8-1-2012

Influence of coffee vermicompost on growth and nutrient quality of greenhouse spinach and field grown green bell peppers

April Lorraine Vigardt
Southern Illinois University Carbondale, avigardt@siu.edu

INFLUENCE OF COFFEE VERMICOMPOST ON GROWTH AND NUTRIENT QUALITY OF GREENHOUSE SPINACH AND FIELD GROWN GREEN BELL PEPPERS

by

April Vigardt

B.S., Southern Illinois University Carbondale, 2007

A Thesis
Submitted in Partial Fulfillment of the Requirements for the
Masters of Science Degree

Department of Plant, Soil, and Agricultural Systems
in the College of Agricultural Sciences
Southern Illinois University Carbondale
August, 2012

THESIS APPROVAL

INFLUENCE OF COFFEE VERMICOMPOST ON GROWTH AND NUTRIENT QUALITY OF GREENHOUSE SPINACH AND FIELD GROWN GREEN BELL PEPPERS

by

April Vigardt

A Thesis Submitted in Partial

Fulfillment of the Requirements

for the Degree of

Master of Science

in the field of Plant and Soil Science

Approved by:

S. Alan Walters, Chair

Brian P. Klubek

Ruplal Choudhary

Graduate School
Southern Illinois University Carbondale
2 July, 2012

AN ABSTRACT OF THE THESIS OF

APRIL VIGARDT, for the Master of Science degree in PLANT, SOIL AND AGRICULTURAL SYSTEMS, presented on JULY 29, 2012, at Southern Illinois University Carbondale.

TITLE: INFLUENCE OF COFFEE VERMICOMPOST ON GROWTH AND NUTRIENT QUALITY OF GREENHOUSE SPINACH AND FIELD GROWN GREEN BELL PEPPERS

MAJOR PROFESSOR: Dr. Stuart A. Walters

Coffee vermicompost (VC) was used to evaluate the yield and quality of spinach. Coffee VC was added to a media mix v/v (0, 25, 50 and 75 %). In 2011, yield, ascorbic acid (AA) and nitrate contents were highest at 75% VC. In 2012, yield was highest at 50% VC, nitrate content was highest at 75% VC, and no difference in AA content was detected.

A field study was conducted to compare four treatments [coffee VC, dairy compost, standard fertility (SFT) and no treatment (control)] for their effects on growth, yield and AA content of peppers. Coffee VC and dairy compost were applied at the rate of 22 t/ha and SFT was based on 212 kg/ha N. Yield, as well as AA content was not affected by the treatments evaluated; however, plant height and chlorophyll index were higher for VC and SFT treatments compared to dairy compost and the control.

ACKNOWLEDGMENTS

I would like to express appreciation to my advisors Dr. Alan Walters, Dr. Brian Klubek, and Dr. Ruplal Choudhary for their help and guidance throughout this project.

Thanks to my fellow graduate students, Nick Ouellette, Travis Killion and Nick Gerfen and student workers Meredith Churchman, Jessica Brunner and Megan Ewers for their tremendous help in the field.

I also thank John Miller for his help in the greenhouse and Doug Bogard for his help in field preparation.

To my husband Aaron and my daughters Agnes and Ella, thank you for your help and patience throughout these two years.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 1

LITERATURE REVIEW

About 50% of our waste stream in the U.S is composed of organic materials (Edwards 1996). These include animal manures (Atiyeh et al. 2001, Llaven et al. 2008), sewage sludge (Atiyeh et al. 2000, Hashemimajd et al. 2004), yard and household wastes (Atiyeh et al. 2000, Hashemimajd et al. 2004), and industrial byproducts such as paper pulp (Arancon et al. 2003, 2005), coffee grounds (Adi et al. 2009, Orozco et al. 1996, Jamaludin et al. 2010), potato peels and many others (Edwards 1996). The benefits of biological processing and stabilization of these wastes into vermicompost (VC) (earthworm manure) by epigeic earthworms (such as *Eisenia fetida*) for use in horticultural and agricultural based industries are tremendous. The use of VC in organic and integrated nutrient management systems has been shown to enhance plant growth, yields and quality through improved soil physical, chemical and biological properties (Alderfasi et al. 2010, Arancon et al. 2003, 2005; Atiyeh et al. 2000, 2001, 2002, Canellas et al. 2002, Edwards 1996, 2007, 2010, 2011; Hashimimajd et al. 2004, Muscolo et al. 1999, Orozco et al. 1996, Quaggiotti et al. 2004, Szczech et al. 1999, Singh et al. 2008, Tognetti et al. 2005, 2008; Tomati et al. 1990).

The vermicomposting of coffee grounds shows great promise (Adi et al. 2009, Orozco et al. 1996, Jamaludin et al. 2010); as it tends to be high in nitrogen, does not attract rodents or supply weed seeds, and is abundant in most areas. Previous research has focused primarily on the suitability of coffee grounds as a composting substrate, rather than coffee VC as a plant amendment. It has been shown that vermicomposting of coffee grounds often increases nutrients such as N, P, Mg and Ca, converting them to more plant available forms, which may have positive effects for plant growth and quality (Orozco et al. 1996). Furthermore, with the recent developments in VC technology, production systems are now better suited to smaller, indoor spaces, which have the potential to recycle coffee grounds in more urban areas and on university campuses where coffee grounds are plentiful. This coffee VC could then be utilized in flower and vegetable beds, container gardens, greenhouses, green roofs, and in other urban food production systems (Edwards et al. 2011).

Vermicompost has been shown to positively influence plant growth. The most consistent results have occurred in greenhouse studies, where increased growth and yield have been most pronounced for many vegetables, fruits and ornamentals (Arancon et al. 2003, Atiyeh et al. 2000, 2001; Bachman et al. 2008). Optimum application rates vary and seem to be specific to the VC starting materials and plant species (and possibly cultivar) used. In a comparison between VC produced from food waste or pig solids and several thermogenic composts, Atiyeh et al. (2000) observed that while marigolds and tomatoes had higher shoot weight with compost, marigolds grew better in food waste VC compared to pig solids VC, and tomatoes grew better in pig solid VC compared to food waste VC. However, raspberry plants had the lowest shoot weight in compost and grew best in pig manure VC. When all nutrients were supplied with inorganic fertilizer at recommended rates, tomatoes responded well to 25-50% v/v pig manure VC (Atiyeh et al. 2000, 2001), 10t/ha cow manure VC (Arancon et al. 2003), and bell peppers grew best at 40% v/v pig manure VC (Arancon et al. 2004). When inorganic nutrients were not supplied, bell peppers in sheep manure VC had higher marketable fruits at 75% v/v VC. However, research on the effect of VC on several vegetables including (greenhouse spinach) is lacking. Peyvast et al. (2008), found that spinach responded best to 10% cattle manure VC in sandy loam soil under alkaline pH conditions.

Studies using thermogenic composts have shown positive results not only on spinach yield, but on nutritional qualities, such as increased ascorbic acid (AA) and decreased nitrate content (Alderfasi et al. 2010, Citak et al. 2010). However, little is known about the influence that VC may have on AA and nitrate content in spinach. It has been well established that as nitrogen fertilization levels increase, AA content decreases in many fruits and vegetables (Mozafar 1994, Wittwer et al. 1945). Furthermore, leafy vegetables such as spinach tend to accumulate nitrates when the N supply in the soil is high (Elias et al. 1998, Wittwer et al. 1945), which has caused concern and debate about implications for human health (Dykhuizen et al. 1996, Stoewsand et al. 1973, Tannenbaum et al. 1987). Substitutions of inorganic fertilizer for compost, in part or in full, has had positive results in increasing AA and decreasing nitrate content in spinach (Alderfasi et al. 2010, Citak et al. 2010), and when VC is substituted in greenhouse lettuce and tomatoes (Premuzic et al. 1998, 2002). Composts and VC, while similar in many ways, have differences that may affect vegetable growth (Atiyeh, et al. 2000, Anastasi et al. 2004, Ferreras et al. 2005, Hashemimajd et al. 2004, Kalantari et al. 2010, and Tognetti et al. 2005). Spinach is known to prefer N in the form of nitrate (Cruz et al. 2006, Elias et al. 1998), which is found in higher concentrations in VC than in composts (Edwards 1996, Tognetti et al. 2008); this may lead to differences in nitrate accumulation and assimilation and AA content (Cruz et al. 2006, Quaggiotti et al. 2004). Therefore, spinach greenhouse studies using VC as a medium amendment is needed, and in particular, VC produced from coffee grounds which are often higher in percent N than other VC substrates (Jamaludin et al 2010, Orozco et al. 1996).

In contrast to greenhouse studies, field study results with VC have been inconsistent with increased growth found in strawberries and tomatoes (Arancon 2003, Paul et al. 2005) and conflicting results found in grapes and bell peppers (Arancon 2003, 2005; Buckerfield et al. 1999, Edwards et al. 2010, Paul et al. 2005). In these studies, inorganic fertilizers were used to equalize nutrients to reveal other biological influences of VC on plant growth. However, no field study has been conducted to determine suitability of VC to produce bell peppers in organic farming systems. Likewise, differences in ascorbic acid content in bell peppers between organic and conventional farming methods have not been found (Chassy et al. 2006, Mozafar 1994). Greenhouse studies have shown increased growth and yield for bell peppers grown in media amended with VC (Arancon et al. 2004, Llaven et al. 2008), with and without supplementation with inorganic fertilizers. However, further research is needed to determine the effect VC may have on bell peppers in field conditions.

Characteristics of vermicompost (VC). Vermicompost is very high in organic matter (20 – 80%) and humic substances that contain numerous carboxyl, hydroxyl and ketonyl functional groups. It forms a water stable aggregate, held together by polysaccharide gums from the earthworm's digestive system and associated microbial activity, plant fibers, fungal hyphae and organo-mineral bonds. These aggregates tend to improve the physical structure of soil or plant media when used as an amendment to the growth medium (Edwards 1996). In a comparison of VC derived from different animal manures and solid household waste, water stable aggregates and water holding capacity increased in a linear manner with the addition of increasing rates of VC (Ferreras et al. 2006). In substitutions made with VC to a standard peat based greenhouse medium such as Metro-Mix 360, a significant increase was seen in bulk density, water holding capacity, and the number of micropores, while the number of macropores, total percent porosity and total percent air space decreased (Atiyeh et al. 2001). However, in studies combining VC with soils, bulk density and particle density decreased while total percent pore space and water holding capacity increased (Hashimimajd et al. 2004, Kalantari et al. 2010, and Llaven et al.

2008). Good aggregate structure in a growing medium is important as it provides numerous microsites for microbial activity and the adsorption and release of plant available nutrients, extracellular enzymes and Plant growth regulators (PGRs) through interaction with plant root exudates (Aira et al. 2007, Nannipieri 1996, Nardi et al. 1996).

The chemical characteristics of VC depend largely on the composition of the starting material. The pH of VC derived from cattle manure and plant residues, coffee pulp, pig manure and sheep manure was 6.0-6.5, 7.0, 5.3, 8.6, respectively (Atiyeh et al. 2000, 2001; Jordao et al. 2002, Micelli et al. 2007, Orozco et al. 1996). Nitrate is the form of N most prevalent in VC (Atiyeh et al. 2000, Chaoui et al. 2003, Elias 1998, Micelli et al. 2007, Orozco et al. 1996, Tognetti 2010). Total percent N is usually under 2.5% (Buckerfield et al. 1999, Adi et al. 2009) although higher values have been reported; such as, 3.2% N for coffee grounds mixed with cow dung at a 70/30 ratio (Jamaludin et al 2010) and between 3.2-3.6% N for coffee pulp (Orozco et al. 1996). The C:N ratio is usually under 20:1 (Hashemimajd 2004, Orozco et al. 1996, Edwards 1996). The electrical conductivity (EC) tends to increase with increasing addition of VC which can possibly inhibit germination and have a detrimental influence on plant growth (Chaoui et al. 2003), (Atiyeh et al. 2000).

Most plant available nutrients were found to increase during the vermicomposting process. In coffee pulp, an increase in soluble P, Ca and Mg was found, and N increased from 3.0% to 3.2-3.6% (Orozco et al. 1996); however, there was a decrease in K, possibly due to leaching in outdoor worm beds, and the Mg/K ratio was considered to be too low (Orozco et al. 1996). In raw dairy manure, there was a decrease in N and K, possibly due to volitization of ammonia and leaching of these nutrients in outdoor beds, and an increase in all micronutrients and total P, although soluble P decreased (Hashimimajd et al. 2004). An increase in phosphorus is most consistently seen in VC, possibly due to phosphatase enzymatic activity in the worm gut (Albiach et al. 2000, Edwards 1996). Nutrients for VC usually fall within the range of (based on dry weight) 2.2%-3.0% N, 0.4%- 2.9% P, 1.7%-2.5% K and 1.2%-9.5% Ca (Edwards et al. 2011).

For organic growers, VC shows promise as a fertility source, although some have argued that for optimum plant growth, some other nutrient source should also be added along with VC (Orozco 1996, Atiyeh 2000, Arancon 2003). However, there is evidence that the effect of VC on plant growth cannot be attributed to fertility alone. Enhanced microbial activity in soils with the addition of VC is known to increase the production of PGRs and extracellular enzymes such as urease, phosphotase and protease (Aira et al. 2007, Atiyeh et al. 2002, Bernitez et al. 2004, Canellas et al. 2002, Muscolo et al. 1999, Quaggiotti et al. 2004), which can be adsorbed together with ions, onto negatively charged sites on humus surfaces, and protected in aggregate microsites from microbial degradation (Canellas et al. 2002, Nannipieri et al. 1996, Nardi et al. 1996). This allows extracellular enzymes, ions and PGRs to persist in the soil or growth medium until they can be released by organic acids in plant root exudates (Nannipieri et al. 1996, Nardi et al. 1996). Plants grown with humic fractions derived from VC, especially of low molecular weight (<3500 MW), have increased root elongation and lateral root formation (Canellas et al. 2002, 2010), stimulated H^+ ATPases and plasma membrane permeability (Quaggiotti et al. 2004), and enhanced nitrogen assimilatory enzymes (Muscolo et al. 1999, Tomati et al. 1990). This may account for the increased plant growth and yield observed with additions of VC when all recommended nutrients are supplied inorganically (Arancon et al. 2003, 2005; Atiyeh et al. 2000, 2001).

This biological influence observed in VC is attributed to its microbial population which is rich in actinomycetes and fungi (Anastasi et al. 2004, Muscolo et al. 1999), both sources of PGRs (Frankenberger et al. 1993, Arshad et al. 1995). This positive influence of VC on plant growth can also be attributed to a more direct microbial effect; and in recent experiments, some biological activity against horticultural diseases and pests has been found. In several studies, adding VC to the soil or greenhouse medium decreased the incidence of fusarium wilt in tomatoes (Szczech et al. 1999), gray mold and botrytis on strawberries (Singh et al. 2008), suppressed parasitic nematodes and anthropoid pests with compost teas (Edwards et al. 2007), and suppressed cucumber beetles, tomato hornworms and aphids with soil drenches of compost tea (Edwards et al. 2010).

Ascorbic acid (AA) biochemistry and functions. The inability to synthesize ascorbic acid (AA) is shared by humans with other primates and guinea pigs, and we are dependent on obtaining this important vitamin from fruits and vegetables in our diet. AA is most known for its antioxidant properties, but has other important functions including maintaining healthy cartilage, bones, gums, skin and teeth; enhancing leucocyte activity in the immune system; and, as an co-enzyme co-factor (Smirnoff et al.1996). Recent research suggests that AA may have a role in preventing the formation of potentially harmful N-nitrosoamines from dietary nitrate. When proline and nitrate were ingested in human subjects, nitrosoamine formation increased; however, when AA was given together with proline and nitrate, nitrosoamine formation decreased (Tannenbaum 1987).

The biochemical functions and metabolic pathways of AA in plants are not as clear. There is biochemical and molecular evidence that AA is produced in plants through the D-mannose-L-galactose pathway, using hexose for C-skeletons. It's synthesized in the inner membrane of the mitochondria, transported through the xylem and found in all cell types, especially chloroplasts, in the thylakoid lumen. The primary function of AA is that of antioxidant, capable of reducing superoxide, singlet oxygen, ozone and hydrogen peroxide, which is generated during aerobic metabolism, and as an electron donor for photosynthetic and mitochondrial electron transport. Oxidation of AA first forms monodehydroascorbate (MDA) followed by the formation of ascorbic acid and partially oxidized dehydroascorbate (DHA); AA is linked to another important antioxidant glutathione which is used as a reductant by DHA and is known as the ascorbic acid-glutathione cycle. More research into other pathways and functions is still needed (Smirnoff et al. 1996, 2000; Davey et al. 2000, Foyer et al. 1991).

The AA content in fruits and vegetables is determined to some degree by genetic variation, but environmental conditions, such as, season, time of day, temperature, light, water stress, herbivore or pathogen pressure, and nutritional status also play an important role. Plants grown under reduced light, or shaded fruits and leaves often have a lower AA content compared to those positioned to receive more light, and AA content is often higher at the end of the day when carbohydrates from photosynthesis are abundant. The effect of season and temperature is more varied, depending on the life cycle and temperature requirements of the plant. In spinach, higher AA content is often seen in winter and early spring when temperatures are cool, especially under sunny conditions. However, for bell peppers, AA is highest when temperatures and light are high; and, AA content is dependent on the maturity of the plant (late season peppers are higher in AA) and fruit (red is higher than green) (Citak et al. 2010, Mozafar 1996). In drought conditions and other times of water stress, AA can also concentrate in plant tissues while flood irrigation and large rain events can cause a reduction (Mozafar 1996). During an herbivore or pathogen attack, AA production is often suppressed to allow the temporary increase of reactive oxygen species (ROS) used to fight pathogens, and H_2O_2, which induces cell wall strengthening through the cross-linking of wall polymers (Davey et al. 2000). These environmental factors may explain variations in AA content reported for many fruits and vegetables; however, there is evidence that the nutritional status of the plant is also important.

To understand the effect that mineral fertilizers have on vitamin content, interactions between plant, environment, nutrient and vitamin must be considered. Although most studies provide conflicting results, some consistent trends have been observed. There is increasing evidence that nitrogen fertilizers generally reduce AA content while P, K and several micronutrients increase AA content in many fruits and vegetables (Mozafar 1996, Wittwer et al. 1945). This is most pronounced in leafy greens such as spinach, where an inverse relationship between AA and nitrate content in leaf tissue is well established (Alderfasi et al. 2010, Citak et al. 2010, Mozafar 1996, Wittwer et al. 1945). However, in other vegetables, results of the effects of N fertilization on AA content are more varied, with no differences detected in some studies and increases in AA observed in others (Mozafar 1996).

The accumulation of nitrate in leafy vegetables, such as spinach, occurs during times of high N supply, and its ability for N uptake for later reduction and assimilation into plant tissue gives it an advantage over other plants that are unable to accumulate high amounts of N. However, the suppression of AA in plant tissues by nitrate is an important issue, especially for spinach, which is a good dietary source of AA. To prevent this problem, the use of slow release N or reduced N fertilization before harvest is often used (Cruz et al. 2006, Mozafar 1996). As the interest in composting and organic farming methods increased, spinach studies (Table 1) began to show that this relationship between nitrate accumulation and AA suppression could be changed by using organic N sources alone or in conjunction with lower amounts of N from inorganic sources (Alderfasi et al. 2010, Citak et al. 2010, Mozafar 1996, Muramoto et al. 1999). However, results in organic fertilization systems have been inconsistent for other vegetables with increases in AA also observed in lettuce (Mozafar 1996, Worthington 2001), potato (Ansari et al. 2008, Singh et al. 2008, Worthington 2001), and cabbage (Mozafar 1996, Worthington 2001), while no differences were detected for bell pepper (Chassy et al. 2006), potato (Mozafar 1996) and carrots (Mozafar 1996).

These results are promising for organic growers and others interested in reducing organic waste through composting and maintaining vitamin content in vegetable products. However, many questions remain and precise fertilization recommendations cannot be made at this time. There remains a need to have a better understanding of how different organic fertilizers (especially VC) affect AA content in different plant species, since VC has been shown to influence N uptake and assimilation in plants.

Nitrate content in spinach. Spinach and other leafy greens tend to accumulate nitrate in their petioles to be reduced and assimilated as needed (Cruz et al. 2006, Wittwer et al. 1945). Although this may be beneficial for the plant, suppression of AA content in fruit and vegetables is detrimental for human health. Furthermore, the toxicity of high nitrate levels in fresh foods has sparked debate regarding regulations of nitrate levels in foods.

Nitrate levels in drinking water have been regulated for some time, but the health implications for nitrate content in vegetables is widely debated. Toxic effects of nitrates are the formation of possible carcinogenic nitrosoamines and nitric oxide during digestion (Tannenbaum et al 1987), and methemoglobinemia (blue baby syndrome) which can result in hypoxia (or the inability of red blood cells to carry oxygen) (Chan et al. 2011). Besides cured meats, the largest source of nitrates in our diet is from vegetables, particularly leafy vegetables such as lettuce and spinach.

In 1995, the European Commission's Scientific Committee for Food (SCF) established an

Acceptable Daily Intake (ADI) for nitrate at 3.65 mg/kg body weight (about 219 mg/day for 60 kg person) (The Commission of the European Communities 1997). This limit includes all possible nitrate intakes while the ADI established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which is similar at 3.7 nitrate mg/kg body weight, only includes drinking water as a source (JECFA 1995). The health benefits of vegetables are considered by the JECFA to outweigh any carcinogenic risks. Leafy vegetables with the highest nitrate content such as spinach are also high in AA which offers some protection against the formation of nitrosoamines (Tannenbaum et al. 1987). In 1995, the European Union also established limits on nitrate levels in vegetables. For spinach, levels cannot exceed 2,500 mg/kg in summer and 3,000 mg/kg in winter. The U.S currently has no such regulation, but other countries such as South Korea and some in the Middle East are conducting research to determine if regulation is necessary (Chung et al. 2003, Shokrzadeh et al. 2007).

Nitrate levels in spinach can vary considerably (Fig. 1) and are dependent on many factors, including, light, diurnal rhythms, season, and rate of N fertilization as well as N source (inorganic/organic) (Muramoto 1999, Citak et al.2010, Alderfasi et al. 2010, Chung et al. 2003). Nitrate reductase (NR) is induced by light. This creates a diurnal effect with nitrate content highest in the morning and lowest at sunset and a seasonal effect with nitrate content higher in winter than in summer (Marschner 2012, Muramoto 1999). Nitrate content is also highly correlated with N fertilization rate (Cruz et al. 2006, Marschner 2012, Wittwer et al. 1945) and N fertilization source (Alderfasi et al. 2010, Citak et al. 2010, Muramoto 1999). Organic N sources have slower mineralization rates and provide available N at a slower rate for plant uptake (Tognetti et al. 2008). Furthermore, low molecular weight (LMW) (<3500) humic fractions isolated from grassland and VC humus have also increased nitrate assimilatory enzymes, such as, NR, glutamine synthetase (GS), glutamate dehydrogenase (GDH and malic dehydrogenase (MDH) in barley and carrot cells (Albuzio et al. 1986, Muscolo et al. 1999). These factors may help reduce leaf nitrate accumulation in leaves in favor of assimilation into other tissues. Nitrogen in composts and VC may also be taken up in organic form in LMW humic fractions and as amino acids (Nannipieri et al. 2009). This may explain results obtained by Peyvast et al. (2007), who found that as the percentage of VC increased in soil (10-30%), total N increased in spinach leaf tissue, although nitrate content remained the same.

This effect of composts and other organic amendments on nitrate content in vegetables is encouraging. However, nitrate content in vegetables is highly variable and dependent on many environmental, genetic and nutritional factors, making rate recommendations difficult at this time. Furthermore, while organic growers may benefit from such research, conventional growers, possibly facing regulation, may also benefit from the addition of organic composts into their fertilization systems. This integrative approach could open up new markets for compost and VC and further encourage organic waste recycling.

There is compelling evidence that VC can be used to transform and stabilize much of our organic waste. Coffee VC is especially important in urban environments where it can be produced and utilized in small areas without rodent and weed pressure. The use of VC positively effects plant growth through a synergism of physical, chemical and biological properties in the growth medium which increases the uptake of nutrients, enhances the assimilation of nitrogen, and increases the presence of PGRs. Vermicompost may also protect AA content in vegetables, especially spinach, by reducing the accumulation of nitrates in leaves.

It was the objective of this research to determine the effect of coffee VC on the yield and nutrient quality (AA and nitrate content) of greenhouse spinach in a two year study; and, the optimum application rate of coffee VC to obtain the highest AA and lowest nitrate content without compromising spinach yield. In exploration of the possible effects of coffee VC on the growth and AA content of vegetables, a bell pepper study under field conditions for three years was conducted to compare the influence of coffee VC, dairy manure compost, a standard inorganic fertility program and no treatment on pepper plant growth, fruit yield and fruit AA content.

Table 1. A comparison of ascorbic acid mg/100g and nitrate mg/kg content in spinach leaves as influenced by various factors.

AA mg/100g FW [z]	**nitrate mg/kg**	**Nitrate tissue type**	**Fertilization**	**Season**	**Study Location**	**Author**
44.9	451.0	FW	inorganic [x]	winter	field	Citak et al. 2010 Turkey
30.5-154.3	14.1-163.2	FW	organic [w]	winter	field	Citak et al. 2010 Turkey
21.1	477.47	FW	inorganic	autumn	field	Citak et al. 2010 Turkey
15.9-48.3	20.3-76.0	FW	organic	autumn	field	Citak et al. 2010 Turkey
12.7	5100	DW [y]	inorganic	-	greenhouse	Alderfasi et al. 2010 Saudi Arabia
28.4	1200	DW	organic	-	greenhouse	Alderfasi et al. 2010 Saudi Arabia
29.0	942	DW	biofertilizer [v]	-	greenhouse	Alderfasi et al. 2010 Saudi Arabia
44.7	4250	DW	org. + inorg.	-	greenhouse	Alderfasi et al. 2010 Saudi Arabia
41.1	4500	DW	bio + inorg.	-	greenhouse	Alderfasi et al. 2010 Saudi Arabia
38.0	1150	DW	bio + org.	-	greenhouse	Alderfasi et al. 2010 Saudi Arabia
52.2	2825	DW	bio+org.+inorg	-	greenhouse	Alderfasi et al. 2010 Saudi Arabia
-	1500-2900	FW	inorganic	winter	market	Muramoto 1999 California, U.S.A
-	890-2600	FW	organic	winter	market	Muramoto 1999 California, U.S.A
-	2000-3400	FW	inorganic	summer	market	Muramoto 1999 California, U.S.A
-	600-3000	FW	organic	summer	field	Muramoto 1999 California, U.S.A
-	1500-2300	FW	organic	summer	field	Muramoto 1999 California, U.S.A

[z] FW = Fresh leaf weight. [y] DW = Dry leaf weight.

[x] inorganic (inorg.)= fertilizer from inorganic sources.

Citak et al. 2010 – ammonium sulfate, triple phosphate, potassium sulfate.

Alderfasi et al. 2010 – potassium nitrate, super phosphate

[w] organic (org.) = fertilizer from organic sources.

Citak et al. 2010 – farmyard manure, chicken manure, bone meal.

Alderfasi et al. 2010 – commercial compost

[v] biofertilizer (bio.)= Alderfasi et al. 2010 – commercial product (Nitro-pen) with Azobacter sp., Azospirillum sp., phosphoren (containing a phosphate dissolving bacteria, PDB.

CHAPTER 2

INFLUENCE OF COFFEE VERMICOMPOST ON GROWTH AND NUTRIENT QUALITY OF GREENHOUSE SPINACH AND FIELD GROWN GREEN BELL PEPPERS

ABSTRACT

The vermicomposting of coffee grounds shows great promise for urban areas and university campuses. Several studies have examined using coffee grounds as a substrate for vermicomposting, however, little is known about its effect on plant growth, yield and quality. Therefore, two studies were conducted to assess these effects on greenhouse spinach and field grown bell peppers. Coffee vermicompost (VC) was utilized in a greenhouse spinach study over two spring growing seasons (2011 and 2012). Coffee VC was added to a 1:1:1 (peat, soil, sand) medium by volume (0, 25, 50 and 75%) in 4.5 L clay pots and seeded with 'Bloomsdale Longstanding' spinach (*Spinacia oleracea*). Growth parameters evaluated were fresh leaf weight (FLW), leaf area (LA), spinach plant height, number of leaves, fresh leaf ascorbic acid (AA) and dry leaf nitrate (DLN). In 2011, the highest FLW, LA, number of leaves, AA and nitrate content were seen at the 75% VC application rate. Many parameters were correlated: The FLW and the VC application rate ($r=0.41$ $P<0.0001$); the AA content in leaves and the VC application rate ($r=0.60$ $P<0.0001$); and the AA and nitrate content in leaves ($r=0.45$ $P=0.011$). In 2012, the greatest FLW, height and number of leaves were observed at the 50% VC application rate; the highest nitrate content at 75% VC application rate, with no difference in AA content. A field study was conducted over three seasons (2009-2011) to compare four treatments (coffee VC, dairy compost, standard fertility (SFT) and no treatment) for their effects on growth, yield, and AA content of bell peppers. Coffee VC and dairy compost were applied to beds at the rate of 22

t/ha and SFT was applied as 212 kg/ha 12:12:12 (N:P:K). Parameters evaluated were total number and weight of marketable and cull (unmarketable) pepper fruits, plant height, leaf chlorophyll index, and fresh fruit AA content. There were no differences detected for pepper fruit yields or AA content, however, plant height and chlorophyll index were greater for the VC and SFT treatments than for the compost and control treatments. These results indicated that coffee VC can improve the yield of greenhouse spinach and that the AA content does not decrease with higher VC application rates, even as nitrate content increases. Results of the bell pepper field study indicated that the coffee VC treatment produces similar growth, yield and AA content as SFT.

INTRODUCTION

Vermicomposting is the mesophillic processing of organic wastes by epigeic earthworms (*Eisenia fetida* L.*)* into a stabilized product, which is high in organic matter, plant available nutrients, beneficial bacteria, plant growth regulators (PGRs), and extracellular enzymes (Aira et al. 2007, Albiach et al. 2000, Anastasi et al. 2004, Atiyeh et al. 2002, Bernitez et al. 2004, Canellas et al. 2002, Edwards 1996, 2007; Muscolo et al. 1999, Quaggiotti et al. 2004, Tognetti et al. 2005, 2008, Tomati et al. 1990). Vermicompost (VC) has been shown to enhance plant growth, yields and quality through a synergism of physical, chemical and biological properties in the growth medium. With the addition of 25 to 50% VC to a peat based greenhouse medium, an increase in plant growth and yield was observed for greenhouse lettuce, tomatoes, peppers, strawberries, marigolds and petunias, while a decrease in plant growth and yield was observed for higher VC application rates (Arancon et al. 2003, 2005; Atiyeh et al. 2000, 2001, 2002; Edwards et al. 2010). This quadratic growth response has been attributed to the presence of PGRs produced from microbial activity, which may enhance plant growth in small doses, but inhibit it at higher rates (Arancon et al. 2002, 2006). Many organic materials have been vermicomposted successfully, i.e., animal manures (Atiyeh et al. 2001, Llaven et al. 2008), sewage sludge (Atiyeh et al. 2000, Hashemimajd et al. 2004), paper pulp (Arancon et al. 2003, 2005), and coffee grounds (Adi et al. 2009, Jamaludin et al. 2010, Orozco et al. 1996). Although the addition of VC produced from these different substrates has similarly improved plant growth and yield among a broad spectrum of plant species and cultivars, the chemical and biological characteristics of VC is influenced by the substrate and the VC rate recommendations specific to selected plant species may be needed (Arancon 2003, 2005; Atiyeh et al. 2000, Edwards et al. 2010, Hashemimajd et al. 2004). Although coffee VC has been produced in several studies (Adi et al. 2009, Jamaludin et al. 2010, Orozco et al. 1996), further research on the influences of this form of VC on plant growth and yield is still needed, particularly for greenhouse crops such as spinach, lettuce and other fast growing salad greens which may be grown in more urban landscapes and universities where coffee grounds are abundant. Recent VC technology has produced systems able to process wastes in small, urban areas where coffee grounds may be a more suitable substrate than food or animal wastes which may attract pests and produce strong odors. Coffee VC is also high in nitrogen (3.2-3.6%) (Jamaludin et al. 2010, Orozco et al. 1996), as well as soluble P, Ca and Mg (Orozco et al. 1996).

In addition to improving plant growth and yield, VC has been found to increase the ascorbic acid (AA) content of some fruits and vegetables, such as, tomatoes (Premuzic et al. 1998, strawberries (Singh et al.2008), and lettuce (Premuzic et al.2002). The AA content in fruits and vegetables is influenced by the interaction of environmental, genetic, and nutritional factors.

Light quality and duration plays a significant role and is responsible for many of the diurnal and seasonal differences observed regarding AA content (Mozafar 1996, Muramoto 1999); and, the application of high rates of inorganic nitrogen (N) has been known to suppress AA content in many fruits and vegetables, particularly leafy greens such as spinach. Nitrate accumulation and assimilation in leafy greens is affected by rate and source, with higher rates resulting in higher leaf content due to luxury accumulation, and organic sources resulting in lower leaf content due to slower N mineralization (Tognetti et al. 2008) as well as the possible use and assimilation of organic forms of N (Nannipieri et al. 2009). The application of low molecular weight fractions (LMW) (3500 MW) of VC has increased nitrate assimilatory enzymes, such as, nitrate reductase (NR) in maize and carrot cells (Canellas et al. 2002, Albuzio et al. 1986, Muscolo et al. 1999). This is promising for growers that want to reduce nitrate content in leafy greens due to consumer pressures or production regulations, which could directly reduce AA content in leafy greens and other fruits and vegetables.

To determine the effect of VC (especially coffee VC), on vegetable growth, yield and nutrient quality, further research is needed. Therefore, it is the objective of this research to determine the effect of coffee VC on the yield and nutrient quality (AA and nitrate content) of greenhouse spinach in a two year study; and, the optimum application rate of coffee VC to obtain the highest AA and lowest nitrate content without compromising spinach yield. A green bell pepper study was also conducted under field conditions for three years to compare the influence of coffee VC, dairy manure compost, a standard inorganic fertility program and no treatment on pepper plant growth, fruit yield and fruit AA content.

MATERIALS AND METHODS

Spinach study. A spinach greenhouse study was conducted from 16 March, 2011 to 19 April, 2011 and 17 March, 2012 to 16 April, 2012 at the Horticultural Research Center at Southern Illinois University in Carbondale, Illinois. Four treatments were used to determine their influence on growth, yield, AA and nitrate content of 'Bloomsdale long-standing' spinach. The experiment set up was as a randomized complete block design with eight replications. The VC treatments were 0% VC, 25% VC, 50% VC and 75% VC by volume mixed with a 1(sand): 1(soil): 1(peat) media mix. Clay pots (4.5 L) each were filled with a specific VC/1:1:1: soil mix by volume according to each treatment. Proportions were based on 3000 ml with 0% VC (3000 ml 1:1:1 mix), 25% VC (750 ml VC/2250 ml 1:1:1), 50% VC (1500 ml VC/1500 ml 1:1:1), and 75% VC (2250 ml VC/750 ml 1:1:1). A compost analysis of the 100% VC sample (Table 2) and a greenhouse soil analysis of the 1:1:1 soil mix (Table 3) was obtained from Brookside laboratories, New Knoxville, Ohio.

The soil used in the 1:1:1 mix is classified as a Belnap silt loam (Coarse-silty mixed, mesic Aeric Fluvaquent) (Herman 1979) and the VC was obtained from the SIU Vermicomposting Center from red worms (*Eisenia fetida*) that fed on coffee grounds in an indoor system. These coffee grounds were obtained from Starbucks in the SIU Student center, and then precomposted for a month with about 5 cm applied to the worm bed every five to seven days for four months. In 2011, beds were prepared using 5 cm of shredded, wet office paper from SIU offices with about 10 cm of worms and coffee grounds placed on top. In 2011, beds were prepared using coffee grounds (some landscaping materials were adjoining the bed and may have been included) to produce VC. All compost was screened with a rotating trommel to homogenize the VC, and in 2011, to also separate out the worms.

At planting, six spinach seeds were placed into each pot and covered with 0.6cm of soil and placed under 12 hours of supplemental lighting (P.L Light Systems 400W HPS, Hortilux Shreider Group, Canada). At the 2-3 leaf stage, spinach seedlings were thinned to three in each pot. The pots were watered daily as needed and the greenhouse temperature averaged 14 C (low) to a 28 C (high). Data were taken at harvest (34 and 30 days after planting in 2011 and 2012, respectively, on plant height (cm), number of leaves, leaf area (cm^2) and fresh leaf weights (g). Leaf area was determined using a portable leaf area meter (Li-Cor Portable Area Meter L1-3000, Lambda Instruments Corporation; Lincoln, Nebraska). For ascorbic acid analysis, newly matured leaves were harvested from the center of each plant, and placed in a cooler at 5 C. Leaves were ground in a metaphosphoric acid solution with a mortar and pestle with the resulting solution titrated with 2, 6 dichloroindophenol (AOAC 2007) within 24 hours. For nitrate analysis, the remaining leaves were washed in deionized water, oven dried at 60 C for 60 hours and then sent to Brookside Laboratories for analysis where samples were shaken with 2% acetic acid and analyzed on a Lachat Quick Chem 8000 auto analyzer.

Data were analyzed using analysis of variance (ANOVA) procedures and mean separation was performed by the Student's T Multiple Range test. Data analysis was done using JMP Statistical Discovery Software (JMP, 2012, Cary, NC).

Green bell pepper study. A field study was conducted in 2009-2011 at the SIUC Horticultural Research Center comparing the effects of four fertility treatments on the growth, yield and AA content of 'Red Knight X3R' bell peppers. There 4 treatments were: 1) coffee VC, 2) dairy manure compost, 3) standard fertility and 4) control – no treatment. The VC and compost application rates were based upon dry weight at 22 t/ha and adjusted for moisture content. For each plot, 5 kg of VC or compost were added (dry wt.). In 2011, the moisture content was 41% for compost and 73% for VC. Therefore, for compost, 1-0.41 water= 0.59 compost x 5 kg = 8.47 kg compost and for VC, 1-0.73 water = 0.27 compost x 5 kg = 18.52 kg VC was added to the plots. Calculations adjusting for moisture content were performed the same for 2009 and 2010 but data is not available. The standard fertility treatment was based on an application rate of 212 kg/ha of 12:12:12 fertilizer to be applied 106 kg/ha at planting, 53 kg/ha four weeks after transplant, and 53 kg/ha eight weeks after transplant.

The experiment was set up as a randomized complete block with three replications. Six plants were in each plot on double rows, at 0.3 m in-row spacing on 1 m wide raised beds. The transplants were started in March in the greenhouse using the standard method for bell pepper production and transplanted into the field in mid-May.

VC was obtained from the SIU Vermicomposting Center from red worms (*Eisenia fetida*) fed coffee grounds as previously described. The Compost was obtained from The SIU Agricultural Research Center and produced thermogenically from dairy manure in an outdoor system. All applications were worked into the top 20 cm of a Hosmer silt loam soil (Fine-silty mixed, mesic Typic Frgiudalfs) (Herman 1979). A sample of the VC (Table 4) compost (Table 5) and the selected soil (Table 6) were sent to Brookside laboratories for analysis. Drip irrigation was used and after transplanting, the beds were covered with straw mulch for water conservation and weed control.

Data was taken on plant height (cm) and chlorophyll index, using a Minolta SPAD-502 chlorophyll meter (Special Products Analysis Division, Konica Minolta Sensing, Inc., Osaka, Japan) three times during the season; one month after transplanting, at first harvest, and at final harvest. The number and weight of marketable and unmarketable fruits were recorded every 2 weeks beginning 8-10 weeks after transplanting for a total of 7-10 harvests (2009 and 2011 had ten harvests and 2010 had seven). For ascorbic acid determination, three pepper fruits of similar maturity, size and plant position were collected from each plot at time of first harvest and in late October, between 21-24 weeks after transplanting. They were quickly placed in a cooler and titration using the 2, 6 dichloroindophenol method (AOAC 2007) was performed within 24 hours. For the pepper leaf analysis, 25-50 recently matured leaves were chosen at the time of final harvest, oven dried at 60 C, and sent to Brookside laboratories for determination of N, P, K, Ca, and Mg. Analysis of leaf tissue was determined only for the 2011 season.

Data was analyzed using analysis of variance (ANOVA) and mean separation was performed by the Student's T Multiple Range test. Data analysis was done using JMP Statistical Discovery Software (JMP, 2012, Cary, NC).

RESULTS AND DISCUSSION

Vermicompost rate and spinach growth. Interactions ($P \leq 0.05$) were detected for all parameters evaluated between year and vermicompost rate for the two-year greenhouse study. Therefore, the two years are reported separately.

In 2011, spinach fresh leaf wt. (g) (FLW) and leaf area (cm^2) (LA) increased as the percentage VC in the media increased, with the highest amount of growth occurring at 75% VC (7.1 g and 123.0 cm^2 for FLW and LA, respectively) (Table 7). The FLW at 75%VC was higher than 0%, 25%, and 50% VC rates with 63%, 37%, and 31% increases observed respectively; and, FLW was correlated with percent VC rate (r = 0.41, P< 0.0001). For LA, 75% VC differed from 0% VC with a 51% increase detected. However, spinach leaf number and plant height did not increase as the percent VC increased in the media. There were more numbers of leaves in the 0% and 75% VC rates than in the 25% and 50% VC rates, although the average leaf size data not shown) was only 7.5 cm^2 for 0% VC compared to 12.2 cm^2 for 75% VC. Spinach plant height at 25% VC differed from 0% and 50% VC but not from 75% VC; and, this was due to longer leaf petioles observed in the 25% VC compared to the 0% and 50% VC, but not the 75% VC (data not shown).

In 2012, FLW was found to increase from 4.5 g at 0% VC to 13.8 g for 50% VC and then decrease to 11.0 g 75% VC, with 50% VC producing greater FLW than 0% VC (Table 3). For leaf number and height, the 50% VC rate was higher compared to all other treatments. In many studies, additions of VC to a growth medium have tended to show highest growth at 50% with a decline observed at higher percentages (Atiyeh et al. 2001, Atiyeh et al. 2002). This may be explained by plant growth hormones (PGRs) that adsorb onto the VC humic fraction, which increases plant growth at low concentrations but cause inhibition at higher concentrations (Arancon 2005, Hopkins et al. 2004). The application of humic acids (extracted from VC using an alkali/acid fractionation procedure) above the rate of 500mg/kg to a peat based commercial greenhouse medium, inhibited cucumber and tomato plant growth (Atiyeh et al. 2002). In 2012, VC was harvested from an active worm bed with a moisture content of 71% compared to 2011 in which the bed was not active and the moisture content was 43%. Once worms have been removed from a bed, microbial activity has been shown to decline (Aira et al. 2007). This higher microbial activity of the 2012 VC could have increased PGRs to levels that inhibit plant growth above the 50% VC rate. These higher rates of VC may also negatively affect the physical structure of the growth medium by increasing water retention at the expense of root aeration. The 75% VC treatments were noticeably more saturated than other treatments and generally took longer for water to drain from pots.

Spinach FLW was higher in 2012 compared to 2011 (Table 3) with increases of 19.5% (0% VC), 15.1% (25% VC), 90.5 % (50% VC), and 43% (75% VC) observed. Some aphid damage occurred and some plants had started to bolt in 2011 which may partially explain this difference in FLW. In 2012, although some caterpillar damage was observed it was light and not isolated to any one treatment and no bolting was observed.

The growth increase observed in 2012 could also be due to the higher total N (4.2%) in the 2012 coffee VC compared to 2011 (2.8%) (Table1). However, plant available nitrogen (PAN) based on organic matter content was calculated to be 1,619 mg/kg N in 2011 and 1,872 mg/kg N in 2012. The critical nitrate concentration for spinach leaves at a 10% growth restriction is 1,700 mg/kg (dry wt.) with spinach considered deficient at 4,000 mg/kg and sufficient at 8,000 mg/kg (Maynard et al. 1976). In 2011, 0% and 25% VC would be considered deficient at 1397 and 2205 mg N/kg respectively, and 50% and 75% VC would be sufficient at 5046 and 6587 mg N/kg respectively. However, in 2012, although nitrate levels were deficient, FLW was numerically higher than in 2011, particularly for 50% VC, in which a 90.5% increase was observed. Microbial activity and PGRs may have enhanced nitrate assimilation in spinach leaves, reducing accumulation without having a detrimental impact on yield.

During the first 3 weeks in 2012, spinach growth appeared to be slow, which was attributed to an immobilization of inorganic N through microbial activity. Although a C/N ratio of 12 was determined (Table 1), N mineralization in coffee VC may be slower due to a higher amount of phenolic C present. It was found that even with similar C/N ratios around 12, the chemical composition of the composting substrate may have significant effects on N mineralization through influencing the type and abundance of extracellular enzymes such as urease, protease and microbial activity (Tognetti et al. 2005). Nitrification is the main form of N mineralization in VC with nitrate accounting for 99% of the inorganic N after a 16 week soil incubation period (Tognetti et al. 2005). The rate of N mineralization for VC was found to be linear with about half of total inorganic N available at 4 weeks within this incubation period (Tognetti 2005). Fungal species tend to dominate VC, which increases nitrate mineralization compared to ammonium mineralization (Anastasi 2004). In 2011, the N mineralization rate may have stabilized due to decreased microbial activity (VC not harvested from an active worm bed), while in 2012 these biological processes may have been more active. An uptake of organic nitrogen in the form of amino acids may also be a possibility, allowing for increased growth without nitrate accumulation (Marschner 2012, Nannipieri et al. 2009).

Spinach is a crop that is sensitive to iron deficiencies (Marschner 2012). The poor germination and stunted seedling growth detected at the 75% VC rate in 2012 may also be explained by an iron deficiency that was observed on leaves between the second and third week after seedling emergence, which may have been due to a high percentage of organic matter and/or an alkaline pH in the media. Iron content for the VC used in 2012 was 97% lower (734 mg/kg) than in 2011 (3998 mg/kg) (Table 2). This may have caused an initial iron deficiency due to a synergism between carbonates of unknown origin in the soil or the VC, which could have raised the pH higher than it was for either substrate alone. The soil analysis indicated that the pH of 75% VC was 7.4 compared to 7.0 and 7.1 in the 100% VC and no VC, respectively (data not shown). At this pH, iron is not as available compared to lower pH levels (Marschner 1986). The higher percent organic matter (based on dry wt.) in 2012 (91.3 %) compared to 49.1 % in 2011, may have initially complexed iron (III) in humic substances. Iron (III) would then become more available through chelation in the interaction of plant root exudates, microbial activity and organic acids (Chen et al. 1996). Low molecular weight humic substances, in particular fulvic acids have been shown to complex with soluble iron (Cesco et al. 2002, Chen et al. 1996) and then be absorbed and translocated within the plant (Chen et al. 1996, Nardi et al. 1996), which will allow partial recovery from iron deficiency symptoms even at pH 7.5 (Cesco et al. 2002, Pinton et al. 1999).

The caffeic acid present in the 2012 VC may have contributed to an allelopathic response in spinach. However, the C/N ratio was 12 (Table 1), indicative of finished compost, and soil humic and fulvic acid have been shown to reduce root and shoot inhibition of lettuce seedlings exposed to caffeic acid (Loffredo et al. 2005). The limited adsorption of caffeic acid onto these humic fractions is thought to partially explain how negative effects of caffeic acid may be regulated. Furthermore, caffeic acid is also rapidly assimilated and degraded in the presence of seedlings (Loffredo et al.2005). Despite these findings, it may be possible that caffeic acid within coffee grounds may persist longer in VC, which can have a negative effect on germination and growth.

The addition of VC to soil or soil-less growing media has been shown to positively increase growth of many fruits, vegetables and ornamental plants. This has been attributed to an increase in plant available nutrients, improved soil/media physical structure, increased microbial activity, humic fractions and the presence of plant hormones (Atiyeh et al. 2002, Theunissen 2010). Plant available P, K, Ca and Mg was higher in VC of diverse parent materials (Albiach et al. 2000, Chaoui 2003, Edwards 1996, and Orozco 1996, Tognetti 2008), and N mineralization rates were higher in VC processed municipal waste compared to thermogenic composting

(Tognetti et al. 2005, 2008). Soil and compost analysis showed that all nutrients, except possibly for iron in 2012 in the 75% VC treatment (data not shown), were within the optimum range needed for spinach growth, with phosphorus, potassium and sulfate higher than the optimum. However, the estimation of adequate nitrogen release based on organic matter content and microbial activity has been difficult. Yield was higher in 2012 than 2011, although a nitrate deficiency was revealed through spinach leaf tissue analysis. This suggests that the positive effect of VC on spinach growth cannot entirely be attributed to an increase in mineral nutrition. The improvement of soil physical structure by the addition of VC should also be considered. When VC is added to soil, there is an increase in water stable aggregates, total porosity and water holding capacity (Atiyeh et al. 2001). The soil used was a Belnap silt loam, characterized as poorly drained with moderate to low water and air flow (Herman 1979), and the addition of VC would have improved water and air infiltration, plant root growth, microbial activity and nutrient availability.

Plant nutrient uptake is enhanced when VC is present. This may be partially explained by the increased activity of H+ ATPase and the subsequent increase of plasma membrane permeability found in studies using humic substances extracted from VC. Canellas et al. (2002) found that humic acid extracted from VC stimulated ATPase activity, root elongation and lateral root formation of maize roots. The release of protons from ATPase would contribute to the acidification of the root rhizosphere and the formation of an electrochemical gradient across the plasma membrane, which could positively influence plant nutrient uptake (Canellas et al. 2010, Nardi et al. 1996). Low molecular weight (LMW) humic fractions (<3500 MW) extracted from VC also stimulated an increase in H+ ATPase activity, which related to 70% increased nitrate uptake in maize roots and 50% increased nitrate accumulation in leaves (Quaggiotti et al. 2004);

and, humic fractions (<9000 MW) and unfractionated humic substances extracted from grassland soil stimulated nitrate uptake of barley seedlings with a 100 mg/L application (Albuzio et al. 1986).

On a molecular level, VC LMW humic fractions have increased the transcript accumulation of the Mha2 transcript (maize H+ ATPase isoform) eight fold in maize roots, and induced the high affinity nitrate transporter gene (ZmNrt2.1) in shoots. Indole acetic acid (IAA), a PGR, was also isolated from the VC LMW humic fractions (<3500 MW) and may be the mechanism by which the Mha2 gene was induced (Quaggiotti et al. 2004). The IAA in humic substances extracted from diverse soils has also been found in other studies; in humic acid (Canellas et al. 2002) and in LMW humic substances (<3500 MW) (Muscolo et al. 1998, 1999). Nitrogen assimilatory enzymes such as nitrate reductase (NR), glutamine synthatase (GS), glutamate dehydrogenase (GDH), glutamate synthase (GOGAT) and malic dehydrogenase (MDH) were also shown to increase in barley and carrot cells with the addition of grassland and VC LMW humic fractions (Albuzio et al. 1986, Muscolo et al. 1996, 1999, 2007). The increased protein synthesis in lettuce (24%) and radish (32%) observed with the addition of VC is thought to be influenced by improved N metabolism and NR activity (Tomati et al. 1990), which can be induced by increased nitrate concentrations and the presence of PGRs (Hageman 1979).

The increase of plasma membrane permeability and nitrate assimilatory enzymes are both thought to be induced by PGRs (especially IAA), adsorbed on and encapsulated within humic substances, which may be released by organic acids produced through microbial activity and plant roots (Nardi et al. 1996, Piccolo et al. 1992, Pinton et al. 1999) Low molecular weight humic acids (<3500 MW) extracted from VC exhibited the same isoenzyme profile as auxin, and displayed similar hormonal activity. However, it was determined that PGR activity of humic acids, while being similar to auxins and other plant growth regulators, is more complex (Muscolo et al. 1999). In cucumber, changes in root morphology in plants treated with humic acids were only partially affected by inhibitors of ethylene, auxin and nitric oxide (Mora et al. 2012). These results may indicate that the presence of PGRs or other biologically active materials in humic substances such as humic acid and LMW humic fractions are able to produce changes observed in plasma membrane permeability, nitrate assimilatory enzymes and root morphology. Microorganisms such as bacteria, fungi, yeasts, actinomycetes are known to produce PGRs (Frankenberger et al. 1993, Arshad et al. 1995); and, many of these, such as actinomycetes and fungi (Anastasi et al. 2004, Muscolo et al. 1999), and free-living nitrogen fixing bacteria (Vallini et al. 1997) are present in VC. Furthermore, PGRs such as cytokinins and auxins were found in the intestines of large earthworm populations, with significant positive correlations ($r=0.97$, $P< 0.05$) detected between earthworm populations and the levels of auxins and cytokinins in ten different soils (Krishnamoorthy et al. 1986). The PGRs present in VC are thought to result from microbial activity in the VC and the earthworm gut, although the endogenous production of PGRs by earthworms has not been determined (Edwards et al. 1996).

The positive effect of VC on spinach growth observed is not likely due to any one of these factors alone, but rather a synergism between improved soil physical, biological and chemical properties. However, in 2011, due to the older age of the VC, we believe improved growth with increasing VC rate was due more to improved physical properties of the soil and increased mineral nutrition; while in 2012, the growth improvement in spinach suggests more of a biological system involvement.

Vermicompost and spinach nutritional composition. In 2011, spinach AA content was higher at 75% VC (65.17 mg/100g) compared to all other treatments, and significantly correlated with percentage of VC applied ($r = 0.60$, $P<0.0001$) (Table 3). Spinach leaf nitrate concentration increased in a linear manner with percentage of VC [$r^2= 0.59$, $P<0.0001$; y=1047+ 73.6 (VC %)], with a range of 1397 mg/kg at 0% VC to 6587 mg/kg at 75% VC, and AA and nitrate were positively correlated ($r=0.45$, $P<0.011$. In 2012, AA content did not differ between treatments; and, nitrate concentration was more variable than in 2011, with a decrease between 0% VC and 25% VC and an increase between 25% VC and 75% VC. Similar to 2012, spinach leaf nitrate content was found to be correlated with percentage of VC ($r = 0.57$, $P<0.0016$).

AA and nitrate content are known to be influenced by light quality and diurnal rhythms Mozafar 1996). Thus, in our studies, light was supplemented for 12 hours daily to minimize the effect of cloudy weather on AA and nitrate content, and samples were taken during the early afternoon to minimize diurnal effects. The synthesis of AA is light dependent because of its need for glucose produced in photosynthesis as a source of C- skeletons, and the role of AA as a reducing agent for H_2O_2 and reactive oxygen species (ROS) produced during photosynthesis and photoprotection (Smirnoff et al. 2000). Reduced AA levels for spinach have been reported under low light conditions (Mozafar 1996, Muramoto 1999). Nitrate content is diurnal with highest accumulations occurring in the morning and under low light conditions due to the light dependent activity of NR (Marschner 2012). Spinach AA content has been reported within the range of 15-54 mg/100g (Mozafar 1996, Muramoto 1999), and found to increase in winter under high light, with an optimum temperature range of 12-15 C (Mozafar 1996). The range of AA for this study was 43.4-65.2 mg/kg in 2011 and 47.6-53.9 mg/kg in 2012. The average high/low greenhouse temperature for the duration of this study was 16-29 C, which was higher than the optimum and may have decreased AA content. The variability of nitrate content found in this study reflects that reported in previous studies (Muramoto 1999). Some of this variability may be explained by differences in time of collection (day and season) and methodology. However, we have controlled diurnal, light and seasonal effects in this study and attributed this variation to other factors.

AA content in fruits and vegetables has also been shown to have an inverse relationship with nitrogen fertilization. As nitrogen fertilization rates increase, AA content tends to decrease in many fruits and vegetables (Mozafar 1994, Wittwer et al. 1945). Leafy vegetables, such as spinach, tend to also accumulate nitrates in their petioles when nitrate reserves are high for possible later reduction. This reduction by NR can be rapid, and nitrate stores can be depleted within 48 hours (Marschner 2012). Therefore, slow release fertilizers or an intermittent nutrient supply (where fertilization is disrupted before harvest) has been suggested to reduce nitrate content in fruits and vegetables (Cruz et al. 2006, Mozafar 1994). In this study, AA content did not decrease as nitrate content increased in spinach leaf tissue. Although, spinach fertilized with an inorganic N source provides N that is immediately available for plant uptake, fertilization with an organic source provides N that will be released more slowly. Thus, nitrate would be reduced and taken up at a similar rate, decreasing luxury accumulation. Another possible factor for reduced nitrate composition may be the stimulation of NR previously discussed which would further reduce and assimilate nitrate into plant tissues that are complimentary with plant uptake (Muscolo et al. 1999, Quaggiotti et al. 2004, Tomati et al. 1990). Also, the differences in the range of nitrate composition seen between 2011 and 2012 may be explained by the difference in VC age. In 2011, more mineralized total N may have occurred than in 2012 allowing for plant available N; whereas microbial activity may have been more pronounced in 2012, possibly stimulating a hormonal trigger of N metabolism and assimilation.

One explanation for this inverse relationship observed between AA and nitrate content in spinach, concerns the use of AA as a precursor for oxalate (Smirnoff et al.2000), since it can be synthesized in the cytosol and stored in the vacuole in order to maintain cation – anion balance and intracellular pH during nitrate assimilation (Marschner 2012). Spinach is known to be high in oxalates, especially with high rates of N fertilization (Alderfasi et al. 2010, Elias et al. 1998). Therefore, a change in N metabolism may affect oxalate and AA content in spinach. However, we did not test for oxalates, so this cannot be determined from this study.

This study indicated that spinach AA content remains the same whether nitrate content is high or low. Surprisingly, AA and nitrate content were both at high levels with the 75% VC treatment in 2011 and 2012. It appears that VC may be an important medium addition with properties that stimulate nitrate uptake and assimilation, allowing for the preservation of AA composition during high rates of nitrate accumulation in spinach.

Vermicompost and green bell pepper growth. Interactions ($P<0.05$) were not detected for yield and ascorbic acid content between treatments and year for the green pepper field study (2009-2011). Therefore, data was combined for the three years. However, interactions ($P<0.05$) were detected for mid-season height, and mid and late season chlorophyll indices between treatment and year (2009-2011). Therefore, data were reported separately.

Differences were not detected between the four treatments evaluated for green bell pepper yield during all three years (Table 8). For mid-season height (cm) and mid and late season chlorophyll index, differences were detected in 2009 and 2010, with VC and Standard fertility treatment (SFT) higher ($P<0.05$) than the compost and control treatments (Table 9). Ascorbic acid content in fresh bell peppers was not different among treatments and its range was 102-106 mg/100g (Table 10), which is within the range reported in the literature (95-173 mg/100g)

(Mozafar 1994). Leaf tissue analysis also revealed no nutrient deficiencies or differences in nutrient uptake among treatments (Table 11).

Previous bell pepper field studies using composts and VC have provided variable results. Differences in plant growth and yield appear to be significantly influenced by the nature of the compost and VC material (Atiyeh, et al. 2000, Anastasi et al. 2004, Ferreras et al. 2006, Hashemimajd et al. 2004, Kalantari et al. 2010, Tognetti et al. 2005). Although, the addition of 10 or 20t/ha cow manure VC, paper waste VC or leaf compost increased marketable pepper yields compared to inorganic fertilization alone, food waste VC did not, and yield did not differ between 10 and 20t/ha application of VC. Furthermore, in the following year, 5 and 10t/ha application rates of cow manure VC, paper waste VC, food waste VC, or leaf compost increased marketable pepper yields compared to the inorganic fertilizer. The only consistent treatment in both years was the 10t/ha application rate, yet at that rate, there was a 2 fold difference between the two years for marketable pepper yields. Therefore, it is difficult to ascertain from these studies what the optimum application rate or composting material is for field production of bell peppers. Furthermore, in those studies, compost and VC rates were equalized by the recommended N rate for bell pepper production. This was to demonstrate that the positive effects of composts and VC on plant growth and yield is not due to nutrition as much as other biological factors, such as increased microbial activity and the presence of PGRs (Arancon et al. 2003, 2005). Our results indicate that the application of two different composts at the 22.4 t/ha application rate did not produce higher yields than the SFT or control, despite an increase in height and chlorophyll index found in SFT and VC treatments in 2009 and 2010. Possible explanations may be that our application rate of 10t/acre (22t/ha) was too low, or the soil to which they were applied had a fertility level high enough to obscure discernible differences.

Vermicompost and ascorbic acid content of green bell peppers. There is no strong evidence linking AA content in bell peppers to mineral nutrition or cultural practices used (organic/inorganic). Nitrogen and phosphorus fertilization have provided conflicting results for AA content; and, comparisons of organic and conventional methods for AA content have generally not differed. Ascorbic acid production in fruits increase as they mature and is more influenced by carbohydrate production (Table 10). Sunlight and cultivar selection have also shown to be more influential in the ascorbic acid content of bell peppers than nutrition or cultural practices (Mozafar 1994).

Our research indicates that the addition of compost or VC does not improve marketable yield or AA content compared to the standard nutritional program recommended for bell peppers. For producers wanting to reduce their application of inorganic fertilizers, there is some evidence that compost and VC may substitute or be used together with inorganic fertilizers without compromising yield. The application of compost and VC to soil can increase soil organic matter content, improve soil structure, reduce fertilizer leaching and increase microbial activity while recycling organic wastes back into the soil (Edwards et al 2011). However, the economic feasibility for the production and application of compost and VC to soil at rates above 22t/ha is questionable. Many farms have added thermogenic composting to their production activities to recycle animal wastes that may pollute local areas and waterways; and, the application of this compost to vegetable or fruit fields can resolve waste removal issues and supply organic matter back to the soil. Organic growers also produce and rely on compost as an important fertility source. Over time, fewer compost inputs may be required or desired as the soil structure and fertility is improved, and to prevent the increase of phosphorus and potassium recommended levels. However, VC at this time is expensive due to production requirements and costs. Its use in greenhouse production of food and transplants seems more feasible compared to applying large amounts in field situations. At this time, we cannot claim that VC or compost will improve growth, yield or AA content in bell peppers. Different effects may be seen in other soil types of lower fertility or poorer structure or higher application rates may be needed. The AA content of fruits also does not appear to be directly affected by VC or compost soil amendments. (Edwards et al. 2011).

Table 2. Coffee vermicompost analysis (mg/kg dry weight) used in a spinach greenhouse study during 2011and 2012.

Year	pH	C/N	OM	N	P	K	Ca	Mg	Na	S	C	B	Fe	Mn	Cu	Zn
2011	6.38	8.8	49.1[z]	28400	3500	8200	101400	9000	400	3200	250700	32.3	3998	503.2	53.3	182.4
2012	7.02	12.1	91.3	41600	2800	8800	72400	2700	400	2100	505300	9.0	734	91.7	25.8	65.4

[z] OM (organic matter) is in percent dry weight.

Table 3. Soil mix (1:1:1) (sand, soil, and peat) analysis (mg/kg dry weight) used in a spinach greenhouse study during 2011 and 2012.

pH	CEC	OM	N	P	K	Ca	Mg	Na	S	B	Fe	Mn	Cu	Zn
7.1	7.0	1.9[z]	28.5	31	142	1080	122	32	54	0.43	127	55	0.9	1.62

[z] OM (organic matter) is in percent dry weight.

Table 4. Vermicompost analysis (mg/kg dry weight) used in 'Red Knight X3R' bell pepper field study, 2009-2011.

Year	pH	C/N	OM [z]	N	P	K	Ca	Mg	Na	S	C	B	Fe	Mn	Cu	Zn
2009	5.2	11.8	52.9	25000	6000	5000	41000	5000	1000	4000	294000	34	4632	683.5	47.9	301.8
2010	5.6	11.5	46.9	25000	6000	4000	72400	4600	400	3200	289100	29	6910	672	119	700
2011	6.4	8.8	49.1	28000	3500	8200	101400	9000	400	3200	250700	32	3998	503.2	53.3	182.4

[z] OM (organic matter) is in percent dry weight

Table 5. Dairy manure and leaf waste compost analysis (mg/kg dry weight) used in 'Red Knight X3R' bell pepper study, 2009-2011.

Year	pH	C/N	OM [z]	N	P	K	Ca	Mg	Na	S	C	B	Fe	Mn	Cu	Zn
2009	8.1	20.6	23.4	10000	2000	4700	174100	9700	500	2400	213000	28	3113	513	9.5	73
2010	7	11	28.8	17700	4500	10200	169000	12200	1100	3800	194900	39	4390	608	46.4	183
2011	7.9	17	23.3	11900	4600	4800	200000	14100	400	3000	201000	33	3570	493	52.6	201

[z] OM (organic matter) is in percent dry weight

Table 6. Average research site soil analysis (mg/kg dry weight) used in ‘Red Knight X3R’ bell pepper study, 2009-2011.

pH	CEC [z]	OM [y]	N	P	K	Ca	Mg	Na	S	B	Fe	Mn	Cu	Zn
	meq/100g	% dry wt.	t/ha											
7.2	19.1	3.5	180	240	365	3069	227	22	14	69	402	140	3.2	17.4

[z] CEC is the cation exchange capacity of the soil.
[y] OM is organic matter.

Table 7. The effects of vermicompost application on spinach growth parameters and quality for the 2011 and 2012 greenhouse study.

VC[y] (v/v)	Leaf number	Height (cm)	Fresh leaf wt (g)	Leaf area (cm^2)	Ascorbic Acid (mg/100g) fresh wt	Nitrate (mg/kg) dry wt
2011						
0	9.7 a [x]	8.8 c	3.7 c	73.0 c	43.4 b	1397 b
25	7.5 b	11.9 a	4.9 bc	95.2 bc	43.3 b	2205 b
50	8.3 b	10.3 b	5.2 b	99.4 ab	53.4 b	5046 a
75	10.0 a	11.4 ab	7.1 a	122.6 ab	65.2 a	6587 a
2012						
0	13.2 b	9.1 d	4.5 c	-[w]	53.9 a	188 bc
25	11.2 c	11.3 c	5.7 c	-	47.6 a	42 c
50	15.8 a	14.5 a	13.8 a	-	53.0 a	1709 ab
75	10.4 c	12.7 b	11.0 b	-	53.3 a	2695 a

[z] g = 0.0353 oz, 1 cm^2 = 0.1550 inch^2.

[y] VC = coffee vermicompost

[x] Means within a column not followed by the same letter are significantly different at P≤0.05 according to the Student's T multiple range test.

[w] Data not collected.

Table 8. The effects of coffee vermicompost, dairy manure compost, and standard fertility on 'Red Knight X3R' bell pepper yield in a field study combined for 2009-2011.

	Marketable[z]		Cull[y]		Total	Total
Treatment[x]	(kg[w])	(fruits)	(kg)	(fruits)	(kg)	(fruits)
VC	15.7a[v]	118.7a	8.3a	87.4a	18.3a	149a
Compost	13.2a	107.3a	7.4a	82.8a	15.2a	133a
SFT	15.3a	117.0a	8.8a	94.4a	18.0a	152a
Control	14.4a	108.0a	7.5a	80.0a	16.8a	138a

[z] Marketable is fruit without malformations or blemishes.
[y] Cull is fruit with malformations or blemishes which cannot be sold.
[x] Treatments: VC (coffee vermicompost), Compost (dairy manure compost), SFT (standard fertility treatment, 100lbs/Acre (212 t/ha) 12:12:12 N, P and K), and Control (no treatment).
[w] kg = 2.2 lbs.
[v] Means within a column not followed by the same letter are significantly different at P<0.05 according to the Student's T multiple range test.

Table 9. The effects of coffee vermicompost, dairy manure, and standard fertility on 'Red Knight X3R' bell pepper height and chlorophyll index in a 3 year field study.

	height (cm)[z]	Chlorophyll Index[y]	
Treatment[x]	1st harvest	1st harvest	final harvest
2009			
VC	59.8 a [w]	67.4 a	62.2 a
Compost	46.0 c	48.8 b	49.1 b
SFT	58.5 a	67.1 a	68.6 a
Control	51.0 b	47.8 b	50.9 b
2010			
VC	55.1 a	68.0 a	61.0 a
Compost	50.0 b	56.2 b	54.3 ab
SFT	54.1 a	68.6 a	63.9 a
Control	51.8 ab	52.7 b	46.2 b
2011			
VC	53.7 a	59.0 a	44.0 a
Compost	53.3 a	61.1 a	43.4 a
SFT	55.7 a	59.7 a	45.6 a
Control	54.3 a	61.1 a	47.5 a

[z] cm= 0.394 inches

[y] Measured using a Minolta SPAD-502 chlorophyll meter, Osaka, Japan

[x] Treatments: VC (coffee vermicompost), Compost (dairy manure compost), SFT (standard fertility treatment, 100 lbs/acre (212 t/ha) of 12:12:12 (N, P and K), and Control (no treatment).

[w] Means within a column not followed by the same letter are significantly different at $P<0.05$ according to the Student's T multiple range test.

Table 10. The influence of coffee vermicompost, dairy compost, and standard fertility on ‘Red Knight X3R’ green bell pepper ascorbic acid content in a field study combined for 2009-2011.

	Ascorbic acid mg/100g[z]	
Treatment[y]	1st harvest	final harvest
VC	83 a[x]	104 a
Compost	80 a	103 a
SFT	78 a	102a
Control	80 a	106 a

[z] Based on fresh leaf weight.

[y] Treatments: VC (coffee vermicompost), Compost (dairy manure compost), SFT (standard fertility treatment, 100 lbs/acre (212 t/ha) of 12:12:12 (N, P and K), and Control (no treatment).

[x] Means within a column not followed by the same letter are significantly different at $P < 0.05$ according to the Student’s T multiple range test.

Table 11. The effects of coffee vermicompost, dairy manure, and standard fertility on ‘Red Knight X3R’ bell pepper leaf analysis at final harvest for 2011 season.

	N	P	K	Ca	Mg
Treatment [z]	Percentages % dry wt.				
VC	3.4 a[y]	0.8 a	5.4 a	1.8 a	0.3 a
Compost	3.3 a	1.0 a	5.6 a	1.7 a	0.3 a
SFT	3.4 a	0.6 a	5.3 a	1.4 a	0.2 a
Control	3.0 a	1.1 a	5.7 a	1.9 a	0.3 a

[z] Treatments: VC (coffee vermicompost), compost (dairy manure compost), SFT (standard fertility treatment, 100lbs/Acre (212 t/ha) 12:12:12; N, P and K), and Control (no treatment).

[y] Means within a column not followed by the same letter are significantly different at $P<0.05$ according to the Student’s T multiple range test.

CONCLUSION

In this study, coffee VC improved the growth and quality of greenhouse spinach. The optimum application rate appeared to be 50% VC by volume in a 1:1:1 (sand, soil, peat) media in which AA and nitrate content were considered to be within acceptable levels and yield was not compromised. The harvesting of VC from an active worm bed in 2012 as opposed to an inactive bed in 2011, may explain the differences in plant growth and nitrate content observed between the two years, with microbial activity more pronounced in 2012. If this was the case, then increased microbial activity in the 2012 VC could be responsible for the reduced nitrate levels observed compared to 2011. Furthermore, the spinach AA content in this study did not differ appreciably despite the wide range of nitrate content observed. This may indicate that the use of VC may change this well documented, inverse relationship between AA and nitrate content in spinach, possibly through a change in nitrate accumulation and assimilation, or an increased uptake of organic forms of N.

While greenhouse VC studies have shown positive results, we cannot justify the expense of VC in the field production of organic bell peppers using the method of whole bed application. No differences in yield or ascorbic acid content in bell pepper fruits were found between coffee VC, dairy compost, standard fertility treatment, and the control, although the plant height and chlorophyll index was greater in the VC and SFT treatments compared to the compost and control. The field conditions may have been too diverse, or the soil too fertile, to reproduce the same effects observed in greenhouse VC studies, in which an increase of organic marketable bell pepper fruits was seen (Llaven et al. 2008). If VC is available, a more precise application in regards to spatial placement and developmental stage of the plant may be an economical approach. Foliar application or fertigation with VC tea (the leachate of VC) may also be beneficial, but more research is needed before recommendations can be made to organic growers. For conventional growers, VC application in addition to an inorganic plant nutrition program has increased fruit and vegetable growth and yield in the greenhouse and in the field. However, there is no evidence that it may increase AA content of field grown bell peppers.

REFERENCES

Adi, A.J., Z.M. Noor. 2009. Waste recycling: utilization of coffee grounds and kitchen waste in vermicomposting. Bioresource Technol. 100: 1027-1030.

Aira, M., F. Monroy, J. Domínguez. 2007. Microbial Biomass Governs Enzyme Activity Decay during Aging of Worm-Worked Substrates through Vermicomposting. J. Environ. Qual. 36: 448–452.

Albiach, R., R. Canet, F. Pomares, F. Ingelmo. 2000. Microbial biomass content and enzymatic activities after the application of organic amendments to a horticultural soil. Bioresource Technol. 75: 43-48.

Albuzio, A., G. Ferrari, S. Nardi. 1986. Effects of humic substances on nitrate uptake and assimilation of barley seedlings. Can. J. Soil Sci. 66: 731-736.

Alderfasi, A.A., A.E. Moftah, A.M. Aljuaed. 2010. Prospective study of using bio-organic farming system on growth, nitrate, oxalate and ascorbic acid contents in spinach. World Appl. Sci. J. 9 (1): 49-54.

Anastasi, A., G.C. Varese, S. Voyron, S. Scannerini, V.F. Marchisio. 2004. Characterization of fungal biodiversity in compost and vermicompost. Compost Sci. and Utilization. 12 (2):185-191.

Ansari, A.A. 2008. Effect of vermicompost on the productivity of potato (*solanum tuberosum*), spinach (Spinacia oleracea), and turnip (Brassica campestris). World J. Agi. Sci. 4(3):731-735.

AOAC International (2007) AOAC method 967.21. In: Official methods of analysis, 18th ed. AOAC International, Gaithersburg, MD.

Arancon, N.Q., C.A. Edwards, P. Bierman, J.D. Metzger, S. Lee, C. Welch. 2003. Effects of vermicomposts on growth and marketable fruits of field-grown tomatoes, peppers and strawberries. Pedobiologia. 47: 731-735.

Arancon, N.Q., Edwards, C.A., Atiyeh, A., Metzger, J.D., 2004. Effects of vermicomposts produced from food waste on the growth of greenhouse peppers. Bioresource Technol. 93, 139-144.

Arancon, N., C.A. Edwards, P. Bierman, J.D. Metzger, C. Lucht. 2005. Effects of vermicomposts produced from cattle manure, food waste and paper waste on the growth and yield of peppers in the field. Pedobiologia. 49: 297-306.

Arancon, N., C.A. Edwards, A. Babenko, J. Cannon, P. Galvis, J.D. Metzger. 2008. Influences of vermicomposts, produced by earthworms and microorganisms from cattle manure, food waste and paper waste, on the germination, growth and flowering of petunias in the greenhouse. Appl. Soil Ecol. 39:91-99.

Arshad, M., W.T. Frankenberger. 1993. Microbial production of plant growth regulators. In: F.B. Metting, Jr., (ed). Soil Microbial Ecology. Marcel Dekker, New York.

Atiyeh, R.M., S. Subler, C.A. Edwards, G. Bachman, J.D. Metzger, W. Shuster. 2000. Effects of vermicompost and composts on plants growth in horticultural container media and soil. Pedobiologia. 44:579-590.

Atiyeh, R.M., C.A. Edwards, S. Subler, J.D. Metzger. 2001. Pig manure vermicompost as a component of a horticultural bedding plant medium: effects on physicochemical properties and plant growth. Bioresource Technol. 78:11-20.

Atiyeh, R.M., S. Lee, C.A. Edwards, N.Q. Arancon, J.D. Metzger. 2002. The influence of humic acids derived from earthworm- processed organic wastes on plant growth. Bioresource Technol. 84: 7-14.

Bachman, G.R., J.D. Metzger. 2008. Growth of bedding plants in commercial potting substrate amended with vermicompost. Bioresource Technol. 99: 3155-3161.

Bernitez, E., H. Sainz, R. Nogales. 2004. Hydrolytic enzyme activities of extracted humic substances during the vermicomposting of a lignocellulosic olive waste. Bioresource Technol. 96(7):785-790.

Bukerfield, J.C., T.C. Flavel, K.E. Lee, K.A. Webster.1999. Vermicompost in solid and liquid forms as a plant growth promoter. Pedobiologia. 43:753-759.

Canellas, L.P., F.L. Olivares, A.L. Okorokova-facanha, A.R. Facanha. 2002. Humic acids isolated from earthworm compost enhance root elongation, lateral root emergence, and plasma membrane H^+ - ATPase activity in maize roots. Plant Physiol. 130:1951-1957.

Canellas, L.P., A. Piccolo, L.B. Dobbss, R. Sapccini, F.L. Olivares, D.B. Zandonadi, A.R. Facanha. 2010. Chemosphere. 78:457-466.

Cesco, S., M. Nikolic, V. Römheld, Z. Varanini, R. Pinton. 2002. Uptake of 59Fe from soluble 59Fe-humate complexes by cucumber and barley plants. Plant and Soil. 241:121-128.

Chan, T.Y.K., 2010. Vegetable-born nitrate and nitrite and the risk of methaemoblobinaemia. Toxicology letters. 200:107-108.

Chaoui, H.I., L.M. Zibilske, T. Ohno. 2003. Effects of earthworm casts and compost on soil microbial activity and plant nutrient availability. Soil Biol. and Biochem. 35:295-302.

Chassy, A.W., L. Bui, E.C. Renaud, M. van Horn, A.E. Mitchell. 2006. Three-Year Comparison of the Content of Antioxidant Microconstituents and Several Quality Characteristics in

Organic and Conventionally Managed Tomatoes and Bell Peppers. J. Agric. Food Chem. 54:8244-8252.

Chen, Y., 1996. Organic matter reactions involving micronutrients in soils and their effects on plants, p. 507-529. In: A. Piccolo (ed.). Humic substances in terrestrial ecosystems. Elsevier, Oxford.

Chung, S.Y., J.S. Kim, M. Kim, K. Hong, J.O. Lee, C.M. Kim, I.S. Song. 2003. Survey of nitrate and nitrite contents of vegetables grown in Korea. Food Additives and Contaminants 20(7):621-628.

Citak, S., S. Sonmez. 2010. Effects of conventional and organic fertilization on spinach (*Spinacea oleracea* L.) growth, yield, vitamin C and nitrate concentration during two successive seasons. Scientia Horticulturae. 126:415-420.

Cruz, C., A.F.M Bio, M.D. Dominguez-Valdivia, P.M. Aparicio-Tejo, C. lamsfus, M.A. martins-Loufo. 2006. How does glutamine synthetase activity determine plant tolerance to ammonium? Planta. 223:1068-1080.

Davey, M.W., M. Van Mantagu, D. Inze, M. Sanmartin, A. Kaneliis, N. Smirnoff, I.J.J. Benzie, J.J. Strain, D. Flavell, J. Fletcher. 2000. Plant L-AA: chemistry, function, metabolism, bioavailability and effects of processing. J. Sci. food and Agr. 80:825-860.

Dykhuizen, R.S., R. Frazer, C. Duncan, C.C. Smith, M. Golden, N. Benjamin, C. Leifert. 1996. Antimicrobial effect of acidified nitrite on gut pathogens: Importance of dietary nitrate in host defense. Antimicrobial Agents and Chemotherapy. 40(6):1422-1425.

Edwards, C.A., 1977. Biology and Ecology of Earthworms. 3rd ed. Chapman and Hall Press, London.

Edwards, C.A., N.Q. Arancon, E. Emerson, R. Pulliam. Dec. 2007. Suppressing plant parasitic nematodes and anthropod pests with vermicompost teas. BioCycle.

Edwards, C.A., N.Q. Arancon, M. Vasko-Bennet, G. Keeney. 2010. Effects of aqueous extracts from vermicomposts on attacks by cucumber beetles (*Acalymna vittatum) (*Fabr.) on cucumbers and tobacco hornworm (*Manduca sexta) (L.)* on tomatoes. Pedobiologia. 53(2):141-148.

Edwards, C.A., N.Q. Arancon, R. Sherman. 2011. Vermiculture Technology: Earthworms, organic wastes, and environmental management. CRC Press, Boca Raton, Fl.

Elia, A., P. Santamaria, F. Serio. 1998. Nitrogen Nutrition, Yield and Quality of Spinach. J Sci. Food Agric. 76:341-346.

Ferreras, L., E. Gomez, S. Toresani, I. Firpo, R. Rotondo, R. 2006. Effect of organic amendments on some physical, chemical and biological properties in a horticultural soil. Bioresource Technol. 97:635-640.

Foyer, C., M. Lelandals, E.A. Edwards, P.M. Mullineaux. 1991. The role of ascorbate in plants, interactions with photosynthesis, and regulatory significance. Current topics in plant physiol. 6:131-144.

Frankenberger, W.T., Jr., M. Arshad. 1995. Phytohormones in soil: Microbial production and function. Marcel Dekker, New York.

Hashemimajd, K., Kalbasi, M., Golchin, A., Shariatmadari, H. 2004. Comparison of vermicompost and composts as potting media for growth of tomatoes. Journal of Plant Nutrition 27 (6), 1107-1123.

Havlin, J.L., J.D. Beaton, S.L. Tisdale, W.L. Nelson. 2005. Soil fertility and fertilizers: An introduction to nutrient management. 7th ed. Prentice hall, Upper Saddle River, New Jersey.

Herman, R.J. 1979. Soil Survey of Jackson County, Ill. Ill. Ag. Exp. Stat. Soil Report no. 106.

Hopkins, W.G., N.P.A. Hüner. 2004. The plant hormones: Control and developments, p. 341-365. In. Introduction to Plant Physiology, Wiley, New York.

Jamaludin, A.A., N.Z. Mahmod. 2010. Effects of vermicomposting duration to macronutrient elements and heavy metals concentrations in vermicompost. Sains Malaysiana 39 (5):711-715.

Joint FAO/WHO Expert Committee on Food Additives, JEFCA. 1995. Evaluation of certain food additives and contaminants'. 29-35. World Health Organization.

Jordão, C.P., D.G. Pererira, R. Einloft, M.B. Santana, C.R. Bellato, J.W.V. Mello. 2002. Removal of Cu, Cr, Ni, Zn, and Cd from electroplating wastes and synthetic solutions by vermicompost of cattle manure. J. Environ. Sci. health, Part A. 37:875-892.

Kalantarti, S., S. Hatami, M.M Ardalan, H.A. Aikhani, M. Shorafa. 2010. The effect of compost and vermicompost of yard leaf manure on growth of corn. African J. of Agri. Res. 5(11):1317-1323.

Krishnamoorthy, R. V., S.N. Vajrabhiah. 1986. Biological activity of earthworm casts: An assessment of plant growth promoter levels in casts. Proc. Indian Acad. Sci. (Anim. Sci.) 95:341-351.

Llaven, M.A.O., J.L.G. Jimenez, B.I.C. Coro, R.R. Rosales, J.M. Molina, G. Micelli. 2008. Fruit characteristics of bell pepper cultivated in sheep manure vermicompost substituted soil. J. of Plant Nutr. 31:1585-1598.

Loffredo, E., L. Monaci, N. Senesi. 2005. Humic substances modulate the allelopathic potential of caffeic, ferulic and salicyclic acids for seedlings of lettuce (*lactuca sativa* L.) and tomato (*Lycospersicon ensulentum* Mill.). J. Agric. Food Chem. 53:9424-9430.

Marshner, P. 2012. Mineral nutrition of higher plants. 3rd ed. Academic Press, Elsevier, San Diego, CA.

Micelli, F.A.G., Borraz, J.S., Molina, J.A.M., Nafata, C.C., Archila, M.A., Llaven, M.A.O., Rosales, R.R., Dendooven, L. 2007. Vermicompost as a soil supplement to improve growth, yield and fruit quality of tomato (lycospersicum enculentum). Bioresource Technol. 98, 2781-2786.

Mora, V., R. Baigorrib, E. Bacaicoab, A.M. Zamarre˜nob, J.M. Garcĩa-Minaa. 2012. The humic acid-induced changes in the root concentration of nitric oxide, IAA and ethylene do not explain the changes in root architecture caused by humic acid in cucumber. Environ. Expt. Bot. 76:24-32.

Mozafar, A. 1994. Plant vitamins: Agronomic, physiological, and nutritional aspects. CRC Press, Boca Raton, FL.

Muramoto, J. Comparison of nitrate content in leafy vegetables from organic and conventional farms in California. Center for Agroecology and Sustainable Food Systems, University of California, Santa Cruz. June 1999.

Muscolo, A., M.R. Panuccio, M.R. Abenavoli, G. Concheri, S. Nardi. 1996. Effect of molecular complexity and acidity of earthworm faeces humic fractions on glutamate dehydrogenase, glutamine synthase, and phosphoenolpyruvate carboxylase in *Daucus carota* cells. Biol. Fertile. Soils. 22:83-88.

Muscolo, A., S. Cutrupi, S. Nardi. 1998. IAA detection in humic matter. Soil. Biol. Biochem. 30:1199-1201.

Muscolo, A., F. Bovalo, F. Gionfriddo, S. Nardi. 1999. Earthworm humic matter produces auxin-like effects on *Daucus carota* cell growth and nitrate metabolism. Soil. Biol. Biochem. 31:1303-1311.

Muscolo, A., M. Sidari, E. Attina, O. Francioso, V. Tugnoli, S. Nardi. 2007. Biological activity of humic substances is related to their chemical structure. Soil. Sci. Am. J. 71:75-85.

Nannipieri P., P. Sequi, P. Fusi. 1996. Humus and enzyme activity, p. 293-329. In: Humic substances in terrestrial ecosystems. Elsevier Science, Amsterdam, The Netherlands.

Nannipieri P., P. Eldor. 2009. The chemical and functional characterization of soil N and its biotic components. Soil Biol. Biochem. 41:2357-2369.

Nardi S., G. Concheri, G. Dell’Agnola. 1996. Biological activity of humus, 361-407. In: Humic substances in terrestrial ecosystems. Elsevier Science, Amsterdam, The Netherlands.

Orozco, F.H., Cegarra, J., Trujillo, L.M., Roig, A. 1996. Vermicomposting of coffee pulp using the earthworm *Eisenia fetida*: Effects on C and N contents and the availability of nutrients. Biol. Fertil. Soils 22, 162-166.

Paul, L.C., J.D. Metzger. 2005. Impact of vermicompost on vegetable transplant quality. HortScience 40(7), 2020-2023.

Peyvast, Gh., J.A. Olfati, S. Madeni, A. Forghani. 2008. Effect of vermicompost on the growth and yield of spinach (*Spinacia oleracea* L.). J. Food, Agr. Environ. 6(1):110-113.

Piccolo, A., S. Nardi. G. Concheri. 1992. Structural characteristics of humic substances as related to nitrate uptake and growth regulation in plant systems. Soil Biol. Biochem. 24(4):373-380

Pinton R. S., Cesco, S. Santi, F. Agnolon Z. Varanini.1999 Water extractable humic substances enhance iron deficiency responses by Fe-deficient cucumber plants. Plant Soil. 210:145–157.

Premuzic, Z., M. Bargiela, A. Garcia, A. Rendina, A. Iorio. 1998. Calcium, iron, potassium, phosphorus and vitamin C content of organic and hydroponic tomatoes. HortScience 33(2):255-257.

Premuzic, Z., A. Garate, I. Bonilla. 2002. Production of lettuce under different fertilization treatments, yield and quality. Acta Hort. 571, ISHS.

Quaggiotti, S., B. Ruperti, D. Pizzeghello, O. Francioso, V. Tugnoli, S. Nardi. 2004. Effect of low molecular size humic substances on nitrate uptake and expression of genes involved in nitrate transport in maize (*Zea mays* L.). J. Expt. Bot. 55(398):803-813.

Shokrzadeh, M., M. Shokravie, A.G. Ebadi, Z. Babaee, A. Tarighati. 2007. The measurement of nitrate and nitrite content in leek and spinach sampled from central cities of Mazandaran State of Iran. World Appl. Sci. J. 2(2):121-124.

Singh, R., R.R. Sharma, S. Kumar, R.K. Gupta R.T Patil. 2008. Vermicompost substitution influences growth, physiological disorders, fruit yield and quality of strawberry (*Fragaria x ananassa* Duch.). Bioresource Technol. 99:8507-8511.

Smirnoff, N., 1996. The function and metabolism of AA in plants. Annals Bot. 78:661-669.

Stoewsand, G.S., J.L. Anderson, C.Y. Lee. 1973. Nitrite-induced methemoglobinaemia in guinea pigs: influence of diets containing beets with varying amounts of nitrate, and the effect of AA, and methione. J. plant Nutr. 103:419-424.

Szczech, M.M. 1999. Suppressiveness of vermicompost against Fusarium Wilt of tomato. J. Phytopatho. 147:155-161.

Tannenbaum, S.R., J.S. Wishnok, C.D. Leaf. 1991. Inhibition of nitrosoamine formation by Ascorbic acid. Am. J. Clin. Nutr. 53:247S-50S.

The Commission of the European Communities. Jan. 1997. Commission regulation (EC) no. 197/97. Setting maximum levels for certain contaminants in foodstuffs. Offic. J. of the European Communities. L31:48-50.

Theunissen, J., P. A. Ndakidemi, C. P. Laubscher. 2010. Potential of vermicompost produced from plant waste on the growth and nutrient status in vegetable production. Intl. J. Phys. Sci. 5(13):1964-1973.

Tognetti, C., F. Laos, M.J. Mazzarino M.T. Hernandez. 2005. Composting vs. vermicomposting: A comparison of end product quality. Compost Sci. and Utilization. 13(1):6-13.

Tognetti, C., M.J. Mazzarino, F. Laos. 2008. Compost of municipal organic waste: Effects of different management practices on degradability and nutrient release capacity. Soil Biol. Biochem. 40:2290-2296.

Tomati, U., E. Galli, A. Grapelli, G. Dihena. 1990. Effects of earthworm casts on protein synthesis in radish (*Raphanus sativum*) and lettuce (*lactuca sativa*) seedlings. Biol. Fertil. Soils 9:288-289.

Vallini, A. Pera, M. Agnolucci, M.M. Valdrighi. 1997. Humic acids stimulate growth and activity of in vitro tested axenic cultures of soil autotrophic nitrifying bacteria. Biol. Fertil. Soils. 24:243-248.

Wittwer, S.H., R.A. Schroeder, Wm. A. Albrecht. 1945. Vegetable crops in relation to soil fertility: Vitamin C and nitrogen fertilizers. Soil Sci. 59:329-336.

Worthington, V. 2001. Nutritional quality of organic versus conventional fruits, vegetables, and grains. J. Alternative and Complementary Medicine. 7(2):161-173.

VITA

Graduate School
Southern Illinois University

April Vigardt

ajvigardt@yahoo.com

Southern Illinois University Carbondale
Bachelor of Science, Plant and Soil Science, August 2007

THESIS Title:
INFLUENCE OF COFFEE VERMICOMPOST ON GROWTH AND NUTRIENT QUALITY OF GREENHOUSE SPINACH AND FIELD GROWN GREEN BELL PEPPERS

Major Professor: Dr. Stuart A. Walters

.