount of research in biochar on container-grown plant growth, which shows the potential as replacement for commonly used soilless substrates. However, the effect of biochar in soilless substrates is variable, which depends on a lot of factors. There are distinct interactions between biochar and different substrates components. Different biochars used, biochar percentage and fertilization status can also contribute to different results. Besides, plants' responses to biochar also vary. Many mechanisms of biochar are not fully understood.

A lot of research has shown that mixing biochar in soilless substrates could have positive effects on plant growth. Tian et al. (2012) found that mixing biochar made from green waste with peat (50% each, by vol.) increased total biomass and leaf surface of *Calathea rotundifola cv. Fasciata* when compared to that of peat substrates alone, because of more nutrient release from biochar. Replacing 10% (by vol.) of peat to sewage sludge biochar showed enhanced lettuce biomass production by 184%-270% when compared to

100% peat-based substrates (Méndez et al., 2017). Addition of biochar produced from pruning waste at 300 °C and 500 °C in peat substrates at the ratio of 50% and 75% (by vol.) increased the lettuce biomass when compared to those in peat alone (Nieto et al., 2016). Mixing 20% or 35% (w/w) biochar made from coir with 0.5% or 0.7% humic acid in composted green waste medium showed increased biomass of *Calathea insignis* when compared to that grown in the composted green waste medium without biochar and humic acid amendments (Zhang et al., 2014). Some forms of biochars can result in increased P and K availability and support plant growth with less fertilizer (Altland and Locke, 2013).

Biochars may also have no or negative effects on plants. Biochar made from tomato crop green waste had no effect on tomato plant growth, fruit number and fruit yield when applied in sawdust soilless substrates at the ratio of 0, 25, 50, 75 and 100 (w/w) (Dunlop et al., 2015). Another study tested the effects of mixing three different acidified biochars (wood pellet biochar, pelletized wheat straw biochar and pennycress presscake biochar) with potato anaerobic digestate at the ratio of 1:1 (v:v). It showed that fresh weight and dry weight of tomato plants in mixes with wood pelletized biochar increased, while tomatoes in mixes with wheat straw biochar showed no significant difference and the fresh weight of tomatoes in mixes with pennycress presscake biochar decreased, when compared to the peat vermiculite control (Vaughn et al., 2015a). Another study showed that straw gasification biochar has the potential to increase both roots and shoot growth in coarse sandy soil, while wood gasification biochar have no effect on it (Hansen et al., 2016).

The impact of biochar on plant growth differs in different plants. Kadota and Niimi (2004) investigated that adding 10% or 30% (by vol.) biochar to peat, vermiculite, soil and sand substrates caused enhanced shoot growth for zinnia but no positive effects in marigold or scarlet sage (Kadota and Niimi, 2004). Vaughn et al. (2013) concluded that height of tomato plants was all increased after replacing peat moss with 5%-10% (by vol.) pelletized biochar, but the dry weights were almost the same when compared to the peat: vermiculite substrates. For marigold, plant height was also increased but not significantly. Graber et al. (2010) tested the effects of mixing three ratios of citrus wood biochar (1%, 3% or 5% by weight) with commercial soilless substrates (a mixture of 70% coconut fiber and 30% tuff by vol.) on the growth of pepper (*Capsicum annuum L.*) and tomato (*Lycopersicum esculentum Mill.*), and the results showed increased leaf area, canopy dry weight and yields of buds, flowers and fruit of pepper and increased plant height and leaf size of tomato plants, but no significant difference on yields of flower and fruit in biochar mixes when compared to the commercial soilless substrates (Graber et al., 2010). In addition, another research also showed that mixing potato anaerobic digestate with acidified wood pellet biochar (1:1, by vol.) lead to higher fresh and dry weight of tomatoes than the peat: vermiculite control, however it resulted in lower fresh and dry weight of marigold plant than the control (Vaughn et al., 2015a)

The percentage of biochar in substrates plays an important role on its impact on plant growth. As biochar percentage increased to 80% and 100% (by vol.) when mixed with bark, the growth index of tomato plants in these mixes decreased, while the tomato plants in mixes with 40% and 60% biochar showed slightly higher growth index than the control (Yu et al., 2012). The aboveground dry weight of pansy (*Viola* var. *hybrida*) increased with 2.5% (w/w) *Eucalyptus saligna* wood chip biochar mixed with growing medium containing pine bark, coir, clinker ash and coarse sand, while decreased when mixing 10% (w/w) biochar, compared to the control (Housley et al., 2015). Ruqin et al. (2015) investigated the effects of mixed wheat straw biochar with super absorbent polymer on the substrates containing spent pig litter compost, vermiculite, perlite and peat. The germination rate of water spinach (*Ipomoea aquatica*) decreased as the percentage of biochar added increased. However, there was not significant difference between different percentage of biochar (from 0 to 160 mL L^{-1}) applied on the germination rate when biochar was applied together with super absorbent polymer (Ruqin et al., 2015). Conversa et al. (2015) also showed that mixing peat with biochar at the ratio of 70:30 (by vol.) with slow released fertilizer at a rate of 140 and 210 mg L^{-1} did not affect the dry weight of leaves and total aerial parts of *Pelargonium* growth, while mixing peat with biochar at the ratio of 30:70 (by vol.) with high rate of slow release fertilizer (210 mg L^{-1}) decreased

Pelargonium plant growth and flowering traits (Conversa et al., 2015).

The other substrate components we use to mix with biochar could affect plant growth. Yu et al. (2012) demonstrated that when biochar was mixed with bark at the ratio of 20%, 40%, 60%, 80% or 100% ratio (by vol.), the growth index of chrysanthemum (*Chrysanthemum nankingense*) grown in these mixes were similar to that in 100% bark mixes, while the growth index of chrysanthemum grown in mixes with 20%, 80% or 100% biochar with the rest being Sunshine #1 Mix was higher than that in 100% Sunshine #1 Mix. Also, Gu et al. (2013) showed that gomphrena (*Gomphrena* 'Firework') grown in pinewood biochar mixed with the commercial peat-based substrates at the ratio of 5%, 10%, 15%, 20%, 25% and 30% (by vol.) had greater width, higher fresh weight and dry weight than those grown in biochar mixed with bark substrates (Gu et al., 2013). Méndez et al., (2015) demonstrated that the total biomass, shoot and root weight of lettuce were higher in biochar from deinking sludge with peat (50:50 by vol.) than those in biochar mixed with coir (50:50 by vol.).

Potentially Toxic Contaminants in Biochar

Biochar may contain potentially toxic substances, such as heavy metals and organic contaminants (polycyclic aromatic hydrocarbons (PAH) and dioxin). The addition of biochar with high content of these contaminants is a concern. When applying biochar in soil or soilless substrates, the toxic substance could be detrimental when absorbed by plants and leaching to groundwater and may have noxious effects on soil function and soil organisms (Kołtowski and Oleszczuk, 2015).

Biochar could contain heavy metals, from contaminated feedstocks used to produce biochar. Lievens et al. (2009) found that the biochar made from pyrolysis of heavy metal (Cd, Cu, Pb, Zn)-contaminated willow leaves and branches at 349.85°C still contained large portion of the heavy metals from the feedstock (Lievens et al., 2009). Jin et al (2016) found that the total content of Cu, Zn, Pb, Cr, Mn and Ni in biochar produced from sludge increased as the temperature of pyrolysis increased from 400 to 600°C, however, most of the heavy metals were in their oxidized and residual forms, which had low bioavailability and risks (Jin et al., 2016). Buss et al. (2016) investigated 19 biochars produced from marginal biomass containing contaminants. It was shown that the percentage of potential toxic elements was the highest in biochar produced at the highest temperature (750°C) in the research, but there was not significant difference on the amount of the available heavy metal such as Cu, Cr, Ni and Zn. When applying 5% of biochar in sand, only 5 of 19 biochars showed suppressive effects on plant growth. And the reason of growth suppression was because of the high K and pH of the biochars, and not the heavy metals.

Other potential toxic compounds found in biochars are polycyclic aromatic hydrocarbons (PAHs). Most of PAHs are formed at high temperatures, especially over 750°C (Shackley et al., 2010). And there is also evidence that a small quantity of PAHs could be formed between 400 and 600°C (McGrath et al., 2001; Shackley et al., 2010). In contrast, it was also found that the PAH concentration in biochar produced from pine wood at 900°C was significantly lower than the ones produced at lower temperatures from 250 to 500°C, and the PAH concentration in biochar made from switchgrass at 900°C was lower than that produced from 250 to 700 °C (Hale et al., 2012). The content of PAHs in biochar depends on the feedstocks and the conditions of the biochar production. Wiedner et al. (2013) investigated the PAH amount of various biochars made from different production conditions and feedstocks, which were biochars made from gasification of poplar, wheat straw, woodchips, sorghum and olive and from pyrolysis of draff (the waste product from the beer production process after separating liquid malt) and miscanthus. The results showed that although biochars made from different feedstock and production conditions had different percentages of PAH compounds with diverse structures and total PAH amount, all investigated biochars contained low content of PAH (below 1.7 mg kg^{-1}) except biochar (PAH 15 mg kg^{-1}) made from woodchip gasification. It is still under the threshold values recommended by International Biochar Initiative (IBI; between 6 and 20 mg kg^{-1}) (Wiedner et al., 2013).

Dioxins could be formed in biochar in certain conditions. Dioxins are persistent organic pollutants, including polychlorinated dibenzo dioxins and polychlorinated dibenzo furans (Wilson and Reed, 2012). Dioxins could be formed only in biochar made from feedstock containing chlorine under specific conditions (Shackley et al., 2010). Feedstock sources, such as straws, grasses, halogenated plastics and food waste containing sodium chloride, can be sources of chlorine and dioxin formation (Shackley et al., 2010; Wilson and Reed, 2012). Dioxins could be produced during two pathways, "precursor" pathway and "de novo" pathway (Garcia-Perez and Metcalf, 2008). The precursor pathway begins with the synthesis of dioxin precursors, such as polychlorophenols and polychlorobenzenes, from feedstock containing chlorine at temperatures between 300 and 600°C (Everaert and Baeyens, 2002). The de novo pathway occurs in a catalytic reaction with oxygen and carbon at temperatures between 200 and 400 °C (Everaert and Baeyens, 2002; Wilson and Reed, 2012). Hale et al. (2012) investigated the biochars produced at 250 to 900°C and found that total dioxin concentrations were very low (up to 92 pg g^{-1}) and the bioavailable concentrations were below detection limit (Hale et al., 2012). Wiedner et al. (2013) found that the dioxins in four biochars produced from gasification of poplar and olive residues and pyrolysis of draff and wood chips and two other hydrochars made from leftover food and sewage sludge were all under the limit of detection except the one made from sewage sludge (14.2 ng kg^{-1}). And the recommended threshold values given by IBI and European Biochar Certificate (EBC) are below 9 (IBI) or 20 (EBC) ng kg^{-1} (Wiedner et al., 2013). Therefore, caution is needed when selecting feedstock for biochar production.

CHAPTER II

EFFECTS OF BIOCHAR AND VERMICOMPOST ON CONTAINER-GROWN BASIL AND TOMATO PLANTS[*]

Introduction

Biochar (BC), attracting a lot of interest in recent years for its use in agriculture, can be used as a replacement for commonly used container substrates (Dumroese et al., 2011; Northup, 2013; Vaughn et al., 2015b; Vaughn et al., 2013; Yu et al., 2012). Biochar refers to the carbon-rich material derived from biomass (Hansen et al., 2016; Lehmann, 2007). Biochars could be made from different feedstocks, such as green waste (Buss et al., 2016; Tian et al., 2012), wheat straw (Vaughn et al., 2013; Xu et al., 2016), wood (Hansen et al., 2016; Spokas et al., 2009; Vaughn et al., 2013), and rice hull (Locke et al., 2013). Biochar is renewable and fast to generate (Yu et al., 2012), compared to peat moss.

Biochar incorporation in container substrates has a lot of benefits including increased water holding capacity (Dumroese et al., 2011) and pH (Dumroese et al., 2011; Vaughn et al., 2013) and improved plant growth (Vaughn et al., 2013). Tian et al. (2012) found

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[*] Part of this chapter is reprinted with permission from "Effects of Biochar and Vermicompost on Container-grown Basil (*Ocimum basilicum*) and Tomato (*Solanum lycopersicum* 'Roma')" by Lan Huang, Xiuli Liu and Mengmeng Gu, 2017. TNLA Green Magazine November/ December issue: 27-28.

that the incorporation of the BC made from green waste in peat substrates (50% each, by vol.) increased the total biomass and leaf area of *Calathea rotundifola* cv. *Fasciata* when compared to peat substrates alone, because of improved substrate properties, which lead to increased nutrient retention after biochar incorporation. Replacing 10% (by vol.) peat with sewage sludge BC showed enhanced lettuce biomass production by 184%-270% when compared to 100% peat-based substrates due to increased N, P, and K concentrations and microbial activities (Méndez et al., 2017).

The effects of BC on container substrates depend on many factors such as feedstock sources, BC production conditions, the percentage of BC applied, other substrate components mixed with BC, and the plant type. The straw BC has higher pH, exchangeable cations, and potassium (K) content compared to the wood BC (Vaughn et al., 2013). The BCs pyrolyzed at temperatures lower than 400 °C could retain more P while as temperature increased, more water soluble and organic P are converted to unavailable P (Xu et al., 2016). Higher temperatures can produce BCs with higher pH (Butnan et al., 2015). The physical and chemical properties of BCs caused by different feedstocks and production conditions would affect plant growth after BC incorporation in container substrate. Kadota and Niimi (2004) concluded that adding 10% or 30% (by vol.) BC to peat, vermiculite, soil and sand substrates resulted in enhanced shoot growth for zinnia (*Zinnia linearis*) but no positive effects on marigold (*Tagetes patula*) or scarlet sage

(*Salvia splendens*). Housley et al. (2015) pointed that different percentage of BC mixed with substrates components could lead to diverse results. They found that the aboveground dry weight of pansy (*Viola var. hybrida*) increased with 2.5% (w/w) *Eucalyptus saligna* wood chip BC mixed with growing medium containing pine bark, coir, clinker ash and coarse sand, but decreased with 10% (w/w) BC, compared to the control. Yu et al. (2012) showed that the growth index of tomato plants in pine bark mixes with 80% and 100% (by vol.) BC incorporation decreased, while the tomato plants in pine bark mixes with 40% and 60% BC incorporation showed slightly higher growth index than the control. Yu et al. (2012) also found that there was no significant difference on tomato plant dry weight between 40% and 60% BC mixed with Sunshine # 1 Mix (Sun Gro® Horticulture, Agawam, MA, USA) and the control. Similarly, Méndez et al. (2015) demonstrated that the total biomass and the shoot and root dry weight of lettuce were higher in the deinking sludge BC mixed with peat substrates (50/50 by vol.) than those in the BC mixed with coir substrates (50/50 by vol.). All BCs are not equal when being incorporated as container substrate.

Vermicomposts (VC) are the end products of using earthworms to break down organic wastes, such as sewage sludge (Mitchell et al., 1980), animal waste (Chan and Griffiths, 1988; Edwards, 1985; Hartenstein and Bisesi, 1989), and crop residues (Manna et al., 2005). Vermicomposts are finely textured and rich in nutrients (Atiyeh et al., 2000; Sinha et al., 2010), and have good water holding capacity (Edwards and Burrows, 1988). Atiyeh et al. (2000) concluded that VC addition in container substrates could enhance plant growth. When pine bark was amended with 20% (by vol.) VC, VC could provide adequate nutrients (P, Ca, Mg, S, Fe, Mn, Zn, Cu and B) needed for the container-grown plants, and *Hibiscus moscheutos* 'Luna Blush' plants grown in the containers with VC showed improved plant dry weight and growth (McGinnis et al., 2009). Vermicompost could be mixed with coir at a ratio of 2:1 (w/w) as container substrates to grow Swiss chard (*Beta vulgaris*), with increased plant height and fresh weight (Abbey et al., 2012).

The majority of the past research has tested and reported the impact of compost with commonly used container substrates or using BC and compost as soil amendments. A limited amount of research exists on using combinations of BC with composts, especially VCs, as container substrates. The purpose of this experiment was to test the potential of the BC and VC mixes as replacements for commercial peat-based container substrates. The specific objectives were to 1) investigate the physical and chemical properties of the BC and VC mixes; and 2) compare impacts of different rates of BC with VC on container-grown basil *(Ocimum basilicum*) and tomato (*Solanum lycopersicum* 'Roma') plants.

Materials and Methods

Plant materials and substrates. Tomato (*Solanum lycopersicum* 'Roma') (Morgan County Seeds, Barnett, MO, USA) and basil (*Ocimum basilicum*) seeds (Johnny's

Selected Seeds, Winslow, ME, USA) were sown in commercial propagation mix (Propagation mix; Sun Gro® Horticulture, Agawam, MA, USA) in plug trays on 28 Oct 2016. One tomato seed and four basil seeds was sown per cell (hexagon with side length of 2.6 cm; height: 4.2cm; volume: 20ml), respectively. Uniform basil and tomato seedlings were selected and transplanted into the experimental substrates in 6-inch azalea pots (depth: 10.8cm; top diameter: 15.5cm; bottom diameter: 11.3cm; volume: 1,330ml) on 16 Nov 2016 after true leaves emerged. Each container contained one tomato seedling and four basil seedlings. Sixteen biochar: vermicompost (BC:VC) substrates were formulated by mixing four rates of BC (20%, 40%, 60% and 80%, by vol.; Proton Power, Inc., Lenior City, TN, USA) with four rates of VC (5%, 10%, 15% and 20%, by vol.; Pachamama earthworm castings; Lady Bug Brand, Conroe, TX, USA) (Fig. 2.1). The commercial substrate (BM 7; Berger, Saint-Modeste, QC, Canada) made up the rest of the volume when the BC and VC did not add up to 100%. The commercial substrates used in this research consisted of 55% coarse peat moss, 35% pine bark and 10% horticultural perlite. The pH of the commercial substrates, BC and VC were measured using a handheld pH-EC meter (HI 98129, Hanna Instruments, Woonsocket, RI, USA) and electrical conductivity (EC) of the commercial substrates, BC and VC were measured using the Bluelab Combo Meter (Bluelab Corporation Limited, Tauranga, New Zealand) according to the pour-through extraction method (LeBude and Bilderback, 2009). The pH of the commercial substrate was 7.1. Electrical conductivity (EC) was around 1.3 dS m^{-1}. The commercial substrate was used as the control (Fig. 2.1). The BC used in this experiment was made from fast pyrolysis of mixed hardwood. The pH of the BC is 11.18 and the EC is 2.0 dS m^{-1} measured using pour-through extraction method (pH=10.5, soluble salts=4.6 dS m^{-1} measured by North Carolina State University Horticultural Substrates Laboratory, method unknown). The pH of the VC is 4.8. The EC of the VC is 6.7 dS m^{-1}. The total porosity (TP), container capacity (CC), air space (AS) and bulk density (BD) of the BC is 84.7%, 60.3%, 24.4% and 0.15 g cm^{-3}, respectively. Particle size distribution of the BC was determined by passing 40 g BC through 2.8, 2, 1, 0.425 and 0.25 mm sieves. Weight on each sieve was measured to calculate the proportion of each size. Percentages of the BC particles ranging from more than 2.8 mm, 2.0 mm to 2.8 mm, 1.0 mm to 2.0 mm, 0.425 mm to 1.0 mm, 0.25 mm to 0.425 mm, and less than 0.25 mm in diameter were 47.9%, 19.4%, 19.4%, 9.1%, 2.0% and 2.2% (w/w), respectively.

Six replications of the seventeen treatments were arranged in completely randomized blocks in the greenhouse located on Texas A&M University campus, College Station, TX. During the experimental period, the temperature, humidity and dew point in the greenhouse were monitored using Watchdog (Spectrum Technologies Inc., Paxinos, PA). The average greenhouse temperature, relative humidity and dew point are 20.5 °C (68.8 °F), 76.0% and 15.4 °C (59.7 °F), respectively. The basil plants were irrigated with 200 mg N L^{-1} (20N-4.3P-16.6K) Peters® Professional (Everris NA Inc., Dublin, OH, USA) nutrient solution. The total N in Peters® Professional contains 8.1% ammoniacal N and 11.9% nitrate N. The tomato plants were irrigated with 200 mg N L^{-1} (20N-4.3P-16.6K) Peters® Professional nutrient solution from 0 to 3 weeks after transplanting (WAT) and changed to 300 mg N L^{-1} from 4 WAT.

Substrate physical properties, leachate pH and electrical conductivity. Four replications of each substrate were tested to determine physical properties including BD, TP, AS and CC of the seventeen substrates using the North Carolina State University Horticultural Substrates Laboratory porometers (Fonteno et al., 1995). The substrate leachate pH and EC were measured at 0, 2, 4, 6 and 9 WAT using a handheld pH-EC meter (HI 98129, Hanna Instrument, Woonsocket, RI, USA) according to the pour-through extraction method (LeBude and Bilderback, 2009). The upper limit of the EC meter is 3,999 µS cm^{-1}. The value of 3,999 µS cm^{-1} was used in data analysis when the EC readings exceeded the upper limit.

Plant growth and development. Plant growth index (GI) was measured at 0, 2, 4, 6 and 9 WAT. The height of the plant was measured from the medium surface to the highest point of the plants. The widest plant canopy width and its perpendicular width were measured. The plant GI was determined by the following formula: GI= (plant height/2+ (plant width 1+plant width 2))/4. The leaf greenness, which was abbreviated as SPAD

(Soil Plant Analysis Development), was measured at 2, 4, 6 and 9 WAT using a portable SPAD 502 Plus Chlorophyll Meter (Spectrum Technologies, Inc., Plainfield, IL, USA). Plant leaves were too small for measurement at 0 WAT. Leaf greenness of each plant was determined using the average of readings from three mature leaves.

At the end of 9 WAT, plants were harvested to measure dry weight (DW). For the tomato plants, the stems, leaves, root and combined fruits and flowers were harvested separately. For the basil plants, the shoot and root were harvested separately. All the plant parts were oven-dried at 80 °C to constant weight before the DW measurements. The total plant DW was calculated by adding DW of all parts of the plants.

Statistical analysis. The experiment was set up in a completely randomized block design with the type of substrate being the main factor. Data were analyzed with one-way analysis of variance (ANOVA) using JMP Statistical Software (version Pro 12.2.0; SAS Institute, Cary, NC, USA) and means were separated using Dunnett's test when treatments were significant at $P<0.05$.

Results and Discussion

Physical properties of the container substrates. There was no significant difference on the total porosity (TP) between any biochar: vermicompost (BC:VC) mixes (except 80BC:10VC) and the control (Fig. 2.2A). The container capacity (CC) of the BC (20% or 40%) and VC (5%, 10% or 15%) mixes (Treatment 1, 2, 5, 6, 9 and 10) was similar to the control (Fig. 2.2B). Mixes with 80% BC or 20% VC (Treatment 4, 8, 12, 13, 14, 15 and 16) had lower CC when compared to the control. The air space (AS) of 60BC:5VC, 60BC:20VC and all 80% BC mixes (Treatment 4, 8, 12 and 16) was higher than the control due to high incorporation rate of BC while the other mixes were similar to the control (Fig. 2.2C). Tian et al. (2012) reported that substituting peat with 50% BC (by vol.) made from green waste had no effect on TP and CC, while others found that the TP and CC of the substrates increased with the increasing BC rate (Méndez et al., 2015; Ruqin et al., 2015; Zhang et al., 2014). Méndez et al. (2015) concluded that the addition of 50% (by vol.) BC produced from deinking sludge to peat increased the AS. Nieto et al. (2016) also found that the incorporation of 75% or 50% (by vol.) pruning waste BC produced at 500 °C to peat increased the AS compared to the peat substrates. However, Méndez et al. (2017) showed that the low incorporation of 10% (by vol.) sewage sludge BC to peat showed similar AS compared to the peat substrates.

The bulk density (BD) of the mixes with 15% VC (Treatment 9, 10, 11 and 12) were significantly higher than the control (Fig. 2.2D). The BD of 40BC:10VC, 60BC:10VC and 60BC:20VC was also higher than the control. The increased BD could be due to the high BD of VC (0.38 g cm^{-3}) and BC (0.15 g cm^{-3}) compared to the control (0.10 g cm^{-3}). The BD of the other mixes was similar to that of the control. Research has shown that many BCs have higher BD than commonly used substrate components, such as peat moss

(Dumroese et al., 2011; Tian et al., 2012; Vaughn et al., 2015a; Vaughn et al., 2013) and vermiculite (Vaughn et al., 2015a; Vaughn et al., 2013) and using the BCs to replace certain percentage of commonly used substrates could increase the BD (Bilderback et al., 2005; Dumroese et al., 2011; Tian et al., 2012; Vaughn et al., 2015a; Vaughn et al., 2013).

The effects of BC on container physical properties are variable, contributed by diverse properties of BC. All the TPs and BDs of the BC:VC mixes in this experiment were in the acceptable ranges to grow plants in containers, when compared to the optimal physical properties (50% to 80% for TP and less than 0.4 g cm^{-3} for BD) indicated by Abad et al. (2001) and Méndez et al. (2015). Mixes with 80% BC or those with 20% VC all had CC below the ideal range (60% to 100%) (Méndez et al., 2015).

Substrate leachate pH and electrical conductivity. The substrates leachate pH in all BC:VC mixes were higher than that of the control, except 20BC:5VC, 20BC:10VC, and 20BC:15VC with tomato plants at 9 WAT (Fig. 2.3). The increased pH was due to the high pH of the BC (11.18) used in this experiment. The liming effect of BC was found in a lot of research, reflected as increased pH after the BC incorporation (Chan et al., 2008; Ducey et al., 2015; Dumroese et al., 2011; Steiner and Harttung, 2014; Streubel et al., 2011). The reason of the similar pH of mixes of 20BC:5VC, 20BC:10VC, and 20BC:15VC with tomato plants at 9 WAT with the control could be due to the low percentage of the BC incorporation rate and the relative large amount of nutrient solution applied to the tomato plants for nine weeks 'washing down' the substrate leachate pH.

At 0 WAT, substrate leachate EC in mixes of 20BC:5VC, 40BC:10VC, 60BC:15VC and 80BC:15VC were similar to the control (Fig. 2.4). For basil plants, at 2 WAT, EC of all the BC:VC mixes (except 60BC:5VC and 80BC:5VC) were similar to or higher than the control (Fig. 2.4A). At 4 WAT, EC of all the BC:VC mixes were similar to or lower than the control. At 6 and 9 WAT, EC of the 60% BC (Treatment 3, 6, 9 and 12) or 80% BC (Treatment 4, 8, 12 and 16) mixes were all lower than that of the control. And at 9 WAT, EC of mixes of 20BC:5VC, 20BC:10VC, 20BC:20VC and 40BC:20VC were similar to the control, while that of the other BC:VC mixes was lower than the control (Fig. 2.4A). For tomato plants, at 2 WAT, EC of the all BC:VC mixes (except 20BC:15VC, 40BC:15VC and 40BC:20VC) were similar to or lower than the control (Fig. 2.4B). At 4 or 6 WAT, EC of the 80% BC mixes (Treatment 4, 8, 12 and 16) were all lower than that of the control. And at 9 WAT, ECs of mixes of 80BC:5VC, 60BC:15VC and 80BC:15VC were lower than the control, with the others all similar to the control. The different results of the EC of the substrate leachates growing tomato and basil plants could be due to different concentrations and amount of N nutrient solutions applied after 4 WAT and also different nutrient uptake of tomato and basil plants. The general trend of the decreased EC caused by the increased high percentage of BC incorporation rates was similar to the results found by Steiner and Harttung (2014), who pointed out that ECs were lower in mixes with 50%, 75% or 100% BC incorporation than mixes with 25% BC incorporation in peat. The lower leachate EC of the substrate with higher percentage of BC may be caused by BC's effective moderating effect on extreme fluctuations of macronutrients (Altland and Locke, 2012). However, this result was not consistent with the results of Ruqin et al. (2015), who described that EC increased with increased incorporation rate of wheat straw BC from 20 to 160 mL L^{-1} to mixes with spent pig litter compost, vermiculite, perlite and peat (3:2:3:2 by vol.). Tian et al. (2012) also found that adding 50% (by vol.) BC made from green waste to peat moss media significantly increased EC. Effects of BC incorporation on EC could be different in every individual condition when different BCs were incorporated in the container substrates. EC of the BCs were strongly related to the functional groups of the BC (Li et al., 2013). Diverse functional groups, metal oxide precipitates and metals binding on the surface affect the EC of BCs (Lehmann et al., 2011) and thus affect the EC of the substrate leachate. Therefore, effects of BC on EC are closely related to the BC used.

Plant growth and development. For basil plants, there was no significant difference on the SPAD (Soil Plant Analysis Development) readings between the ones grown in BC:VC mixes and the control at 2, 4, 6 or 9 WAT (Fig. 2.5A). For tomato plants, the SPAD readings of the tomato plants in BC:VC mixes were similar to or higher than the ones grown in the control at 2, 4 and 6 WAT, while at 9 WAT leaf SPAD readings of tomato plants grown in 60BC:5VC, 20BC:10VC and 60BC:10VC and all the 80% BC mixes (Treatment 4, 8, 12 and 16) were lower than the control (Fig. 2.5B). Similar to this result, Nair and Carpenter (2016) also found that pepper (*Capsicum annuum*) leaf SPAD readings decreased with increasing BC incorporation rate in container. Research has showed that the leaf N content is related to SPAD reading (Gholizadeh et al., 2017), which indicated the decreased plant N uptake with the high percentage (80%) of BC incorporation in this study. According to Tian et al. (2012), the available N decreased after substituting 50% peat substrates with the BC. The decreased N could be caused by the BC's N-binding effect (Altland and Locke, 2012). And the reason for the decreased leaf SPAD readings of the tomato plants only shown at 9 WAT could be due to the deficiency of nutrient of the leaves caused by the strong nutrient sinks fruits and flowers at that stage since all the tomato plants had flowers and fruits at 9 WAT. However, possible N binding of BC did not decrease plant GI and DW of either tomato or basil plants in this research. The GIs of both basil and tomato plants grown in BC:VC mixes were similar to that in the control at 2, 4, 6 and 9 WAT (Data shown in Appendix A), except that the tomato plants grown in 20BC:15VC had higher GI at 9 WAT (Fig. 2.6). All basil plants grown in BC:VC mixes had higher or similar DW (the shoot, root and total DW) when compared to the control (Fig. 2.7). Similarly, all tomato plants grown in BC:VC mixes had higher or similar

DW (the combined flower and fruit, leaf, stem, root and total DW) compared to the control, except the tomato root DW of plants in 40BC:10VC (Fig. 2.8). These results were consistent with that of Yu et al. (2012) who reported that when substituting Sunshine #1 Mix with 60% or 80% pinewood BC (by vol.), the DW of basil plants grown in mixes with BC was similar to or higher than the control. Incorporation of biochar produced from pruning waste at 300 °C and 500 °C in peat substrates at the ratio of 50% and 75% (by vol.) increased the lettuce biomass when compared to that in peat alone (Nieto et al., 2016).

Conclusions

The biochar (BC; 20%, 40%, 60% or 80%, by vol.) and vermicompost (VC; 5%, 10%, 15% or 20%, by vol.) mixes had the potential to replace the commercial container substrates to grow basil and tomato plants. All the total porosity and bulk density of the BC:VC mixes were in the acceptable range to grow plants in container. Mixes with 80% BC could reduce leachate electrical conductivity of substrates growing tomato and basil, and leaf SPAD readings of tomato plants at 9 WAT. However, this did not cause suppressive effects on plant growth index (GI) or dry weight (DW). The GI and total DW of basil and tomato plants in BC:VC mixes were similar to or even higher than the ones in commercial substrates. Our results found difference on the substrate leachate pH between the commercial substrate and BC:VC mixes except for 20BC:5VC, 20BC:10VC and 20BC:15VC with tomato plants at 9 weeks after transplanting, which caused by the high pH of BC used in this research. Concerning of the cost of the alternative substrates, mixes with the lowest percentage of VC (5% VC; Treatment 1, 2, 3 and 4) in this experiment could be selected as the suitable one to grow plants. More BC incorporation percentages and other potential amendment candidates need to be tested for economical concern.

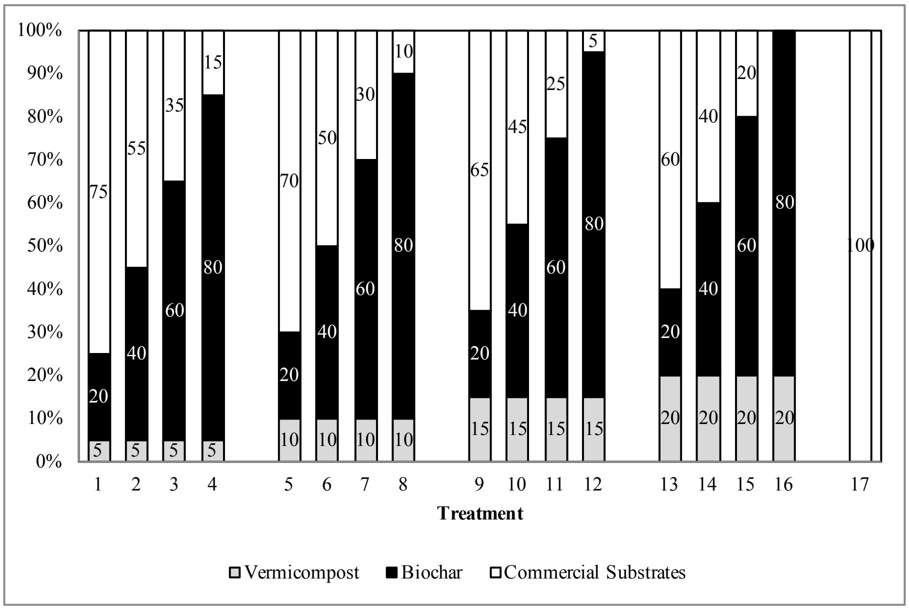


Fig. 2.1. Seventeen formulated substrates including mixes of biochar (20%, 40%, 60% or 80%, by vol.) with vermicompost (5%, 10%, 15% or 20%, by vol.) and the control.

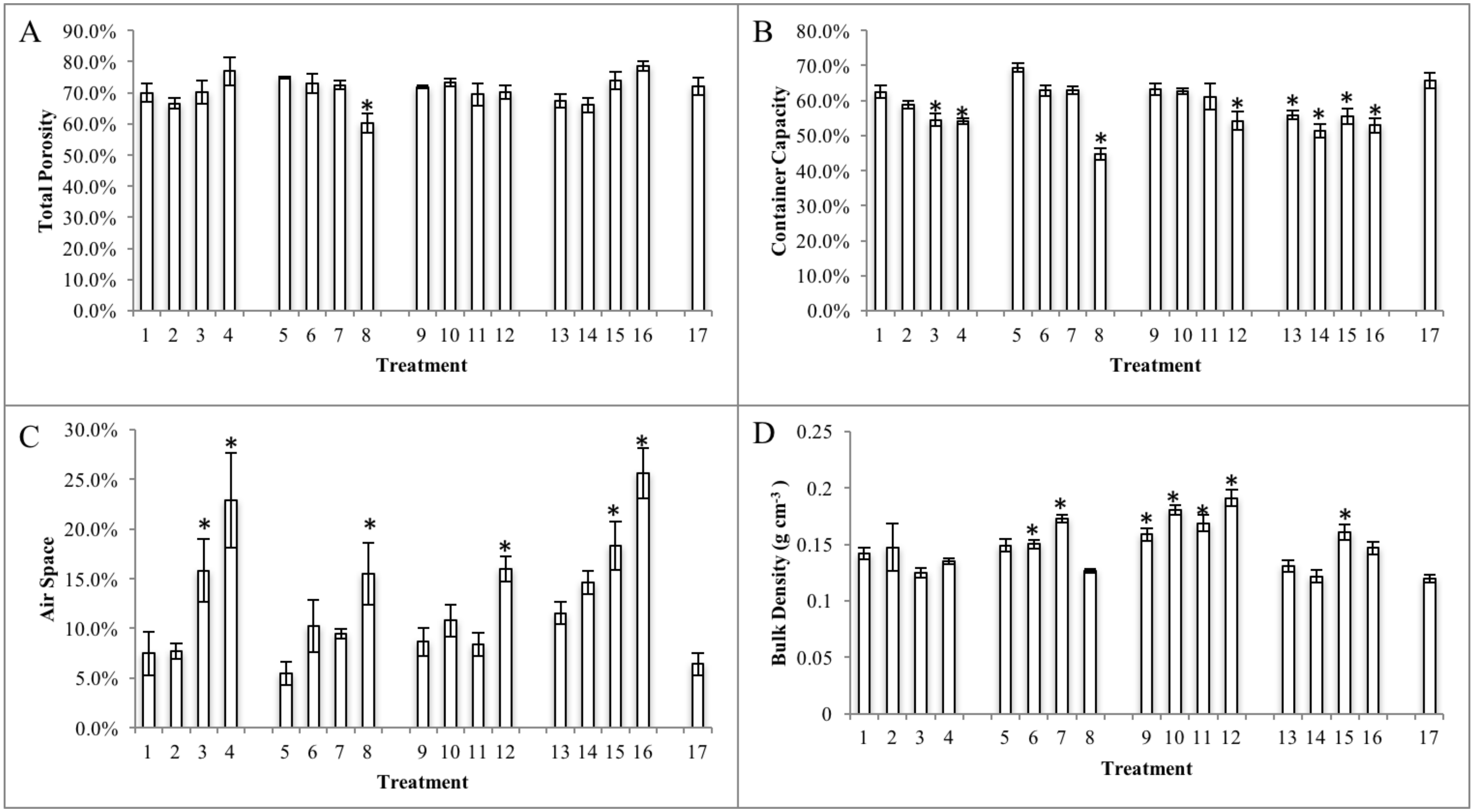


Fig. 2.2. Total porosity (A), container capacity (B), air space (C) and bulk density (D) (mean ± standard error) of the 17 different substrates including mixes of biochar (BC) with vermicompost (VC) and the control. The asterisks indicated significant difference from the control using Dunnett's test at *P*<0.05. Treatment 1-20BC:5VC, 2-40BC:5VC, 3-60BC:5VC, 4-80BC:5VC, 5-20BC:10VC, 6-40BC:10VC, 7-60BC:10VC, 8-80BC:10VC, 9-20BC:15VC, 10-40BC:15VC, 11-60BC:15VC, 12-80BC:15VC, 13-20BC:20VC, 14-40BC:20VC, 15-60BC:20VC, 16-80BC:20VC and 17-control.

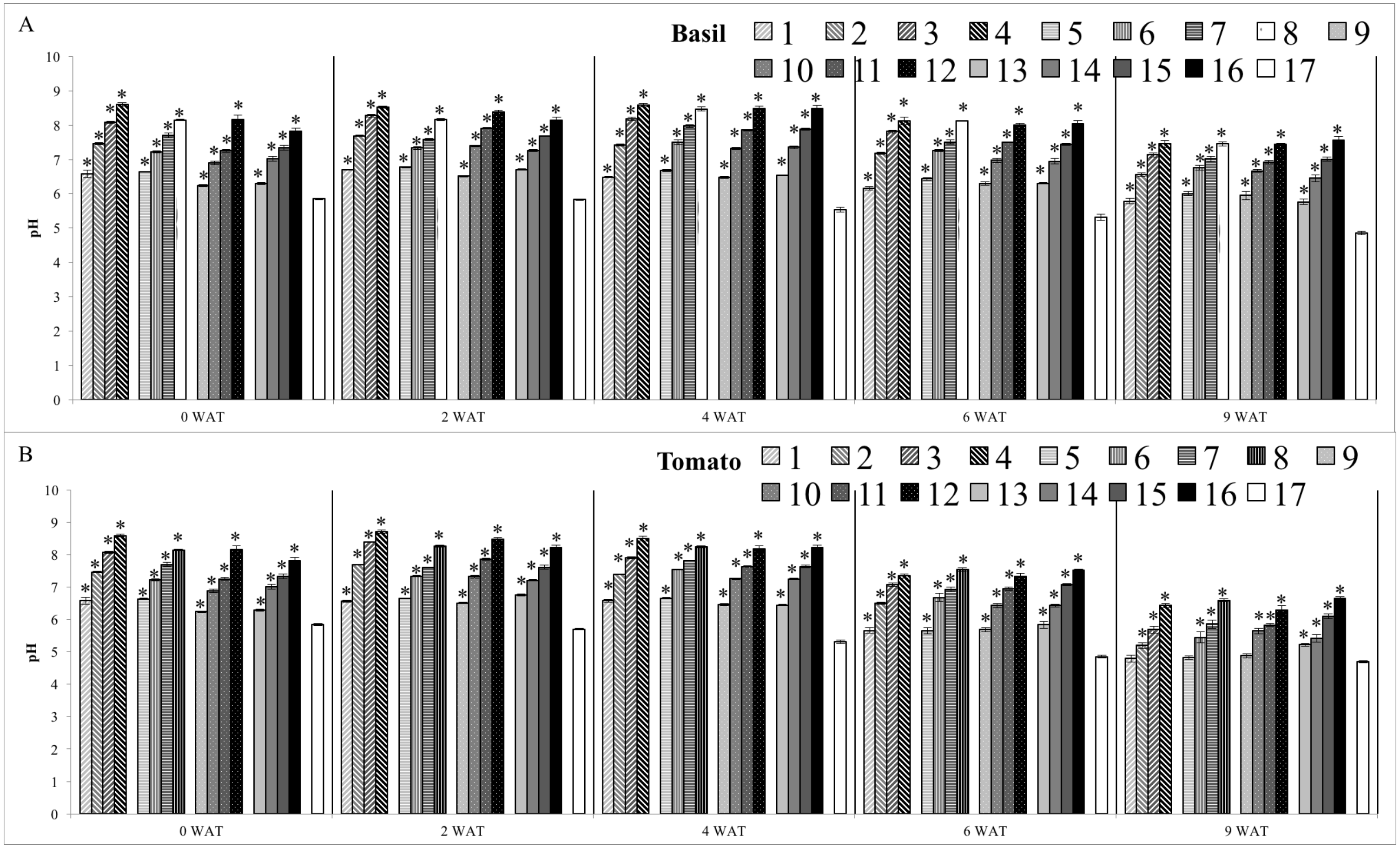


Fig. 2.3. Leachates pH (mean ± standard error) of mixes of biochar (BC) with vermicompost (VC) and the control growing basil (A) and tomato (B) at 0, 2, 4, 6 and 9 weeks after transplanting (WAT). The asterisks indicated significant difference from the control using Dunnett's test at *P*<0.05. Treatment 1-20BC:5VC, 2-40BC:5VC, 3-60BC:5VC, 4-80BC:5VC, 5-20BC:10VC, 6-40BC:10VC, 7-60BC:10VC, 8-80BC:10VC, 9-20BC:15VC, 10-40BC:15VC, 11-60BC:15VC, 12-80BC:15VC, 13-20BC:20VC, 14-40BC:20VC, 15-60BC:20VC, 16-80BC:20VC and 17-control.

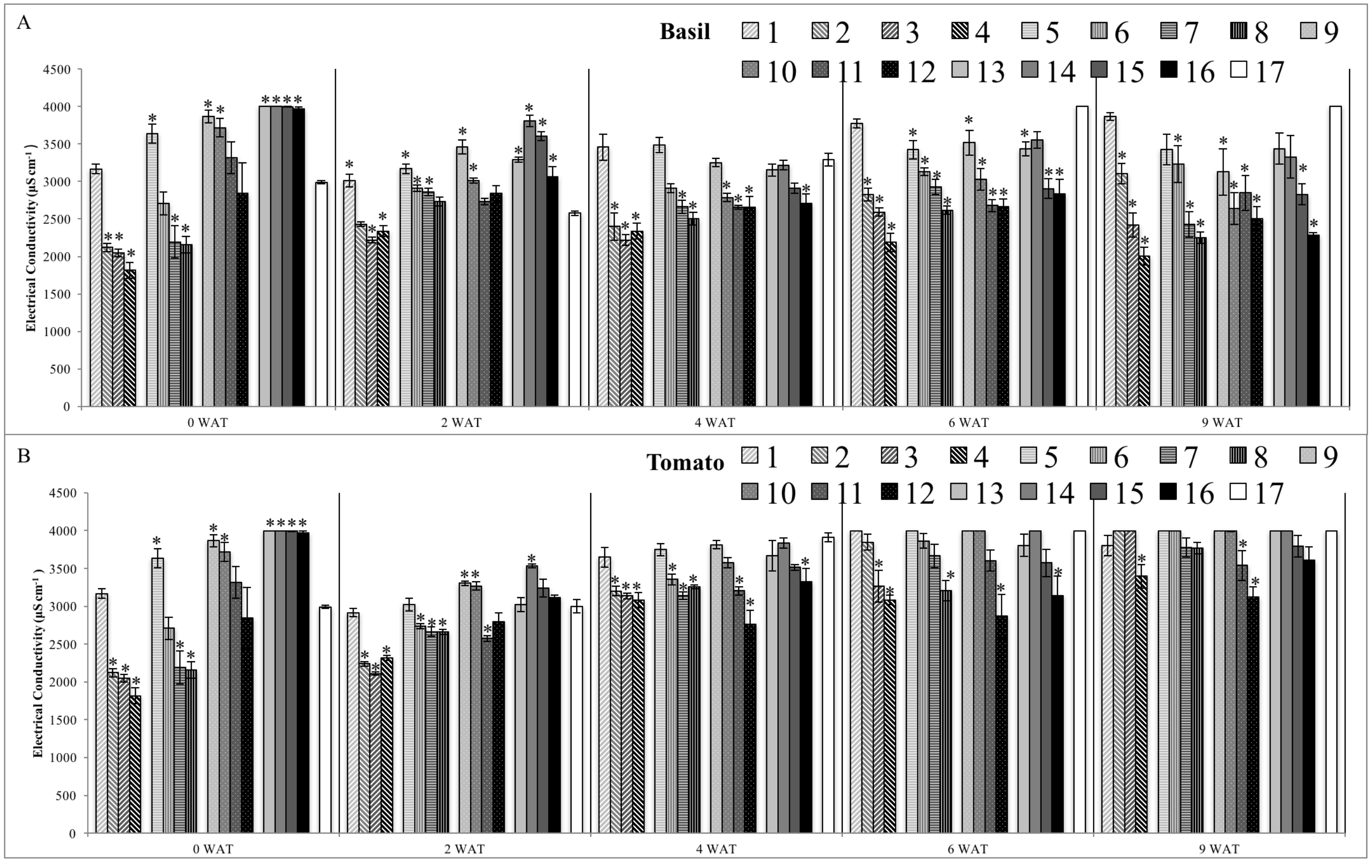


Fig. 2.4. Leachates electrical conductivity (EC) (mean ± standard error) of mixes of biochar (BC) with vermicompost (VC) and the control growing basil (A) and tomato (B) at 0, 2, 4, 6 and 9 weeks after transplanting (WAT). The upper limit of the EC meter 3,999 µS cm^{-1} was used in data analysis when the EC readings exceeded the upper limit. The asterisks indicated significant difference from the control using Dunnett's test at *P*<0.05. Treatment 1-20BC:5VC, 2-40BC:5VC, 3-60BC:5VC, 4-80BC:5VC, 5-20BC:10VC, 6-40BC:10VC, 7-60BC:10VC, 8-80BC:10VC, 9-20BC:15VC, 10-40BC:15VC, 11-60BC:15VC, 12-80BC:15VC, 13-20BC:20VC, 14-40BC:20VC, 15-60BC:20VC, 16-80BC:20VC and 17-control.

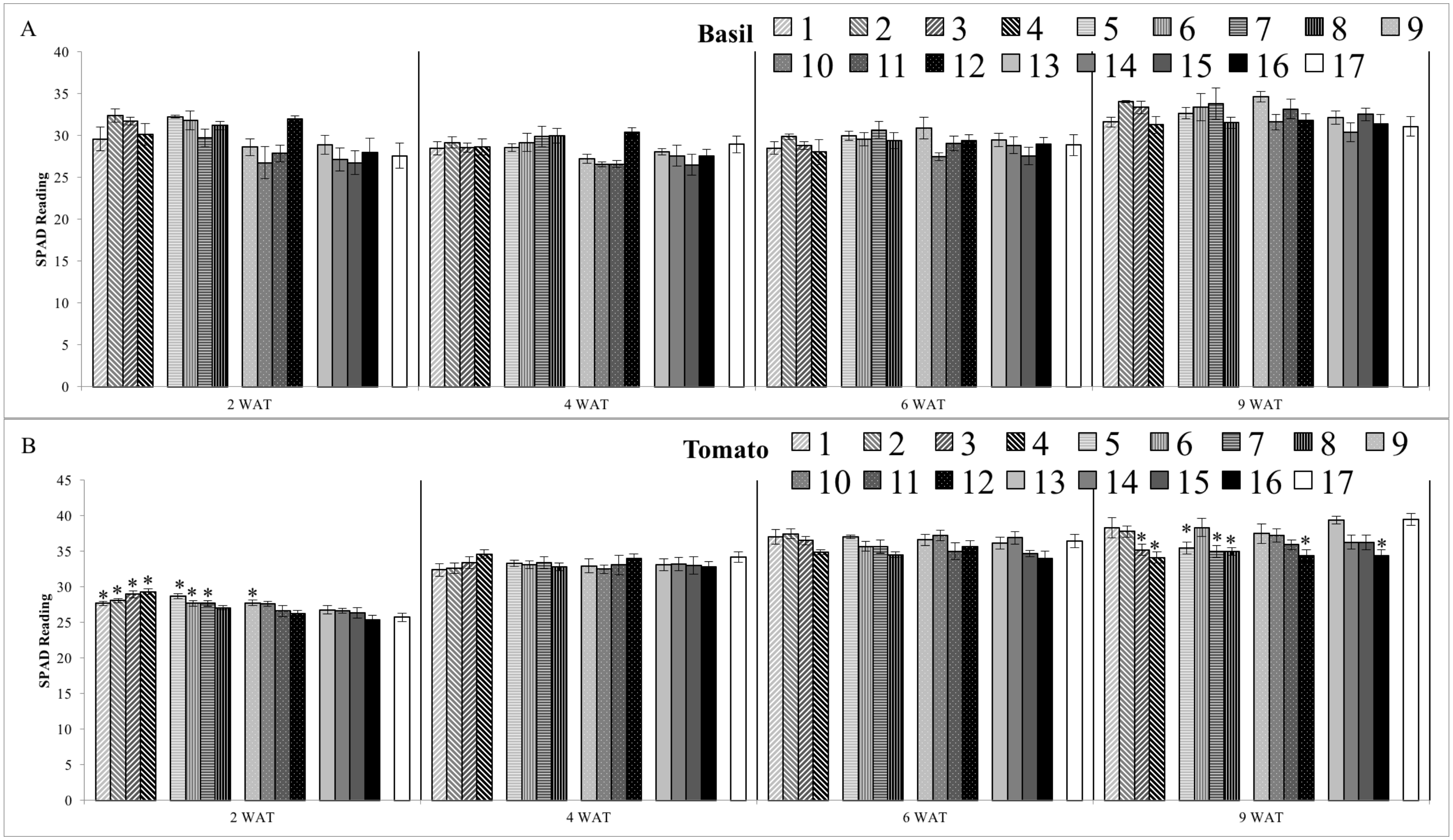


Fig. 2.5. SPAD readings (mean ± standard error) of basil (A) and tomato (B) plants grown in seventeen substrates including mixes of biochar (BC) with vermicompost (VC) and the control at 0, 2, 4, 6 and 9 weeks after transplanting (WAT). The asterisks indicated significant difference from the control using Dunnett's test at *P*<0.05. Treatment 1-20BC:5VC, 2-40BC:5VC, 3-60BC:5VC, 4-80BC:5VC, 5-20BC:10VC, 6-40BC:10VC, 7-60BC:10VC, 8-80BC:10VC, 9-20BC:15VC, 10-40BC:15VC, 11-60BC:15VC, 12-80BC:15VC, 13-20BC:20VC, 14-40BC:20VC, 15-60BC:20VC, 16-80BC:20VC and 17-control.

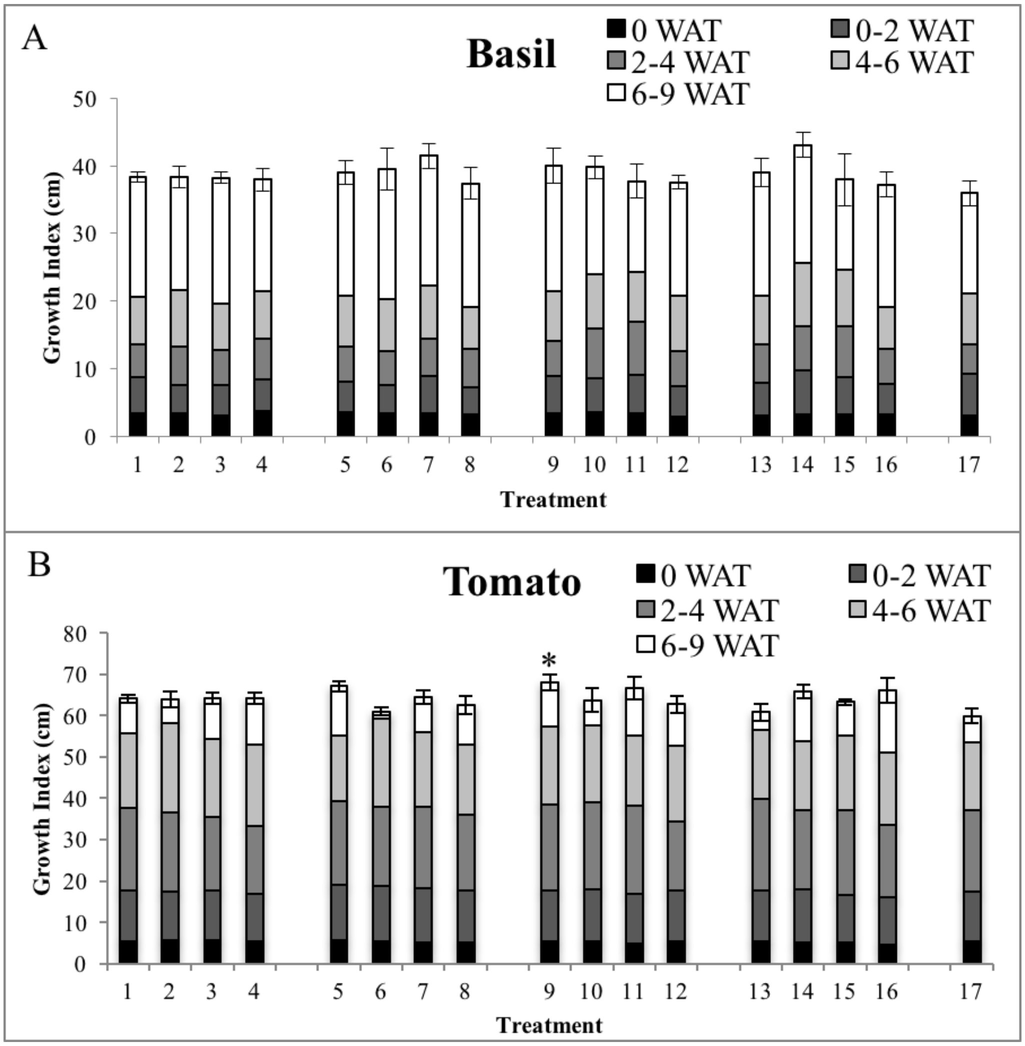


Fig. 2.6. Cumulative growth indexes (mean ± standard error) of basil (A) and tomato (B) plants grown in seventeen substrates including mixes of biochar (BC) with vermicompost (VC) and the control at 0, 2, 4, 6 and 9 weeks after transplanting (WAT). The asterisks indicated significant difference from the control using Dunnett's test at *P*<0.05. Treatment 1-20BC:5VC, 2-40BC:5VC, 3-60BC:5VC, 4-80BC:5VC, 5-20BC:10VC, 6-40BC:10VC, 7-60BC:10VC, 8-80BC:10VC, 9-20BC:15VC, 10-40BC:15VC, 11-60BC:15VC, 12-80BC:15VC, 13-20BC:20VC, 14-40BC:20VC, 15-60BC:20VC, 16-80BC:20VC and 17-control.

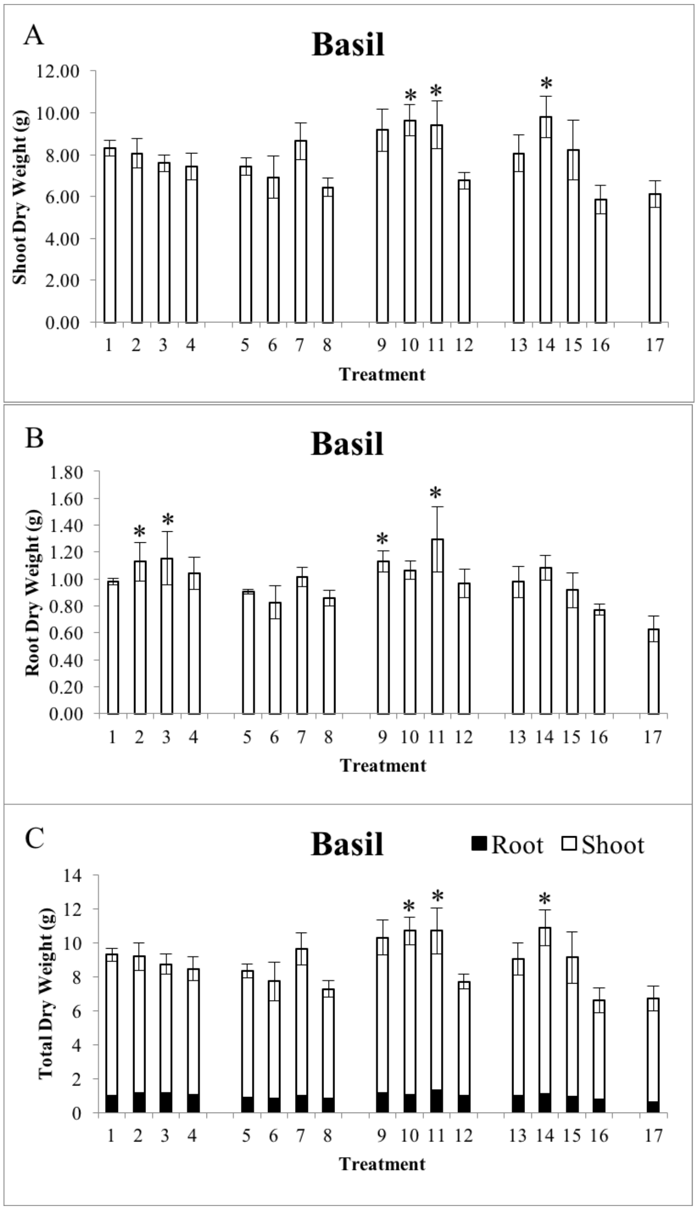


Fig. 2.7. Shoot (A), root (B) and total (C) dry weight (mean ± standard error) of basil plants harvested at 9 weeks after transplanting. The asterisks indicated significant difference from the control using Dunnett's test at $P<0.05$. Treatment 1-20BC:5VC, 2-40BC:5VC, 3-60BC:5VC, 4-80BC:5VC, 5-20BC:10VC, 6-40BC:10VC, 7-60BC:10VC, 8-80BC:10VC, 9-20BC:15VC, 10-40BC:15VC, 11-60BC:15VC, 12-80BC:15VC, 13-20BC:20VC, 14-40BC:20VC, 15-60BC:20VC, 16-80BC:20VC and 17-control.

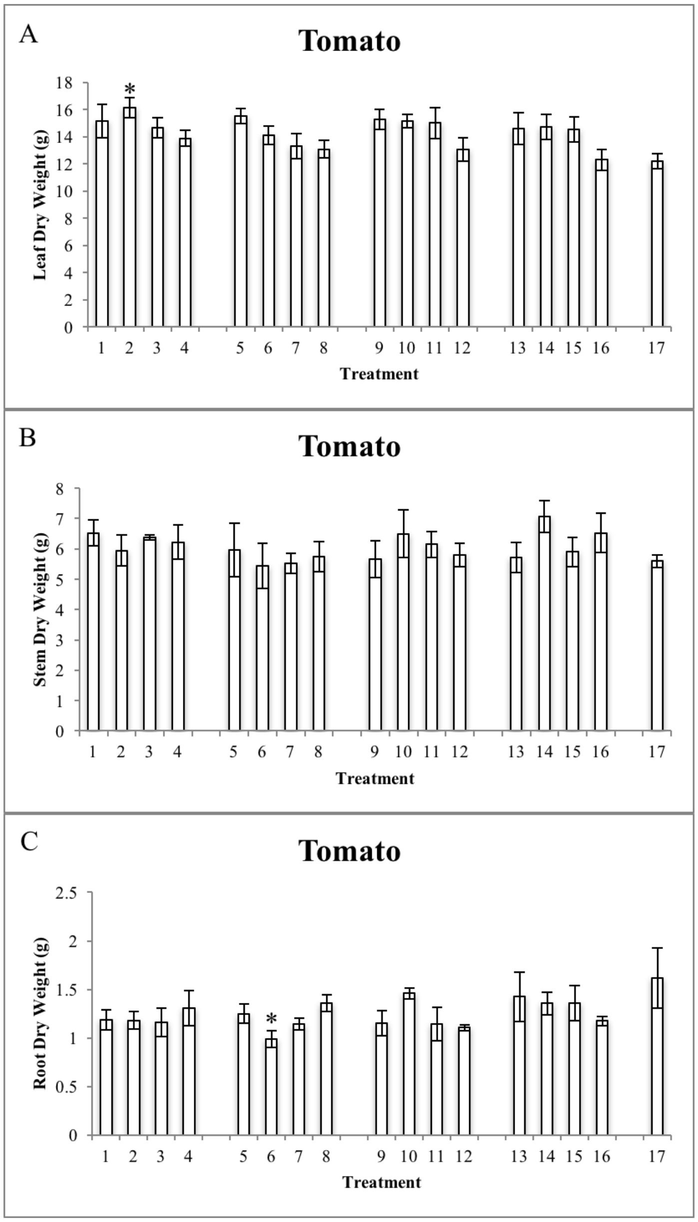


Fig. 2.8. Leaves (A), stem (B), root (C), combined flower and fruit (D), and total (E) dry weight (mean ± standard error) of tomato plants harvested at 9 weeks after transplanting. The asterisks indicated significant difference from the control using Dunnett's test at $P<0.05$. Treatment 1-20BC:5VC, 2-40BC:5VC, 3-60BC:5VC, 4-80BC:5VC, 5-20BC:10VC, 6-40BC:10VC, 7-60BC:10VC, 8-80BC:10VC, 9-20BC:15VC, 10-40BC:15VC, 11-60BC:15VC, 12-80BC:15VC, 13-20BC:20VC, 14-40BC:20VC, 15-60BC:20VC, 16-80BC:20VC and 17-control.

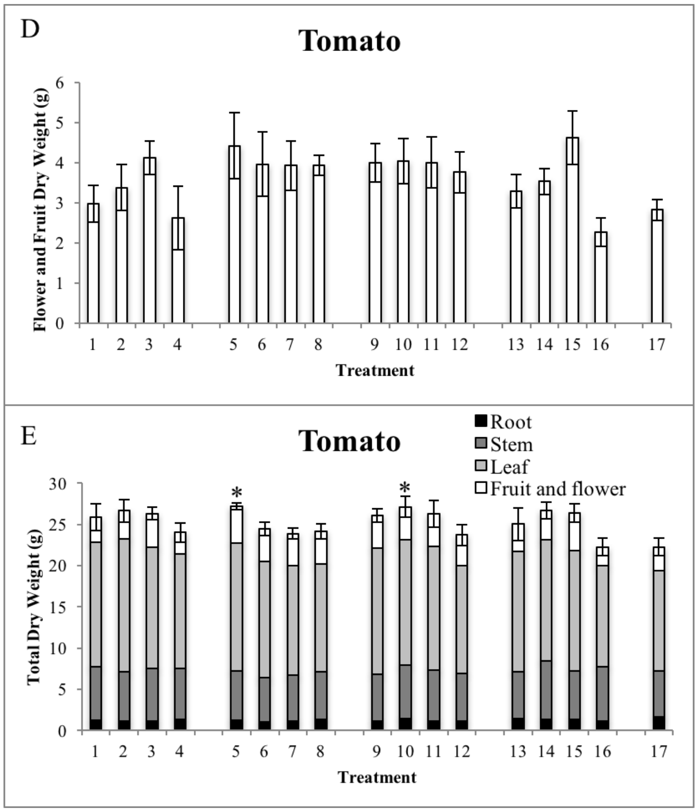


Fig. 2.8 Continued.

CHAPTER III

EFFECTS OF HIGH PERCENTAGE OF BIOCHAR AND TWO COMPOSTS ON CONTAINER-GROWN BASIL AND TOMATO PLANTS

Introduction

The field of container horticultural plant production has increased dramatically (Judd et al., 2015). Peatmoss, bark and coconut coir are major container substrate components (Jackson et al., 2009). And they are normally combined with other components such as vermiculite, perlite and compost (Landis and Morgan, 2009). Recently, several factors including cost and availability of the commonly used substrates (Landis and Morgan, 2009; Lu et al., 2006), the desire to use environmentally friendly substrates (Landis and Morgan, 2009) and the ecological concern of excess harvest of peatmoss (Verhagen and Blok, 2007) have caused growers to consider new container substrates.

Biochar (BC) showed great potential to be used as container substrates. Biochar refers to the carbon-rich material derived from biomass (Hansen et al., 2016; Lehmann, 2007). A lot of research has shown that using BC incorporation in container substrates could increase plant growth at certain conditions (Headlee et al., 2014; Méndez et al., 2017; Nieto et al., 2016; Tian et al., 2012; Zhang et al., 2014). The incorporation of BC to container substrate could increase water holding capacity (Dumroese et al., 2011), increase pH (Dumroese et al., 2011; Vaughn et al., 2013) and improve plant growth (Vaughn et al., 2013). Many factors such as feedstock sources, production conditions, BC incorporation rate, the other substrate components mixed with BC, plant type, and fertility could lead to different results after BC incorporation.

Preliminary trials have shown that the growth indexes, shoot dry weight and total dry weight of basil and tomato plants grown in mixes of BC (20%, 40%, 60% or 80%; by vol.) and vermicompost (VC; 5%, 10%, 15% or 20%; by vol.) were similar to or higher than those in a commercial peat-based substrate at 9 weeks after transplanting. These positive results indicated that the BC could be used as alternative container substrate. The wholesale price for the peat-based commercial substrates is approximately $5 per cubic feet. Due to the relatively high cost of the VC (wholesale price: $17.2 per cubic feet), the lowest VC percentage (5%) in the preliminary trial would be more appropriate than high percentages as amendment for the BC container substrate. Chicken manure compost (CM) has relatively similar fine texture to VC, and is cheaper (retail price: $5.5 per cubic feet) and more readily available than VC. Chicken manure, the waste resulting from the poultry industries (Li et al., 2017a), is widely used in horticulture. Although chicken manure without being properly treated can cause unpleasant environmental problems such as odor and greenhouse gas emissions (Wu et al., 2016), properly treated chicken manure can be suitable compost adding to substrates due to its rich nutrients readily available to plants. With proper treatments, CM may contain 8.85% N, 8.22% P and K, and 86.62% organic matter (Chen et al., 2017). Compost incorporation in container substrates had a lot of benefits including disease suppression and nutrient addition (Barker and Bryson, 2006).

Based on the positive results obtained from the preliminary trials with the BC and VC mixes, the purpose of this experiment was to evaluate the feasibility of mixes with either CM or VC (5%, by vol.) and high percentages of the BC (60%, 70%, 80% or 90%, by vol.) with the rest being commercial substrates as replacements for commercial peat-based container substrates to grow tomato and basil plants.

Materials and Methods

Plant materials and container substrate treatments. Basil (*Ocimum basilicum*) (Johnny's Selected Seeds, Winslow, ME) seeds were sown in commercial propagation mix (Propagation mix; Sun Gro® Horticulture, Agawam, MA) in 288-cell plug trays (cell depth: 2.5cm; cell top length & width: 2cm; volume: 6ml) on March 19, 2017. Tomato (*Solanum lycopersicum* 'Tumbling Tom Red') (Fred C. Gloeckner & Company Inc., Harrison, NY) seeds were sown in commercial propagation mix (Propagation mix; Sun Gro® Horticulture, Agawam, MA) in 200-cell plug trays (cell depth: 4.5cm; cell top length & width: 2.2cm; volume: 10ml) on March 30, 2017. Eight substrates were formulated by mixing biochar (BC; 60%, 70%, 80% or 90%, by vol.; Proton Power, Inc., Lenoir City, TN) with either 5% (by vol.) composted chicken manure (CM; Back to Nature, Inc., Slaton, TX) or vermicompost (VC; Pachamama Earthworm Castings™; Lady Bug Natural Brand, Conroe, TX). The remaining volume in each BC-compost mix was commercial substrate

(BM 7; Berger, Saint-Modeste, QC, Canada). The commercial substrate was used as the control (Fig. 3.1). The commercial substrates used in this research consisted of 55% coarse peat moss, 35% pine bark and 10% horticultural perlite. The pH of the commercial substrates, BC and VC were measured using a handheld pH-EC meter (HI 98129, Hanna Instruments, Woonsocket, RI, USA) and electrical conductivity (EC) of the commercial substrates, BC and VC were measured using the Bluelab Combo Meter (Bluelab Corporation Limited, Tauranga, New Zealand) according to the pour-through extraction method (LeBude and Bilderback, 2009). The pH of the commercial substrate was 7.1. The EC was 1.3 dS m^{-1}. The BC used in this experiment was made from fast pyrolysis of mixed hardwood. The pH of the BC is 11.18 and the EC is 2.0 dS m^{-1} (pH=10.5, soluble salts=4.6 dS m^{-1} measured by North Carolina State University Horticultural Substrates Laboratory, method unknown). The pH of the VC is 4.8. The EC of the VC is 6.7 dS m^{-1}. The pH of the CM is 7.5 and the EC is 32.9 dS m^{-1}. The nutrient contents of the BC, VC and CM were tested by the Texas A&M AgriLife Extension Service Soil, Water and Forage Testing Laboratory in College Station, TX. The total N was determined by a combustion process and determined spectrophotometrically (Parkinson and Allen, 1975) and the mineral contents (including P, K, Ca, Mg, S, B, Ca, Cu, Fe, Mn, Na and Zn) were determined by inductively coupled plasma (ICP) analysis of a nitric acid digest (Havlin and Soltanpour, 1980). The BC, VC and CM had similar or higher concentration of P, K, Ca, S, Fe, B, Cu, Mn and Zn compared to the control (Table 3.1). The BC had lower N percentage, while

CM and VC had higher N percentage than that of the control. The concentration of Mg in CM and VC was higher or similar to the control but that of BC was lower than that of the control. The BC and VC had lower Na concentration, while CM had significantly higher Na concentration (5.8 times of the control). Particle size distribution of BC, commercial substrate, CM and VC was determined by passing 40g BC and 20g commercial substrate, CM and VC through 2.8, 2, 1, 0.425 and 0.25mm sieves. Weight on each sieve was measured to calculate the proportion of each size (Table 3.2).

Uniform basil and tomato seedlings were transplanted in the experimental substrates in 6-inch azalea pots (depth: 10.8cm; top diameter: 15.5cm; bottom diameter: 11.3cm; volume: 1,330ml) on May 6, 2017. Each container contained one seedling of tomato or basil plant. Seven replications of the nine treatments were arranged in completely randomized blocks in the greenhouse located on Texas A&M University campus, College Station, TX. During the experimental period, the temperature, humidity and dew point in the greenhouse were monitored using Watchdog (Spectrum Technologies Inc., Paxinos, PA). The average greenhouse temperature, relative humidity (from 6am to 6pm) and dew point were 27.3 °C, 85.4% and 25.6 °C, respectively. The average photosynthetic photon flux density in the 400 to 700 nm waveband (photosynthetically active radiation) from June 27 to July 07, 2017 was 207.3 μmol m^{-2} s^{-1}, which was measured using LightScout quantum light sensor (Spectrum Technologies Inc., Paxinos, PA).

The basil and tomato plants were irrigated with 100 and 200 mg L^{-1} N Peters® Professional (20N-4.4P-16.6K; Everris NA Inc., Dublin, OH) nutrient solution, respectively. The total N in Peters® Professional contains 8.1% ammoniacal N and 11.9% nitrate N. To determine the actual water use, the plants were thoroughly irrigated and allowed to drain for at least 2 hours until there was no more leachate. The average weight of the containers with the saturated substrate was determined. When plants needed to be irrigated after transplanting, three containers of each treatment were weighed to get an average value. The decrease in weight compared to the original containers with saturated substrate was the amount of nutrient solution to be applied to the plant. Saucers were placed underneath the containers to hold the extra leachate, which could be then re-absorbed by the plants.

Physical properties of the substrates. Physical properties including the total porosity (TP), container capacity (CC), air space (AS) and bulk density (BD) of VC, CM and the nine formulated substrates were determined using the North Carolina State University Horticultural Substrates Laboratory porometers (Fonteno et al., 1995). The TP, CC and AS of the VC was similar to that of the control (Table 3.3). Total porosity of the BC was higher than the control, while that of the CM was lower than the control. The CC of BC and CM were all lower than the control. The AS of VC and CM were similar to the control, while that of BC was higher than the control. Six replications of the nine substrates were tested to determine the physical properties. The BD of VC and CM were higher than the control.

Leachate electrical conductivity, pH and nutrients analysis. Substrate leachate pH and electrical conductivity (EC) were measured at 0, 2, 4, 6 and 8 weeks after transplanting (WAT) using a handheld pH-EC meter (HI 98129, Hanna Instruments, Woonsocket, RI) according to the pour-through extraction method (LeBude and Bilderback, 2009). The upper limit of the EC meter is 3,999 μS cm^{-1}. The value of 3,999 μS cm^{-1} was used in data analysis when the EC readings exceeded the upper limit. At 0, 4 and 8 WAT, 4 replications of leachates of each of the nine substrates were filtered through VWR Grade 415 filter paper (quantitative) with 11cm diameter (VWR International, LLC, Randor, PA). Filtered leachate was sent to the Texas A&M AgriLife Extension Service Soil, Water and Forage Testing Laboratory for nutrient analysis (P, K, Ca, Mg, S, Fe, Cu, Mn, Na and Zn) using ICP analysis (Franson, 1989). Substrate leachate NO_3-N was measured using HQ440d Benchtop Meter and ISENO3181 nitrate electrode (Hach Company, Loveland, CO).

Plant growth and development. Plant growth index (GI) and leaf greenness, which was abbreviated as SPAD (Soil Plant Analysis Development), were measured at 0, 2, 4, 6 and 8 WAT. Plant height was determined from the base to the top of the plant. The widest plant canopy width and its perpendicular width were measured. The plant GI was determined by the following formula: GI=plant height/2+(plant width 1+plant width 2)/4. Leaf greenness of each plant was determined by using the average of readings from three mature leaves per plants using a portable SPAD 502 Plus Chlorophyll Meter (Spectrum Technologies, Inc, Plainfield, IL).

The basil plants were harvested approximately 15cm above the base of the plant at 5 WAT to measure the first harvest fresh weight (FW) and dry weight (DW). The second FW and DW were determined by cutting the entire aboveground part of the basil plants from the substrates surface at 8 WAT. The shoot FW and DW were determined by adding these two FWs and DWs, respectively. The roots were cleaned to measure the root DW. Red fruits on the tomato plants were harvested twice at 7 and 8 WAT, respectively. Red fruit FW and DW were calculated by adding these two harvests together. In addition to the red fruits, at 8 WAT, leaves, stems, roots, flowers and green fruits were harvested separately. The total fruit FW and DW were calculated by adding all the red and green fruits together. The total plant DW was calculated by adding DW of all parts of the plants. Dry weight of plant parts was measured after being oven-dried at 80 ºC until constant weight.

Photosynthesis. Photosynthetic rates were measured on five replicates of each treatment on a sunny day at 5 and 8 WAT using LI-COR6400 (LI-6400XT, LI-COR Inc., Lincoln, NE). The environment of the leaf chamber was set at 30 °C, 400 µmol m^{-2} s^{-1} CO_2 concentration, and 800 µmol m^{-2} s^{-1} quantum flux density.

Statistical analysis. The experiment was set up in a completely randomized block design with the substrate type being the main factor. Data were analyzed with one-way analysis of variance (ANOVA) using JMP Statistical Software (version Pro 12.2.0; SAS Institute, Cary, NC) and means were separated using Dunnett’s test when treatments were significant at $P < 0.05$. Coefficient of determination to assess the relationship between nutrient concentration at 0 WAT and plant GI at 4 and 8 WAT and DW were computed.

Results and Discussion

Physical properties of the container substrates. The total porosity (TP) of mixes of 90% biochar with 5% vermicompost (90BC:5VC), 70% BC with 5% chicken manure compost (70BC:5CM) and 80BC:5CM were lower than the control, while the other BC-compost mixes were not significantly different from the control (Fig. 3.2A). The lower TP was due to the lower container capacity (CC) since TP is the sum of CC and air space (AS) (Fig. 3.2B). The CCs of the BC-compost mixes were lower than that of the control (Fig. 3.2B), mainly caused by the low CC of the BC used in the formulated substrates in this study (60%, 70%, 80%, or 90%, by vol.) (Table 3.3). The AS of the mixes of 70BC:5VC, 80BC:5VC, 90BC:5VC and 90BC:5CM were higher than the control (Fig. 3.2C), caused by the incorporation of BC with higher fraction of larger particles compared to the commercial substrates (Table 3.2), increasing the percentage of macropores. Research has shown physical properties (CC, AS and TP) of the substrates are related to the particle size distribution of the substrates (Abad et al., 2005). Compared to the substrates with higher proportion of fine particles, substrates with high proportions of large particles will have larger AS and lower CC (Evans et al., 1996). This was consistent with our results that incorporation of the BC with higher fraction of large particles increased AS in 70BC:5VC, 80BC:5VC, 90BC:5VC and 90BC:5CM and decreased CC in all BC-compost mixes.

Substrates with higher fraction of fine particles tend to have more micropores (Tilt et al., 1987), which will have higher CC due to the attraction of water to the walls of small pores (McCarty et al., 2016). Robinson et al. (1975) also showed that when the substrate particle size was between 0.01 to 0.8 mm, the CC was high, while as the particle size increased from 0.8 to 6mm, the proportion of large noncapillary pores increases thus increasing the AS.

The BD of the BC-compost mixes was significantly higher than the control (Fig. 3.2D) because of the high BDs of the two composts and the decreased pore spaces of larger-particle component (i.e., the BC) filled by smaller-particle components (i.e., the VC and CM). Research has showed that when different dry substrates are blended, BD increased if they fit with each other tightly (Bilderback et al., 2005). Compared to the optimal physical properties indicated by Méndez et al. (2015) and Abad et al. (2001), the TP and BD of the mixes of BC with compost in this research were in the ideal ranges of the corresponding physical properties (50% to 80% for TP and less than 0.4 g cm^{-3} for BD) to grow plants in containers, but the CC of these mixes was below the acceptable range (60% to 100%).

Substrate leachate pH and electrical conductivity (EC). The substrate leachate pH in 60BC:5VC, 60BC:5CM and 70BC:5CM with tomato plants at 8 WAT was similar to the control, while that of the other BC-compost mixes at 0, 2, 4, 6 and 8 weeks after transplanting (WAT) was higher than the control (Fig. 3.3), which was caused by the initial high pH of the BC (pH=11.18). Higher pH of the leachate after BC incorporation was found in a lot of research (Chan et al., 2008; Ducey et al., 2015; Dumroese et al., 2011; Steiner and Harttung, 2014; Streubel et al., 2011). The leachate pH of 60BC:5VC, 60BC:5CM and 70BC:5CM with tomato plants being similar to the control at 8 WAT could be due to the large amount of N nutrient solution applied, which 'washed down' the pH of the BC mixes.

The BC-compost mixes had a significant effect on the leachate EC. At 0 WAT, the substrate leachate EC of 90BC:5VC and all BC:CM mixes was significantly higher than that of the control, while the substrate leachate EC of mixes with 60BC:5VC was lower than the control (Fig. 3.4). The EC value can indicate the total inorganic ion concentration in the container substrate leachate (Awang et al., 2009). The increased leachate EC in 90BC:5VC mixes could be due to increased release of weakly bound nutrients (cations and anions) of BCs. In addition to this, the higher EC in BC:CM mixes was due to the high nutrient content of CM (Table 3.1). It was shown that the presence of high soluble salts in the BCs could lead to increased EC after BC incorporation (Chintala et al., 2014). The increased EC value was consistent with Tian et al. (2012), who found that adding 50% (by vol.) BC made from green waste to peat moss media significantly increased EC due to nutrient release of the BC.

All the BC:CM mixes with basil plants at 2 and 4 WAT had higher EC than the control, while all BC:VC mixes had lower or similar EC compared to the control (Fig. 3.4A). After the first harvest at 5 WAT, the EC of BC-compost mixes was similar to the control, except that of mixes of 80BC:5CM at 6 and 8 WAT and 70BC:5CM at 6 WAT (Fig. 3.4A). The

EC of the BC:VC mixes with tomato plants at 2 and 4 WAT was lower than that of the control, while the EC of BC:CM mixes was similar to or higher than the control (Fig. 3.4B). At 6 and 8 WAT, the EC of BC-compost mixes was similar to the control except the 90%BC mixes at 6 WAT and 90BC:5CM at 8 WAT. Steiner and Harttung (2014) also found that the EC was lower in mixes with higher BC incorporation rates (50%, 75% or 100%) than mixes with lower BC rate (25%) at 6 WAT. Lower leachate EC of the substrate with higher percentage of BC may be caused by BC's effective moderating effect on extreme fluctuations of macronutrients (Altland and Locke, 2012). The reason for the lower leachate EC of 90BC:5CM with tomato plants at 6 and 8 WAT could be less nutrient solution applied for plants with reduced growth. The different results of the EC of the substrates growing tomato and basil plants could be due to different concentration of N nutrient solutions and water needs at different plant growth and development stages.

Substrate leachate nutrients analysis. For basil plants, the leachate NO_3^--N concentration of all the BC-compost mixes was similar to or lower than the control (Fig. 3.5A). The leachate P concentration of all the BC:CM mixes was higher than the control at 0, 4 and 8 WAT, while that of BC:VC mixes were lower than the control at 0 WAT, but higher or similar to the control at 4 and 8 WAT (Fig. 3.5B). All the BC-compost mixes had higher or similar leachate K concentration when compared to the control at 0, 4 and 8 WAT (Fig. 3.5C). The BC-compost mixes had lower leachate Ca and Mg concentrations when compared to the control at 0, 4 and 8 WAT except for Ca concentration of 70BC:5CM and

80BC:5CM at 8 WAT (Fig. 3.5D and E). The leachate S concentration of BC:VC mixes was lower than the control at 0 and 4 WAT and similar to the control at 8 WAT, and that of BC:CM mixes was similar to or higher than the control (Fig. 3.5F). The leachate Fe concentration of all BC-compost mixes (except 90BC:5VC) were higher than or similar to the control at 0 WAT (Fig. 3.5G). And the leachate Fe concentration in all BC-compost mixes was lower than or similar to the control at 4 and 8 WAT. The leachate B concentration of the BC-compost mixes was similar to or lower than the control except that of mixes of 80BC:5CM and 90BC:5CM at 0 WAT (Fig. 3.5H). The leachate Cu concentration of BC:VC mixes was similar to the control and that of BC:CM mixes was higher than or similar to the control (Fig. 3.5I). The leachate Mn concentration in the BC:VC mixes was similar to the control and that in the BC:CM mixes was higher than the control at 0 and 4 WAT (Fig. 3.5J). At 8 WAT, the BC-compost mixes had lower leachate Mn concentration than the control except 70BC:5CM and 80BC:5CM. The leachate Na concentration of BC:VC mixes was lower than or similar to the control, while that of BC:CM mixes were all higher than the control (Fig. 3.5K). The leachate Zn concentration of BC:VC mixes was lower than or similar to the control, while that of BC:CM mixes was higher than or similar to the control at 0, 4 and 8 WAT (Fig. 3.5L).

Leachate NO_3^--N concentration of all the BC-compost mixes with tomato plants was similar to or lower than the control at 0, 4 and 8 WAT (Fig. 3.6A). The leachate P concentration of all BC-compost mixes was lower than or similar to the control except that of mixes of 70BC:5CM and 80BC:5CM at 0 WAT (Fig. 3.6B). All the BC-compost mixes had higher or similar leachate K concentration when compared to the control at 0, 4 and 8 WAT (Fig. 3.6C). The leachate Ca and Mg concentrations were lower in the BC-compost mixes compared the control at 0 and 4 WAT and higher than or similar to the control at 8 WAT (Fig. 3.6D and 3.6E). The leachate S concentration of the BC-compost mixes was higher than or similar to the control except that of BC:VC mixes at 0 WAT (Fig. 3.6F). The leachate Fe concentration of 70BC:5CM, 80BC:5CM and 90BC:5CM at 0 WAT were higher than the control, while that of other BC-compost mixes was lower than or similar to the control (Fig. 3.6G). The leachate B concentration of all the BC-compost mixes was similar to or lower than the control except that of mixes of 80BC:5CM and 90BC:5CM at 0 WAT (Fig. 3.6H). The leachate Cu concentration in BC:CM mixes at 0 and 4 WAT was higher than the control, while that of other BC-compost mixes was similar to or lower than that of the control (Fig. 3.6I). The leachate Mn concentration of BC-compost mixes was similar to or lower than the control except that of BC:CM mixes at 0 WAT and 80BC:5CM and 90BC:5CM at 4 WAT (Fig. 3.6J). The leachate Na concentration of BC-compost mixes was lower than or similar to the control except that of BC:CM mixes at 0 WAT and mixes of 60BC:5CM, 70BC:5CM and 80BC:5CM at 4 WAT (Fig. 3.6K). The leachate Zn concentration of BC-compost mixes was lower than or similar to the control except that of mixes of 80BC:5CM and 90BC:5CM at 0 WAT (Fig. 3.6L).

Plant growth and development. High percentage of BC incorporation in BC-compost reduced the SPAD (Soil Plant Analysis Development) readings of basil plants from 4 WAT and tomato plants from 2 WAT (Fig. 3.7). For basil plants, the SPAD readings in mixes of 70BC:5VC, 80BC:5VC, 90BC:5VC and 80BC:5CM were lower than the control at 4 WAT. In the first week (at 6 WAT) after the first harvest, the SPAD readings of the basil plants in all BC-compost mixes (except 80BC:5VC) were similar to the control. At 8 WAT, all the basil plants grown in BC-compost mixes had lower SPAD readings than the control. The SPAD readings of tomato plants in all BC-compost mixes were lower than the control at 2 WAT. At 4 WAT, the SPAD readings of tomato plants in all BC:VC mixes and 90BC:5CM were lower than the control. At 6 and 8 WAT, the SPAD readings of the tomato plants in all BC-compost mixes (except 90BC:5VC) were similar to those in the control. This confirmed the results of Nair and Carpenter (2016), who found that pepper (*Capsicum annuum*) leaf SPAD readings decreased with increasing BC incorporation rate in container. Since the SPAD reading is related to the leaf N content (Gholizadeh et al., 2017), the lower SPAD reading at the high BC incorporation rate might indicated decreased foliar N level. Therefore, the lower SPAD readings of basil and tomato plants in BC-compost mixes could be due to the lower foliar N level when compared to the control. According to Tian et al. (2012), the available N decreased after substituting 50% peat with the BC. The decreased N could be caused by N-binding of the BC's (Altland and Locke, 2012).

Photosynthetic rates of the basil and tomato plants at 5 WAT and 8 WAT in the BC-compost mixes were similar to the one grown in the control, respectively, except that the tomato plants in 90BC:5CM at 8 WAT were higher than the control (Fig. 3.8). Tomato plants in 90BC:5CM grew slower than the control. At 8 WAT, tomato plants in 90BC:5CM were still in the flowering stage while plants in all the other mixes had a lot of mature fruits. High photosynthetic rates of tomato plants in 90BC:5CM at 8 WAT could be due to its delayed developmental stage, as the photosynthetic rates of the tomato plants decreases during the late developmental and senescence stage due to the decreased content of the ribulose-1,5-bisphosphate carboxylase/oxygenase, an important enzyme in photosynthesis (Xu et al., 1997).

At 4 and 8 WAT, the growth index (GI) of basil plants grown in the BC-compost mixes was similar to that of the control except those grown in mixes of 90BC:5VC, 80BC:5CM, and 90BC:5CM (Fig. 3.9A and B). The GI of tomato plants in all the BC-compost mixes (except 90BC:5CM) was similar to those in the control (Fig. 3.9C). The effect of BC-compost mixes on basil plant fresh weight (FW) and dry weight (DW) showed trend similar to the basil plant GI. The shoot FW and DW, root DW and total DW of basil plants grown in the BC-compost mixes (except 80BC:5CM, 90BC:5VC and 90BC:5CM) were similar to the control (Fig. 3.10). Leaf DW of tomato plants in 90BC:5VC and 90:5CM was lower than the control, while that of others in BC-compost mixes was similar to the control (Fig. 3.11A). The stem and root DW, total fruit FW and DW, combined flower and fruit DW and red fruit FW and DW of tomato plants in all BC-compost mixes (except 90BC:5CM) were similar to or higher than the control, respectively (Fig. 3.11B, C, D, E and F). Green fruit FW and DW in all BC mixes were higher than or similar to the control (Fig. 3.11G). And total DW of tomato plants in all BC-compost mixes (except 90BC:5CM) was similar to higher than the control (Fig. 3.11H). The higher total fruit FW and DW in 60BC:5CM, 70BC:5CM and 80BC:5CM and total DW in 60BC:5CM and 80BC:5CM could be associated with the high amount of nutrients from the CM (Table 3.1). The BC incorporation can improve nutrient retention due to its porous structure and high adsorption ability caused by BC's high surface area and intraporosity (Laird et al., 2010a). It was shown that mixing 20% or 35% BC made from coir with 0.5% or 0.7% humic acid (by dry weight) in composted green waste medium showed increased biomass of *Calathea insignis* when compared to that grown in the composted green waste medium without BC and humic acid amendments due to increased water retention and nutrients by BC (Zhang et al., 2014).

The difference on GI and DW between the plants in BC-compost mixes and the control was related to leachate nutrient concentration. Because leachate nutrient may have a delay effect on plant GI and DW, the correlation of leachate nutrient concentrations at 0 WAT with basil and tomato plant GI at 4 and 8 WAT and DW were investigated (Table 3.4). Basil plant GI at 4 and 8 WAT and DW were positively correlated with leachate NO_3^--N at 0 WAT. And there was no correlation between P concentration at 0 WAT and the basil plant GI or DW. However, leachate K at 0 WAT was negatively correlated with basil GI at 4 and 8 WAT and DW. There was also negative correlation between leachate Na concentration and the basil GI at 4 WAT and DW. Thus, the reason of the decreased basil plant GI at 4 WAT and total DW in 80BC:5CM and 90BC:5CM could be caused by the high concentrations of K and Na in BC:CM mixes (Table 3.4, Fig. 3.5C and 3.5K, Fig. 3.9 and 3.10). Salinity stress could be present when the BC percentage is high and mixed with the CM. The CM had higher concentrations of salt compared to the control (Table 3.1). The leachate Na concentration at 0 WAT was significantly higher in BC:CM mixes when compared to the control (Fig. 3.5K). Although K has no direct toxicity effect on plants, high K concentration may cause Mg and Ca uptake deficiency (Landis, 2005), which could thus decrease plant growth. In addition, it was also shown that basil plant is sensitive to high concentration of fertilizer as increasing the fertilizer (20N-8.7P-16.6K) solution from 100 to 500 mg N L^{-1} stunted basil plant growth (Tesi et al., 1994). The stunted basil plant growth reflected by GI, shoot FW and DW and root and total DW in 90BC:5VC mixes, 80BC:5CM and 90BC:5CM could be caused by the high nutrient content in the container substrates caused by VC or CM combined with nutrient solution applied and BC's nutrient holding capacity. For tomato plants, leachate NO_3^--N positively affect tomato plant GI at 4 WAT, but had no effect on GI at 8 WAT and DW (Table 3.4). Leachate K concentration negatively affect tomato plant GI at 4 and 8 WAT. All leachate nutrient concentrations at 0 WAT didn't have any correlation with tomato plant DW. There was no correlation of the leachate P and Na concentrations with tomato plant GI at 4 and 8 WAT and DW. Tomatoes have been shown to be relatively salt tolerant (Jones Jr, 2007), while basil are regarded as moderately salt tolerant (Scagel et al., 2017). Therefore, salinity could suppress basil growth in 80BC:5CM and 90BC:5CM, but had no effect on tomato plant growth.

Conclusions

The incorporation of BC in container substrate shows promise as an ecological and economical way to be used to replace commonly used the peat-based commercial substrate. When comparing mixes of 5% composts (either VC or CM) and 60%, 70%, 80% or 90% BC by vol. with the rest being commercial substrates to the control (100% commercial substrates), TP and BD were all in the ideal ranges of the physical properties to grow plants in container. At the end of the experiment, GI, shoot DW and FW, and root and total DW of basil plants in all BC-compost mixes were similar to those in the control except the ones in 80BC:5CM, 90BC:5VC and 90BC:5CM. The GI, stem, root and total DW, red, green and total fruit FW and DW of tomato plants in all BC-compost mixes (except 90BC:5CM) were similar or higher than the control. The reduced plant growth could be caused by substrate high pH and salt. The improved plant growth was due to improved nutrient level provided by composts and improved nutrient retention by BC. Photosynthetic rates of plants in BC-compost mixes were similar to or higher than the control. Leachate Na nutrients concentrations at 0 WAT were negatively correlated with basil plant GI at 4 WAT and DW, while had no effect on tomato plants. Therefore, 5% (by vol.) CM and VC can be mixed with 60% and 70% BC to grow container basil and tomato.

The results of this study are important for future use of BC in container substrates. However, careful consideration should be taken in using BC in container. All the BCs are not equal. Physical and chemical properties of BCs might be different which are related to their original feedstock sources and production conditions. And the effects of BC and composts on plant growth depends on the percentage of BC incorporated, other components mixed with BC and plant type.

The results in this study can be only suitable for these specific BC, CM and VC. The recommended mixture is mix of 5% CM with 70% BC with the rest being the peat-based commercial substrates since CM is cheaper than the VC and more BC in the container is preferred. Future research should be done to evaluate the impact of this specific BC and composts on many other plants for broad use.

Table 3.1. Nutrient analysis of the commercial substrate, biochar, chicken manure compost and vermicompost.

	N	P	K	Ca	Mg	S	Fe	B	Cu	Mn	Na	Zn
Substrate	(%)	(ppm)										
Commercial substrates	0.70	540	1265	25108	4237	1744	1508	11	17	98	953	46
Biochar	0.23*[z]	456	6362*	27507	1299*	231	2039	15	9	905*	107*	13
Chicken manure compost	2.03*	17315*	28565*	71239*	11513*	7169*	3703*	49*	119*	424*	5497*	453*
Vermicompost	2.43*	4901*	3714*	25841	3819	5996*	4835*	42*	165*	374*	351*	385*

[z] Means within a column under each main factor followed by an asterisk were significantly different from the control according to Dunnett's test at *P*<0.05 (n=4).

Table 3.2. Particle size distribution of the commercial substrates, biochar, chicken manure compost and vermicompost.

	Size fraction (%)					
Substrate	<0.25mm	0.25-0.425mm	0.425-1.0mm	1.0-2.0mm	2.0mm-2.8mm	>2.8mm
Commercial Substrates	8.3	8.8	26.4	21.7	9.4	25.4
Biochar	2.2	2.0	9.1	19.4	19.4	47.9
Chicken Manure Compost	27.3	13.8	17.9	22.5	9.5	9.0
Vermicompost	11.6	13.9	41.3	22.6	9.4	1.2

Table 3.3. Physical properties of the container substrates.

Substrate	Total porosity (%)	Container Capacity (%)	Air Space (%)	Bulk density (g cm^{-3})
Commercial Substrates	74.0	70.7	3.3	0.10
Biochar	84.7*[z]	60.3*	24.4*	0.15
Chicken Manure Compost	64.4*	60.0*	4.4	0.62*
Vermicompost	75.0	72.2	2.8	0.38*

[z] Means within a column under each main factor followed by an asterisk were significantly different from the control according to Dunnett's test at $P<0.05$.

Table 3.4. Correlations (R^2) of the substrates NO_3^--N, P, K, Ca, Mg, S, Fe, B, Cu, Mn, Na and Zn concentrations at 0 weeks after transplanting (WAT) with the growth index (GI) at 4 and 8 WAT and dry weight (DW), respectively.

	Nutrient Concentration at 0 WAT											
	NO_3^--N	P	K	Ca	Mg	S	Fe	B	Cu	Mn	Na	Zn
						Basil						
GI at 4 WAT	0.21**	NS	-0.53***	NS	0.11*	-0.17*	-0.41***	-0.37***	-0.45***	-0.31**	-0.24**	-0.41***
GI at 8 WAT	0.14*	NS	-0.31**	NS	0.14*	NS	-0.23**	-0.19**	-0.27**	-0.18**	NS	-0.24**
DW at 8 WAT	0.17*	NS	-0.54***	0.17*	0.23**	-0.14*	-0.34**	-0.25**	-0.40***	-0.27**	-0.22**	-0.32**
						Tomato						
GI at 4 WAT	0.28**	NS	-0.18*	NS	NS	NS	-0.32**	-0.34**	-0.33**	-0.26**	NS	-0.29**
GI at 8 WAT	NS	NS	-0.11*	NS	NS	NS	-0.12*	-0.15*	-0.12*	NS	NS	-0.12*
DW at 8 WAT	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS (nonsignificant) or significant correlation at $P \leq 0.5$ (*), $P \leq 0.01$ (**), or $P \leq 0.0001$ (***). Negative sign in front of the correlation means negative correlation.

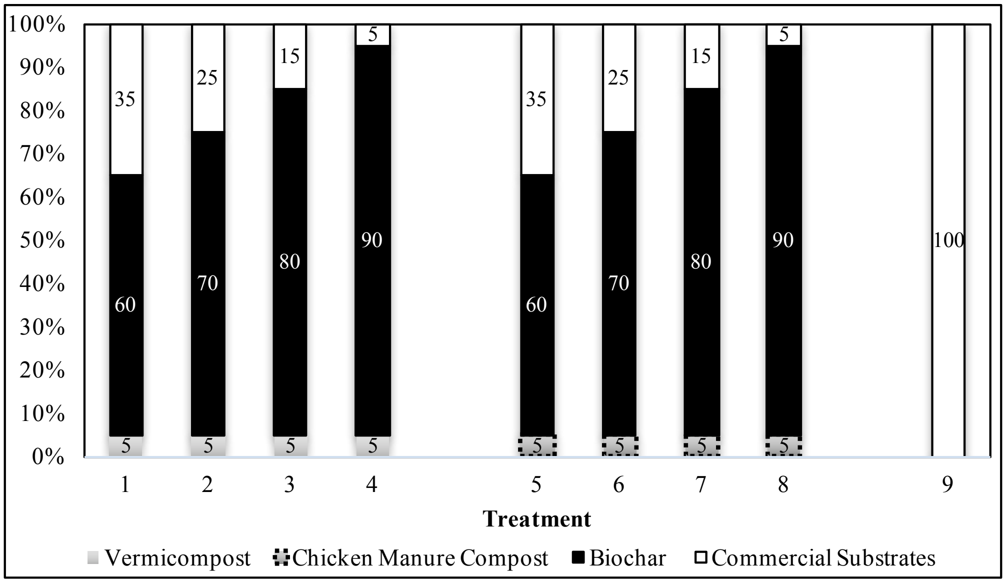


Fig. 3.1. Nine formulated substrates including mixes of biochar (60%, 70%, 80% or 90%, by vol.) with either 5% (by vol.) composted chicken manure or vermicompost and the control.

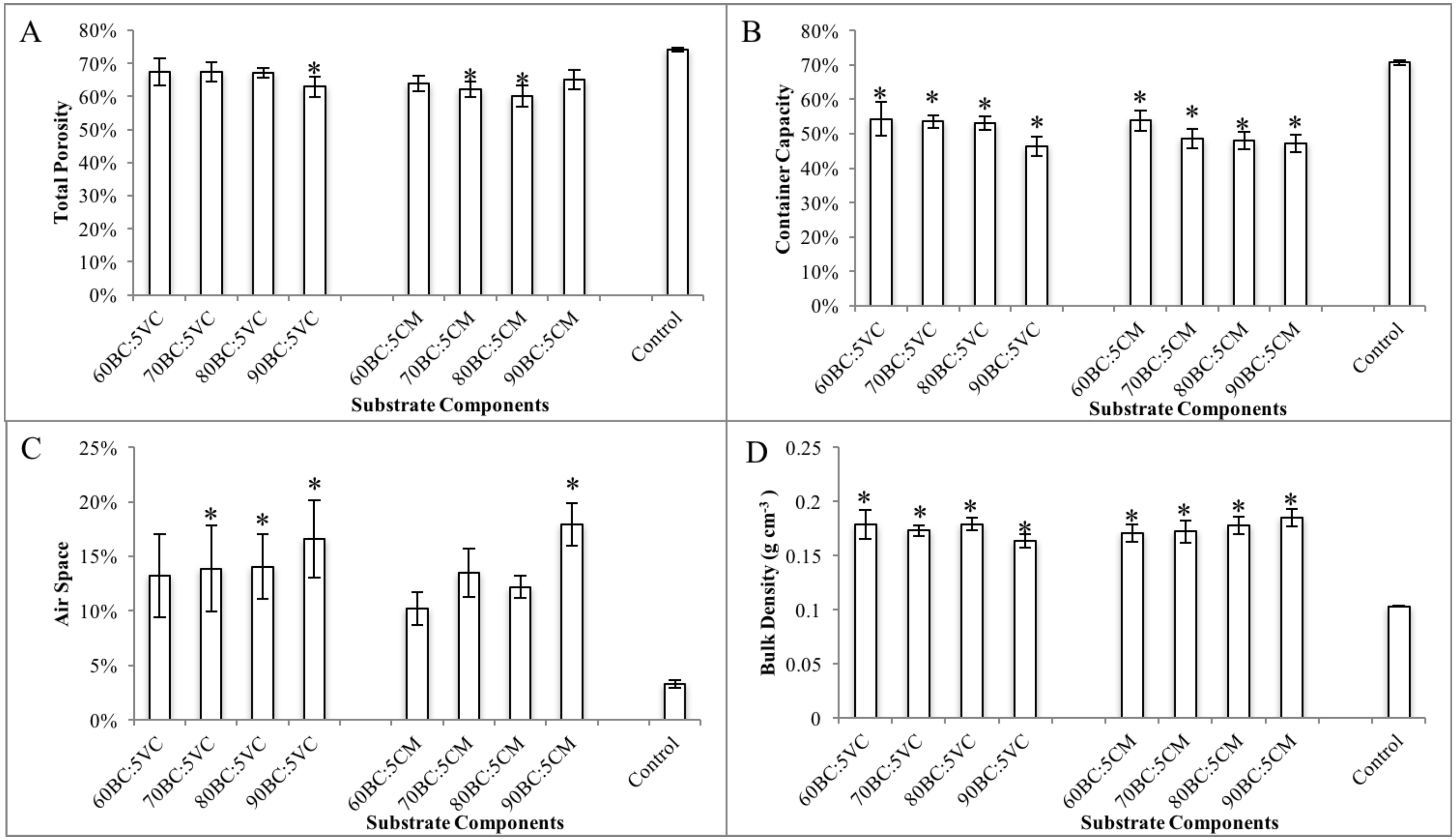


Fig. 3.2. Total porosity (A), container capacity (B), air space (C) and bulk density (D) (mean ± standard error) of the eight different substrates formulated by mixing biochar (BC; 60%, 70%, 80% or 90%, by vol.) with either 5% (by vol.) composted chicken manure (CM) or vermicompost (VC) and the control. The asterisks indicated significant difference from the control using Dunnett's test at $P < 0.05$.

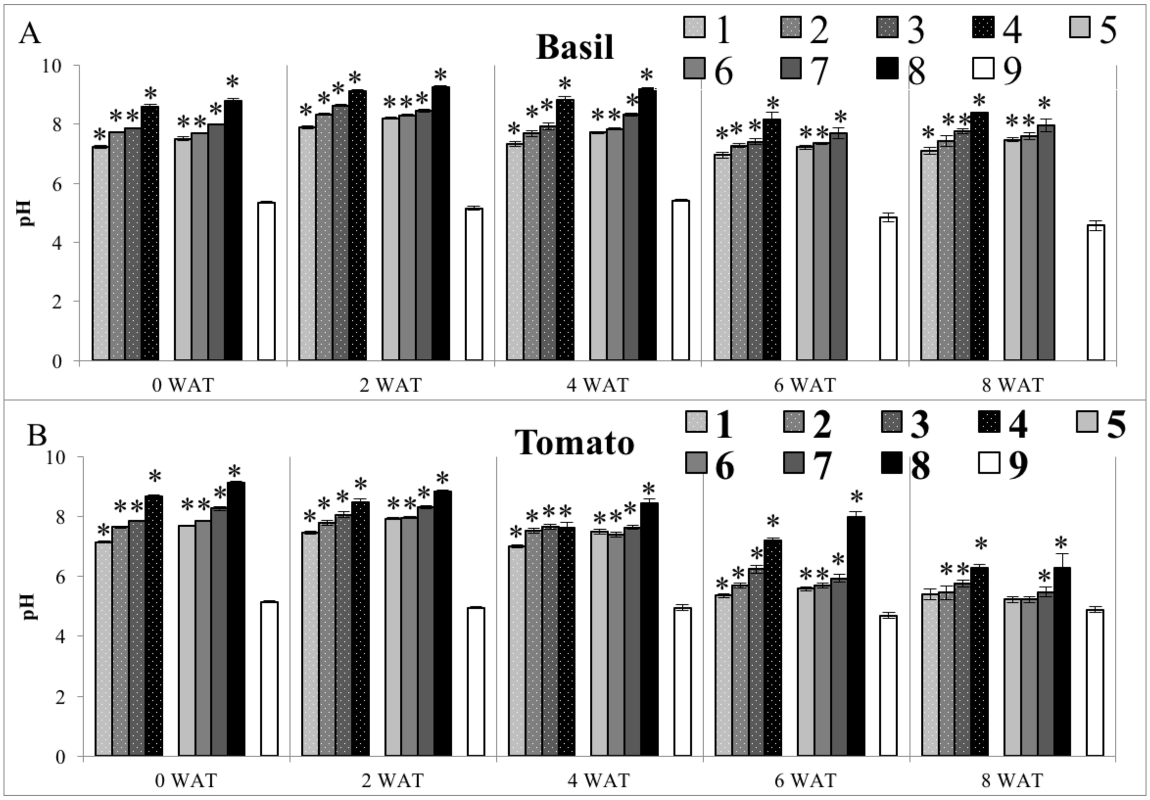


Fig. 3.3. Leachates pH (mean ± standard error) of mixes of biochar (BC) with either composted chicken manure (CM) or vermicompost (VC) and the control growing basil (A) and tomato (B) at 0, 2, 4, 6 and 8 weeks after transplanting (WAT). Treatment 8 does not display due to dead basil plants after 4 WAT. The asterisks indicated significant difference from the control using Dunnett's test at *P* <0.05. Treatment 1-60BC:5VC, 2-70BC:5VC, 3-80BC:5VC, 4-90BC:5VC, 5-60BC:5CM, 6-70BC:5CM, 7-80BC:5CM, 8-90BC:5CM and 9-control.

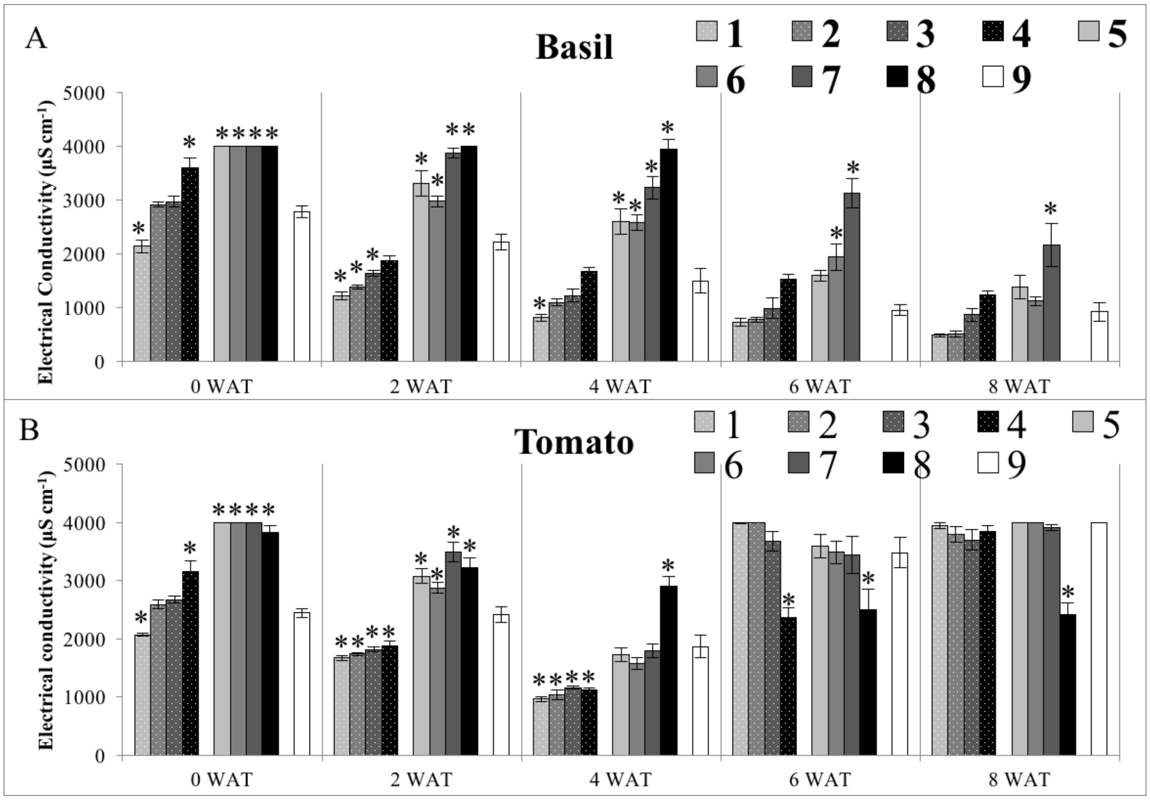


Fig. 3.4. Leachates electrical conductivity (EC) (mean ± standard error) of mixes of biochar (BC) with either composted chicken manure (CM) or vermicompost (VC) and the control growing basil (A) and tomato (B) at 0, 2, 4, 6 and 8 weeks after transplanting (WAT). The upper limit of the EC meter is 3,999 μS cm^{-1}. Data was analyzed using the upper limit when it exceeded the upper limit and cannot be measured. Treatment 8 does not display due to dead basil plants after 4 WAT. The asterisks indicated significant difference from the control using Dunnett's test at $P < 0.05$. Treatment 1-60BC:5VC, 2-70BC:5VC, 3-80BC:5VC, 4-90BC:5VC, 5-60BC:5CM, 6-70BC:5CM, 7-80BC:5CM, 8-90BC:5CM and 9-control.

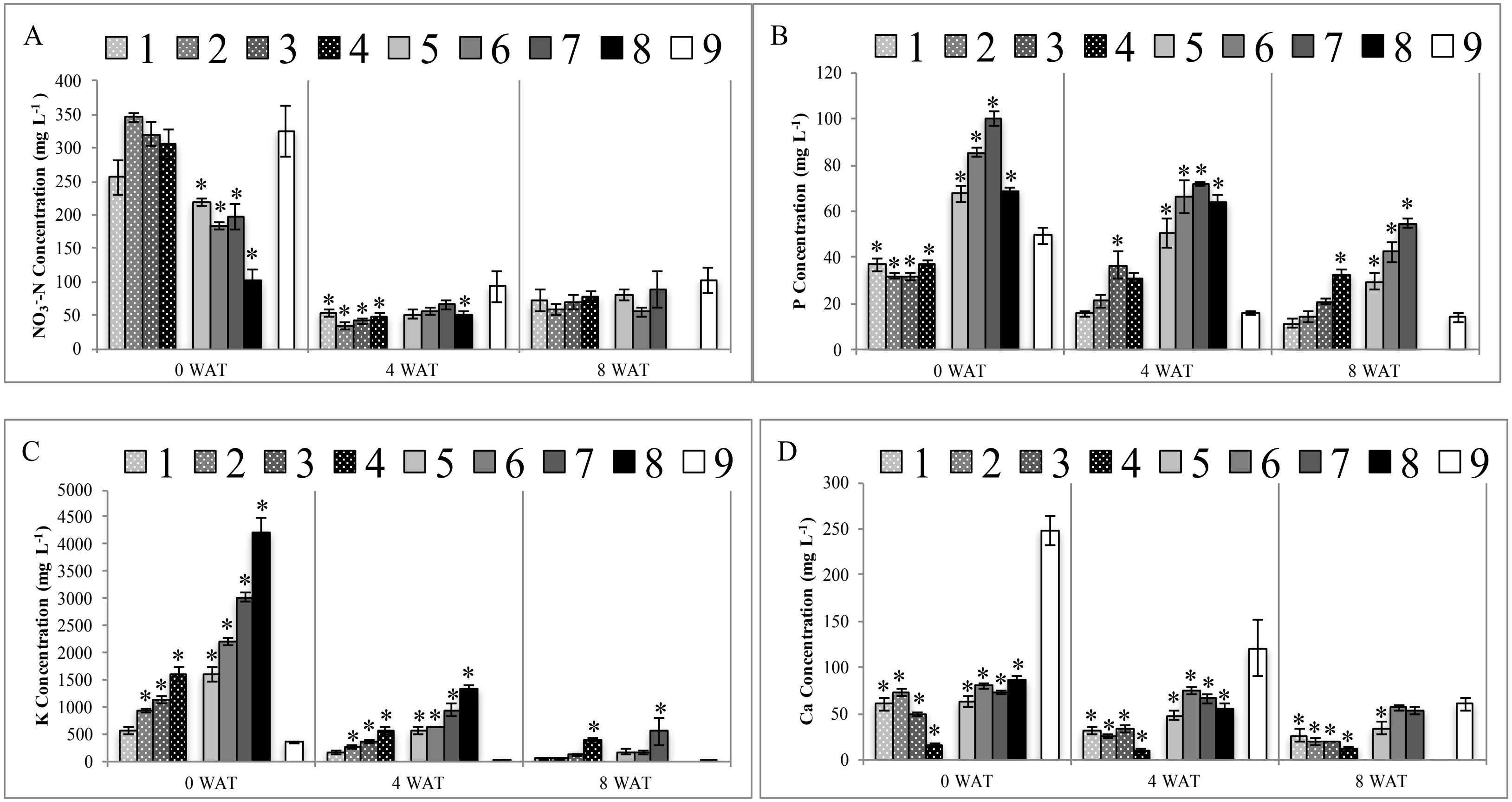


Fig. 3.5. NO_3^--N (A), P (B), K (C), Ca (D), Mg (E), S (F), Fe (G), B (H), Cu (I), Mn (J), Na (K) and Zn (L) concentrations (mean ± standard error) of the 9 substrates growing basil plants at 0, 4 and 8 weeks after transplanting (WAT). Treatment 8 at 8 WAT does not display due to dead basil plants. The asterisks indicated significant difference from the control using Dunnett's test at $P < 0.05$. Treatment 1-60BC:5VC, 2-70BC:5VC, 3-80BC:5VC, 4-90BC:5VC, 5-60BC:5CM, 6-70BC:5CM, 7-80BC:5CM, 8-90BC:5CM and 9-control.

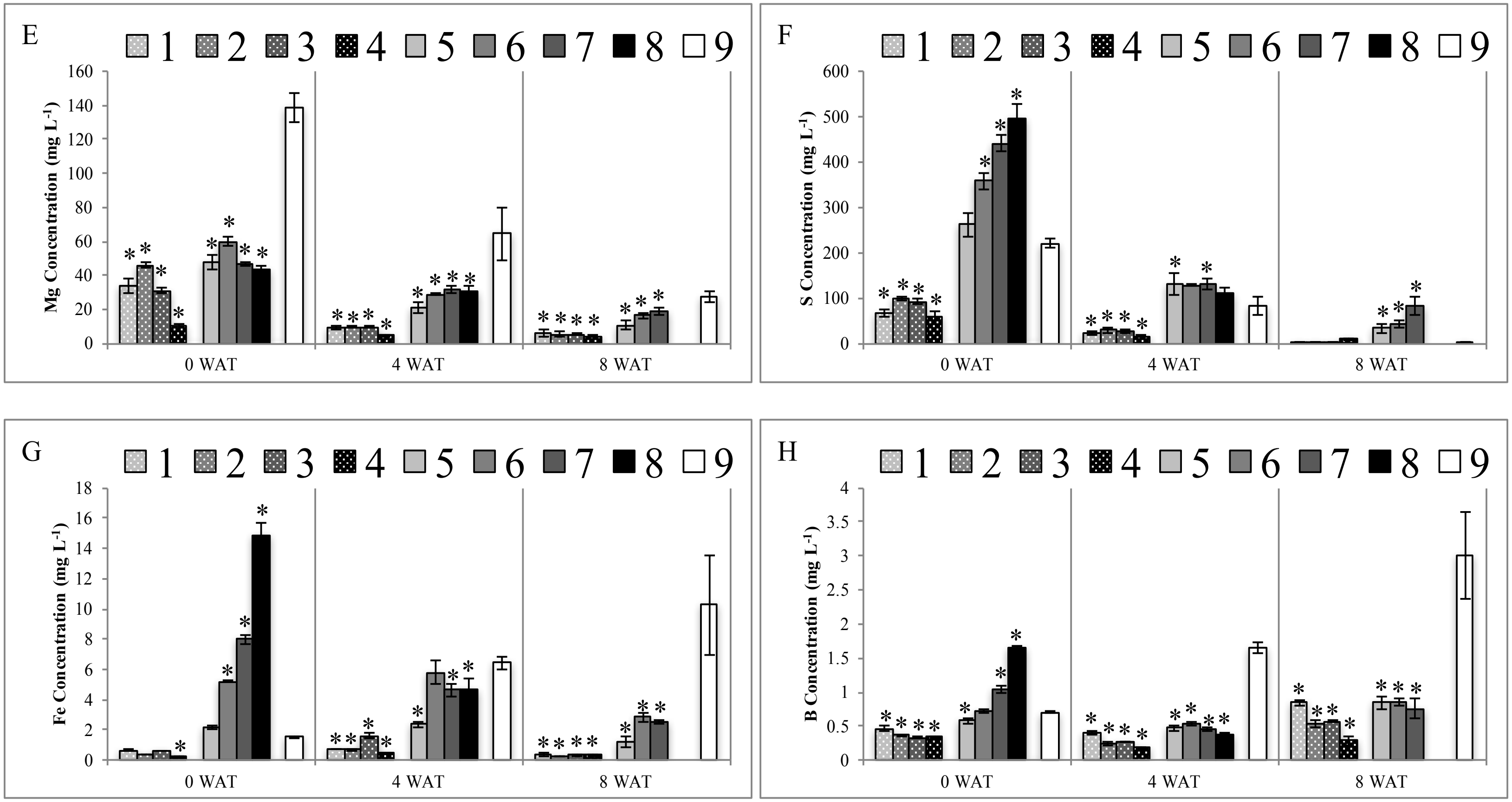
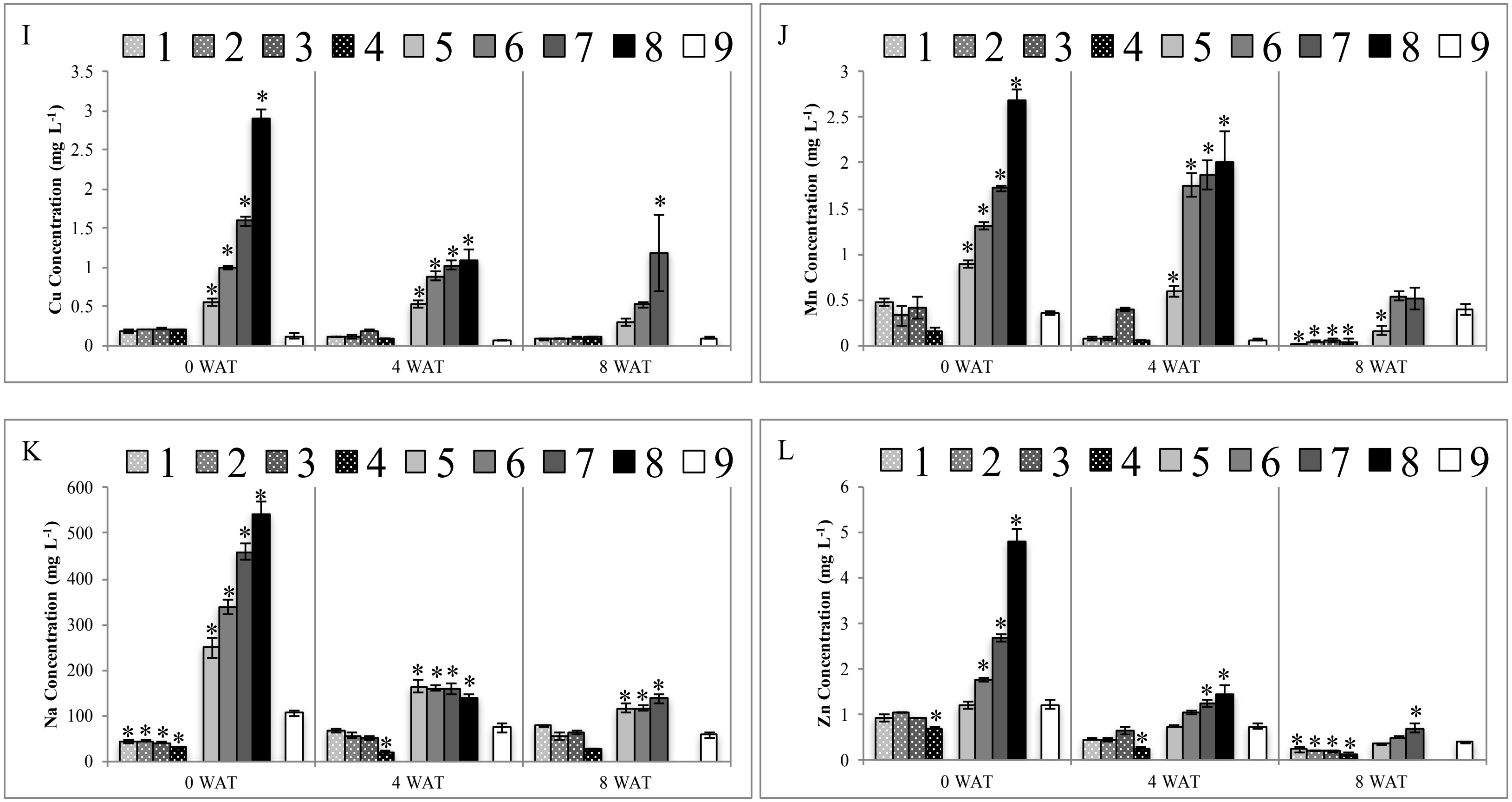


Fig. 3.5 Continued.



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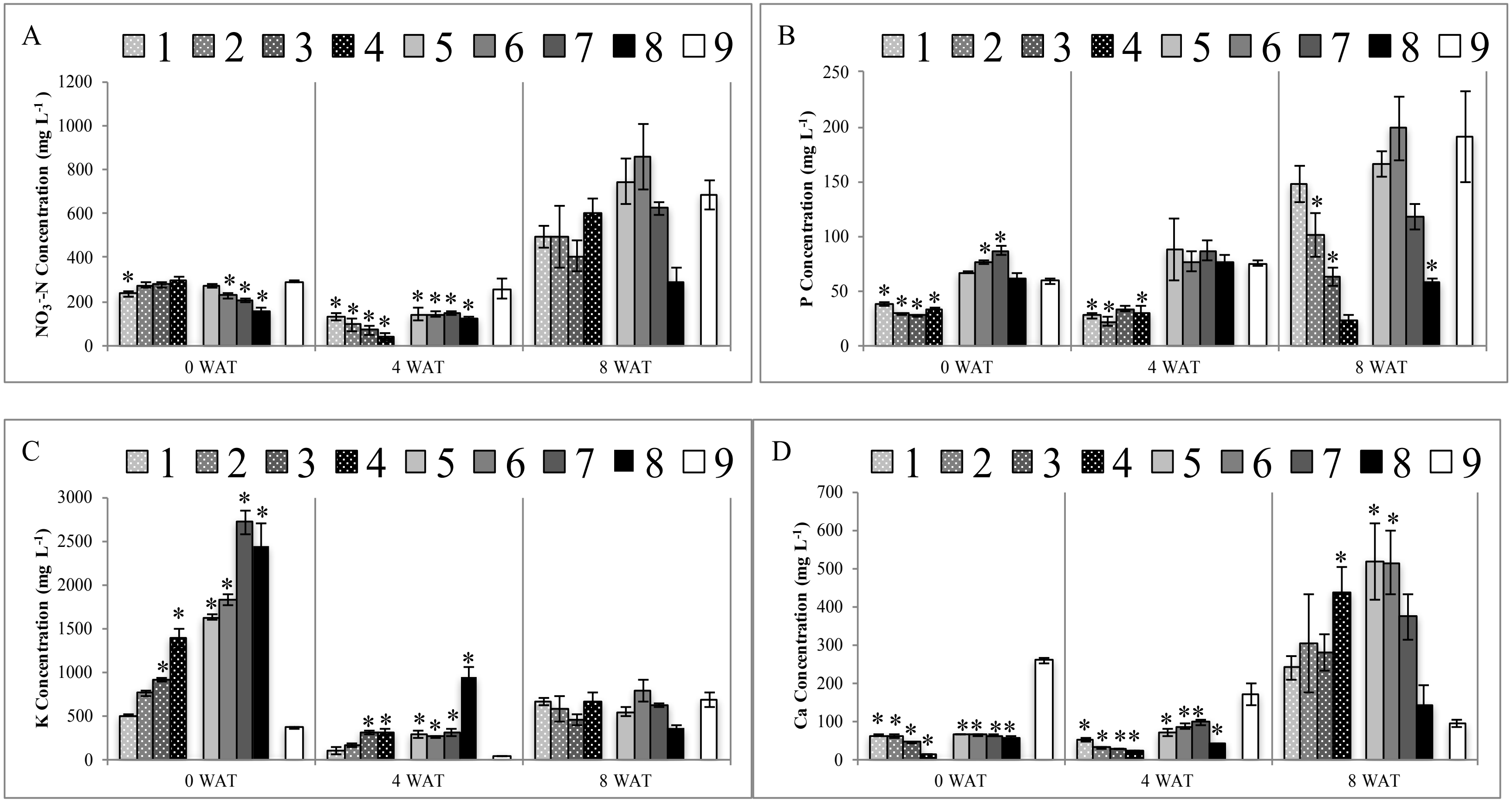
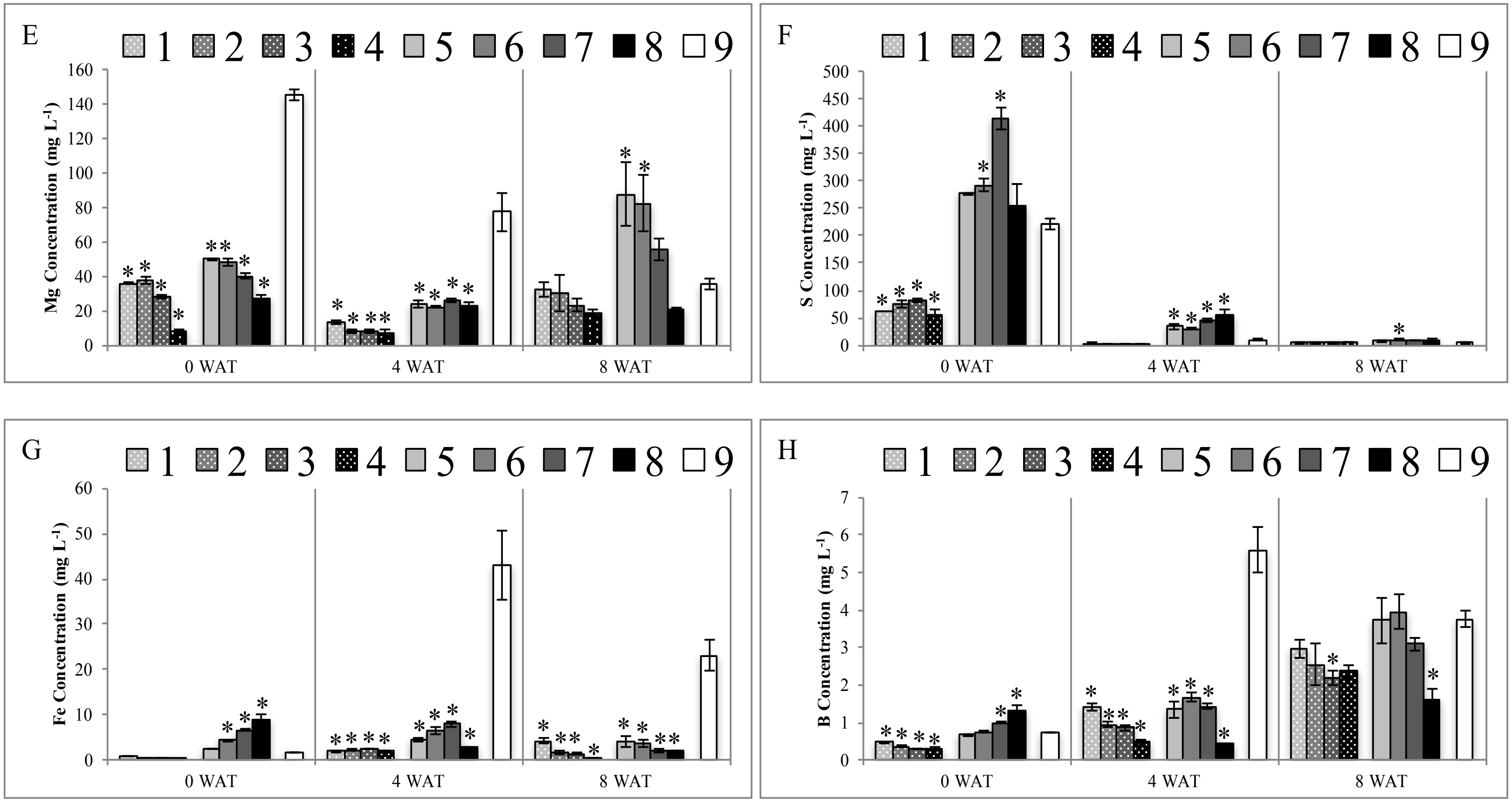


Fig. 3.6. NO$_3^-$-N (A), P (B), K (C), Ca (D), Mg (E), S (F), Fe (G), B (H), Cu (I), Mn (J), Na (K) and Zn (L) concentrations (mean ± standard error) of the 9 substrates growing tomato plants at 0, 4 and 8 weeks after transplanting (WAT). The asterisks indicated significant difference from the control using Dunnett's test at $P < 0.05$. Treatment 1-60BC:5VC, 2-70BC:5VC, 3-80BC:5VC, 4-90BC:5VC, 5-60BC:5CM, 6-70BC:5CM, 7-80BC:5CM, 8-90BC:5CM and 9-control.



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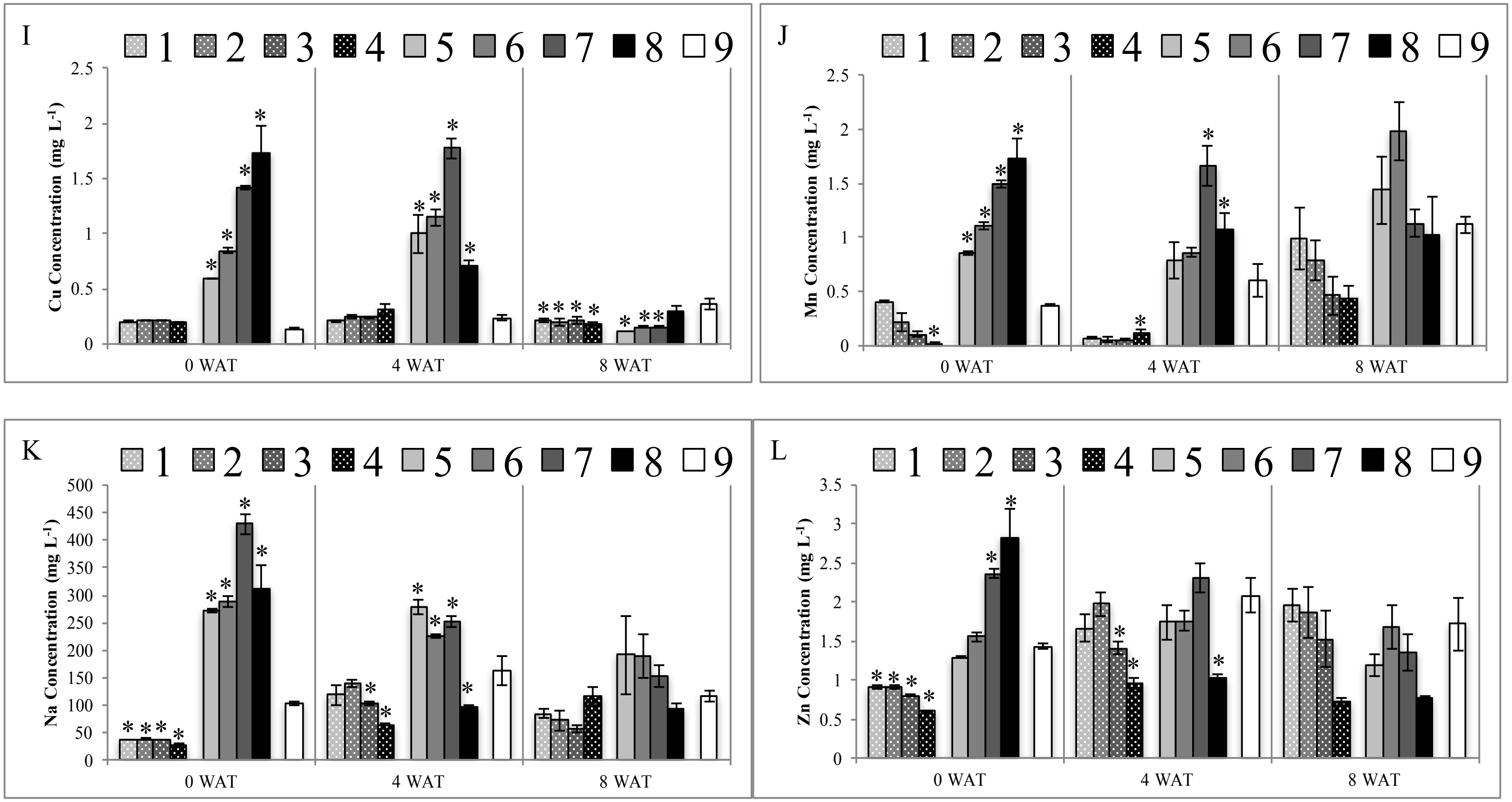

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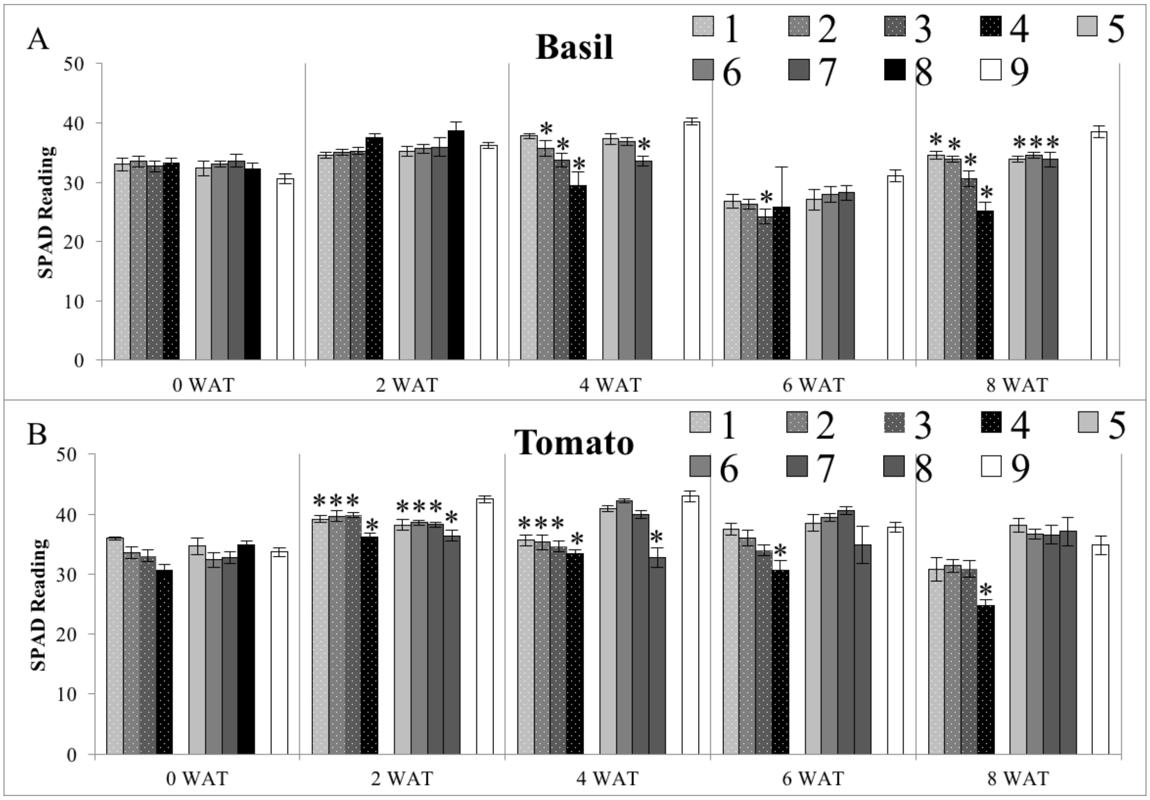

Fig. 3.7. SPAD readings (mean ± standard error) of basil (A) and tomato (B) plants grown in nine substrates including mixes of biochar (BC) with either composted chicken manure (CM) or vermicompost (VC) and the control at 0, 2, 4, 6 and 8 weeks after transplanting (WAT). Treatment 8 does not display due to dead or wilting basil plants starting at 4 WAT. The asterisks indicated significant difference from the control using Dunnett's test at *P* <0.05. Treatment 1-60BC:5VC, 2-70BC:5VC, 3-80BC:5VC, 4-90BC:5VC, 5-60BC:5CM, 6-70BC:5CM, 7-80BC:5CM, 8-90BC:5CM and 9-control.

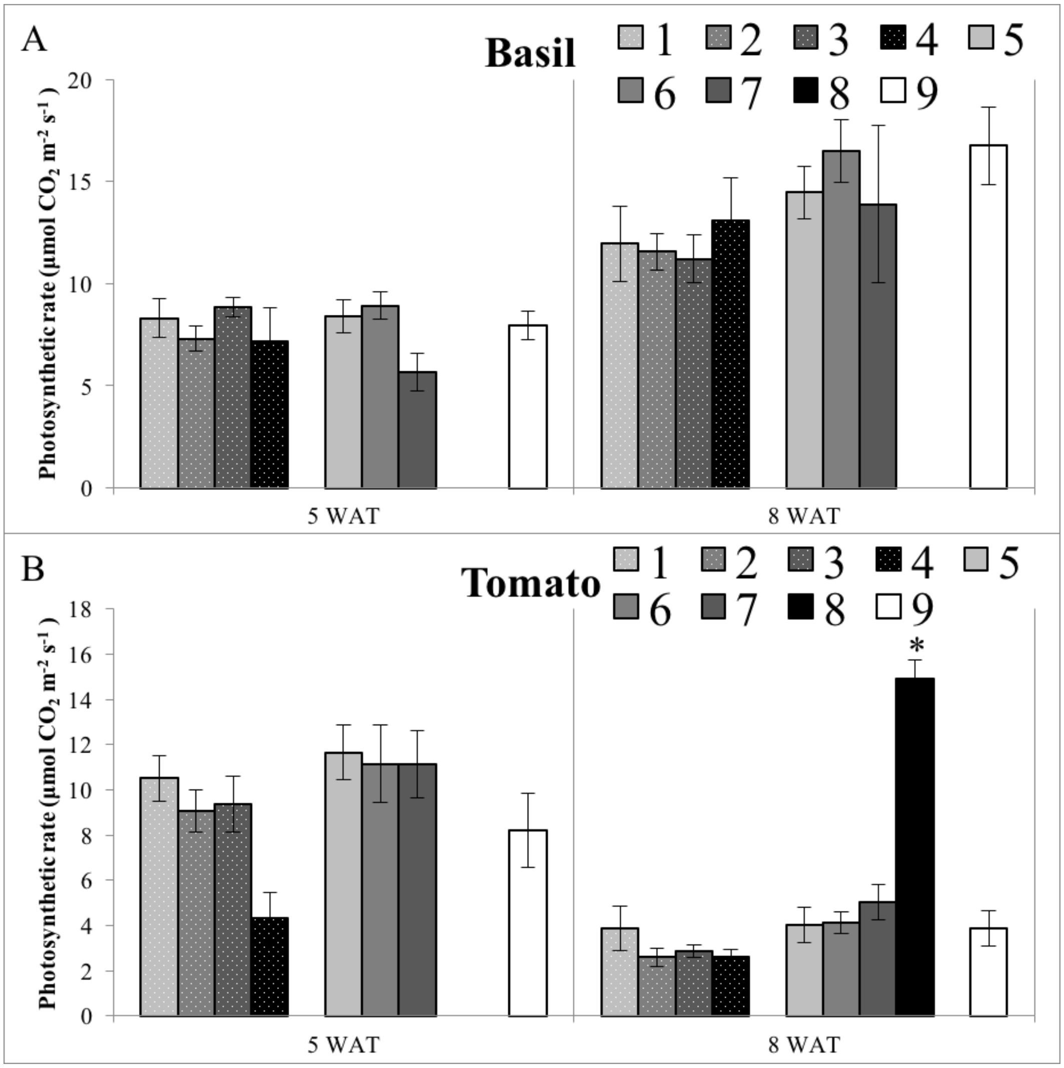

Fig. 3.8. Photosynthetic rates (mean ± standard error) of basil (A) and tomato (B) plants at 5 and 8 weeks after transplanting (WAT). Treatment 8 does not display because of dead plants or wilting leaves too small to have photosynthesis test. The asterisks indicated significant difference from the control using Dunnett's test at *P* <0.05. Treatment 1-60BC:5VC, 2-70BC:5VC, 3-80BC:5VC, 4-90BC:5VC, 5-60BC:5CM, 6-70BC:5CM, 7-80BC:5CM, 8-90BC:5CM and 9-control.

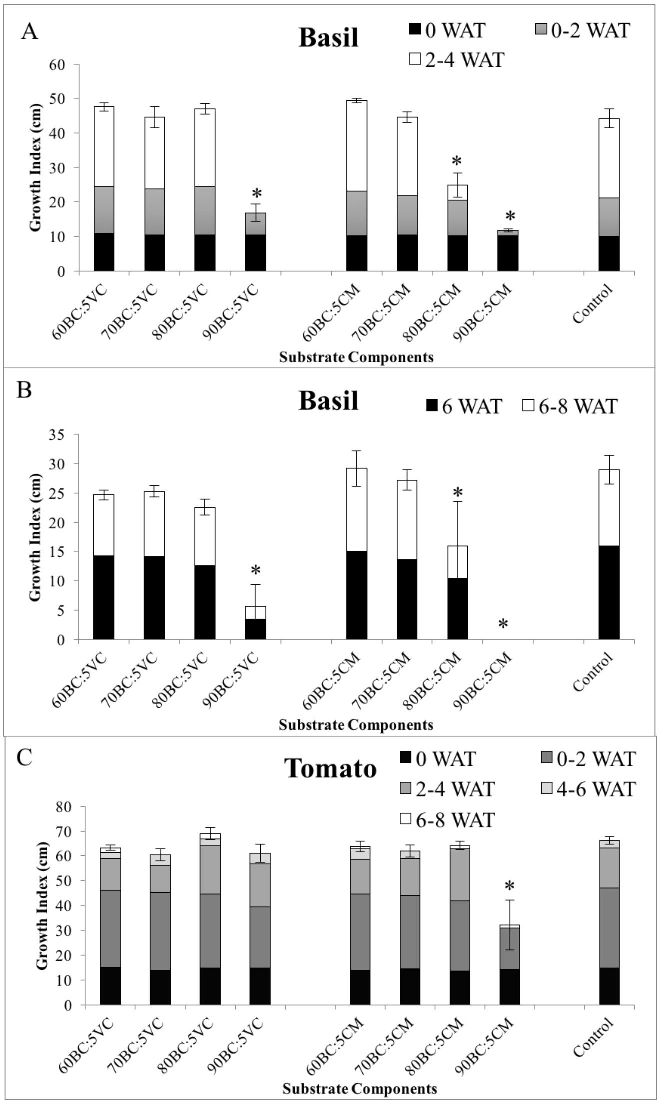

Fig. 3.9. Cumulative growth indexes (mean ± standard error) of basil before (A) and after (B) the first harvest at 5 weeks after transplanting (WAT), and tomato (C) plants grown in substrates formulated by mixing biochar (BC; 60%, 70%, 80% or 90%, by vol.) with either 5% (by vol.) composted chicken manure (CM) or vermicompost (VC) and the control. Basil plants growth indexes in 90B:5CM in Fig. 3.9B does not display due to dead basil plants. The asterisks indicated significant difference of the cumulative growth index from the control using Dunnett's test at $P < 0.05$.

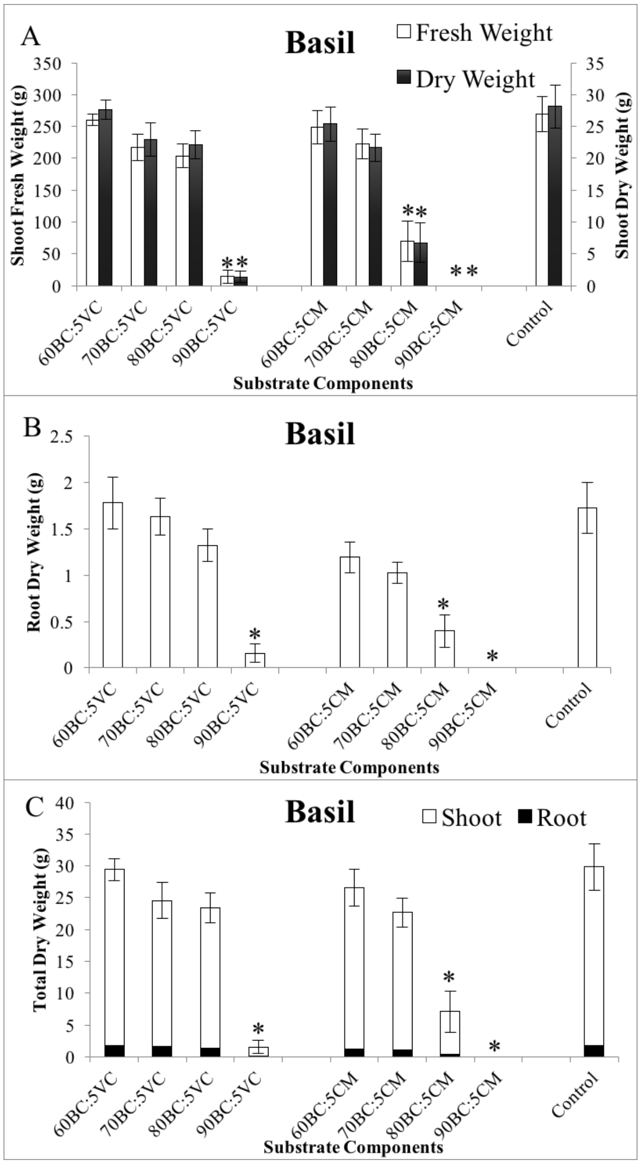

Fig. 3.10. Shoot fresh and dry weight (A) and root (B) and total dry weight (C) (mean ± standard error) of basil plants in eight substrates formulated by mixing biochar (BC; 60%, 70%, 80% or 90%, by vol.) with either 5% (by vol.) composted chicken manure (CM) or vermicompost (VC) and the control. Basil plants in 90BC:5CM does not display due to dead basil plants. The asterisks indicated significant difference from the control using Dunnett's test at *P* <0.05.

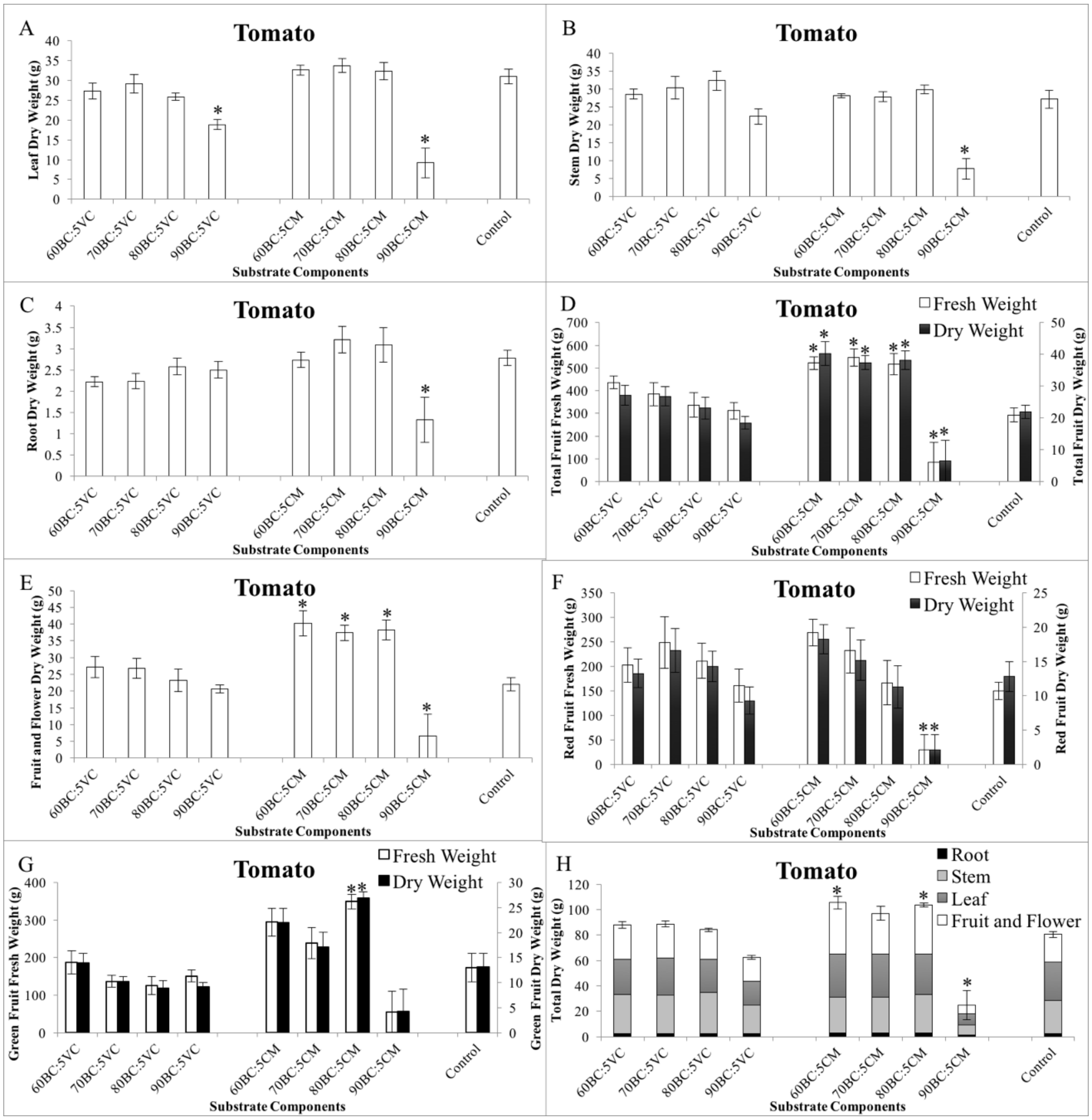

Fig. 3.11. Leaf (A), stem (B), root (C), total fruit FW and DW (D), fruit and flower (E), red fruit fresh weight (FW) and dry weight (DW) (F), green fruit FW and DW (G), and total DW (H) (mean ± standard error) of tomato plants in eight substrates formulated by mixing biochar (BC; 60%, 70%, 80% or 90%, by vol.) with either 5% (by vol.) composted chicken manure (CM) or vermicompost (VC) and the control. The asterisks indicated significant difference from the control using Dunnett's test at $P < 0.05$.

CHAPTER IV

SUMMARY

Biochar (BC), a carbon-rich material mainly made from pyrolysis or gasification of biomass, has shown the potential to be used as replacement for container substrates. Several benefits including ameliorating acidity, improvement of the physical and chemical properties of the substrates, providing nutrients as fertilizers, and improved plant growth after the incorporation of BC have been found in a lot of research studies. However, the effects of BC on container-grown plants vary among feedstock sources, BC production conditions, percentage of BC applied, other substrate components mixed with BC and plant type. There is no universal standard for BC use for all plants. The purpose of this study was to find the optimal combinations of BC with compost to be used as the alternatives to the commercial container substrates.

In the first experiment, we found that all the sixteen mixes with BC (20%, 40%, 60% and 80%, by vol.) and vermicompost (VC; 5%, 10%, 15% and 20%, by vol.) could be used as the alternative container substrates to grow tomato and basil plants. Total porosity (TP) of all the BC:VC mixes (except 80BC:10VC) were similar to the control. The bulk density (BD) of 20BC:10VC, 40BC:10VC, 20BC:15VC, 40BC:15VC, 60BC:15VC, 80BC:15VC and 60BC:20VC were higher than the control. And the TP and BD of all the BC:VC mixes in this experiment were in the acceptable ranges to grow plants in container. High incorporation rate of BC could reduce leachate electrical conductivity (EC). All the 80% BC mixes reduced leachate EC of substrates with basil at 4, 6 and 9 weeks after transplanting (WAT) and leachate EC of substrates with tomato at 4 and 6 WAT due to BC's effective moderating effect on extreme fluctuations of macronutrients. The BC incorporation increased substrate leachate pH except for 20BC:5VC, 20BC:10VC and 20BC:15VC with tomato plants at 9 WAT, indicating the liming effect of the BC. Leaf SPAD readings of tomato plants in 60BC:5VC, 20BC:10VC and 60BC:10VC and all the 80% BC mixes at 9 WAT were reduced due to BC's N-binding effect, while those of other tomato plants and all the basil plants at 2, 4, 6 and 9 WAT were similar to or higher than the control. However, this N-binding effect did not have an effect on the plant growth or dry weight (DW). Growth index (GI), shoot, root and total DW of basil and leaf, stem and total DW of tomato plants grown in all the BC:VC mixes were similar to or higher than the ones in commercial substrates at 9 WAT.

The wholesale price for the peat-based commercial substrates is approximately \$5 per cubic feet. Due to the relatively high cost of the VC (wholesale price: \$17.2 per cubic feet), the lowest VC percentage (5%) from the first experiment was selected for the second experiment. Chicken manure compost (CM) has relatively similar fine texture to VC, and is cheaper (retail price: \$5.5 per cubic feet) and more readily available than VC. Therefore, the incorporation of 5% (by vol.) CM in BC container substrate was also tested to compare to the commercial substrates in the second experiment.

In the second experiment, further evaluation of mixes with high incorporation of BC (60%, 70%, 80%, 90%, by vol.) and either 5% (by vol.) VC or CM with the rest being peat-based commercial substrate was conducted to compare to the container substrates. The TP of all the BC-compost mixes (except 90BC:5VC, 70BC:5CM and 80BC:5CM) were similar to the control. The BD of all the BC-compost mixes were higher than the control. Same as the experiment one, the TPs and BDs of the BC-compost mixes in this research were in the ideal ranges of the corresponding physical properties. At 6 and 8 WAT, leachate EC of all BC-compost mixes with tomato plants was similar to the control, except for 90% BC mixes at 6 WAT and 90BC:5CM at 8 WAT, while leachate EC of all BC-compost mixes with basil was higher than or similar to the control. Similar to the preliminary trial, SPAD readings of basil and tomato plants in all BC-compost mixes were lower than or similar to the control at 8 WAT caused by BC's N-binding effect. The photosynthetic rates of plants in the BC mixes were similar to or higher than the control. At 8 WAT, there was no significant difference on the GI, shoot DW and fresh weight (FW), and root and total DW between the basil plants in BC-compost mixes (except 80BC:5CM, 90BC:5VC and 90BC:5CM) and the control. The GI, stem, root, combined fruit and flower and total DW, and red and total fruit FW and DW of tomato plants in BC-compost mixes (except 90BC:5CM) were similar to or higher than the control. High salinity caused by incorporation of CM could be the reason of the decreased growth of basil in 80BC:5CM and 90BC:5CM due to significant negative correlation between leachate Na concentration at 0 WAT and basil GI at 4 WAT and DW. The leachate NO_3^--N concentration at 0 WAT were positively correlated with basil GI at 4 and 8 WAT, tomato GI at 4 WAT and basil DW, while the leachate K concentration at 0 WAT were negatively correlated with basil and tomato GI at 4 and 8 WAT and basil DW. Based on the results, 5% (by vol.) CM and VC can be mixed with 60% and 70% BC made from pyrolysis of mixed hardwood to grow container basil and tomato.

Since VC is more expensive than CM and higher incorporation rate of BC is preferred for practical use, the recommended treatment should be mixes of 5% CM and 70% BC with the rest being peat-based commercial substrate. And these results are just suitable for these specific BC, VC and CM.

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APPENDIX A

EFFECTS OF BIOCHAR AND VERMICOMPOST ON CONTAINER-GROWN BASIL AND TOMATO PLANTS

Table A-1. Physical properties of the 17 substrates.

	Total porosity (%)	Container Capacity (%)	Air Space (%)	Bulk density (g cm^{-3})
20BC:5VC	69.9	62.4	7.5	0.14
40BC:5VC	66.6	58.9	7.7	0.15
60BC:5VC	70.3	54.5	15.8	0.12
80BC:5VC	76.9	54.0	22.9	0.14
20BC:10VC	74.9	69.4	5.5	0.15
40BC:10VC	73.0	62.8	10.2	0.15
60BC:10VC	72.5	63.1	9.4	0.17
80BC:10VC	60.2	44.8	15.5	0.13
20BC:15VC	71.9	63.3	8.6	0.16
40BC:15VC	73.4	62.6	10.8	0.18
60BC:15VC	69.5	61.0	8.4	0.17
80BC:15VC	70.2	54.2	16.0	0.19
20BC:20VC	67.4	55.9	11.5	0.13
40BC:20VC	66.0	51.4	14.6	0.12
60BC:20VC	73.9	55.6	18.3	0.16
80BC:20VC	78.5	52.9	25.6	0.15
Control	72.1	65.7	6.4	0.12

Table A-2. The leachate pH of the 17 substrates with basil and tomato plants at 0, 2, 4, 6 and 9 weeks after transplanting (WAT).

	pH				
	0 WAT	2 WAT	4 WAT	6 WAT	9 WAT
			Basil		
20BC:5VC	6.58	6.70	6.49	6.16	5.78
40BC:5VC	7.47	7.69	7.43	7.18	6.56
60BC:5VC	8.08	8.28	8.18	7.81	7.14
80BC:5VC	8.60	8.53	8.59	8.12	7.46
20BC:10VC	6.64	6.78	6.67	6.43	6.01
40BC:10VC	7.23	7.33	7.50	7.26	6.76
60BC:10VC	7.70	7.59	7.97	7.51	7.02
80BC:10VC	8.15	8.17	8.47	8.13	7.46
20BC:15VC	6.24	6.52	6.48	6.31	5.95
40BC:15VC	6.89	7.40	7.33	6.98	6.66
60BC:15VC	7.26	7.90	7.86	7.49	6.92
80BC:15VC	8.17	8.39	8.49	8.00	7.44
20BC:20VC	6.29	6.71	6.54	6.30	5.76
40BC:20VC	7.03	7.25	7.36	6.94	6.45
60BC:20VC	7.34	7.68	7.89	7.44	7.01
80BC:20VC	7.82	8.14	8.49	8.05	7.56
Control	5.85	5.83	5.54	5.31	4.85
			Tomato		
20BC:5VC	6.58	6.57	6.59	5.66	4.80
40BC:5VC	7.47	7.70	7.40	6.51	5.20
60BC:5VC	8.08	8.40	7.90	7.08	5.69
80BC:5VC	8.60	8.71	8.50	7.35	6.43
20BC:10VC	6.64	6.65	6.66	5.65	4.82
40BC:10VC	7.23	7.34	7.55	6.68	5.45
60BC:10VC	7.70	7.61	7.82	6.94	5.86
80BC:10VC	8.15	8.27	8.25	7.54	6.60
20BC:15VC	6.24	6.52	6.47	5.69	4.89
40BC:15VC	6.89	7.33	7.26	6.44	5.65
60BC:15VC	7.26	7.87	7.64	6.96	5.83

Table A-2. Continued

	pH				
	0 WAT	2 WAT	4 WAT	6 WAT	9 WAT
			Tomato		
80BC:15VC	8.17	8.48	8.19	7.34	6.29
20BC:20VC	6.29	6.76	6.45	5.84	5.22
40BC:20VC	7.03	7.21	7.26	6.43	5.43
60BC:20VC	7.34	7.62	7.64	7.08	6.10
80BC:20VC	7.82	8.23	8.23	7.52	6.65
Control	5.85	5.70	5.32	4.85	4.70

Table A-3. Leachate electrical conductivity of the 17 substrates with basil and tomato plants at 0, 2, 4, 6 and 9 weeks after transplanting (WAT).

	Electrical Conductivity (µS cm^{-1})				
	0 WAT	2 WAT	4 WAT	6 WAT	9 WAT
			Basil		
20BC:5VC	3167.50	3014.17	3458.67	3771.83	3867.17
40BC:5VC	2122.33	2429.50	2400.83	2825.00	3106.83
60BC:5VC	2049.00	2218.83	2219.67	2591.83	2417.83
80BC:5VC	1814.67	2337.00	2337.33	2188.67	2005.67
20BC:10VC	3634.67	3169.50	3486.67	3424.67	3429.50
40BC:10VC	2707.17	2909.50	2911.67	3128.33	3234.00
60BC:10VC	2193.67	2857.33	2663.17	2924.67	2426.17
80BC:10VC	2157.33	2732.33	2507.17	2616.83	2253.17
20BC:15VC	3865.83	3457.67	3252.17	3515.67	3129.50
40BC:15VC	3717.83	3009.00	2786.00	3026.33	2642.83
60BC:15VC	3316.83	2735.50	2654.83	2678.33	2846.83
80BC:15VC	2844.17	2839.33	2660.17	2662.83	2501.67
20BC:20VC	3999.00	3288.50	3153.00	3436.83	3438.33
40BC:20VC	3999.00	3807.00	3216.17	3555.00	3328.17
60BC:20VC	3994.20	3605.67	2908.83	2904.50	2828.83
80BC:20VC	3966.80	3065.83	2707.17	2836.33	2286.00
Control	2989.17	2578.67	3293.17	3999.00	3999.00
			Tomato		
20BC:5VC	3167.50	2914.17	3646.83	3999.00	3799.67
40BC:5VC	2122.33	2239.33	3199.33	3846.50	3999.00
60BC:5VC	2049.00	2109.67	3135.33	3267.00	3999.00
80BC:5VC	1814.67	2317.00	3081.00	3082.50	3398.17
20BC:10VC	3634.67	3023.67	3747.83	3999.00	3999.00
40BC:10VC	2707.17	2735.33	3355.00	3863.67	3999.00
60BC:10VC	2193.67	2663.50	3140.33	3664.50	3775.67
80BC:10VC	2157.33	2661.17	3257.17	3207.33	3767.80
20BC:15VC	3865.83	3303.67	3812.17	3999.00	3999.00
40BC:15VC	3717.83	3267.67	3576.33	3999.00	3994.60
60BC:15VC	3316.83	2574.17	3204.33	3604.83	3539.17

Table A-3. Continued

	Electrical Conductivity ($\mu S\ cm^{-1}$)				
	0 WAT	2 WAT	4 WAT	6 WAT	9 WAT
			Tomato		
80BC:15VC	2844.17	2799.00	2760.00	2869.67	3120.00
20BC:20VC	3999.00	3021.17	3666.17	3805.00	3999.00
40BC:20VC	3999.00	3532.00	3835.50	3999.00	3999.00
60BC:20VC	3994.20	3239.83	3514.67	3571.83	3792.83
80BC:20VC	3966.80	3110.20	3323.00	3139.50	3610.83
Control	2989.17	3000.50	3907.17	3999.00	3999.00

Table A-4. The SPAD reading of basil and tomato leaves at 2, 4, 6 and 9 weeks after transplanting (WAT).

	SPAD Readings			
	2 WAT	4 WAT	6 WAT	9 WAT
		Basil		
20BC:5VC	29.6	28.5	28.5	31.6
40BC:5VC	32.4	29.2	29.9	34.0
60BC:5VC	31.7	28.5	28.8	33.3
80BC:5VC	30.1	28.6	28.0	31.3
20BC:10VC	32.2	28.5	30.0	32.7
40BC:10VC	31.8	29.2	29.6	33.4
60BC:10VC	29.7	29.9	30.7	33.8
80BC:10VC	31.2	30.0	29.4	31.5
20BC:15VC	28.6	27.2	30.9	34.6
40BC:15VC	26.7	26.6	27.5	31.6
60BC:15VC	27.9	26.6	29.0	33.1
80BC:15VC	32.0	30.3	29.4	31.8
20BC:20VC	28.9	28.1	29.4	32.1
40BC:20VC	27.1	27.6	28.8	30.4
60BC:20VC	26.8	26.5	27.5	32.5
80BC:20VC	28.0	27.6	29.0	31.4
Control	27.6	28.9	28.9	31.1
		Tomato		
20BC:5VC	27.7	32.4	37.0	38.3
40BC:5VC	28.1	32.6	37.5	37.8
60BC:5VC	29.0	33.4	36.5	35.2
80BC:5VC	29.3	34.6	34.9	34.1
20BC:10VC	28.7	33.3	37.1	35.5
40BC:10VC	27.7	33.1	35.7	38.3
60BC:10VC	27.7	33.4	35.7	35.0
80BC:10VC	27.1	32.8	34.5	35.0
20BC:15VC	27.8	33.0	36.6	37.5
40BC:15VC	27.6	32.5	37.2	37.2
60BC:15VC	26.6	33.1	35.0	35.9

Table A-4. Continued

	SPAD Readings			
	2 WAT	4 WAT	6 WAT	9 WAT
		Tomato		
80BC:15VC	26.3	34.0	35.6	34.4
20BC:20VC	26.8	33.1	36.1	39.3
40BC:20VC	26.6	33.2	36.9	36.3
60BC:20VC	26.4	33.0	34.7	36.3
80BC:20VC	25.4	32.8	34.0	34.3
Control	25.7	34.2	36.4	39.4

Table A-5. Growth index of basil and tomato plants at 0, 2, 4, 6 and 9 weeks after transplanting (WAT).

	Growth index (cm)				
	0 WAT	2 WAT	4 WAT	6 WAT	9 WAT
			Basil		
20BC:5VC	3.4	8.7	13.6	20.6	38.3
40BC:5VC	3.4	7.5	13.3	21.6	38.3
60BC:5VC	3.0	7.5	12.7	19.6	38.2
80BC:5VC	3.8	8.5	14.4	21.5	37.9
20BC:10VC	3.6	8.2	13.3	20.7	38.9
40BC:10VC	3.4	7.6	12.6	20.3	39.5
60BC:10VC	3.4	8.9	14.4	22.3	41.4
80BC:10VC	3.3	7.3	12.9	19.1	37.4
20BC:15VC	3.4	8.9	14.2	21.5	40.0
40BC:15VC	3.6	8.6	15.9	24.0	39.8
60BC:15VC	3.4	9.1	16.9	24.3	37.7
80BC:15VC	3.0	7.5	12.5	20.8	37.6
20BC:20VC	3.0	8.0	13.6	20.8	39.0
40BC:20VC	3.3	9.7	16.2	25.6	43.1
60BC:20VC	3.3	8.7	16.3	24.6	37.9
80BC:20VC	3.3	7.7	12.9	19.1	37.2
Control	3.1	9.2	13.6	21.1	35.9
			Tomato		
20BC:5VC	5.4	17.6	37.5	55.8	64.1
40BC:5VC	5.5	17.5	36.7	58.1	63.8
60BC:5VC	5.6	17.8	35.4	54.3	64.2
80BC:5VC	5.4	16.8	33.4	53.1	64.2
20BC:10VC	5.7	19.1	39.2	55.3	67.1
40BC:10VC	5.4	18.7	38.0	59.3	61.0
60BC:10VC	5.2	18.1	37.9	56.0	64.5
80BC:10VC	5.2	17.6	36.1	53.0	62.5
20BC:15VC	5.2	17.8	38.6	57.4	68.0
40BC:15VC	5.2	18.0	39.1	57.6	63.7
60BC:15VC	4.8	16.9	38.1	55.2	66.6

Table A-5. Continued

	Growth index (cm)				
	0 WAT	2 WAT	4 WAT	6 WAT	9 WAT
			Tomato		
80BC:15VC	5.4	17.7	34.4	52.7	62.7
20BC:20VC	5.4	17.6	39.8	56.4	60.8
40BC:20VC	5.1	17.9	37.2	53.8	65.9
60BC:20VC	5.0	16.7	37.2	55.1	63.2
80BC:20VC	4.6	16.1	33.5	51.0	66.1
Control	5.3	17.4	37.2	53.4	59.9

Table A-6. Basil dry weight at 9 WAT.

	Basil Dry Weight (g)		
	Shoot	Root	Total
20BC:5VC	8.32	0.98	9.31
40BC:5VC	8.07	1.13	9.20
60BC:5VC	7.60	1.15	8.76
80BC:5VC	7.43	1.04	8.47
20BC:10VC	7.43	0.91	8.34
40BC:10VC	6.93	0.83	7.75
60BC:10VC	8.64	1.01	9.66
80BC:10VC	6.44	0.86	7.30
20BC:15VC	9.18	1.13	10.31
40BC:15VC	9.64	1.07	10.71
60BC:15VC	9.43	1.30	10.72
80BC:15VC	6.76	0.97	7.73
20BC:20VC	8.07	0.98	9.05
40BC:20VC	9.80	1.08	10.88
60BC:20VC	8.23	0.92	9.15
80BC:20VC	5.86	0.77	6.63
Control	6.12	0.63	6.74

Table A-7. Tomato dry weight at 9 WAT.

	Tomato Dry Weight (g)				
	Stem	Leaf	Fruit and flower	Root	Total
20BC:5VC	6.52	15.15	2.98	1.19	25.84
40BC:5VC	5.95	16.13	3.38	1.18	26.64
60BC:5VC	6.38	14.67	4.12	1.16	26.33
80BC:5VC	6.22	13.87	2.62	1.31	24.02
20BC:10VC	5.97	15.52	4.42	1.25	27.15
40BC:10VC	5.44	14.09	3.96	0.99	24.48
60BC:10VC	5.52	13.29	3.92	1.14	23.88
80BC:10VC	5.74	13.08	3.93	1.36	24.12
20BC:15VC	5.67	15.28	4.00	1.15	26.11
40BC:15VC	6.50	15.14	4.04	1.46	27.14
60BC:15VC	6.15	15.01	4.01	1.14	26.30
80BC:15VC	5.79	13.04	3.76	1.11	23.70
20BC:20VC	5.72	14.59	3.29	1.43	25.02
40BC:20VC	7.06	14.71	3.53	1.36	26.67
60BC:20VC	5.90	14.52	4.62	1.36	26.39
80BC:20VC	6.53	12.30	2.27	1.17	22.27
Control	5.59	12.20	2.82	1.62	22.23

APPENDIX B

EFFECTS OF HIGH PERCENTAGE OF BIOCHAR AND TWO COMPOSTS ON CONTAINER-GROWN BASIL AND TOMATO PLANTS

Table B-1. Physical properties of the 9 substrates.

	Total porosity (%)	Container Capacity (%)	Air Space (%)	Bulk density (g cm^{-3})
60BC:5VC	67.5	54.3	13.2	0.18
70BC:5VC	67.4	53.6	13.9	0.17
80BC:5VC	67.0	53.0	14.1	0.18
90BC:5VC	62.9	46.3	16.6	0.16
60BC:5CM	64.0	53.8	10.2	0.17
70BC:5CM	62.0	48.5	13.5	0.17
80BC:5CM	60.1	47.9	12.2	0.18
90BC:5CM	65.1	47.2	17.9	0.19
Control	74.0	70.7	3.3	0.10

Table B-2. The leachate pH of the 9 substrates with basil and tomato plants at 0, 2, 4, 6 and 8 weeks after transplanting (WAT).

	pH				
	0 WAT	2 WAT	4 WAT	6 WAT	8 WAT
			Basil		
60BC:5VC	7.23	7.91	7.33	6.96	7.10
70BC:5VC	7.72	8.35	7.69	7.28	7.44
80BC:5VC	7.87	8.63	7.94	7.41	7.76
90BC:5VC	8.59	9.13	8.84	8.16	8.41
60BC:5CM	7.51	8.20	7.72	7.22	7.48
70BC:5CM	7.70	8.31	7.84	7.35	7.60
80BC:5CM	8.00	8.47	8.33	7.70	7.97
90BC:5CM	8.79	9.26	9.18		
Control	5.36	5.15	5.42	4.85	4.57
			Tomato		
60BC:5VC	7.15	7.45	7.01	5.36	5.40
70BC:5VC	7.64	7.80	7.53	5.70	5.46
80BC:5VC	7.86	8.06	7.66	6.25	5.76
90BC:5VC	8.68	8.47	7.64	7.19	6.28
60BC:5CM	7.68	7.93	7.50	5.59	5.23
70BC:5CM	7.86	7.96	7.40	5.69	5.23
80BC:5CM	8.26	8.31	7.64	5.94	5.48
90BC:5CM	9.12	8.82	8.46	7.98	6.30
Control	5.16	4.96	4.96	4.70	4.89

Table B-3. Leachate electrical conductivity of the 9 substrates with basil and tomato plants at 0, 2, 4, 6 and 8 weeks after transplanting (WAT).

	Electrical Conductivity (us cm^{-1})				
	0 WAT	2 WAT	4 WAT	6 WAT	8 WAT
			Basil		
60BC:5VC	2138.71	1221.57	814.29	727.43	486.67
70BC:5VC	2918.29	1388.43	1100.00	778.17	509.50
80BC:5VC	2970.71	1635.57	1228.57	992.33	866.86
90BC:5VC	3602.00	1870.57	1671.43	1537.60	1232.00
60BC:5CM	3999.00	3312.57	2600.00	1593.83	1386.86
70BC:5CM	3999.00	2974.71	2585.71	1940.14	1123.00
80BC:5CM	3999.00	3873.86	3228.57	3125.14	2172.60
90BC:5CM	3999.00	3999.00	3950.00		
Control	2787.71	2219.71	1500.00	955.17	920.57
			Tomato		
60BC:5VC	2071.71	1671.00	966.67	3989.00	3947.50
70BC:5VC	2593.71	1742.14	1042.86	3999.00	3793.33
80BC:5VC	2675.57	1819.00	1166.67	3680.43	3698.00
90BC:5VC	3155.43	1884.00	1133.33	2373.14	3846.71
60BC:5CM	3999.00	3077.71	1728.57	3592.14	3999.00
70BC:5CM	3999.00	2876.29	1571.43	3484.43	3999.00
80BC:5CM	3999.00	3493.00	1800.00	3440.71	3910.86
90BC:5CM	3829.43	3225.57	2900.00	2500.67	2414.20
Control	2444.86	2412.33	1871.43	3483.00	3999.00

Table B-4. Leachate nutrient concentrations in the 9 substrates with basil and tomato at 0 week after transplanting.

	NO_3-N	P	K	Ca	Mg	S	Fe	B	Cu	Mn	Na	Zn
	(ppm)											
	Basil											
60BC:5VC	257	37	562	61	34	67	0.7	0.5	0.2	0.5	42	0.9
70BC:5VC	345	32	939	73	46	99	0.4	0.4	0.2	0.3	45	1.1
80BC:5VC	321	32	1129	49	31	93	0.6	0.3	0.2	0.4	41	0.9
90BC:5VC	306	37	1621	15	10	60	0.2	0.3	0.2	0.2	31	0.7
60BC:5CM	220	68	1605	63	48	262	2.2	0.6	0.6	0.9	249	1.2
70BC:5CM	183	86	2210	80	60	359	5.2	0.7	1.0	1.3	339	1.8
80BC:5CM	197	100	3023	72	46	441	8.0	1.0	1.6	1.7	460	2.7
90BC:5CM	102	69	4219	86	43	494	14.8	1.7	2.9	2.7	541	4.8
Control	324	50	359	248	139	221	1.5	0.7	0.1	0.4	105	1.2
	Tomato											
60BC:5VC	237	38	503	64	36	61	0.9	0.5	0.2	0.4	39	0.9
70BC:5VC	275	30	758	62	38	77	0.5	0.4	0.2	0.2	39	0.9
80BC:5VC	277	28	917	46	28	81	0.6	0.3	0.2	0.1	38	0.8
90BC:5VC	294	33	1382	13	9	54	0.1	0.3	0.2	0.0	28	0.6
60BC:5CM	273	67	1624	66	50	277	2.5	0.7	0.6	0.9	272	1.3
70BC:5CM	227	76	1821	66	48	292	4.3	0.8	0.8	1.1	289	1.6
80BC:5CM	203	87	2716	63	40	414	6.7	1.0	1.4	1.5	430	2.4
90BC:5CM	155	61	2430	56	27	255	8.8	1.3	1.7	1.7	311	2.8
Control	292	59	370	260	145	223	1.7	0.7	0.1	0.4	104	1.4

Table B-5. Leachate nutrient concentrations in the 9 substrates with basil and tomato at 4 weeks after transplanting.

	NO_3-N	P	K	Ca	Mg	S	Fe	B	Cu	Mn	Na	Zn
							(ppm)					
Basil												
60BC:5VC	53	16	161	32	9	24	0.7	0.4	0.1	0.1	68	0.5
70BC:5VC	35	21	275	26	9	29	0.7	0.2	0.1	0.1	57	0.5
80BC:5VC	42	36	369	34	10	27	1.6	0.3	0.2	0.4	51	0.6
90BC:5VC	48	31	569	10	5	16	0.5	0.2	0.1	0.1	21	0.3
60BC:5CM	51	50	567	48	21	131	2.4	0.5	0.5	0.6	165	0.8
70BC:5CM	56	66	634	75	29	128	5.8	0.5	0.9	1.8	161	1.1
80BC:5CM	66	72	940	66	31	130	4.6	0.5	1.0	1.9	159	1.3
90BC:5CM	51	64	1326	54	31	112	4.7	0.4	1.1	2.0	137	1.5
Control	93	16	20	121	65	81	6.4	1.7	0.1	0.1	73	0.7
Tomato												
60BC:5VC	132	28	106	52	14	3	1.9	1.4	0.2	0.1	120	1.7
70BC:5VC	96	22	170	31	8	3	2.2	0.9	0.2	0.1	140	2.0
80BC:5VC	70	34	303	27	8	2	2.4	0.9	0.2	0.1	104	1.4
90BC:5VC	42	30	314	22	7	1	1.9	0.5	0.3	0.1	63	1.0
60BC:5CM	144	88	293	72	24	34	4.4	1.4	1.0	0.8	280	1.7
70BC:5CM	141	77	259	86	22	29	6.5	1.7	1.1	0.9	226	1.8
80BC:5CM	149	87	313	98	26	45	8.0	1.4	1.8	1.7	253	2.3
90BC:5CM	121	77	940	41	23	57	2.7	0.4	0.7	1.1	96	1.0
Control	258	75	44	171	77	9	42.9	5.6	0.2	0.6	163	2.1

Table B-6. Leachate nutrient concentrations in the 9 substrates with basil and tomato at 8 weeks after transplanting.

	NO_3-N	P	K	Ca	Mg	S	Fe	B	Cu	Mn	Na	Zn
							(ppm)					
							Basil					
60BC:5VC	72	12	58	26	6	2	0.4	0.8	0.1	0.0	77	0.2
70BC:5VC	59	14	64	20	5	1	0.3	0.5	0.1	0.0	55	0.2
80BC:5VC	69	21	125	20	5	2	0.3	0.6	0.1	0.1	63	0.2
90BC:5VC	77	32	401	13	4	10	0.4	0.3	0.1	0.0	29	0.2
60BC:5CM	80	30	182	34	11	33	1.2	0.9	0.3	0.2	116	0.4
70BC:5CM	55	43	171	56	17	43	2.9	0.9	0.5	0.5	117	0.5
80BC:5CM	88	55	560	54	19	83	2.5	0.8	1.2	0.5	137	0.7
90BC:5CM												
Control	102	14	20	61	27	4	10.3	3.0	0.1	0.4	57	0.4
							Tomato					
60BC:5VC	492	148	663	240	33	4	4.3	3.0	0.2	1.0	85	2.0
70BC:5VC	496	100	585	303	30	4	1.7	2.5	0.2	0.8	73	1.9
80BC:5VC	408	63	453	281	23	4	1.5	2.2	0.2	0.5	57	1.5
90BC:5VC	604	24	674	437	19	4	0.6	2.4	0.2	0.4	118	0.7
60BC:5CM	746	166	548	518	88	8	4.2	3.7	0.1	1.4	192	1.2
70BC:5CM	857	199	795	514	82	9	3.5	4.0	0.2	2.0	190	1.7
80BC:5CM	623	117	621	373	56	8	2.0	3.1	0.2	1.1	153	1.4
90BC:5CM	290	59	366	141	21	9	2.0	1.6	0.3	1.0	95	0.8
Control	682	191	679	94	36	4	23.1	3.8	0.4	1.1	116	1.7

Table B-7. The SPAD reading of basil and tomato leaves at 2, 4, 6 and 8 weeks after transplanting (WAT).

	SPAD Readings			
	2 WAT	4 WAT	6 WAT	8 WAT
		Basil		
60BC:5VC	33.04	34.59	37.77	26.86
70BC:5VC	33.51	34.99	35.76	26.26
80BC:5VC	32.67	35.27	33.71	24.16
90BC:5VC	33.29	37.60	29.50	25.75
60BC:5CM	32.33	35.24	37.34	27.06
70BC:5CM	33.07	35.67	36.89	27.96
80BC:5CM	33.63	35.93	33.50	28.20
90BC:5CM	32.20	38.74		
Control	30.63	36.24	40.23	31.14
		Tomato		
60BC:5VC	35.93	39.19	35.63	37.50
70BC:5VC	33.59	39.69	35.33	36.00
80BC:5VC	33.07	39.83	34.64	33.93
90BC:5VC	30.70	36.24	33.36	30.64
60BC:5CM	34.63	38.20	40.86	38.50
70BC:5CM	32.39	38.59	42.17	39.47
80BC:5CM	32.69	38.23	39.99	40.63
90BC:5CM	34.88	36.39	32.81	34.82
Control	33.67	42.49	42.99	37.78

Table B-8. The photosynthetic rate of basil and tomato leaves at 5 and 8 weeks after transplanting (WAT).

Photosynthetic rate (μmol CO_2 m^{-2} s^{-1})		
	5 WAT	8 WAT
	Basil	
60BC:5VC	8.32	11.96
70BC:5VC	7.31	11.57
80BC:5VC	8.85	11.21
90BC:5VC	7.18	13.06
60BC:5CM	8.40	14.47
70BC:5CM	8.91	16.50
80BC:5CM	5.66	13.89
90BC:5CM		
Control	7.95	16.76
	Tomato	
60BC:5VC	10.51	3.87
70BC:5VC	9.05	2.59
80BC:5VC	9.36	2.87
90BC:5VC	4.34	2.58
60BC:5CM	11.65	4.00
70BC:5CM	11.15	4.13
80BC:5CM	11.12	5.03
90BC:5CM		14.92
Control	8.19	3.87

Table B-9. Growth index of basil and tomato plants at 0, 2, 4, 6 and 8 weeks after transplanting (WAT).

	Growth index (cm)				
	0 WAT	2 WAT	4 WAT	6 WAT	8 WAT
			Basil		
60BC:5VC	10.80	24.50	47.61	14.28	24.65
70BC:5VC	10.50	23.91	44.60	14.18	25.27
80BC:5VC	10.54	24.54	46.99	12.61	22.58
90BC:5VC	10.36	16.89	15.67	3.46	5.70
60BC:5CM	10.20	23.07	49.36	15.02	29.17
70BC:5CM	10.45	21.86	44.52	13.67	27.18
80BC:5CM	10.18	20.52	24.82	10.41	15.92
90BC:5CM	10.28	11.86	8.92	0.00	0.00
Control	10.10	21.15	44.21	15.95	28.96
			Tomato		
60BC:5VC	15.17	46.04	58.78	61.43	63.33
70BC:5VC	13.90	45.15	56.19	60.42	57.71
80BC:5VC	14.81	44.68	63.96	66.75	69.00
90BC:5VC	14.75	39.49	56.83	61.01	59.48
60BC:5CM	14.04	44.65	58.64	62.85	63.70
70BC:5CM	14.50	44.09	58.78	62.06	60.29
80BC:5CM	13.80	41.77	62.99	60.79	61.98
90BC:5CM	14.36	30.95	30.39	23.66	31.72
Control	15.01	47.01	63.31	66.23	61.99

Table B-10. Basil fresh weight (FW) and dry weight (DW).

	Basil FW and DW (g)			
	Shoot FW	Shoot DW	Root DW	Total DW
60BC:5VC	260.19	27.64	1.78	29.42
70BC:5VC	217.17	22.94	1.63	24.56
80BC:5VC	203.48	22.13	1.32	23.45
90BC:5VC	14.18	1.37	0.16	1.53
60BC:5CM	248.55	25.38	1.19	26.57
70BC:5CM	222.73	21.66	1.03	22.69
80BC:5CM	69.82	6.70	0.40	7.10
90BC:5CM	0.00	0.00	0.00	0.00
Control	269.89	28.13	1.73	29.85

Table B-11. Tomato fresh weight (FW) and dry weight (DW).

	Tomato FW and DW (g)										
	Leaf DW	Stem DW	Root DW	Total Fruit FW	Total Fruit DW	Fruit and Flower DW	Red Fruit FW	Red Fruit DW	Green Fruit FW	Green Fruit DW	Total DW
60BC:5VC	27.30	28.59	2.22	436.25	27.19	27.19	202.30	13.26	188.03	13.92	87.81
70BC:5VC	29.12	30.32	2.24	384.68	26.73	26.80	248.45	16.58	136.23	10.15	88.48
80BC:5VC	25.86	32.36	2.58	336.62	23.12	23.26	211.03	14.25	125.59	8.87	84.05
90BC:5VC	18.86	22.34	2.50	311.47	18.43	20.63	160.74	9.29	150.73	9.14	62.54
60BC:5CM	32.59	28.18	2.74	521.41	40.24	40.24	268.59	18.23	294.43	22.02	105.41
70BC:5CM	33.70	27.86	3.21	546.55	37.40	37.40	232.90	15.19	238.55	17.04	97.00
80BC:5CM	32.25	29.90	3.09	515.91	38.22	38.23	166.79	11.28	349.11	26.94	103.47
90BC:5CM	9.23	7.64	1.32	85.85	6.48	6.61	30.23	2.16	55.62	4.32	24.83
Control	31.03	27.20	2.78	293.68	22.00	22.08	149.80	12.82	173.06	13.19	80.59