VERMICOMPOST TEA: EFFECTS ON PAK CHOI (*BRASSICA RAPA* CV. BONSAI, CHINENSIS GROUP) GROWTH AND YIELD, PHYTONUTRIENT CONTENT AND SOIL BIOLOGICAL PROPERTIES

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Dedicated to my husband Pradip
and daughter Preetha

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ABSTRACT

Vermicompost tea, an aqueous extract of vermicompost has been reported to improve plant health, yield and nutritional quality. Most of the previous researches on compost tea have investigated the potential of compost tea for control of plant disease. Only limited data exists on the use of vermicompost tea for improving the yield and nutritional quality of vegetable crops and altering soil biological properties. Therefore, laboratory, greenhouse and field experiments were conducted in order to identify and describe: 1) the effects of vermicompost tea extraction methods [(i) non-aerated (NCT), (ii) aerated (ACT), and (iii) aerated with additives (ACTME)], 2 fertilizers (Osmocote and vermicompost), and three growth media (Oxisol, Mollisol and a peat-perlite medium) on yield and nutritional quality of pak choi (*Brassica rapa, Chinensis*) as well as soil biological properties; 2) the effects of the ratio of vermicompost to water and different fertilizers on yield and nutritional quality of pak choi as well as soil biological properties; 3) the effect of compost quality on biochemical properties of compost tea; and 4) mechanisms involved in the effects of compost tea on plant growth.

The result showed that applications of vermicompost tea, regardless of extraction method, increased plant yield, mineral nutrients, phytonutrient content of pak choi; and microbial activities of an Oxisol, a Mollisol or a peat-perlite medium and this effect was most prominent under organic fertilization. This finding suggests that vermicompost tea serves both as a supplemental source of plant nutrients and an enhancer of soil biological properties. Similarly, application of vermicompost tea with vermicompost to water ratios of 1:10 - 1:100 (v:v) increased yield, total carotenoids,

total glucosinolates and N content of pak choi; and microbial activities in soil. The responses of these parameters to vermicompost to water ratio was positive and linear. The results also indicated that biochemical properties of compost determined biochemical properties of compost tea, and the resulting quality of tea positively impacted plant growth and tissue mineral nutrient. The positive influence of vermicompost tea or compost tea on plant growth was largely associated with N and gibberellin$_4$ (GA_4) present in the tea and nutrient uptake by plants. Overall, results from these studies improve the understanding of vermicompost tea effects on yield and nutritive quality of pak choi as well as soil biological properties.

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CHAPTER 1

INTRODUCTION

Compost tea, a water-based compost extract containing high levels of beneficial microbes and soluble nutrients, has attracted the attention of growers and researchers in recent years. The most important reason to apply compost tea is to supply microbial biomass, fine particulate organic matter, and soluble chemical components of compost to plant surfaces and soils in a way not possible or achievable with solid compost (NOSB, 2004). Use of compost tea as a foliar spray or soil drench has been demonstrated to improve plant health, yield and nutritional quality by: (i) enhancing beneficial microbial communities and their effects on agricultural soils and plants; (ii) improving mineral nutrient status of plants; and, (iii) inducing the production of plant defense compounds that may have beneficial bioactivities in humans (Weltzien, 1991; Hoitink *et al.*, 1997; Scheuerell and Mahaffee, 2002; Carpenter-Boggs, 2005; Ingham, 2005a; Diver, 2001). The potential benefits of compost tea are substantial and particularly relevant to crop production in low-input agricultural systems.

Although the chemistry and microbiology of compost extract is complex, it is hypothesized that soluble mineral nutrients extracted from compost and applied on foliage and soil have positive effects on plant growth (Ingham, 2005a). It is also postulated that the action of living microorganisms and microbial metabolites stimulate plant growth (Carpenter-Boggs, 2005; Diver, 2001). Water extractable growth regulators or phyto-hormones from compost and vermicompost may also have positive effect on initial root development and plant growth (Keeling *et al.*, 2003; Edwards *et al.*, 2006).

While compost teas are generally low in plant available nutrients and may not substitute for a fertilizer, they can provide supplements for short periods when rapid nutrient adsorption is critical to crop production. Microbial biomass in compost tea provides a source of nutrients and plays an important role in soil organic matter mineralization, improving the synchrony of nutrient release to meet crop demand.

Merrill and McKeon (1998) noted that the potential for compost tea has been hindered by lack of standard protocols, producing varied and often conflicting results from research. This situation is primarily due to variations in the quality of compost used for compost tea extraction and the different extraction methods.

Compost tea can be produced using a variety of composts. Vermicompost, in contrast to conventional compost, is the product of accelerated bio-degradation of organic matter through the use of high densities of earthworms without a thermophilic stage (Dominguez *et al.*, 1997). Vermicompost generally contains higher nutrient concentrations and greater microbial populations and activity than thermophilic compost consequently making greater potential contributions to crop yield (Tognetti *et al.*, 2005). Fine texture along with higher nutrient content and microbial activity apparently contributed to greater market acceptance of vermicomposts than that of composts (Subler *et al.*, 1998; Ndegwa and Thompson, 2000).

Compost tea can be prepared by aerated or non-aerated methods. Scheuerell and Mahaffee (2002) use the terms non-aerated compost tea (NCT) and aerated compost tea (ACT) for these two methods. The non-aerated method results in generally low-oxygen

conditions during tea extraction whereas, the aerated method maximizes oxygen during the extraction of compost. Sugars, grain, fish emulsion, kelp tea, humic acid and other products are often added during extraction of aerated teas to enhance microbial activity of the finished product, but little research work are reported regarding the impact of these additives on tea quality or plant response. It is generally expected that the use of additives to enhance microbial growth in compost tea would be more effective in promoting plant growth. Additives may also have a direct effect on plants via added nutrients. Compost tea produced from the ACT extraction method with the use of additives (microbial enhancer) is called ACTME.

Several investigators have reported that non-aerated compost tea has consistently positive effects on disease control and plant growth in contrast to aerated compost tea (Weltzien, 1991; Cronin *et al.*, 1996; Scheuerell and Mahaffee, 2006). Welke (2005) reported that both aerated and non-aerated compost tea extracted from composted animal manure have similar positive effects on strawberry yield and suppression of *Botrytis cinerea*. In contrast, Arancon *et al.* (2007) reported that aerated vermicompost tea had a greater positive impact on plant growth than non aerated tea extracted for the same period of time (24 hrs). There is no agreement whether aeration is required.

Secondary plant metabolites such as glucosinolates, carotenoids and phenolic compounds have important implications to human health, crop flavor, and commodity value because of their demonstrated biological reactivity and association with anti-carcinogenic activity in humans. Various studies have shown that agricultural practices can significantly affect the concentration of

those plant metabolites in vegetables (Radovich *et al.*, 2005a; Perez-Lopez *et al.*, 2007). Perez-Lopez *et al.* (2007) observed that the use of composted animal manure increased the total carotenoids in sweet pepper (*Capsicum annuum*). Sanwal *et al.* (2006) have reported that increased crop yield and dietary anti-oxidants of broccoli occurred with the use of compost and non-aerated compost tea.

Rhizosphere properties are strongly influenced by management practices and sensitively reflect the change and dynamics in soil quality and health. Various studies have shown that organic fertilization improved mineral nutrient status as well as soil biological and physical properties (Tejada and Gonzalez, 2006; Okur *et al.*, 2008). Application of vermicompost tea potentially can add a huge numbers of active microbial populations and mineral nutrients to the soil. It is also assumed that higher microbial activity and nutrient status contribute to better root development and nutrient uptake. However, there is a lack of scientific study and documentation on the effect of vermicompost tea on plant and soil interaction.

Our ability to effectively employ compost teas to their full advantage is severely limited by our lack of understanding of the interactions between compost tea extraction methods, crop physiology, and environmental factors on plant yield and nutrient contents, especially under tropical conditions. These gaps in our knowledge limit the efficacy of compost tea applications to optimize crop yield, nutritive quality, and soil quality on certified organic farms that predominantly employ this strategy currently. In addition, this lack of knowledge

restricts the extension and adoption of compost tea technology to improve the sustainability of conventional farms.

The primary goal of this dissertation research is to evaluate the effectiveness of vermicompost tea prepared by different extraction methods on plant growth, yield and phytonutrients content in pak choi (*Brassica rapa,* cv Bonsai, Chinensis group) as well as soil biological properties under different soil types and fertilizer regimes. The specific objectives of this study were to:

I. Determine the effects of extraction methods on vermicompost tea quality and subsequent effects on growth, mineral nutrient, phytonutrient and anti-oxidant activity of pak choi grown under organic (vermicompost) and synthetic (Osmocote) fertilization.

II. Investigate the effects of vermicompost tea on (i) growth, mineral nutrient and phytonutrient of pak choi grown in different media under organic (green waste compost) and synthetic (Osmocote) fertilization and (ii) chemical and biological properties of growth media.

III. Evaluate the effects of the concentration of vermicompost tea on pak choi yield, quality, and soil biological properties under greenhouse and field condition.

IV. Investigate the effects of compost quality on compost tea quality and subsequent plant growth response as well as possible mechanisms involved in the compost tea influence on growth and yield of pak choi.

The present dissertation is organized in 7 chapters. The current chapter (Chapter 1) introduces the problem and justification of the study. The literature review (Chapter 2) provides the historical and scientific background on the use of compost/vermicompost tea in agriculture production system. In particular, the expected mechanisms involved in vermicompost tea action in plant production are addressed. The effects of 3 different vermicompost tea extraction methods (non-aerated, aerated, and aerated with additives) and their interactions with 2 fertilizers (Osmocote and vermicompost) on pak choi yield, phytonutrients and mineral nutrients in plant tissue is evaluated in chapter 3. Chapter 4 investigates the effects of vermicompost tea on growth, mineral nutrient and phytonutrient of pak choi grown in different growth media (Oxisol, Mollisol and peat-perlite medium) under organic (green waste compost) and synthetic (Osmocote) fertilization. The impacts of tea application on chemical and biological properties of growth media have been also evaluated. Effects of the concentration of vermicompost tea on pak choi yield and quality, as well as soil biological properties under greenhouse and field conditions are evaluated in chapter 5. The links between compost and compost tea quality and subsequent plant growth response are evaluated in chapter 6. Also, the possible mechanisms involved in the compost tea influence on growth and yield of pak choi are discussed in chapter 6. Chapter 7 summarizes the conclusions of this dissertation research and provides recommendations for future research.

CHAPTER 2

LITERATURE REVIEW

2.1 Background

Aqueous extracts of compost and various plant materials have been used in agricultural production systems since the 1920s. The practice of soaking seeds and drenching plants or soil with a liquid extract of compost for fertilization and plant disease suppression was used in historical gardening systems (Rodale, 1967). Various experimental evidence indicated that applications of water-based compost and vermicompost extract improved plant health, yield and nutritional quality (Elad and Shtienberg, 1994; Al-Dahmani *et al.*, 2003; Scheuerell and Mahaffee, 2004; Welke, 2005; Edwards *et al.*, 2006). In recent years, there has been a rising interest in the potential for improving plant health, yield and nutritional quality with the application of water-based compost extracts, typically called compost teas. As a consequence, compost tea industries have been estimated to be increasing by 25% per year (Carpenter-Boggs, 2005).

The mechanisms by which compost tea is expected to suppress plant disease include induced resistance, antibiosis, and competition (Scheuerell and Mahaffee, 2002). It is also believed that application of compost tea improves plant health, yield and nutritional quality by supplying microbial biomass, fine particulate organic matter, organic acids, plant growth regulator-like substances, and soluble mineral nutrients to plant surfaces and soils in a way that is not possible or realistic with solid compost (NOSB, 2004; Welke, 2005; Edwards *et al.*, 2006).

Most of compost tea research has investigated the potential of compost tea for plant disease control but results have been inconsistent, which may in part be associated with variation among the compost teas tested (NOSB, 2004). The importance of aeration seems to be the most disputed aspect of its efficacy on disease suppression and plant growth promotion; however, conclusive evidence that demonstrates the superiority of aerated compost tea (ACT) over non-aerated tea (NCT) is lacking (Brinton *et al.*, 2004; Kelley, 2004). Merrill and McKeon (1998) noted that the potential for compost tea has been hindered by lack of standard protocols for production, producing varied and often conflicting results from research. There are many influential production decisions including choice of compost feedstock, compost age, brew time, and compost to water ratio (Scheuerell and Mahaffee, 2002). The Compost Tea Task Force Report to the USDA concluded that there is a major data gap in compost tea research and our understanding of compost tea is in its infancy, which warrants more research in this issue (NOSB, 2004).

2.2 Terminology

'Compost tea' has been described as an aqueous extract of compost in which microorganisms, organic matters, and nutrients are transferred from compost to solution either through active aeration or non-aerated extraction at room temperature for a defined period of time (Riggle, 1996; Scheuerell and Mahaffee, 2002; Ingham, 2005a). 'Compost extract' (Weltzien, 1989), 'watery fermented compost extracts'(Weltzien, 1991) and 'steepages' (Hoitink *et al.*, 1997) have been used to refer 1:5 to 1:10 ratio of compost to water (v:v) that is fermented without stirring at room temperature in an open container for

a defined period of time. Weltzein (1991) used the term ‘amended extract’ for the compost extracts that have been fermented with the addition of specific nutrients or combined with isolated microorganisms before application. Ingham (2005a) however, used the term fermentation typically for the extraction of un-decomposed plant materials in water without active aeration. Brinton *et al.* (1996) defines compost extracts or teas as a "deliberate production of specific (water) extracts based on composts of known properties and age" without distinguishing between non-aerated and aerated production. Scheurell and Mahaffee (2002) noted that the use of the term 'compost extract' poses a particular challenge due to the widespread use of this term in studies on the chemical properties of compost. This suggests that the term 'compost extract' should typically be used for describing the filtered product of compost mixed with any solvent when used for analytical or assay work. Several companies have developed different systems of compost tea production under highly aerated conditions and each company describes the product as 'compost tea'. This commercial usage of the term further reinforces the common use of the term ‘compost tea’. The terms compost tea and vermicompost tea referring to the aqueous extract of compost and vermicompost, respectively, will be used in this study.

2.3 Vermicompost and vermicompost tea

Vermicompost is the product of accelerated bio-degradation of organic matter through the use of high densities of earthworms without a thermophilic stage (Dominguez *et al.*, 1997). Digestion process of organic wastes in earthworm’s gizzard facilitates the breakdown of parent organic matter, alters the physical and chemical properties of the

material and further decomposition by microorganisms leading to a humification effect (Albanell *et al.,* 1988; Orozco *et al.,* 1996). The end product, commonly referred to as vermicompost is fine textured peat-like organic material with high porosity, aeration, drainage, water-holding capacities, and low C:N ratios (Edwards and Burrows, 1988; Subler *et al.*, 1998; Domínguez, 2004). Vermicomposts generally hold greater soluble, nutrient concentrations (NO_3^-, Ca, P and K) that are taken up readily by plants and diverse microbial populations than those of conventional thermophilic composts (Tognetti *et al.*, 2005). Fine texture along with higher nutrient content and microbial activity apparently contributed to greater market acceptance of vermicomposts than that of thermophilic composts (Subler *et al.,* 1998; Ndegwa and Thompson, 2000). Scientific studies and documentations regarding the impact of vermicompost on soil fertility and plant disease suppression have focused mainly on solid amendments (Edwards, 1998; Domínguez, 2004), and their use as liquid extracts has not been investigated thoroughly.

Although vermicompost tea and compost tea are similar in terms of production method and their modes of action to plants, it is believed that superior biochemical and physical properties of vermicompost over thermophilic compost would also be reflected in tea quality as well as disease suppression and plant growth response (Edwards *et al.*, 2006; Zaller, 2006). Scientific comparison of vermicompost tea and compost tea on disease suppression and plant growth response is scarce and the few available publications provide inconsistent results (Hoitink *et al.*, 1997; Scheuerell and Mahaffee, 2004; Edwards *et al.*, 2006).

2.4 Compost tea production method

Two dominant approaches of compost tea production are aerated and non-aerated methods. Scheurell and Mahaffee (2002) and Ingham (2005a) used the terms non-aerated compost teas (NCT) and aerated compost teas (ACT) to refer to the extracts produced by these methods. ACT refers to methods in which the mixture is actively aerated during extraction. NCT refer to methods that do not disturb or only minimally disturb the extraction after initial mixing. Other terms used to describe aerated and non-aerated compost teas are: aerobic and anaerobic, or active and passive. Aerated and non-aerated, however, seem to most accurately describe the different processes used in production as well as the end-product (Scheuerell and Mahaffee, 2002).

Both extraction methods involve the steeping of compost in water for a defined period at room temperature. In addition, aerated compost tea requires aeration throughout the extraction period (Weltzien, 1991; Scheuerell and Mahaffee, 2002). Weltzein, as a pioneer in this area, focused primarily on non-aerated method of compost tea production in the late 1980's and early 1990's (Weltzein and Ketterer, 1986; Weltzien, 1991). However, in recent years, interest has shifted to the ACT method (Scheuerell and Mahaffee, 2002). From a grower's perspective, ACT has the distinct advantage that it can be prepared in 1-2 days and results in less odor problems, whereas NCT requires 1-2 weeks steeping time (Ingham, 2005a). Non-aerated compost tea does not require any special technology beyond a steeping vessel and is associated with low cost or low energy input, whereas ACT requires constant stirring and aerating of large volumes of liquid. Proponents of ACT production argue that the risk of contamination by

human pathogens is very low in ACT compared to that of NCT, as the human pathogens including *E coli* are poorly competitive under aerobic conditions, however, there is no documented evidence to substantiate these claims (Brinton *et al.*, 2004).

Several investigators have reported that non-aerated compost tea has consistent and significantly positive effects on plant growth and disease control compared to that of aerated compost tea (Weltzien, 1991; Tranker, 1992; Cronin *et al.*, 1996; Scheuerell and Mahaffee, 2004, 2006). Scheuerell and Mahaffee (2004) reported that aerated compost tea made with different composts and supplemented with kelp and humic acid consistently suppressed damping off of cucumber seedlings caused by *Pythium ultimum* compared to non-aerated teas. However, in another study Scheuerell and Mahaffee (2000) observed no significant difference between ACT and NCT in suppressing powdery mildew of rose and concluded that compost source was more important than aeration for maximizing disease control. Welke (2005) concluded that both aerated and non-aerated teas have similar effect on plant growth and disease suppression. Hargreaves *et al.* (2009) have shown that non-aerated compost tea is as effective a nutrient amendment as compost and inorganic fertilizer for growth promotion of strawberry plants. The use of microbial enhancer (kelp extract and humic acid to promote equal bacterial and fungal growth) along with the aeration is desirable for better result (Ingham, 2005a). These varying reports of the impacts of compost tea production method on plant health and yield are crop specific and inferences about the superiority of one method over another on disease suppression or plant yield cannot be generalized.

2.5 Microbial population in compost tea

Microbial populations and their diversities in compost tea are believed to be the most important factors contributing to its effect on disease suppression and plant growth. A good quality compost tea should contain a large population of beneficial microorganisms such as active and total bacteria, fungi, protozoa and nematodes. Ingham (2005a) suggests that one mL of a compost tea should contain 10-150 µg active bacteria, 150-200 µg total bacteria, 2-10 µg active fungi, 2-20 µg total fungi, 1000 each flagellate and amoeba type protozoa, 20-50 ciliate type protozoa and 2-10 beneficial nematodes. However, standardized protocols for characterizing the biological quality of compost tea have not been well established. This limits our understanding of the microbial species composition of compost tea and how these organisms impact plant growth. Presence of a diverse range of active and total microbial populations is an important characteristic of good quality compost tea; however, possibility of contamination by pathogenic microorganisms such as *Escherichia coli* and some fungal pathogens is always a challenge. It is believed that use of unfinished or not fully decomposed compost is one of the most likely sources of pathogenic microorganisms in compost tea.

2.6 Mineral nutrients, humic acid and phytohormones in compost tea

Compost tea is thought to improve plant health, yield and nutritional quality by the addition of microbial biomass, soluble mineral nutrients, organic acids and water soluble plant growth regulator like substances. Various studies reported that compost tea contains a considerable amount of soluble mineral nutrients that are readily available for

plant uptake and promote crop growth and yield (Welke, 2005; Hargreaves *et al.*, 2009; Pant *et al.*, 2009; Azza *et al.*, 2010). Mineral nutrient concentration in compost tea generally varies with compost source, compost age and compost tea production methods. Hargreaves (2008) stated that NCT produced from ruminant manure compost contains 315:43:122 mg L^{-1} N:P:K, 23 Ca and 13 Mg; whereas NCT produced from municipal solid waste compost contains 58:11:188 mg L^{-1} N:P:K and 68 Ca and 21 Mg. Pant *et al.* (2009) reported that ACT and NCT produced from chicken manure-based vermicompost contain 80:16:180 mg L^{-1} N:P:K, 49 Ca and 43 Mg. Compost tea and vermicompost tea also contain a considerable amount of micronutrients macronutrients (Hargreaves, 2008; Pant *et al.*, 2009).

Previous studies suggest that compost and vermicompost contain a substantial amount of humic acids that stimulate plant growth and yield (Valdrighi *et al.*, 1996; Arancon *et al.*, 2003b; Arancon *et al.*, 2006b). Arancon *et al.* (2006b) reported that cattle manure, food waste and paper waste vermicomposts contained about 2.5 g humate kg^{-1} of vermicompost and the application of that humate improved plant growth as well as fruit yield of pepper and strawberries. It is likely that humic acid present in compost or vermicompost would be extracted in the tea during the brewing cycle. Arancon *et al.*(2006b) also demonstrated that humic acid extracted from food waste vermicompost had an effect on pepper plants similar to the effects of a plant growth regulator such as IAA. Valdrighi *et al.* (1996) stated that amendments with humic acids extracted from green waste compost improved vegetative growth of chicory.

Compost tea may contain phytohormones or plant growth regulator-like substances which contribute to better plant growth and yield. It is believed that greatly increased microbial population during composting would produce plant growth regulator-like substances. Ali *et al.* (2009) demonstrated that various strains of bacteria such as *Bacillus, Pseudomonas, Escherichia, Micrococcus* and *Staphylococcus* genera associated with wild herbaceous flora are able to synthesize indole-3 aceteic acid (IAA). The authors also reported that most of the bacterial strains of *Pseudomonas* and *Bacillus* genera enhanced endogenous IAA content and growth of *Triticum aestivum*. Similarly, Ali and Hasnain (2007) observed that RE1 strain of *Halomonas desiderata* produced IAA that has similar effects to other synthetic and natural auxins on *in vitro* growth of *Brassica oleracia*. Garcia Martinez *et al.* (2002) determined that a compound with molecular structure and biological activity analogous to auxins was present in compost. The authors also reported similar biological activity and growth promotion effect of water based compost extract and IAA treatments on garden cress (*Lepidium sativum*). Leachate from well decomposed compost has been shown to contain cytokinin-like substance, derived from hydrolysis of glucosides by the enzyme β-glucosidase produced by microbes (Arthur *et al.*, 2001). Various studies have postulated that vermicomposts contain a large amount of plant growth regulators such as gibberellins, auxins, and cytokinins produced by the increased microbial populations resulting from earthworm activity (Atiyeh *et al.*, 2000b, a; Arancon *et al.*, 2004b). These studies have concluded that application of vermicompost increases seed germination, seedling growth, flowering of ornamentals, and yield of vegetables even at low substitution rates regardless of nutrient supply. Edwards *et al.* (2006) observed better growth of tomato seedlings treated

with vermicompost tea compared to water (control) and suggested that presence of plant growth regulators in vermicompost tea is responsible for growth promotion effect, but the authors did not report on the amount of any phytohormones present in vermicompost tea. It would be reasonable to believe that phytohormones present in compost or vermicompost would be extracted in the tea during brewing process; however, information on the type and amount of plant growth regulators present in compost tea is anecdotal.

2.7 Factors affecting compost tea quality

2.7.1 Compost quality

Research has shown that compost tea prepared from different sources of compost produced variable results. Weltzien (1990; 1991) reported that compost tea prepared from animal manure composts had better disease suppression effect compared to that of compost tea prepared from composted vegetative material. Composted chicken manure produced the most suppressive compost teas for controlling powdery mildew of rose plants compared to the compost produced from vegetative sources (Scheuerell and Mahaffee, 2002). In contrast, Elad and Shtienberg (1994) determined that compost tea produced from composted vegetative material was equally effective as compost tea produced from manure based thermophilic compost on suppressing *B. cinerea*. None of the studies compare the effect of vermicompost tea over thermophilic compost tea and plant growth. Edwards *et al.* (2006) and Gamaley *et al.* (2001); however, reported that the extract produced from animal manure based vermicompost and food waste vermicompost suppressed plant disease and increased the growth and yield of various

crops. The efficacy of compost tea produced from various compost sources varies with plant type and disease categories.

The quality of compost used in making compost tea is critical. The characteristics that influence the biological, physical, and chemical properties of the compost will in turn impact those properties of the compost tea made from the compost (Scheuerell and Mahaffee, 2002; Ingham, 2005a). In fact, the transformation of compost into compost tea cannot improve the original quality of the compost (Kelley, 2004; Ingham, 2005a). Beneficial microorganisms, mineral nutrients, organic acids, and texture could be the main attributes of good quality compost contributing to the quality of compost tea. Compost for compost tea should be certified free of pathogens and residual herbicides to prevent the possibility of detrimental effects. Therefore, it is suggested that the use of undigested materials and raw manures should be avoided in making compost tea because of potential pathogens (Scheuerell and Mahaffee, 2002; Ingham, 2005a). The compost particle size will also play a significant role in compost tea extraction and final quality.

Compost stability and age are important parameters that affect the quality and efficacy of compost tea. Haug (1991) defined compost stability as the point at which the rate of oxygen consumption is reduced so that anaerobic or odorous conditions are not produced to the extent that they cause problems with storage and end use of the product. Stability prevents nutrient immobilization allowing the nutrients to be available for plant needs. Furthermore, stability prohibits rapid oxygen uptake that can create anaerobic conditions and offensive odors. Inbar *et al.* (1990) and Scheuerell and Mahaffee (2002) reported that composts should be cured for 2 to 6 months before use in compost tea

production. The work of Brinton *et al.* (1996) has indicated that compost made from vegetative materials are less effective after 3 months; whereas, animal manure compost can be used until 9 to 12 months old. Andrews (1993) reported that the efficacy of compost tea declined as cattle manure-straw compost aged beyond 12 months. Weltzien (1991) indicated better effect of NCT produced from 6 month-old horse manure compost over that of 1 year-old compost for suppressing cucumber downy mildew. Well-ventilated cool storage condition generally allows compost to be stable for quite a long period compared to moist and warm storage condition. Feed stocks and storage conditions of the compost are therefore important factors contributing to the effect of compost age on effectiveness of compost tea.

2.7.2 Ratio of compost to water

Optimum ratio of compost to water tends to vary, depending upon the brewing process, compost quality and purpose of compost tea application. Too little compost will result in dilute tea with low amounts of nutrients or organisms; whereas, too much compost may not allow maximum amounts of nutrients and microbial biomass of compost to be extracted (Ingham, 2005a). Studies on compost to water ratio have mainly focused on disease suppression effect of compost tea with diverse results. Most studies have followed the methodology developed by Weltzien (1990) that uses a 1:3 - 1:10 (v:v) compost to water ratio. Weltzien (1990) reported that there was a significant suppression of *Phytopthora infestans* with the application of compost tea, no difference in suppression was observed for compost to water ratios between 1:3 and 1:10. However, the suppression effect of compost tea was lower with 1:50 compared to 1:3 and 1:10

compost to water ratio (Weltzien, 1990). Welke (2005) observed that strawberry plants had higher yield and lower incidence of disease with the application of compost tea prepared with a 1:8 compared to that of 1:4 ratio. Edwards *et al.* (2006) reported a non-significant difference on plant growth response of tomato seedlings with the applications of vermicompost teas with 1:25, 1:12 and 1:10 ratios. Several studies indicated that limiting compost to water ratio to 1:10 is found to be effective on disease suppression and yield improvement although the exact mechanism is unclear (Weltzien, 1991; Touart, 2000; Scheuerell and Mahaffee, 2002).

2.7.3 Compost tea brewing (extraction) period

Brewing (extraction) period is an important factors contributing to compost tea quality and efficacy. Compost tea should be brewed to an extent when most of the soluble nutrients and organisms from the compost are extracted or pulled out into the solution (Ingham, 2005a). Too short brewing period may prevent maximum extraction of nutrients and microbial biomass from the compost whereas too long brewing period may favor microbial immobilization of extracted nutrients leading to microbes become inactive once all the available foods are immobilized (Ingham, 2005a). Similarly, Scheurell and Mahaffee (2002) noted that effectiveness of compost tea increases with increasing brewing time to a maximum and then declines.

Brewing period of compost tea may vary with brewing methods, compost source and purpose of compost tea application. Non-aerated compost tea generally requires a longer brewing period compared to that of aerated tea (Brinton *et al.*, 2004; Ingham, 2005a; Diver, 2001). Weltzien (1991) reported that usually a 5 to 8 day period and up to a

16 day brewing time is needed for NCT, which has been hypothesized to allow sufficient time for facultative anaerobes to dominate and for their metabolites to accumulate. Ketterer *et al.* (1992) examined *Botrytis* suppression on detached grape leaves with 1, 3, 7 and 14-day brewed NCT, and that the maximum suppression was observed with the application of 7-day brewed tea. Ingham (2005a) suggests that the optimum brewing time for ACT coincides with maximum active microbial population in the tea, often 12-24 hours with commercial aerobic compost tea makers. Schurell and Mahaffee (2002) citing Cantisano (1998) stated that one day brewing of ACT would be effective for foliar feeding while longer brewing period up to 14 days is useful for disease control. Research on compost tea brewing period have focused primarily on disease suppression effects; further research work on effect of compost tea brewing period on plant growth is needed.

2.8 Pak choi

A model leafy green, pak choi (*Brassica rapa* cv Bonsai, Chinensis group) was selected as the test crop for this study. This fast-growing vegetable has tender green leaves and crispy green petioles. It is a cool season crop but also tolerates heat. Individual leaves can be harvested early for baby greens or later when the mature plant has grown 15 cm tall. Pak choi has a peppery taste, crunchy texture and often used in stir-fry dishes. As a member of the *Brassicaceae*, pak choi contains significant amounts of carotenoids, polyphenols and nitrogen containing secondary metabolites, such as glucosinolates, which possess important anti-oxidative properties and exert anti-carcinogenic, anti-mutagenic, and anti-viral action (Kopsell *et al.*, 2007; Harbaum-Piayda *et al.*, 2008 ; Verkerk *et al.*, 2009). Pak choi is rich in vitamins A and C and folic acid. Its deep green

leaves have attributed to have more beta-carotene than other cabbages and supplies considerably higher calcium (USDA, 2008).

2.9 Phytonutrients

Secondary plant metabolites such as carotenoids, glucosinolates, and phenolic compounds are often called phytonutrients. These molecules are known to play a major role in the adaptation of plants to their environment and also have important implications to human health, crop flavor, and commodity value because of their demonstrated biological reactivity and association with anti-oxidative and anti-carcinogenic activity in humans (Radovich *et al.*, 2005a). Anti-oxidants(e,g. total phenolic and carotenoids) are vital to prevent damage due to pollution in plants, and to prevent diseases in both plants and animals. They play a very important role in scavenging reactive oxygen species and in the body defense system (Ou *et al.*, 2002). Vegetable crops, particularly cruciferous vegetables, act as good sources of nutritionally important dietary carotenoids, polyphenols and glucosinolates (Kopsell *et al.*, 2007; Ahmed and Beigh, 2009).

Although these phytonutrients at moderate levels generally add commodity value by enhancing crop flavor, improving storage life, and having a positive effect on human health, it should be noted that biodegradation products of some phytonutrients could be toxic. For instance, hydrolysis of glucosinolates by the action of endogenous enzyme myrosinase produce a variety of potentially toxic and goitrogenic products, including isothiocynates, thiocynates, nitriles, elemental sulfur (S) and oxazolidinethiones (Shahidi *et al.*, 1997). It has been reported that goitrin, a cyclization product of an aliphatic glucosinolates, progoitrin interferences with the uptake of iodine by thyroid gland (Butler

et al., 1982). Transformation product of glucobrassicin, an indolyl glucosinolate, in contrast to its aliphatic counterparts may inhibit carcinogenesis induced by polycyclic aromatic hydrocarbons and other initiators (Shahidi *et al.*, 1997). Glucosinolates impart a desirable pungent flavor, such as the biting taste of mustard or the characteristic flavors of radish, broccoli, cabbage and cauliflower.

Studies have demonstrated that plant nutrient relations and environmental factors significantly affect the concentration of those plant metabolites in vegetables (Radovich *et al.*, 2005a; Perez-Lopez *et al.*, 2007). Hussein *et al.* (2006) and Kopsell *et al.* (2007) reported higher carotenoids in plant tissue to correspond with increased plant growth at higher fertilizer rates, particularly levels of available N. Krumbein *et al.* (2002) reported that levels of total glucosinolates were reduced at low S and N fertilizer in broccoli plants whereas, total glucosinolates levels were increased at sufficient N supply and high S levels. In another study, the levels of several glucosinolates decreased in leaves under N deficiency but accumulated in roots of *Arabidopsis thaliana* (Hirai *et al.*, 2004). However, Chen *et al.* (2006) observed lower levels of total glucosinolates in pak choi at high levels of foliar N application. Stress, particularly low N, can induce greater concentrations of phenolics in plant tissues (Brown *et al.*, 1984; Estiarte *et al.*, 1994). Nutrient stresses can reduce growth more than photosynthesis; the excess carbon relative to nutrients will be allocated to carbon-based defensive compounds including phenolics. Asami *et al.* (2003) observed consistently greater levels of total phenolics in organically grown crops than those produced by conventional agricultural practices. Sanwal *et al.* (2006) have reported that increased crop yield and dietary anti-oxidants of broccoli occurred with the use of compost and non-aerated compost tea. However, there is little

information on the effect of vermicompost tea on phytonutrient content of vegetable crops.

2.10 Soil biological properties

Soil chemical and biological properties are indicators of soil quality and health, as strongly influenced by soil management practices. Previous studies have shown that application of vermicompost improved mineral nutrient status as well as soil biological properties (Arancon *et al.*, 2006a; González *et al.*, 2010). Dehydrogenase enzyme activity and soil respiration are often considered to be a good index of total microbial activity (Nannipieri *et al.*, 1990). Arancon (2001) reported significant increases in dehydrogenase activity in soils treated with vermicomposts coinciding with the soil microbial biomass. Various other studies suggested that application of different types of thermophilic compost increased soluble carbon and soil respiration (Sikora and Yakovchenko, 1996; Bernal *et al.*, 1998; Lalfakzuala *et al.*, 2008). These increases would be attributed to the intense activity of the soil microorganisms in degrading easily metabolizable compounds such as active organic carbon added through compost or vermicompost. Application of compost tea or vermicompost tea would also add a large number of active microbial populations, organic acids, and mineral nutrients to the soil (Ingham, 2003). However, studies are limited on the effects of vermicompost tea on chemical and biological properties of soil.

CHAPTER 3

VERMICOMPOST EXTRACTS INFLUENCE GROWTH, MINERAL NUTRIENTS, PHYTONUTRIENTS AND ANTI-OXIDANT ACTIVITY IN PAK CHOI (BRASSICA RAPA CV. BONSAI, CHINENSIS GROUP) GROWN UNDER VERMICOMPOST AND CHEMICAL FERTILIZER

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3.1 Abstract

Multiple studies have reported on the effect of compost tea on suppression of certain plant diseases. However, relatively little work has been done to investigate the effect of vermicompost teas on yield and nutritional quality of vegetable crops. Experiments were conducted to determine the effects of extraction methods on vermicompost tea quality and subsequent effects on growth, mineral nutrient, phytonutrient and anti-oxidant activity of pak choi plant grown under organic (vermicompost) and synthetic (Osmocote) fertilization. Three different vermicompost teas based on extraction methods were applied to plants. The extraction methods were non-aerated vermicompost tea (NCT); aerated vermicompost tea (ACT); aerated vermicompost tea augmented with microbial enhancer (ACTME). Aerated water served as a control. Mineral nutrients were significantly higher in ACTME compared with the other teas, but total microbial population and activity did not differ with extraction method. All vermicompost teas similarly enhanced plant production, mineral nutrient and

total carotenoids, and this effect was most prominent under organic fertilization. Anti-oxidant activity and total phenolics were higher under organic compared to synthetic fertilization. Vermicompost teas generally decreased phenolics under organic fertilization, and increased them under synthetic fertilization compared to control. The vermicompost tea effect on crop growth was attributed largely to additional mineral nutrient uptake by plants. Non significant differences among extraction methods on plant response within fertilizer regimes suggest that aeration and additives are not necessary for growth promotion and nutrient quality under the conditions reported.

3.2 Introduction

Aqueous extracts of vermicompost (vermicompost tea), have been demonstrated to improve plant health, yield and nutritive quality when applied as a foliar spray or soil drench. This improvement is achieved by: (i) enhancing beneficial microbial communities and their effects on agricultural soils and plants; (ii) improving mineral nutrient status of plants; and, (iii) inducing the production of plant defense compounds that have beneficial bioactivities in humans (Weltzien, 1991; Hoitink *et al.*, 1997; Scheuerell and Mahaffee, 2002; Carpenter-Boggs, 2005; Ingham, 2005a; Diver, 2001). Although the chemistry and microbiology of vermicompost extract is complex, it is believed that soluble mineral nutrients extracted from vermicompost have positive effect on plant growth with foliar and soil applications (Ingham, 2005a). It is also postulated that the action of living microorganisms and microbial metabolites stimulate plant growth (Carpenter-Boggs, 2005; Diver, 2001). Water extractable growth regulators or phyto-

hormones extracted from vermicompost may also have positive effect on initial root development and plant growth (Keeling *et al.*, 2003; Edwards *et al.*, 2006).

Substantial work has shown positive responses of the effect of vermicompost tea on suppression of certain plant diseases such as Botrytis on green beans, strawberries, grapes and geraniums, leaf spot on tomatoes, bacterial speck in Arabidopsis and powdery mildew on apples(Weltzein and Ketterer, 1986; Elad and Shtienberg, 1994; Hoitink *et al.*, 1997; Zhang *et al.*, 1998; Al-Dahmani *et al.*, 2003; Scheuerell and Mahaffee, 2004, 2006; Haggag and Saber, 2007). However, little work has been done to investigate the effect of vermicompost teas on yield and nutritional quality of vegetable crops. Even fewer studies have addressed the links between extraction conditions, chemical and biological characteristic of tea and subsequent plant growth and yield.

Vermicompost tea may be extracted under aerated or non-aerated (passive) conditions. During aerated extractions, air is pumped through water containing vermicompost to maintain oxygen levels above 5 ppm (Ingham, 2005a). Sugars, grain, fish emulsion, kelp extract, humic acid and other products are often incorporated as additives during extraction of aerated teas to enhance microbial activity of the finished product, but little work regarding the impact of these additives on tea quality or plant response is reported. For passive extractions, vermicompost is placed in a certain volume of water and allowed to sit for several days, with occasional stirring (Weltzien, 1991). Several investigators have reported that non-aerated compost tea has consistent and significant positive effect on disease control and plant growth compared to aerated compost tea, while other works suggest that non-aerated compost tea can be inconsistent

in quality, may cause phytotoxicity and are generally less preferred than aerated teas (Weltzien, 1991; Tranker, 1992; Cronin *et al.*, 1996; Scheuerell and Mahaffee, 2004, 2006). Increased crop yield and dietary anti-oxidants of broccoli with the use of compost and non aerated compost tea has been reported (Sanwal *et al.*, 2006). Non-aerated compost tea was reported to be as effective as compost and inorganic fertilizer in promoting growth of strawberry plants (Hargreaves *et al.*, 2009). Based on her two years of field experiment, Welke (2005) concludes that both aerated and non-aerated compost tea extracted from composted animal manure for seven days of extraction period has a positive and similar effects on strawberry yield and suppression of *Botrytis cinerea*. In contrast, Arancon *et al.* (2007) reported that aerated vermicompost tea have a more positive impact on plant growth than non aerated tea extracted for the same period of time (24 hrs)

We hypothesize that compost tea extraction method and fertilizer type will independently and interactively affect extract influence on plant growth and nutritive quality. Therefore, the objectives of this study were to determine: (i) the effects of extraction method on mineral nutrient content, chemical quality and biological activity of vermicompost tea, and (ii) the independent effects and interaction between vermicompost tea type and fertilizer regime (vermicompost and Osmocote) on plant yield, mineral nutrient concentration, phytonutrient content and anti-oxidant activity.

3.3 Materials and methods

3.3.1 Experimental set-up and design

A leafy green, pak choi (*Brassica rapa* cv. Bonsai, Chinensis group) was selected as test crop for this experiment. This fast-growing vegetable has tender green leaves and crispy green petioles. As a member of the crucifer family, pak choi is rich in vitamins A and C and folic acid (USDA, 2008). Two greenhouse experiments were conducted on Jan-Feb and April-May 2008. Pak choi plants were grown under both organic (chicken manure-based vermicompost) and chemical (Osmocote 16-16-16: N-P-K) fertilization at the rate of 68 mg N L^{-1} growth media (135 kg N ha^{-1}). Plants were grown in tree tubes (148 cm^3) in the first experiment and in garden pots (865 cm^3) in the second experiment. Three to four pak choi seeds were sown in each pot. After 2 days of the seedling emergence in all tubes or pots, plants were thinned to one plant $tubes^{-1}$ or pot^{-1}. Plants were allowed to grow in the greenhouse on a bench fitted with overhead sprinklers with a frequency of every 6 hours for 5 minutes. Three types of vermicompost tea based on their extraction method as well as the same amount of aerated water (control) were applied weekly at the rate of 25 mL tree $tube^{-1}$ and 150 mL pot^{-1} for four weeks starting 5 days after seedling emergence to the root zone and foliage of plants. The greenhouse experiments were arranged in completely randomized design with 2*4 factorial treatments and 10 replications per treatment.

3.3.2 Vermicompost tea extraction method

Chicken manure-based vermicompost used in this study was obtained from Waikiki Worm Company, HI. Vermicompost teas were prepared in separate events for each week of application using the same batch of vermicompost. Vermicompost teas prepared using three different extraction methods constituted three treatments. They were (i) non-aerated vermicompost tea (NCT), (ii) aerated vermicompost tea (ACT), and, (iii) aerated vermicompost tea augmented with microbial enhancer (ACTME), with aerated water serving as a control. NCT was prepared employing Weltzein's (1991) method. Chicken manure-based vermicompost and tap water were mixed at the ratio of 1:10 (v:v) in a 19 L plastic container. The mixture was left open for 7 days at 20-21^{o}C and stirred once at fourth day of preparation. The tea was filtered with nylon aperture (0.2 mm) before application. ACT was prepared using vermicompost and tap water in the ratio of 1:10 (v:v) in a 19 L plastic container and aerated using a commercial compost tea system constructing of a coiled PVC tubing attached to the air pump (Keep It Simple, Inc. WA). The aerator of the KIS brewer was pressed securely to the bottom of the bucket. Eleven liters of water and 1.1 kg of vermicompost were placed in the bucket, covered and aerated for 12 continuous hours per manufacturer's instructions. The content was filtered with nylon aperture just before application. ACTME was produced similarly to ACT plus the addition of 11.74 g dry humic acid (Peaceful Valley, CA), and 32.4 g kelp (Peaceful Valley, CA) extract into the mixture of chicken manure-based vermicompost and water before extraction to enhance the microbial growth. The amount of humic acid and kelp kg $^{-1}$ of vermicompost was calculated based on manufacturers recommendations and published reports (Ingham, 2005a).

3.3.3 Analysis of vermicompost, kelp, humic acid and vermicompost tea

The pH and electrical conductivity (EC) of the vermicompost were measured in a 1:1 (v:v) mixture of deionized water: vermicompost using a conductivity/pH Meter (SB80PC, sympHony, VWR Scientific Products, MN) (Leege and Thompson, 1997). The pH and EC of humic acid and kelp extract were also measured at 1:1 ratio. The pH and EC of the vermicompost extracts were measured and dissolved oxygen (DO) was recorded at 21-22ºC with a thermo sympHony dissolved oxygen meters (SP70D, VWR Scientific Products, MN). Mineral nutrients in vermicompost and vermicompost teas were analyzed in the Agricultural Diagnostic Service Center, University of Hawaii at Manoa. Ammonium-N and NO_3-N were extracted from fresh vermicompost using 2 M KCl and measured colorimetrically using a discrete analyzer (Easy Chem Plus, Systea Scientific, IL). Total C and N of vermicompost samples were analyzed by dry combustion in a LECO CN-2000 analyzer (Leco Corp., St. Joseph, MI). Other nutrients in the vermicompost samples were measured after wet acid digestion (Hue and Evans, 1986) using an inductively coupled plasma (ICP) spectrophotometer (Jarrel-Ash Division/Fisher Scientific Co., Waltham, MA). Nutrient composition of humic acid and kelp were analyzed using the same method used in the nutrient analysis of vermicompost. Mineral N (NH_4-N, NO_3-N and NO_2-N) of the vermicompost extract were analyzed colorimetrically using a discrete analyzer (Easy Chem Plus, Systea Scientific, IL). Other nutrients of the vermicompost extract were measured using the above mentioned ICP procedure.

3.3.4 Microbial analysis of vermicompost and vermicompost tea

Microbial composition of vermicompost and each vermicompost tea was analyzed on samples from 3 separate events for each extraction methods. Three samples of each treatment were taken filtering the vermicompost tea with nylon aperture (0.2 mm) right after 12 hrs brewing cycle for ACT and ACTME and 7 days of steeping for NCT. A 10-fold serial dilution of each sample was prepared. Active bacteria and active fungi were assessed using a 1:10 dilution under Epifluorescence Microscopy at 40x and 20x objective, respectively (Paul and Clark, 1996). The same dilution and slide, which was used to evaluate active fungi, was used to quantify total fungal biomass under Differential Interference Contrast (DIC) Microscopy using a 20x objective. A 1:100 dilution of liquid samples was used to assess total bacteria under Epifluorescence oil immersion at 100x objective (Ingham and Klein, 1984). A 1:100 dilution was used for all analyses, except a 1:1000 dilution was used for the assessment of total bacteria in vermicompost.

3.3.5 Data collection

Plants were harvested five weeks after emergence. Leaf numbers of each plant were counted. Plant height and fresh weight were measured using an electronic scale (Adventurer SL AS811, Ohaus Corporation, NJ) and leaf area was measured using a portable leaf area meter (CI-202, CID, Inc., WA). Plants were immediately frozen in liquid nitrogen and stored at - 20°C and freeze dried using a lyophilizer (D4A, Leybold-Heraeus Vacuum Products, Inc. PA). Dry weight of each plant was recorded and ground using mortar and pestle and stored in air tight container for further analysis at - 20°C.

3.3.6 Measurement of Phytonutrient and anti-oxidant activities

Phyto-chemical and anti-oxidant were analyzed on 10 lyophilized samples of each treatment from each trial by extracting 25-50 mg in 10 mL of ethanol: acetone (1:1, v:v) in glass vials. All data are reported based on the dry weight of the lyophilized sample. Extraction was aided by a sonic water bath for 1 hr, allowed to soak for 12 hr, followed by an additional 30-min sonication. Following centrifugation and filtration through a Fisherbrand 0.45μm PTFE filter, supernatants were evaluated for total carotenoids at 470 nm using a Thermo-Fisher Helios gamma spectrophotometer. Total carotenoids were calculated according to Gross (1991) using the equation: mg L^{-1} total carotenoids = (AV $\times 10^6$)/(A1% × 100G), where A is the absorbance, V is the total volume of the extract, A% is the extinction coefficient of 2500, and G is the sample weight in grams. Total soluble phenolics were measured using the Folin-Ciocalteu assay according to Swain and Hillis (1959) and the data was reported in mg kg^{-1} equivalents of gallic acid. Anti-oxidant capacity was measured using the oxygen radical absorbance capacity (ORAC) assay with a BGM Labtech FLUOstar Optima fluorescent 96-well micro plate reader with an excitation of 485 nm and an emission of 538 nm (Talcott and Lee, 2002). Aliquot of solvent from the original extracts were evaporated in a 50°C water bath and compounds re-dissolved in water aided by a sonicator to give a 10-fold concentration. The concentrated extracts were evaluated against a standard curve of Trolox and anti-oxidant capacity reported in μmol Trolox equivalents g^{-1}.

3.3.7 Measurement of mineral nutrients in the plant tissue

Composite samples of dried tissue were sub sampled into 3 for each treatment from each trial and analyzed for mineral nutrients. Mineral nutrients in plant tissue were analyzed in the Agricultural Diagnostic Service Center, University of Hawaii at Manoa. Total C and N of dried tissue samples were analyzed by dry combustion in a LECO CN-2000 analyzer (Leco Corp., St. Joseph, MI). Other nutrients in the tissue samples were measured after wet acid digestion (Hue and Evans, 1986) using an inductively coupled plasma spectrophotometer (Jarrel-Ash Division/Fisher Scientific Co., Waltham, MA).

3.3.8 Statistical analysis

Analysis of variance (ANOVA) of growth parameters, mineral nutrients and phytonutrients in plant tissue was performed on treatments and interactions; means were separated using Duncan multiple range test in SAS 9.1 statistical software (SAS Institute Inc., 2003). Statistical significance was obtained at 95% confidence level (α =0.05).

3.4 Results

3.4.1 Chemical properties of vermicompost and vermicompost teas

The chemical properties of vermicompost and additives used in this study are presented in Tables 3.1 and 3.2. The pH of the vermicompost was near neutral and EC was 3.7 dS m^{-1}, with a C:N ratio of 13:1. The pH and EC of humic acid and kelp were higher. The pH, EC, DO and extractable nutrient contents of vermicompost tea varied by extraction methods (Table 3.3). The average pH of ACTME and control treatment was

significantly higher than the pH of ACT and NCT. However, the pH of ACT and NCT was not significantly different from each other. The level of DO in vermicompost tea was influenced by the use of microbial enhancer during production. The DO level in ACTME was significantly lower than ACT and NCT. The DO level in ACT and NCT were not significantly different. EC was highest in ACTME; lowest in control; and no significant difference between ACT and NCT in all trials. Chemical analysis of humic acid and kelp showed that use of these additives added about 48, 19 and 15 mg L^{-1} of total N, NO_3-N and NH_4-N, respectively in ACTME. This is reflected in level of total N, NO_3-N and NH4-N in ACTME compared to that of ACT and NCT (Table 3.4). Nitrate nitrogen content in ACTME was about 33% and 22% higher than that of NCT and ACT respectively. Similarly, NH_4-N content in ACTME was about 13 and 16 times higher than that of NCT and ACT respectively. Phosphorus content in the vermicompost tea was not influenced by the production methods. Potassium content was significantly higher in ACTME than ACT and NCT. Although N and K concentration in vermicompost was lower than P concentration, concentration of both N and K in vermicompost tea was higher than that of P irrespective of extraction methods. Other extractable nutrients were significantly higher in ACTME compared to ACT and NCT while the level of secondary nutrient and micronutrient content was comparable between ACT and NCT (Table 3.5).

3.4.2 Microbial population in vermicompost and vermicompost tea

Microbial population in vermicompost and vermicompost tea is presented in Table 3.6. The total bacterial population present in vermicompost tea and the control were not significantly different. However, the population of active bacteria was

significantly high in all types of vermicompost tea compared to the control. Both the total and active fungal population was high in all types of vermicompost tea compared to the control. There was no significant difference in total and active bacteria and fungi among extraction methods.

3.4.3 Effect of vermicompost tea on plant growth

Effect of vermicompost tea on plant growth is presented in Table 3.7. All types of vermicompost tea increased above ground fresh weight significantly compared to the control treatment across fertilizer regimes. However, there was no significant difference on above ground fresh weight among the three treatments receiving vermicompost tea. Average above ground fresh weight was significantly higher with Osmocote compared to the use of vermicompost. The interaction between vermicompost tea and fertilizer was not significant on above ground fresh weight. The effect of tea type, fertilizer type and their interaction on above ground dry weight was similar to that of fresh weight. The interaction between tea type and fertilizer type on plant height was significant. Vermicompost tea had a greater effect on plant height under vermicompost fertilization and had much smaller effect on plant height under Osmocote fertilization. Leaf numbers increased in vermicompost tea treated plants significantly compared to the control across fertilizer regimes. However, plant height and leaf number were not influenced by the vermicompost tea extraction methods. Average leaf number was significantly higher with Osmocote compared to the use of vermicompost. Leaf area significantly increased in the vermicompost tea treated plants compared to the control across fertilizer regimes. The effect of vermicompost tea extraction methods on leaf area was not significant.

3.4.4 Effect of vermicompost tea on mineral nutrient content of plant

Effect of vermicompost tea on the mineral nutrient uptake plant $^{-1}$ is presented in Tables 3.8 and 3.9. All types of vermicompost tea consistently increased total N content plant $^{-1}$ as of total above ground dry weight (Fig. 3.1) under both fertilizer regimes. Total P and K content plant $^{-1}$ were also higher in vermicompost tea treated plants compared to control across fertilizer regimes. The effect of vermicompost tea extraction methods on total N P and K content plant $^{-1}$ was not significantly different across fertilizer regimes. Similarly, there was a significant positive effect of vermicompost tea on Ca, Mg and S content in plant tissue irrespective of extraction methods under both vermicompost and Osmocote fertilization. Except Fe, all other micronutrients in plant tissue were significantly increased with the application of vermicompost tea irrespective of vermicompost tea extraction methods and fertilizer regimes. The effect of vermicompost tea on Fe content in plant tissue depended on fertilizer types. NCT had significantly greater effect on Fe content in plant tissue compared to other treatments including control under both fertilizer regimes. The effect of ACT, ACTME and control was not different to each other on Fe content in plant tissue under vermicompost fertilization. After NCT, the effect of ACT on Fe content was significantly higher than that of control and ACTME under Osmocote fertilization. Fe content was lowest in the plant treated with ACTME among all treatments under Osmocote fertilization.

3.4.5 Effect of vermicompost tea on phytonutrients

Effect of vermicompost tea on phytonutrients is presented in Table 3.10. Vermicompost tea increased total carotenoids compared to the control across fertilizer

regimes; most notably under vermicompost fertilizer where tea treated plants had 72% higher carotenoids than that of control. However, the tea treated plants grown under Osmocote fertilizer had about 14% higher carotenoids than that of control. There was also a significant interaction effect of vermicompost tea and fertilizers on total phenolics. The effect of ACTME and ACT on total phenolics was significantly higher under Osmocote fertilization, whereas the effect of NCT was intermediate. In contrast, there was a very limited effect of vermicompost tea on total phenolics under vermicompost fertilization. The treatment effect on ORAC was not significant under Osmocote fertilization for all types of vermicompost tea except NCT. The plant treated with NCT appeared to have low level of ORAC under Osmocote fertilization. The effect of all vermicompost tea on anti-oxidant activity was lower than the effect of control treatment under vermicompost fertilization.

3.5 Discussion

The use of kelp extract and humic acid resulted in higher EC, nutrients and lower DO in ACTME. The higher total N, NO_3-N and NH_4-N in ACTME relative to the other extractions (30, 20 and 9 mg L^{-1}, respectively) can be accounted for by that supplied in the additives (Tables 3.1, 3.2 and 3.3). Microbial activity is the most frequent explanation for reduced DO levels during tea extraction (Ingham, 2005a). Although total and active microbial populations in ACTME were not significantly different from that of ACT and NCT, it is possible that the additives did increase microbial populations in ACTME and that microbes remained bound to vermicompost particles that were removed during screening. The chemical reduction of humic acids during extraction is also a possible

explanation for reduced DO in ACTME without corresponding increases in microbial activity. However, tests in the lab run concurrently with the experiments reported here showed no decrease in DO levels when humic acids were aerated in water without vermicompost (data not shown). Total N concentration in vermicompost was lower than P concentration, however; concentration of N in vermicompost tea was significantly higher than that of P irrespective of extraction methods. Use of kelp extract as microbial enhancer attributed to higher level of K in ACTME compared to ACT and NCT.

Vermicompost tea consistently enhanced plant growth and mineral nutrient concentration in plant tissue under both fertilizer regimes, which is consistent with the findings of previous studies (Sanwal *et al.*, 2006 ; Hargreaves *et al.*, 2008). Although the above ground fresh and dry weight was high under Osmocote fertilization compared to vermicompost, the effect of vermicompost tea was most pronounced under vermicompost fertilization. Soluble mineral nutrients and microbial byproducts in vermicompost tea can enhance nutrient uptake from the soil and increase foliar uptake of nutrients (Xu *et al.*, 2001; Ingham, 2005a). Nutrient analysis of vermicompost tea indicates that vermicompost tea supplied a considerable amount of soluble mineral nutrients to the plant compared to control (Tables 3.4 and 3.5). Strong correlation between aboveground dry weight and N uptake by plants explains yield response to vermicompost tea across treatments (Fig. 3.1). However, tissue nitrogen concentration on a dry matter basis was about 1.73% which is below the critical level of 3.2% reported for cabbage (Maynard and Hochmuth, 2007). Increased above ground fresh weight, dry weight, leaf area and extractable mineral element concentration in plant tissue as a result of vermicompost tea treatment was observed in this study. Keeling *et al.* (2003) observed that application of

vermicompost tea on oil seed rape plant at the initial stage of growth increased both root development and plant growth. Siddiqui *et al.* (2008) observed that compost tea enhanced plant growth and increased tap root length of okra plant. Although the experiment did not measure the root growth of the plant, better nutrient uptake by vermicompost tea treated plants compared to control plants suggests that improved root growth or nutrient uptake per unit root might be one of the mechanisms involved to stimulate plant growth. Arancon *et al.* (2007) reported that humic, fulvic and other organic acids extracted or produced by microorganisms in vermicompost tea could induce plant growth. Garcia Martinez *et al.* (2002) investigated that water extract of compost contained a compound with molecular structure and biological activity analogous to auxins. Leachate from well decomposed compost has been shown to contain cytokinin-like substance, derived from hydrolysis of cyanogenic glucosides by the enzyme β-glucosidase produced by microbes (Arthur *et al.*, 2001). Although phytohormones or growth regulators in vermicompost tea were not measured in this study, we suggest they may play a greater role in plant response.

Plant growth was not influenced by the various extraction methods. Although there was higher amount of total N, K and other secondary and micronutrients present in ACTME compared to ACT and NCT, the effect of ACTME on plant growth was not significantly different from ACT and NCT under both fertilizer regimes. In fact, micronutrient uptake in ACTME plants was lower than other treatments (Table 3.9), despite higher levels of Fe, Mn and other micronutrients in ACTME (Table 3.5). This discrepancy may be explained by higher pH in ACTME, reducing solubility of Fe and other metals. Also, higher N, K and micronutrient present in ACTME compared to other

teas is due to the contribution of humic acid and kelp (Table 3.2) and may not be available for plant uptake. Ingham noted that aerated compost tea augmented with microbial enhancer impart a better result by escalating microbial population in the compost tea (Ingham, 1999). Some workers suggested that increased microbial population in compost tea would increase microbial activity in plant and soil, which in turn contributes to better results. However, microbial population in ACTME was neither higher nor its effect on plant growth was more pronounced compared to ACT and NCT in this study. Since the same level of the active and total microbial population was observed in all types of vermicompost tea, contribution of their activities on nutrient uptake and plant growth may be equivalent irrespective of nutrient level in tea. There is debate regarding the efficacy of aeration during compost tea production. Ingham (1999) suggested that ACT would provide better results than that of NCT however; several other investigators have reported that NCT prepared by Weltzien's (1991) method has a more consistent and significantly positive effect than that of ACT on disease control and plant growth (Tranker, 1992; Cronin *et al.*, 1996; Scheuerell and Mahaffee, 2004, 2006). Arancon *et al.* (2007) observed the strong effect of ACT over NCT on disease suppression and plant growth. However, extraction period of NCT adopted by these authors was only 2 days whereas; Weltzien's method recommends 7 days of extraction period (Weltzien, 1991; Arancon *et al.*, 2007). It may be that the lower effect of NCT over ACT observed in the study by Arancon *et al.* (2007) is associated with the shorter extraction period. Welke (2005) has shown that both ACT and NCT have similar effect on plant growth and disease suppression. Result of this study is consistent with the

findings of Welke (2005) that aeration is not essential for plant growth promotion provided extraction period is sufficient.

Vermicompost tea consistently increased total carotenoids content under both fertilizer regimes compared to control although the magnitude of the treatment effect was higher under vermicompost fertilization compared to Osmocote (Table 3.10). The increases in total carotenoids in this study are associated with improved crop growth in the vermicompost tea treatments. This agrees with Hussein *et al.* (2006) who reported higher carotenoids in plant tissue to correspond with increased plant growth at higher fertilizer rates. Better plant growth with the application of vermicompost tea may have contributed to synthesis of carotenoids under both vermicompost and Osmocote fertilization in this study. A higher level of total carotenoids was observed in the tissue of plant grown under chemical fertilizer compared to vermicompost in our study. However, Pérez-López *et al.* (2007) reported a significantly higher content of total carotenoids in organically grown sweet peppers than integrated and conventional peppers.

It has been previously demonstrated that stress, particularly low N, can induce greater concentrations of phenolics in plant tissues (Brown *et al.*, 1984; Estiarte *et al.*, 1994). Nutrient stresses can reduce growth more than photosynthesis; the excess carbon relative to nutrients will be allocated to carbon-based defensive compounds including phenolics (Tuomi *et al.*, 1988). Increased concentrations of total phenolics was associated with lower plant growth (Fig. 3.2) and low mineral N concentration in plant tissue of control plant compared to vermicompost tea treated plants grown under vermicompost fertilization in this study. Comparing two fertilizer regimes, a higher level of total

phenolics was observed in plants grown under vermicompost fertilization compared to Osmocote fertilization. This could be due to a rapid release of plant available nutrient from Osmocote compared to vermicompost. Asami *et al.* (2003) and Wang *et al.* (2002) also observed the consistently higher levels of total phenolics in organically grown crops as compared to those produced by conventional agricultural practices. Higher level of anti-oxidant activity was observed in control plants compared to vermicompost tea treated plants under vermicompost fertilization (Table 3.10). Zhao *et al.* (2007) and Dixon and Paiva (1995) reported that higher level of anti-oxidant capacity of leafy vegetables is associated with reduced pant growth, lower nitrogen concentration and accumulation of higher levels of phenolic compounds in plant tissue. Higher level of anti-oxidant activity is also associated with the high total phenolics and low mineral nutrient content of control plants compared to vermicompost tea treated plants grown under vermicompost fertilization in this study. Although plant fresh and dry weight and tissue nitrogen content of NCT treated plant was not statistically different from that of ACT and ACTME treated plant under Osmocote fertilization, the numerical value of these parameters were high in NCT treated plants. Lower anti-oxidant activity present in NCT treated plant compared to other treatments may be associated with the higher plant growth of NCT treated plant under Osmocote fertilization.

3.6 Conclusion

Chemical properties and mineral nutrient content of vermicompost tea varied across extraction methods but the microbial population and activity in vermicompost tea did not differ with extraction methods. Additions of kelp and humic acids increased

mineral nutrient content in vermicompost tea, but did not affect biological activity after filtering. Application of vermicompost tea enhanced plant production, mineral nutrient content and total carotenoids in plant tissue under both organic and chemical fertilization, however; the effect was more prominent under organic fertilization. Vermicompost tea treated plants had lower anti-oxidant activities and total phenolics compared to control under vermicompost fertilization. Vermicompost tea had non-significant effect on anti-oxidant activities and significantly positive effect on total phenolics compared to control under chemical fertilization. All vermicompost teas based on extraction methods provided equivalent effect on plant growth and nutrient concentration which suggests that aeration is not essential for growth promotion and nutrient quality if extraction period is sufficient. We suggest that the vermicompost tea effect observed here was largely a response to mineral nutrient, particularly N, uptake by plant.

Table 3.1 Chemical properties of vermicompost (n = 3).

pH	EC	Moisture	N	NO_3-N	C	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu	B
	dS m^{-1}	%	---------- mg g^{-1}----------								----------µg g^{-1}----------				
6.9(0) †	3.7(0.3)	67.2(0)	18(1)	2.2(0)	237(16)	23(2)	7(1)	169(6)	11(0)	2(0)	8702(1683)	828(48)	552(73)	91(11)	56(5)

†Standard Error, EC = Electrical Conductivity.

Table 3.2 Chemical properties of humic acid and kelp extract (n = 1).

	pH	EC	N	NO_3-N	NH_4-N	NO_2-N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu	B
		dS m^{-1}	---------- mg g^{-1}----------									----------µg g^{-1}----------				
Kelp	9.3	14.2	12.7	6.0	1.9	0.06	0.1	190.8	2.5	3.3	40.6	42	2	16	4	83.0
Humic acid	10.1	16.3	11.5	1.9	8.9	0.02	0.0	21.3	7.7	0.9	76.4	1850	171	6	5	81.0

EC = Electrical Conductivity

Table 3.3 DO, EC and pH of vermicompost tea across extraction methods at application (n =8).

Extraction method	DO (mg L^{-1})	EC (dS m^{-1})	pH
NCT	7.5(0.4) †	1.2(0.1)	7.5(0.1)
ACTME	5.0(1.0)	2.0(0.2)	8.3(0.1)
ACT	7.9(0.4)	1.2(0.1)	7.8(0.1)
Control	8.7(0.8)	0.4(0.0)	8.1(0.1)

†Standard Error, EC = Electrical Conductivity, DO= Dissolved Oxygen, NCT = Non-aerated vermicompost tea, ACTME = Aerated vermicompost tea with microbial enhancer, ACT = Aerated vermicompost tea, Control = water.

Table 3.4 Macronutrient content in vermicompost tea across extraction methods (n =8).

Extraction method	N	NO_3-N	NH_4-N	NO_2-N	P	K	Ca	Mg	Na
	mg L^{-1}								
NCT	74.9(4.6)†	73.3(4.5)	0.6(0.2)	0.3(0.0)	16.2(1.0)	166.6(10.3)	48.6(2.2)	42.8(2.3)	100.6(2.6)
ACTME	106.9(6.3)	97.5(6.1)	8.3(0.7)	0.5(0.0)	16.5(1.1)	656.1(21.7)	83.4(3.6)	61.5(3.4)	258.7(7.7)
ACT	81.7(4.4)	80.2(4.4)	0.5(0.1)	0.4(0.0)	16.2(1.7)	180.4(5.9)	49.0(2.8)	43.9(2.3)	102.8(3.0)
Control	9.6(1.8)	9.0(1.7)	0.3(0.2)	0.1(0.0)	0.2(0.1)	5.61(1.3)	12.4(0.3)	15.3(0.2)	65.6(1.6)

† Standard Error, NCT = Non-aerated vermicompost tea, ACTME = Aerated vermicompost tea with microbial enhancer, ACT = Aerated vermicompost tea, Control = water.

Table 3.5 Micronutrient content in vermicompost tea across extraction methods (n =8).

Extraction method	Fe	Mn	Zn	Cu	B
	----------µg L^{-1}----------				
NCT	0.0(0.0)†	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.3(0.0)
ACTME	1.5(0.1)	0.3(0.1)	0.6(0.1)	0.4(0.1)	0.6(0.1)
ACT	0.1(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.3(0.0)
Control	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)

† Standard Error, NCT = Non-aerated vermicompost tea, ACTME = Aerated vermicompost tea with microbial enhancer, ACT = Aerated vermicompost tea, Control = water.

Table 3.6 Microbial population in vermicompost and vermicompost tea (n = 3).

Vermicompost/ Extraction method	Log_{10} active bacteria	Log_{10} total bacteria	Active fungi	Total fungi
	------(cells $^{-g}$ dwt or cells $^{-mL}$)-----		-------(µg $^{-g}$ dwt or µg $^{-mL}$)--------	
Vermicompost	8.1(0.1) †	9.9(0.1)	14.3(1.9)	634.9(135.2)
ACT	7.1(0.2)	8.5(0.1)	0.2(0.1)	3.3(1.1)
ACTME	7.1(0.3)	8.6(0.1)	0.5(0.2)	5.8(1.6)
NCT	7.2(0.2)	8.7(0.1)	0.4(0.2)	5.6(1.3)
Control	1.7(1.1)	8. 2(0.2)	0.0(0.0)	0.5(0.3)

† Standard Error, dwt = dry weight, NCT = Non-aerated vermicompost tea, ACTME = Aerated vermicompost tea with microbial enhancer, ACT = Aerated vermicompost tea, Control = water.

Table 3.7 Effect of vermicompost tea on plant growth (n = 20).

Vermicompost

Extraction method	Above ground fresh weight (g plant $^{-1}$)	Above ground dry weight (g plant $^{-1}$)	Plant height (cm)	Leaf area (cm^2 plant $^{-1}$)	Leaf number (plant $^{-1}$)
NCT	13.5(3.3) †	1.1(0.3)	12.1(0.6)	289.7(59.9)	10.3(0.7)
ACTME	11.7(2.2)	1.0(0.2)	12.0(0.7)	275.3(40.6)	10.4(0.6)
ACT	9.6(2.0)	0.8(0.2)	11.9(0.6)	236.0(37.7)	9.8(0.6)
Control	2.3(0.7)	0.2(0.1)	7.0(0.6)	63.0(15.9)	6.0(0.4)

Osmocote

Extraction method	Above ground fresh weight (g plant $^{-1}$)	Above ground dry weight (g plant $^{-1}$)	Plant height (cm)	Leaf area (cm^2 plant $^{-1}$)	Leaf number (plant $^{-1}$)
NCT	23.5(4.1) †	1.7(0.3)	13.5(0.6)	453.8(70.7)	12.6(0.7)
ACTME	22.2(3.9)	1.9(0.3)	13.2(0.6)	431.5(67.6)	12.4(0.7)
ACT	20.7(3.4)	1.6(0.3)	12.7(0.7)	422.3(65.3)	12.3(0.7)
Control	11.0(1.2)	0.9(0.1)	11.8(0.4)	241.7(27.3)	9.8(0.4)

† Standard Error, NCT = Non-aerated vermicompost tea, ACTME = Aerated vermicompost tea with microbial enhancer, ACT = Aerated vermicompost tea, Control = water.

Table 3.8 Effect of vermicompost tea on macronutrient content in plant tissue across fertilizer regimes (n = 6).

Vermicompost

Extraction method	N	P	K	Ca	Mg	S	Na
	(mg plant $^{-1}$)						
NCT	19.3(5.4) †	8.2(2.2)	42.2(11.6)	22.2(5.7)	6.9 (1.8)	7.5(2.0)	10.6(2.9)
ACTME	16.1(3.6)	7.0(1.4)	38.4(8.3)	17.9(3.5)	5.6(1.1)	6.8(1.5)	9.5(2.0)
ACT	14.9(3.8)	5.5(1.3)	29.8(7.4)	16.3(3.6)	4.9(1.1)	5.6(1.3)	7.6(1.9)
Control	2.1(0.8)	2.0(0.8)	5.5(2.2)	4.2(1.6)	1.5(0.6)	1.6(0.6)	2.5(1.0)

Osmocote

Extraction method	N	P	K	Ca	Mg	S	Na
	(mg plant $^{-1}$)						
NCT	33.8(6.8) †	10.1(1.9)	74.8(15.2)	37.9(6.9)	13.5(2.5)	13.0(2.6)	16.0(2.9)
ACTME	27.5(5.4)	9.4(1.7)	66.3(13.1)	30.7(5.4)	12.5(2.4)	12.3(2.4)	19.3(3.6)
ACT	29.2(6.1)	8.8(1.7)	57.4(12.1)	32.5(6.1)	12.4(2.5)	11.3(2.4)	15.8(2.9)
Control	15.3(2.5)	4.9(0.7)	33.9(5.6)	17.3(2.1)	6.7(0.9)	6.5(1.1)	9.1(1.2)

† Standard Error, NCT = Non-aerated vermicompost tea, ACTME = Aerated vermicompost tea with microbial enhancer, ACT = Aerated vermicompost tea, Control = water.

Table 3.9 Effect of vermicompost tea on micronutrient content in plant tissue across fertilizer regimes (n = 6).

Vermicompost					
Extraction method	Fe	Mn	Zn	Cu	B
	----------(µg plant $^{-1}$)----------				
NCT	172.2(48.7) †	130.2(35.4)	116.2(31.9)	18.0(4.0)	30.9(7.8)
ACTME	63.1(10.4)	54.0(7.9)	74.7(14.1)	15.1(1.3)	25.9(4.7)
ACT	50.0(9.3)	42.7(7.7)	55.7(12.6)	11.2(1.5)	21.7(4.5)
Control	12.2(4.6)	7.4(2.5)	22.0(8.9)	6.3(2.5)	6.2(2.4)
Osmocote					
Extraction method	Fe	Mn	Zn	Cu	B
	----------(µg plant $^{-1}$)----------				
NCT	551.7(114.2) †	183.1(37.7)	250.2(54.9)	25.9(4.2)	52.2(9.1)
ACTME	111.1(23.4)	166.4(36.8)	140.4(28.1)	26.8(4.5)	48.8(8.1)
ACT	286.0(54.2)	170.4(35.9)	158.2(35.0)	22.8(3.5)	45.2(8.0)
Control	192.0(12.6)	98.5(14.6)	82.1(14.3)	13.5(1.5)	23.7(3.1)

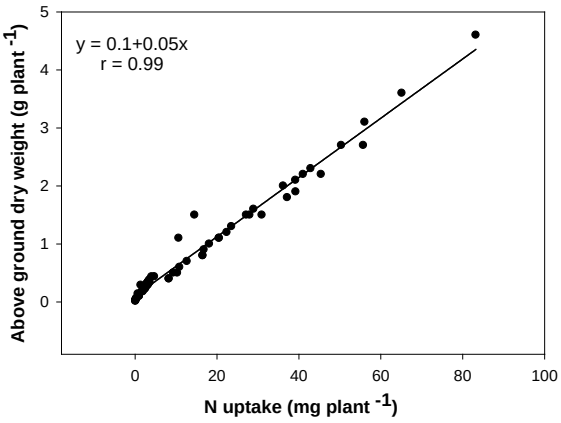
† Standard Error, NCT = Non-aerated vermicompost tea, ACTME = Aerated vermicompost tea with microbial enhancer, ACT = Aerated vermicompost tea, Control = water

Table 3.10 Effect of vermicompost tea on total carotenoids, total phenolics and anti-oxidant activity (n = 20).

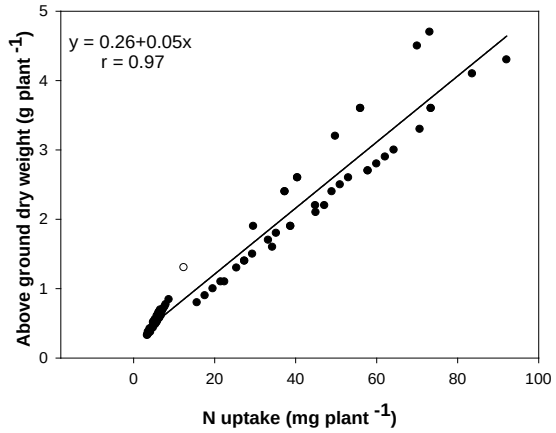
Vermicompost			
Extraction method	Total Carotenoids (mg kg $^{-1}$)	Total Phenolics (mg kg $^{-1}$)	ORAC (µmole TE g $^{-1}$)
NCT	478.5(35.3)†	2313.4(166.5)	202.9(18.8)
ACTME	501.6(38.6)	2561.4(209.0)	178.4(14.8)
ACT	591.1(46.7)	2829.2(231.5)	240.2(18.8)
Control	305.2(72.5)	3398.0(436.6)	341.7(118.4)
Osmocote			
Extraction method	Total Carotenoids (mg kg $^{-1}$)	Total Phenolics (mg kg $^{-1}$)	ORAC (µmole TE g $^{-1}$)
NCT	548.8(45.7) †	2097.9(97.6)	171.2(11.9)
ACTME	517.3(30.9)	2363.6(173.2)	214.7(18.7)
ACT	566.9(53.9)	2433.8(188.8)	238.9(15.9)
Control	477.7(38.2)	1999.6(147.3)	223.3(14.9)

† Standard Error, NCT = Non-aerated vermicompost tea, ACTME = Aerated vermicompost tea with microbial enhancer, ACT = Aerated vermicompost tea, Control = water.

a.



b.



c.

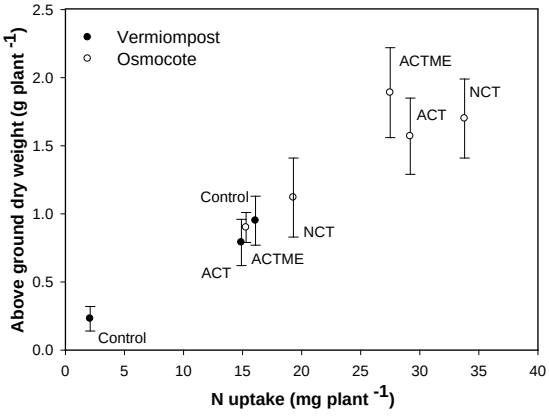


Figure 3.1 a. Above ground dry weight relative to N uptake under vermicompost (n = 80); b. Above ground dry weight relative to N uptake under Osmocote (n = 80); c. Above ground dry weight relative to N uptake across treatments (plotted points are means of 20 samples, and error bars represent standard errors of the mean). Treatments are NCT = Non-aerated vermicompost tea, ACTME = Aerated vermicompost tea with microbial enhancer, ACT = Aerated vermicompost tea, Control = water.

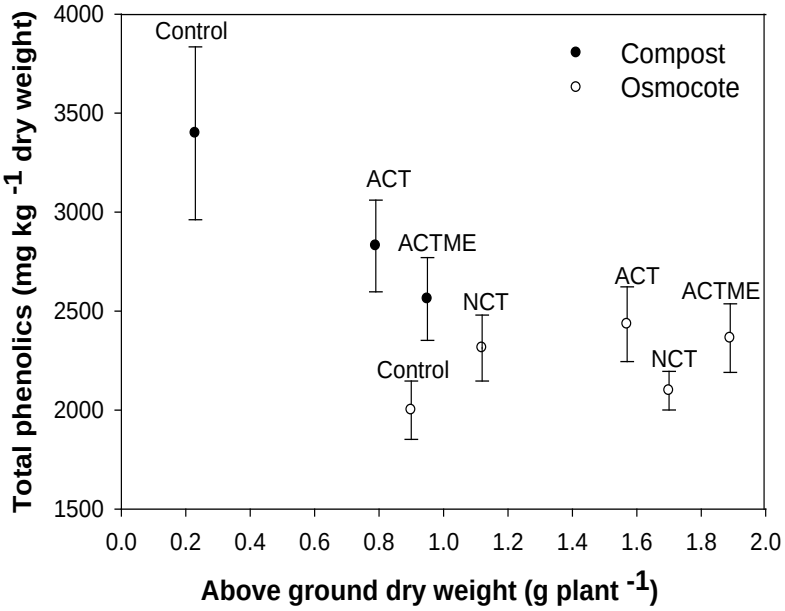


Figure 3.2 Total phenolics relative to above ground dry weight across treatments. Plotted points are means of 20 samples, and error bars represent standard errors of the mean. Treatments are NCT = Non-aerated vermicompost tea, ACTME = Aerated vermicompost tea with microbial enhancer, ACT = Aerated vermicompost tea, Control = water.

CHAPTER 4

EFFECTS OF VERMICOMPOST TEA (AQUEOUS EXTRACT) ON PAK CHOI (*BRASSICA RAPA* CV. BONSAI, CHINENSIS GROUP) YIELD, QUALITY, AND ON SOIL BIOLOGICAL PROPERTIES.

4.1 Abstract

This study investigated the effects of vermicompost tea (aqueous extract) on yield and chemical quality of pak choi (*Brassica rapa* cv Bonsai, Chinensis group) grown in three media (two soils and a peat-perlite medium) under two fertilizer regimes (compost and synthetic fertilizer). The impacts of tea application on the chemical and biological properties of the growth media were also investigated. Vermicompost teas were prepared using various extraction methods (non-aerated, aerated, aerated with additives) with 1:10 (v:v) chicken manure-based vermicompost to water dilution and applied weekly at the rate of 200 mL plant $^{-1}$ for 4 weeks. Application of vermicompost tea regardless of extraction methods increased plant production, total carotenoids and total glucosinolates in plant tissue. Total phenolics was lower in vermicompost tea treated plants compared to those treated with only mineral nutrient (N, P, K) solution and the water control. Vermicompost tea improved mineral nutrient status of plants and media, and enhanced the biological activity of the media. Variability in yield and chemical quality of plants across treatments was explained largely by variability in tissue N uptake and dry matter accumulation. Dehydrogenase activity and soil respiration of vermicompost tea-treated growth media were approximately 50% higher than untreated media. This study

confirmed that vermicompost tea can positively influence plant yield and quality and increase soil biological activity in multiple soil types.

4.2 Introduction

Application of aqueous extract of vermicompost (vermicompost tea) has been shown to improve plant health, crop yield, and nutritive quality (Gamaley *et al.*, 2001; Pant *et al.*, 2009). The primary mechanism of this response is not clearly understood. It is believed that soluble mineral nutrients, organic acids and water-soluble plant-growth regulators extracted in the vermicompost tea have positive effects on initial root development and plant growth with both foliar and soil application (Arancon *et al.*, 2007). Living microorganisms present in vermicompost tea may also induce increased disease resistance as well as stimulate nutrient uptake and plant growth (Ingham, 2005a).

Vermicompost tea can be prepared employing non-aerated or aerated methods. Non-aerated methods result in generally low-oxygen conditions during tea extraction whereas aerated methods maximize oxygen during the extraction of compost. Sugars, grain, fish emulsion, kelp tea, humic acid and other products are often added during extraction of aerated teas to enhance microbial activity of the finished product, but little work regarding the impact of these additives on tea quality or plant response are reported. It is generally expected that the use of additives to enhance microbial growth in compost tea would be more effective in promoting plant growth. Additives may also have a direct effect on plants via added nutrients. Several investigators have reported that non-aerated compost tea has consistently positive effects on disease control and plant growth in contrast to aerated compost tea (Weltzien, 1991; Cronin *et al.*, 1996; Scheuerell and

Mahaffee, 2006). Welke (2005) reported that both aerated and non-aerated compost tea extracted from composted animal manure have similar positive effects on strawberry yield and suppression of *Botrytis cinerea*. In contrast, Arancon *et al.* (2007) reported that aerated vermicompost tea had a greater positive impact on plant growth than non aerated tea extracted for the same period (24 hrs).

Soil chemical and biological properties are indicators of soil quality and health as influenced by management practices. Previous studies have shown that vermicompost improved soil mineral nutrient status and biological properties (Arancon *et al.*, 2006a; González *et al.*, 2010). However, studies on effects of vermicompost tea on soil chemical and biological properties are limited. The objectives of this study were to determine: (i) the effects of vermicompost tea types (based on different extraction methods) applied to different growth media on yield of pak choi, root growth, mineral nutrient concentration and phytonutrient content, and (ii) the effect of vermicompost tea on chemical and biological properties of growth media.

4.3 Materials and Methods

4.3.1 Media

Two soils used in this study were an Oxisol (Wahiawa series soil and Family: clayey, kaolinitic, isohyperthermic, Tropeptic Eutrustox) and a Mollisol (Waialua series soil and Family: very-fine, kaolinitic, isohyperthermic, Vertic Haplustolls). Both soils were air-dried and passed through a 3-mm mesh sieve. A third medium used was a peat-perlite mix (Sunshine mix # 4, Sun Gro Horticulture, Canada Ltd.).

4.3.2 Fertilizer

Two greenhouse experiments were simultaneously conducted between February and April, 2009. A leafy green vegetable, pak choi (*Brassica rapa* cv Bonsai, Chinensis group) was selected as a test crop for the experiments. This fast-growing vegetable has tender green leaves and crispy green petioles and is considered a good source of vitamins A and C and folic acid (USDA, 2008). Plants were grown with compost (green waste thermophilic compost) as the sole fertilizer in one experiment and with solely chemical (Osmocote 14-14-14: N-P-K) fertilization in another experiment. Fertilizer rate was calculated to provide150 mg N L^{-1} growth media (300 kg N ha^{-1}) for both experiments. Since the Oxisol contains very low Ca, gypsum was added to provide Ca at 5 $cmol_c$ kg^{-1} soil. Micronutrients (mg kg^{-1} soil) were added as follows: 50 Mg as $MgSO_4.7H_2O$, 10 Zn as $ZnSO_4.7H_2O$, 10 Fe as $Fe_2SO_4.7H_2O$, 5 Cu as $CuSO_4.5H_2O$ and 2 B as H_3BO_3 to all growth media.

4.3.3 Compost tea treatments

Five tea treatments at the rate of 200 mL pot^{-1} were applied weekly for 4 weeks starting 3 days after transplanting to the root zone and the foliage of plants. These treatments consisted of: three types of vermicompost tea based on extraction method (non-aerated, NCT; aerated, ACT, and aerated augmented, ACTME); a mineral nutrient solution (MNS) prepared by mixing di-ammonium phosphate and potassium nitrate resembling the nutrient content of vermicompost tea (N, P, K); and aerated water (control). The greenhouse experiments were arranged in a randomized complete block

design with 3*5 factorial treatments (media x treatment) and 4 blocks of each treatment combination.

Chicken manure-based vermicompost used in this study was obtained from Waikiki Worm Company, HI. Vermicompost teas were prepared in separate events for each week of application using the same batch of vermicompost. Vermicompost teas were prepared at 1:10 (v:v) ratio of chicken manure-based vermicompost and water using three different extraction methods, as previously described (Pant *et al.*, 2009). They included: (i) non-aerated vermicompost tea (NCT); (ii) aerated vermicompost tea (ACT); and (iii) aerated vermicompost tea augmented with microbial enhancer (ACTME). Aerated water served as the control.

4.3.4 Analysis of chemical properties of vermicompost and vermicompost tea

The pH and electrical conductivity (EC) of the vermicompost and extracts were measured in a 1:1 (v:v) mixture of deionized water: vermicompost, using a conductivity/pH Meter (SB80PC, sympHony, VWR Scientific Products, MN). Dissolved oxygen (DO) was recorded at 21-22ºC with a dissolved oxygen meter (thermo sympHony SP70D, VWR Scientific Products, MN). Mineral nutrients in vermicompost and vermicompost teas were analyzed in the Agricultural Diagnostic Service Center, University of Hawaii at Manoa. Total C and N of vermicompost samples were analyzed by dry combustion (LECO CN-2000 analyzer, Leco Corp., St. Joseph, MI). Mineral N (NH_4-N, NO_3-N and NO_2-N) of the vermicompost tea were analyzed colorimetrically using a discrete analyzer (Easy Chem Plus, Systea Scientific, IL). Other nutrients of the

vermicompost tea were measured using an inductively coupled plasma (ICP) spectrophotometer (Jarrel-Ash Division/Fisher Scientific Co., Waltham, MA).

4.3.5 Analysis of microbial activity in vermicompost tea

Microbial activity of each vermicompost tea was analyzed on 3 samples from 3 separate events for each extraction methods. Tea samples were taken after 12 h of a brewing cycle for ACT and ACTME and 7 days of steeping for NCT. A 10-fold serial dilution of each sample was prepared. Active bacteria and active fungi were assessed using a 1:10 dilution under Epifluorescence Microscopy at 40x and 20x objective, respectively (Vieira *et al.*, 2008). Microbial activity of the mineral nutrient solution and water (control) was also analyzed.

4.3.6 Plant growth, harvest and measurements

Seedlings were grown in peat-perlite medium and one seedling pot $^{-1}$ (volume: 3564 cm^3) was transplanted 10 days after emergence. Plants were grown in the greenhouse on a bench fitted with overhead sprinklers and watered every 4 hours for 3 minutes.

All pak choi plants were harvested four weeks after transplanting. Plant height and above ground plant fresh weight were determined. Plants were immediately frozen in liquid N and stored at - 20°C, and then freeze dried using a lyophilizer (D4A, Leybold-Heraeus Vacuum Products, Inc. PA). Above ground plant dry weight of each plant was recorded, ground using mortar and pestle, and stored in air-tight containers prior to further analysis.

4.3.7 Root growth

Total root length and surface area of the roots of pak choi plants grown in greenhouse experiments were calculated using WinRHIZO Pro V. 2003b system (Regent Instruments Inc., QC, Canada). The system consists of a scanner and WinRHIZO software. After taking the root fresh weight, roots were oven dried at 70°C for 72 h and dry weight of the roots of each plant was recorded.

4.3.8 Measurement of Phytonutrients

Total carotenoids and total phenolics were analyzed on lyophilized samples of each treatment by extracting 100 mg in 20 mL of ethanol: acetone (1:1, v:v) in glass vials for 24 hrs. All data were reported based on the dry weight of the lyophilized sample. Extracts were evaluated for total carotenoids at 470 nm using Genesys 20 spectrophotometer (Thermo Scientific - Model 4001-000, MA). Total carotenoids were calculated according to Gross (1991) using the equation: mg kg^{-1} total carotenoids = $(A*V \times 10^6)/(A\% \times 100G)$, where A is the absorbance, V is the total volume of the extract (mL) A% is the extinction coefficient of 2500, and G is the sample weight in grams. Total soluble phenolics were measured using the Prussian Blue assay as described by Stern *et al.* (1996) and the data was reported in mg kg^{-1} equivalents of gallic acid. Total glucosinolates were extracted and analyzed from lyophilized samples as described by Radovich *et al.* (2005b) .

4.3.9 Measurement of mineral nutrients in the plant tissue

Mineral nutrients in plant tissue were analyzed in the Agricultural Diagnostic Service Center, University of Hawaii at Manoa. Total C and N of dried tissue samples were analyzed by dry combustion as described earlier. Other nutrients in the tissue samples were measured after wet acid digestion using an inductively coupled plasma spectrophotometer (Jarrel-Ash Division/Fisher Scientific Co., Waltham, MA).

4.3.10 Measurement of pH, EC and mineral nutrients in soil

Soil samples were collected from each pot after harvesting the plant. Mineral nutrients in soil were analyzed in the Agricultural Diagnostic Service Center, University of Hawaii at Manoa. Total soil N and C were analyzed by the dry combustion method as described earlier. Other nutrients were extracted from the soil using the Mechlich-3 extractant and measured by the ICP method described earlier.

4.3.11 Soil respiration and Dehydrogenase activity

Soil respiration rate was measured with a portable soil respiration rate measuring system (LI-6400, LI-COR, Lincoln, NB, USA) fitted with a soil respiration chamber (6400-09, LI-COR, NB, USA). The respiration rate was expressed as µmol CO_2 fluxes m^{-2} sec^{-1}. Dehydrogenase activity in soil was measured as described by Alef (1995). Dehydrogenase activity expressed in µg g^{-1} dwt of soil was calculated based on the amount of 1,3,5-triphenyltetrazolium formazan (TPF) formed when 2,3,5-triphenyl tetrazolium chloride (TTC) was reduced by microbes in the soil.

4.3.12 Statistical analysis

Two-way analysis of variance (ANOVA) of plant growth parameters, mineral nutrients and phytonutrients in plant tissues, as well as soil properties was performed on main treatment effects and their interactions. Means were separated using Tukey's pair wise comparison in SAS 9.1 statistical software (SAS Institute Inc.). Statistical significance was obtained at 95% confidence level (α =0.05).

4.4 Results

4.4.1 Chemical properties of vermicompost and vermicompost teas

The pH of the vermicompost was 7.1 and EC 3.4 dS m^{-1}, with a C:N ratio of 12.5:1. The extraction method significantly affected pH, EC, DO, and extractable nutrient concentrations in vermicompost tea (Table 4.1). The average pH levels of ACTME, MNS and control treatment were significantly higher ($p<0.01$) than ACT and NCT. The pH level of ACT was not significantly different from NCT. Dissolved oxygen in vermicompost tea measured at the end of the extraction period was reduced by the use of microbial enhancer during production. Dissolved oxygen level increased in the order ACTME<NCT=ACT<MNS=Control. Electro conductivity level was highest in ACTME, in the reverse order of DO.

Chemical analysis of humic acid and kelp showed that use of these additives added about 48, 19 and 15 mg L^{-1} of total N, NO_3-N and NH_4-N, respectively in ACTME (data not shown). However, the levels of total N, NO_3-N and NH_4-N in ACTME were not significantly different than those in ACT, NCT and MNS. Phosphorus content in the

vermicompost tea was not influenced by the extraction methods. Potassium content was significantly higher ($p<0.05$) in ACTME than in ACT and NCT.

4.4.2 Microbial population in vermicompost tea

The active bacterial and fungal populations were significantly higher ($p<0.01$) in all types of vermicompost tea compared to MNS and the control (Table 4.2). The active bacterial and fungal populations were not affected by the extraction methods.

4.4.3 Effect on plant growth

Compost

Vermicompost tea significantly ($p<0.0001$) increased above ground plant fresh weight, however, there was a significant ($p<0.0001$) interaction between vermicompost tea and growth media. Except for ACTME in the Mollisol, all vermicompost tea significantly increased ($p<0.01$) above ground plant fresh weight compared to MNS and the control treatment across the growth media (Fig. 4.1a). The effect of vermicompost tea extraction method on above ground plant fresh weight was not significant under peat-perlite medium. However, the effect of ACT and NCT on above ground plant fresh weight was significantly higher ($p<0.01$) than that of ACTME in the Oxisol. The effect of tea type, growth media type and their interaction on above ground plant dry weight was similar to that of fresh weight (Fig. 4.1b). Root biomass and total root length increased significantly ($p<0.01$) in vermicompost tea treated plants compared to the MNS and control across growth media (Fig. 4.1c and d). The effect of vermicompost tea extraction methods on

root growth parameters was not significant in the peat-perlite medium. The effect of extraction methods varied among the root growth parameters in the soil media.

Osmocote

Vermicompost tea significantly ($p<0.0001$) increased above ground plant fresh and dry weight, however, there was a significant ($p<0.01$) interaction effect of vermicompost tea and growth media on plant fresh weight. Above ground plant fresh and dry weight was not affected by vermicompost tea application in the Mollisol (Fig. 4.2a and b). Vermicompost tea significantly increased ($p<0.05$) root biomass under peat-perlite medium but the root biomass was not affected under Mollisol and Oxisol (Fig. 4.2c). All vermicompost teas significantly increased ($p<0.05$) total root length under Oxisol and peat-perlite medium (Fig. 4.2d). Only ACT had a significantly positive effect on total root length and root surface area compared to other treatments in Mollisol.

4.4.4 Effect on mineral nutrient content of plant tissue

Compost

Vermicompost tea significantly ($p<0.0001$) increased tissue N, P, K, Ca and Mg content however, there was a significant ($p<0.0001$) interaction of vermicompost tea and growth media. Except for ACTME under the Mollisol, all types of vermicompost tea significantly increased ($p<0.0001$) total N content plant $^{-1}$ across all growth media as for above ground plant fresh weight (Table 4.3a). The effect of ACT and NCT on total N content was significantly higher ($p<0.0001$) than that of ACTME in the Oxisol, however, the effect of ACT and ACTME on total N content was significantly higher ($p<0.0001$)

than that of NCT in the peat-perlite medium. The effect of vermicompost tea on P content plant $^{-1}$ followed the similar trend to that of total N across growth media. All vermicompost teas significantly increased K content plant $^{-1}$ compared to MNS and control across the growth media. The effect of ACT and NCT on K content plant $^{-1}$ was significantly higher ($p<0.05$) than that of ACTME in Oxisol and Mollisol but the effect of ACTME on K content plant $^{-1}$ was significantly higher ($p<0.05$) than that of ACT and NCT in the peat-perlite medium. With the exception of ACTME under Mollisol, all vermicompost tea significantly increased ($p<0.0001$) Ca plant $^{-1}$ across growth media. Also, the effect of ACT and NCT on Ca was greater ($p<0.01$) than ACTME in Oxisol and peat-perlite medium. The effect of vermicompost tea on Mg content plant $^{-1}$ followed a similar trend as that of Ca across growth media.

Osmocote

Vermicompost tea significantly ($p<0.0001$) increased tissue N content however, there was a significant ($p<0.001$) interaction of vermicompost tea and growth media. All vermicompost tea significantly increased ($p<0.05$) total N content of plants in the Oxisol and in the peat-perlite medium; however the treatment effect was not significant in the Mollisol (Table 4.3b). The effect of vermicompost tea on P content was same as of N across the growth media. None of the vermicompost tea increased K content of plants in the Oxisol compared to MNS and control. Application of ACTME increased ($p<0.05$) K content of plants in the peat-perlite medium but decreased (<0.05) K content of plants compared to MNS and control in the Mollisol. Only ACT increased Ca content significantly ($p<0.05$) in plant tissue under Oxisol whereas none of the tea affected Ca

content in plant tissue under Mollisol. All vermicompost teas irrespective of extraction methods significantly ($p<0.05$) increased Ca in plant tissue compared to MNS under peat-perlite medium. The effect of vermicompost tea on Mg content was same as of Ca.

4.4.5 Effect on phytonutrients

Compost

Vermicompost tea significantly increased ($p<0.0001$) total carotenoids content compared to MNS and control across growth media. This increase was most notable in the peat-perlite medium, where tea treated plants had about 4 times more carotenoids than the control (Fig. 4.3a). Vermicompost tea treated plants grown on Oxisol and Mollisol had about twice as much total carotenoids content as the control. Plants receiving ACTME had lower carotenoids compared to ACT and NCT under Mollisol and peat-perlite medium, however all vermicompost teas had similar effects on total carotenoids under Oxisol. The effect of vermicompost tea type, growth media and their interaction on total glucosinolates was similar to that of total carotenoids (Fig. 4.3b). Total phenolics content was significantly lower ($p<0.0001$) in vermicompost tea treated plants compared to the control and MNS across the growth media (Fig. 4.3c).

Osmocote

Vermicompost tea increased total carotenoids significantly (<0.0001) compared to MNS and control across the growth media (Fig. 4.3d). The level of total carotenoids was not affected by vermicompost tea extraction methods. The effect of vermicompost tea on total glucosinolates was not significant across the growth media; however total

glucosinolates was significantly higher ($p<0.0001$) in peat-perlite medium and Oxisol than in Mollisol (Fig. 4.3e). Vermicompost tea significantly decreased ($p<0.05$) total phenolics, however, there was a significant ($p<0.05$) interaction effect of vermicompost tea and growth media.). Plants receiving NCT had significantly lower ($p<0.01$) total phenolics compared to other treatments under the Mollisol, whereas, only ACTME treated plants had significantly lower ($p<0.01$) phenolics compared to other treatments under peat-perlite medium (Fig. 4.3f). Total phenolics content was not affected by vermicompost tea treatment under the Oxisol.

4.4.6 Effect on microbial respiration and dehydrogenase activities

Compost

Vermicompost tea significantly ($p<0.0001$) increased soil respiration with a significant ($p<0.01$) interaction of vermicompost tea and growth media. Except for ACT in Oxisol, all vermicompost teas significantly increased ($p<0.001$) microbial respiration across growth media; and the magnitude of the effect was greater with the application of NCT (Fig. 4.4a). Dehydrogenase activity, expressed in TPF ($\mu g\ g^{-1}$ soil), was significantly ($p<0.0001$) influenced by the use of vermicompost tea across growth media (Fig. 4.4b). Dehydrogenase activity was not affected by the vermicompost tea extraction methods in Oxisol and peat-perlite medium but ACT had greater effect ($p<0.05$) compared to NCT and ACTME in Mollisol.

Osmocote

Vermicompost tea significantly ($p<0.01$) increased soil respiration and dehydrogenase activity (Fig. 4.4a and b). There was a significant ($p<0.0001$) interaction of vermicompost tea and growth media. Microbial respiration was not affected by the application of vermicompost tea in peat-perlite medium whereas all vermicompost tea significantly increased ($p<0.05$) the microbial respiration in Oxisol. Only ACTME and NCT increased microbial respiration in Mollisol. All vermicompost teas significantly increased ($p<0.05$) dehydrogenase activities compared to control and MNS in Mollisol and peat-perlite medium, however dehydrogenase activity was not affected by vermicompost tea treatments in Oxisol.

4.4.7 Effect on soil chemical properties

Compost

Application of vermicompost tea did not affect the pH across growth media (Table 4.4a). Application of vermicompost tea increased ($p<0.0001$) EC, N and K content of growth media and there was a significant ($p<0.0001$) interaction of vermicompost tea and growth media. All vermicompost teas significantly increased ($p<0.05$) the EC of Oxisol and Mollisol, however, only ACTME and NCT increased the EC of peat-perlite medium. Both ACT and ACTME significantly increased ($p<0.05$) total N across growth media compared to control. NCT had no significant effect on total N across growth media. Total carbon was not affected by the application of vermicompost tea in Oxisol and Mollisol but increased ($p<0.05$) in peat-perlite medium compared to control. Phosphorus content was

not affected by vermicompost teas in Oxisol and peat-perlite medium but all vermicompost teas significantly increased ($p<0.05$) P in Mollisol compared to control. Application of ACTME significantly increased ($p<0.0001$) K content across the growth media compared to other treatments.

Osmocote

Application of vermicompost tea significantly increased ($p<0.0001$) the pH, EC and total N, and there was a significant ($p<0.01$) interaction of vermicompost tea and growth media. Application of NCT significantly increased ($p<0.05$) the pH of Oxisol whereas ACTME increased the pH of Mollisol and peat-perlite medium compared to control (Table 4.4b). All vermicompost teas significantly increased ($p<0.01$) the EC of Mollisol and only ACTME increased the EC of Oxisol and peat-perlite medium. Application of ACTME increased ($p<0.05$) total N in Oxisol whereas both ACT and ACTME increased N content in Mollisol. All vermicompost teas irrespective of extraction method significantly increased ($p<0.0001$) total N compared to control in peat-perlite medium. NCT increased total carbon significantly ($p<0.05$) in Oxisol but ACT and ACTME increased total carbon in Mollisol compared to other treatments. Total carbon was not affected by the application of vermicompost tea in peat-perlite medium. Only NCT significantly increased ($p<0.05$) P content in Oxisol but all the teas significantly increased ($p<0.05$) P content compared to control in Mollisol. None of the teas had significant effect on P and K content of peat-perlite medium. Application of ACTME significantly increased ($p<0.01$) K content of Oxisol and Mollisol compared to control.

4.5 Discussion

The use of kelp extract and humic acid resulted in higher EC, some additional mineral nutrients and lower DO in ACTME. The higher K in ACTME relative to the other extracts can be accounted for by that supplied in the additives. Additives also supplied a considerable amount of N in ACTME as reflected the N, NH_4^+ and NO_3^- content in vermicompost tea but were not statistically significant. Although the extraction condition was uniform for each extraction event there was a great variation in N concentration in ACTME in each extraction event which is responsible for having statistically non significant result. Microbial activity is the general explanation for reduced DO levels during tea extraction (Ingham, 2005a), with neither active bacteria nor active fungi in ACTME were significantly different from that of ACT and NCT. It is possible that the additives increased microbial populations in ACTME and that microbes remained bound to vermicompost particles that were removed during screening. This might be the reason for reduced DO level of ACTME.

Vermicompost tea enhanced plant growth and mineral nutrient content in plant tissue in Oxisol and peat-perlite medium under both fertilizer regimes, consistent with previous studies (Gamaley *et al.*, 2001; Hargreaves *et al.*, 2009; Pant *et al.*, 2009). A lack of plant growth response with ACTME in Mollisol under both fertilizer regimes is possibly due to poor drainage combined with addition of extra salts as suggested by higher EC of Mollisol after vermicompost tea treatment (Table 4.4a and b). Although the above ground fresh and dry plant weights were high under Osmocote fertilization compared to compost, the effect of vermicompost teas was most pronounced under

compost fertilization. Enhanced plant growth with vermicompost tea application in peat-perlite medium compared to Oxisol and Mollisol observed in this study was associated with differences in physical and chemical properties of peat-perlite medium and soils. Although all growth media received the same level of nutrients through fertilizers and vermicompost tea, plants grown in peat-perlite medium likely benefited from good drainage and aeration which improved root development and nutrient uptake, and thereby enhancing plant growth.

Soluble mineral nutrients and microbial byproducts in vermicompost tea can enhance nutrient uptake from the soil and increase foliar uptake of nutrients by plants (Xu *et al.*, 2001; Ingham, 2005a). Nutrient analysis of vermicompost tea indicates that vermicompost tea supplied a considerable amount of soluble N (about 110 mg) to each plant compared to control. A positive correlation between plant fresh weight and N uptake by plants explains yield response to vermicompost tea across treatments. Keeling *et al.* (2003) observed that application of compost tea on oil seed rape plants at an early stage of growth increased both root development and plant growth. Siddiqui *et al.* (2008) reported that compost tea applications enhanced plant growth and increased tap root length of okra plant. Increased root biomass, total root length and root surface area with the application of vermicompost tea observed in this study agrees with the findings of these previous studies. Enhanced overall root development accompanied with better nutrient uptake by vermicompost tea treated plants compared to MNS treated and control plants suggests that improved root growth or nutrient uptake per unit root is one of the mechanisms involved in plant growth stimulation. Arancon *et al.* (2007) reported that humic, fulvic and other organic acids extracted or produced by microorganisms in vermicompost tea could

promote plant growth. Garcia Martinez *et al.* (2002) confirmed that water of compost contained a compound with molecular structure and biological activity analogous to auxins. Leachate from well decomposed compost has been shown to contain cytokinin-like substances, derived from hydrolysis of glucosides by the enzyme β-glucosidase produced by microbes (Arthur *et al.*, 2001). Although phyto-hormones or growth regulators in vermicompost tea were not analyzed in this study, the findings of the previous study support that phyto-hormones they may have played an important role in plant responses.

Plant growth was not influenced by the various extraction methods in peat-perlite medium which is consistent with the results of our previous work (Pant *et al.*, 2009). Ingham (1999) noted that aerated compost tea augmented with a microbial enhancer imparted a better plant response by increasing microbial population densities in the compost tea. Some workers suggested that an increased microbial population in compost tea would increase microbial activity in phyllosphere and rhizosphere, which in turn contributes to better plant growth. However, the active microbial population in ACTME was neither higher nor was the ACTME effect on plant growth was more pronounced compared to ACT and NCT in this study. Since the same level of the active microbial population was observed in all types of vermicompost tea, contribution of their activities to nutrient uptake and plant growth may be similar. Inferior plant growth with ACTME compared to ACT and NCT in the Mollisol is associated with the heavy soil texture and poor drainage in Mollisol accompanied with addition of extra salts through ACTME. There is debate regarding the efficacy of aeration during compost tea production. Ingham (1999) suggested that ACT would provide better results than that of NCT however;

several other investigators have reported that NCT prepared using Weltzien's method has a more consistent and significantly positive than that of ACT on disease control and plant growth (Weltzien, 1991; Cronin *et al.*, 1996; Scheuerell and Mahaffee, 2006). Arancon *et al.* (2007) observed the superior effect of ACT over NCT on disease suppression and plant growth. However, the extraction period for NCT adopted by these authors was only 2 days whereas, Weltzien's method recommends a 7-day extraction period (Weltzien, 1991; Arancon *et al.*, 2007). It may be that the reduced effect of NCT over ACT observed in the study by Arancon *et al.* (2007) is associated with the shorter extraction period. Welke (2005) has shown that both ACT and NCT (extracted for a week) have similar effect on plant growth and disease suppression. Results of this study are consistent with Welke (2005) in that aeration is not essential for plant growth promotion provided the extraction period is sufficient.

Increased total carotenoids level in plant tissue in response to vermicompost tea treatments was associated with improved plant growth (Fig. 4.5a). This agrees with findings of previous studies reporting higher carotenoids in plant tissue to correspond with increased plant growth at higher fertilizer rates (Pant *et al.*, 2009). Better plant growth with the application of vermicompost tea may have contributed to synthesis of carotenoids across growth media under both compost and Osmocote fertilizations.

Various studies have shown an interaction between sulfur and nitrogen fertilizer on glucosinolates concentration of Brassica crops. Krumbein *et al.* (2002) reported that the levels of total glucosinolates were low with low sulfur and nitrogen fertilizer in broccoli plants whereas, total glucosinolates levels were high at sufficient nitrogen supply

and high sulfur levels. In another study, the levels of several glucosinolates decreased in leaves under nitrogen deficiency but accumulated in roots of *Arabidopsis thaliana* (Hirai *et al.*, 2004). However, Chen *et al.* (2006) observed lower levels of total glucosinolates in pak choi at high levels of foliar nitrogen application. Applications of vermicompost tea contributed to increased N availability to plants grown under compost fertilization in this study likely explain the positive relationship between total glucosinolates and plant growth (Fig. 4.5b). In the case of Osmocote fertilization, vermicompost tea did not have any influence on total glucosinolates, presumably because of greater N availability.

Stress, particularly low N, can induce greater concentrations of phenolics in plant tissues (Brown *et al.*, 1984; Estiarte *et al.*, 1994). Nutrient stresses can reduce growth more than photosynthesis; the excess carbon relative to nutrients will be allocated to carbon-based defensive compounds including phenolics. Increased concentrations of total phenolics were associated with lower plant growth and low mineral N concentration in tissue of control plants compared to vermicompost tea treated plants grown under compost fertilization in this study (Fig. 4.5c). Between the two fertilizer regimes, a higher level of total phenolics content was observed in plants grown under compost fertilization compared to Osmocote fertilization. This could be due to a relatively rapid release of plant available nutrient from Osmocote compared to compost. Asami *et al.* (2003) also observed consistently greater levels of total phenolics in organically grown crops than those produced by conventional agricultural practices. Also, greater concentrations of total phenolics in the plants grown under the Mollisol compared to the Oxisol and the peat-perlite medium were linked with lower plant growth and low tissue N concentration in Mollisol grown plants.

Applications of vermicompost tea affected some of the chemical and biological properties of the media. Higher EC in vermicompost tea treated media was associated with an increase in mineral nutrient concentrations, primarily total N and K content. Soil respiration and dehydrogenase activity were higher in the growth media that had received compost than that of Osmocote. The increase in soil respiration with the application of compost may be explained by improved microbial decomposition of soil organic matter. This might be due to the availability of active organic carbon or enrichment of nutrients for the microbes through the addition of high organic carbon content of compost (Sikora and Yakovchenko, 1996; Bernal *et al.*, 1998). Vermicompost tea treatments contributed to increased soil respiration and dehydrogenase activity, particularly under compost fertilization, implying a more efficient of organic matter decomposition and mineralization of nutrients in growth media, and therefore producing better plant growth.

4.6 Conclusions

Application of vermicompost tea enhanced plant yield, mineral nutrient content and total carotenoids in plant tissue under both compost and Osmocote fertilization; the effect however was more prominent under compost fertilization. Vermicompost tea enhanced total glucosinolates under compost fertilization but had no effect under Osmocote fertilization. Vermicompost tea reduced total phenolics compared to that of MNS and control. In general all vermicompost teas regardless of extraction method provided similar effect on plant growth and nutrient concentration in Oxisol and peat-perlite medium substantiating our previous study (Pant *et al.*, 2009). Aeration and additives during extraction are not essential for growth promotion and nutrient quality if

tea extraction period is sufficiently long. Furthermore, the absence of ACTME treatment effects on plant growth in the Mollisol suggests that use of additives in vermicompost tea may not be appropriate when use with a heavy soils with poor drainage. Vermicompost tea improved mineral nutrient concentration and microbial activities in Oxisol, Mollisol and peat-perlite medium. Better root and shoot growth and increased N uptake by vermicompost tea treated plants over MNS treated plants suggest the possibility of microbial and hormonal contributions along with the nutrient effects of the tea. The findings of this study suggest that vermicompost tea may be used to improve plant nutrient status and enhance soil biological properties in vegetable production, and that short-term benefits to plant growth is likely to be most measurable under low-input conditions. Research to understand the possible contribution of phytohormones and organic acids from vermicompost tea on plant growth are ongoing.

Table 4.1 Chemical properties of vermicompost tea.

Extraction method	pH	DO (mg L^{-1})	EC (dS m^{-1})	N	NO_3-N	NH_4-N	P	K	Ca	Mg
				----------(mg L^{-1})----------						
ACT	7.5b	7.9b	1.4b	166.3a	162.3 a	2.2b	5.1b	35.3b	185.9a	80.1a
ACTME	8.2a	6.1c	3.0a	192.8a	158.7 a	31.9ab	6.7b	401.4a	149.6a	61.4ab
NCT	7.4b	7.6b	1.5b	99.5ab	96.7ab	1.9b	3.5b	34.5b	114.6ab	50.2ab
MNS	8.1a	8.4a	1.1c	76.6ab	25.2b	51.2a	48.0a	57.7b	9.7b	14.4b
Control	8.1a	8.7a	0.4d	3.4b	1.2 b	1.9b	0.3b	4.1b	9.8b	14.3b

Means (N=3) followed by the same letter are not significantly different ($p < 0.05$). DO=Dissolved Oxygen, EC= Electrical Conductivity, NCT = Non-aerated vermicompost tea, ACTME = Aerated vermicompost tea with microbial enhancer, ACT = Aerated vermicompost tea, MNS= Mineral Nutrient Solution, Control = water.

Table 4.2 Microbial population in vermicompost tea (n = 3).

Extraction method	Active bacteria (log_{10} cells mL^{-1})	Active bacteria ($\mu g\ mL^{-1}$)	Length of active fungi ($cm\ mL^{-1}$)	Active fungi ($\mu g\ mL^{-1}$)
ACT	7.5a	6.0b	31.9a	0.7a
ACT ME	7.8a	21.8a	29.2a	0.6a
NCT	7.6a	5.7b	29.5a	0.6a
MNS	0.0b	0.0c	0.0b	0.0b
Control	0.0b	0.0c	0.0b	0.0b

Means followed by the same letter are not significantly different ($p < 0.05$).
NCT = Non-aerated vermicompost tea, ACTME = Aerated vermicompost tea with microbial enhancer, ACT = Aerated vermicompost tea, MNS= Mineral Nutrient Solution, Control = water.

Table 4.3 Effect of vermicompost tea on mineral nutrient content in plant tissue grown in growth media fertilized with a. compost b. Osmocote (n = 3).

Extraction method	N	P	K	Ca	Mg
	----------mg plant $^{-1}$----------				
a. Fertilizer: Compost					
Oxisol					
ACT	82.4a	21.9 a	173.1 a	98.4a	15.9a
ACTME	33.5b	8.6 b	54.7 b	44.8b	8.4b
NCT	92.2a	22.8 a	184.9 a	113.3a	18.9a
MNS	15.5bc	4.7 bc	32.4 c	21.9bc	4.2bc
Control	5.9c	2.4 c	13.5 c	13.5c	2.6c
Mollisol					
ACT	61.6a	18.6b	120.4a	60.2b	18.7a
ACTME	11.9b	3.4c	10.8b	16.0c	6.8b
NCT	63.1a	23.7a	133.3a	80.1a	20.3a
MNS	11.0b	4.3c	17.9b	12.8c	4.3b
Control	5.2b	2.5c	8.8b	8.5c	2.9b
Peat-perlite medium					
ACT	115.3a	40.8a	225.2b	168.4a	35.5a
ACTME	101.2a	31.4b	257.6a	127.8b	27.5b
NCT	90.3b	32.9b	189.5c	146.8ab	30.6ab
MNS	9.9c	4.6c	21.7d	19.2c	4.7c
Control	2.7c	1.8c	7.9d	6.6c	1.6c
b. Fertilizer: Osmocote					
Oxisol					
ACT	256.8a	54.5 a	416.0 ab	250.8a	48.2a
ACTME	217.1a	45.5 ab	458.3 ab	218.7ab	41.3ab
NCT	261.2a	57.8 a	469.9 a	225.6ab	44.8ab
MNS	139.2b	30.0 b	284.1 b	148.6b	27.5b
Control	174.2ab	46.1 ab	382.6 ab	172.2b	36.8ab
Mollisol					
ACT	97.5a	25.7 a	132.5ab	78.8a	30.3a
ACTME	53.8a	15.2 a	65.0b	50.6a	21.7a
NCT	116.2a	30.6 a	231.4a	109.4a	33.6a
MNS	48.6a	16.5 a	75.2b	58.1a	24.5a
Control	103.2a	32.2 a	209.0a	115.1a	46.3a
Peat-perlite medium					
ACT	263.6b	76.2 ab	222.3bc	307.1a	93.8a
ACTME	367.3a	98.9 a	770.5a	318.0a	91.5a
NCT	357.1a	95.5 a	360.7b	326.7a	97.6a
MNS	116.1c	40.9 c	207.7c	196.4b	60.5b
Control	198.8bc	61.3 bc	283.9bc	278.7ab	88.9ab

Means followed by the same letter are not significantly different ($p < 0.05$) within each growth medium. NCT = Non-aerated vermicompost tea, ACTME = Aerated vermicompost tea with microbial enhancer, ACT = Aerated vermicompost tea, MNS= Mineral Nutrient Solution, Control = water.

Table 4.4 Effect of vermicompost tea on chemical properties of growth media fertilized with a. compost b. Osmocote (n = 3).

Extraction Method	pH	EC (µS cm $^{-1}$)	Total N (g kg $^{-1}$)	Total C (g kg $^{-1}$)	P (mg kg $^{-1}$)	K (mg kg $^{-1}$)
a. Fertilizer: Compost						
Oxisol						
ACT	7.6a	590.3a	2.5a	25.0a	39.3a	335.6b
ACTME	7.6a	594.7a	2.4a	23.2a	40.7a	655.2a
NCT	7.6a	588.0a	2.2b	26.6a	40.7a	333.7b
MNS	7.5a	544.3b	2.2b	26.1a	40.3a	374.2b
Control	7.6a	542.0b	2.1b	24.9a	42.0a	416.4b
Mollisol						
ACT	7.6a	789.7b	2.9a	31.1a	115.0a	961.6b
ACTME	7.5a	990.0a	2.8a	32.2a	106.7ab	1471.7a
NCT	7.6a	789.0b	2.6a	30.8a	114.3a	1043.9b
M NS	7.5a	773.3b	2.5ab	29.3a	100.7bc	939.9b
Control	7.6a	757.3c	2.3b	26.5a	97.3c	997.0b
Peat-perlite medium						
ACT	7.4a	437.7b	10.7b	418.0a	3.2a	10.9b
ACTME	7.5a	594.3a	12.9a	426.6a	4.1a	92.5a
NCT	7.3a	480.0b	10.3bc	441.4a	2.8a	12.4b
MNS	7.3a	432.7b	9.5bc	422.6a	3.6a	11.7b
Control	7.4a	433.7b	8.9c	376.3b	2.9a	16.1b
b. Fertilizer: Osmocote						
Oxisol						
ACT	5.6ab	518.3b	1.5b	10.6b	45.7b	84.9b
ACTME	5. 7ab	576.7a	1.9a	11.6ab	39.0b	278.5a
NCT	5.9a	520.0b	1.4b	12.4a	54.0a	105.4b
MNS	5.4b	517.7b	0.7b	11.0b	45.3b	142.6b
Control	5.4b	506.0b	0.8b	11.1b	42.3b	124.3b
Mollisol						
ACT	6.2b	883.0b	1.6a	13.4a	102.3a	714. 9b
ACTME	6.6a	1007.0a	1.5a	13.4a	111.0a	1138.4a
NCT	6.2b	990. 7a	1.0ab	11.9b	106.7a	672.8b
MNS	6.1bc	891.3b	0.6b	11.1b	106.0a	743.6b
Control	6.0c	715.0c	0.5b	11.2b	84.5b	646.9b
Peat-perlite medium						
ACT	4.6bc	497.0b	12.9b	335.8a	11.4a	16.2a
ACTME	4.9a	673.3a	13.8a	313.8a	9.4a	16.2a
NCT	4.7ab	494.0b	9.6c	332.9a	10.2a	9.4a
MNS	4.8a	444.7b	12.6c	349.4a	7.1a	10.6a
Control	4.4c	421.0b	8.6d	309.9a	11.8a	15.7a

Means followed by the same letter are not significantly different ($p < 0.05$) within each growth medium. NCT = Non-aerated vermicompost tea, ACTME = Aerated vermicompost tea with microbial enhancer, ACT = Aerated vermicompost tea, MNS= Mineral Nutrient Solution, Control = water.

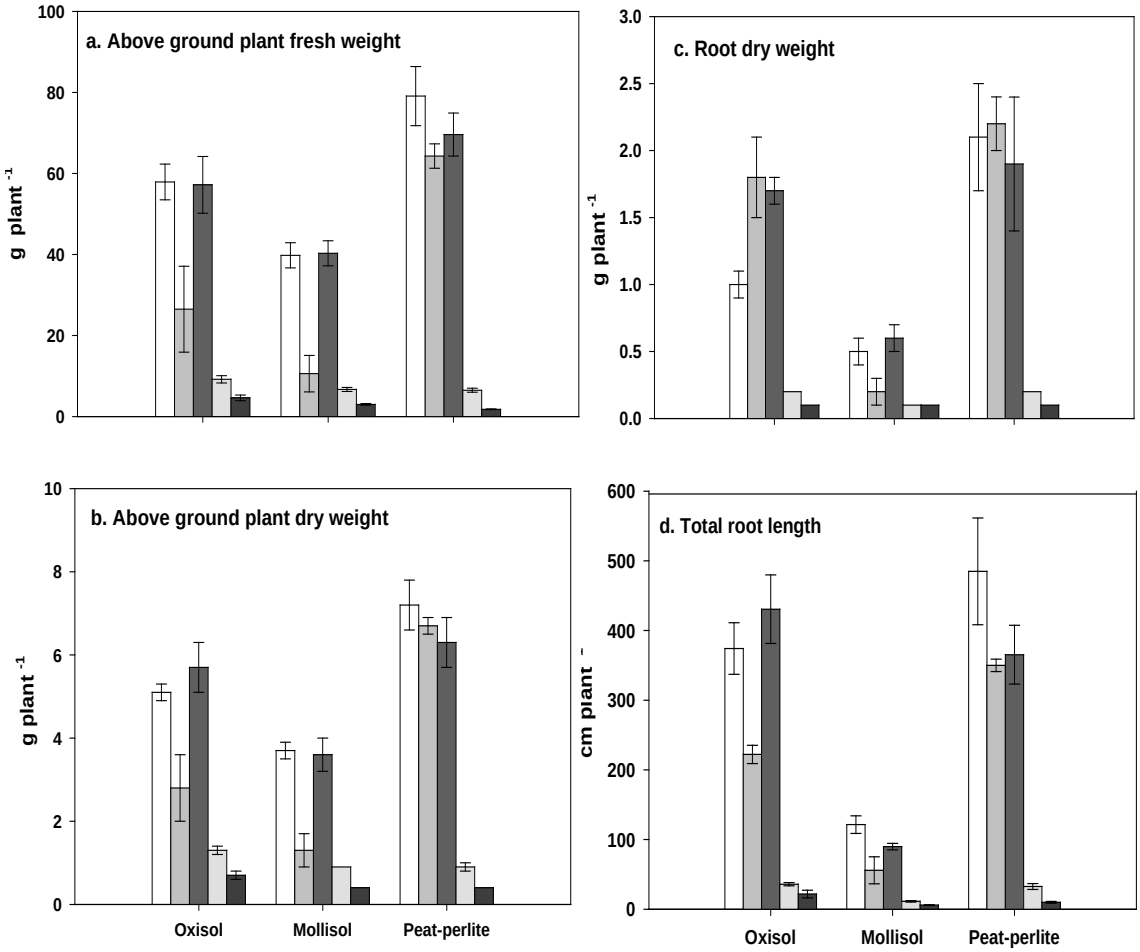


Figure 4.1 Extract effect on a. above ground plant fresh weight, b. above ground plant dry weight, c. root dry weight and d. total root length under compost fertilization. Plotted bars are means of 4 samples, and error bars represent standard errors of the mean. ACT = Aerated vermicompost tea, ACTME = Aerated vermicompost tea with microbial enhancer, NCT = Non-aerated vermicompost tea, MNS = Mineral nutrient solution, Control = water.

ACT ACTME NCT MNS Control

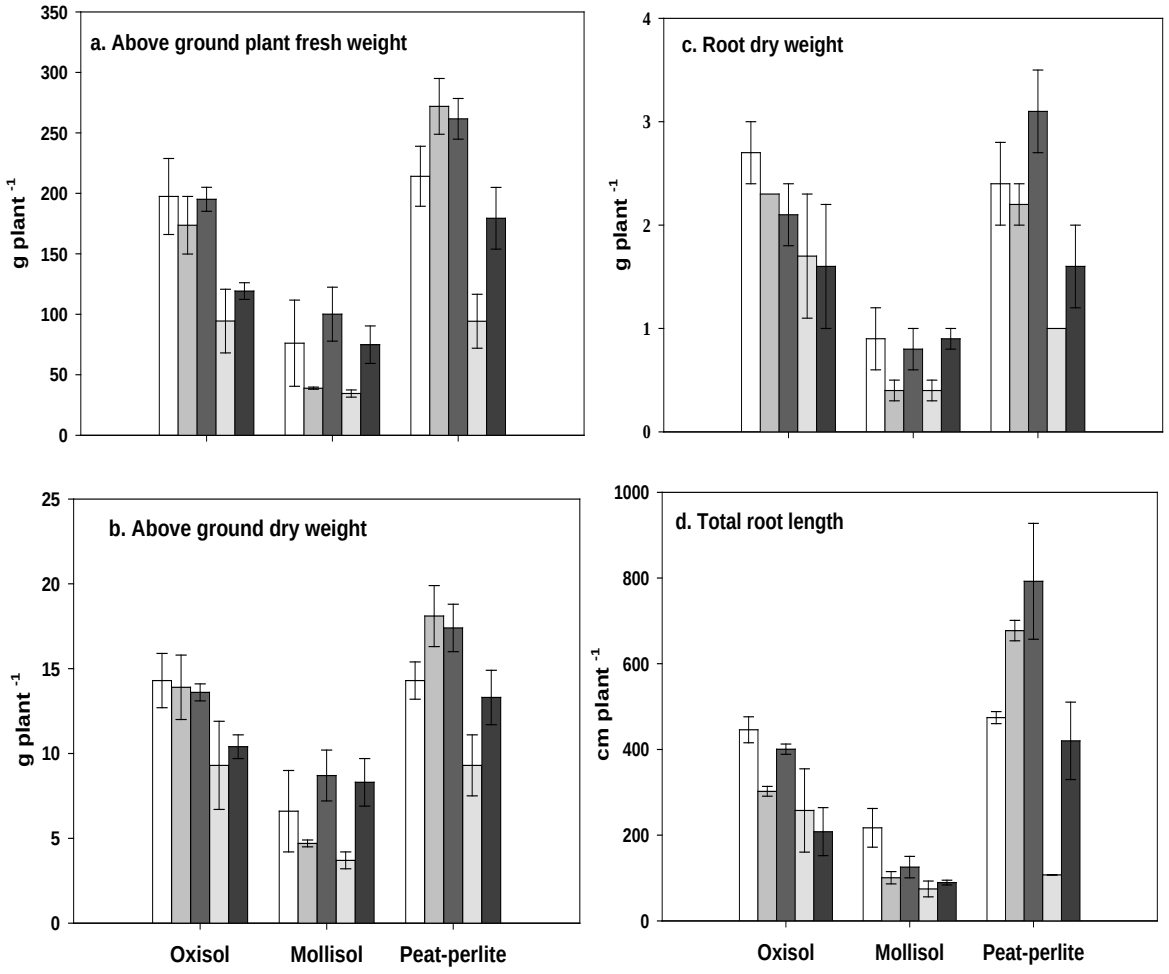


Figure 4.2 Extract effect on a. above ground plant fresh weight, b. above ground plant dry weight, c. root dry weight and d. total root length under Osmocote fertilization. Plotted bars are means of 4 samples, and error bars represent standard errors of the mean. ACT = Aerated vermicompost tea, ACTME = Aerated vermicompost tea with microbial enhancer, NCT = Non-aerated vermicompost tea, MNS = Mineral nutrient solution, Control = water.

ACT ACTME NCT MNS Control

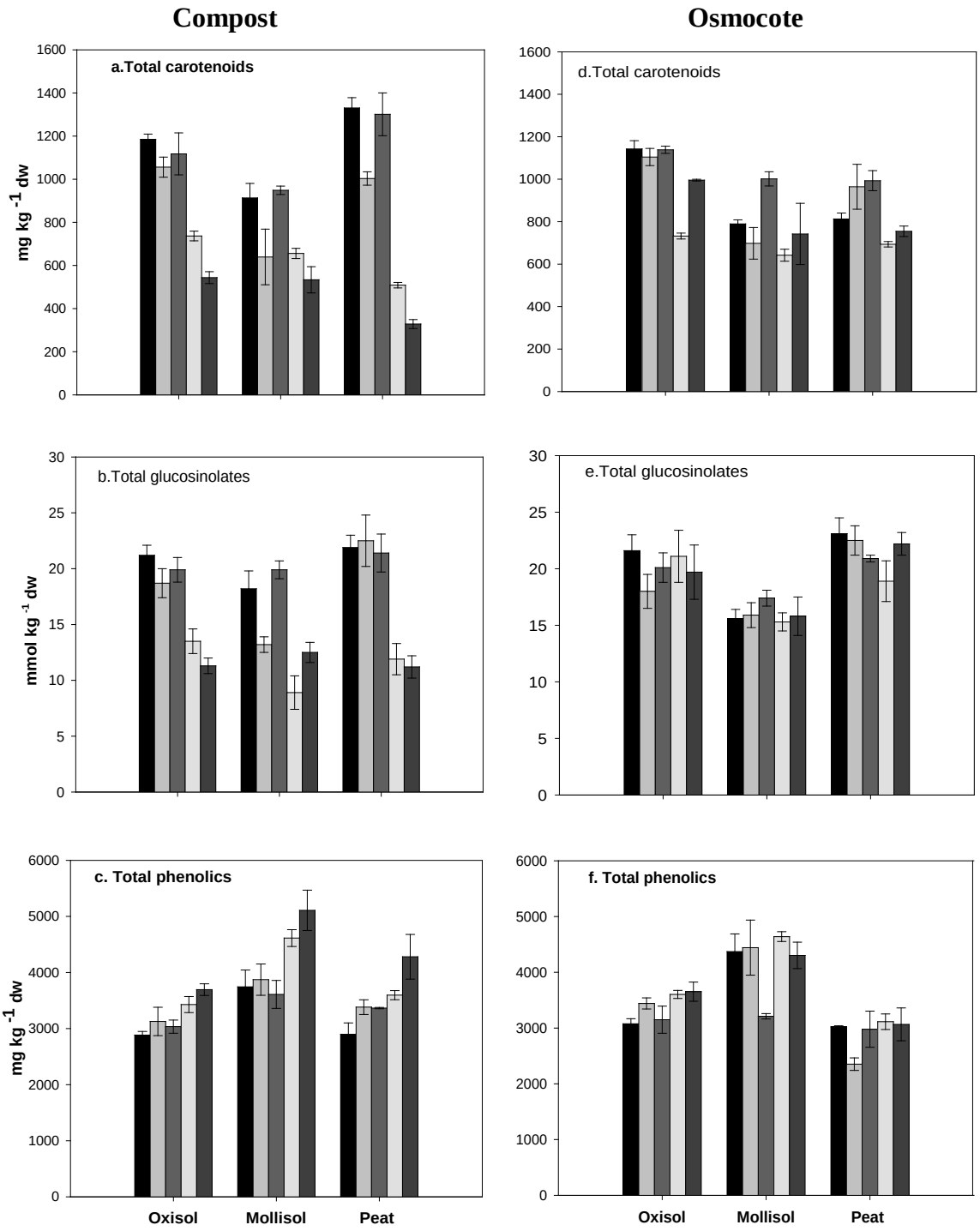


Figure 4.3 Extract effect on total carotenoids, total glucosinolates and total phenolics across the treatments. Plotted bars are means of 4 samples, and error bars represent standard errors of the mean. ACT = Aerated vermicompost tea, ACTME = Aerated vermicompost tea with microbial enhancer, NCT = Non-aerated vermicompost tea, MNS = Mineral nutrient solution, Control = water.

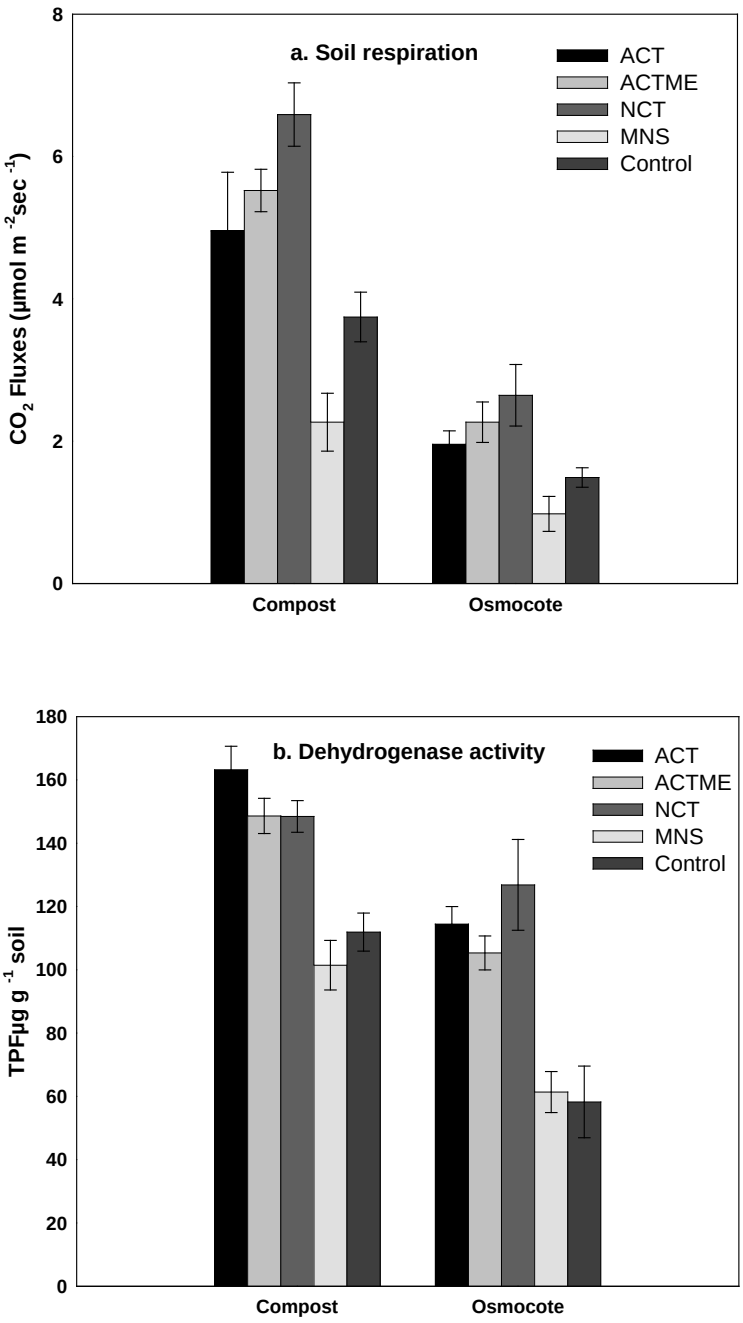


Figure 4.4. a. Soil respiration (CO_2 fluxes µmol m^{-2} s^{-1}) and b. Dehydrogenase activity (TPF µg g^{-1} of media) across the growth media. Plotted bars are means of 9 samples, and error bars represent standard errors of the mean. ACT = Aerated vermicompost tea, ACTME = Aerated vermicompost tea with microbial enhancer, NCT = Non-aerated vermicompost tea, MNS = Mineral nutrient solution, Control = water. TPF = 1,3,5-Triphenyltetrazolium formazan

ACT ACTME NCT MNS Control

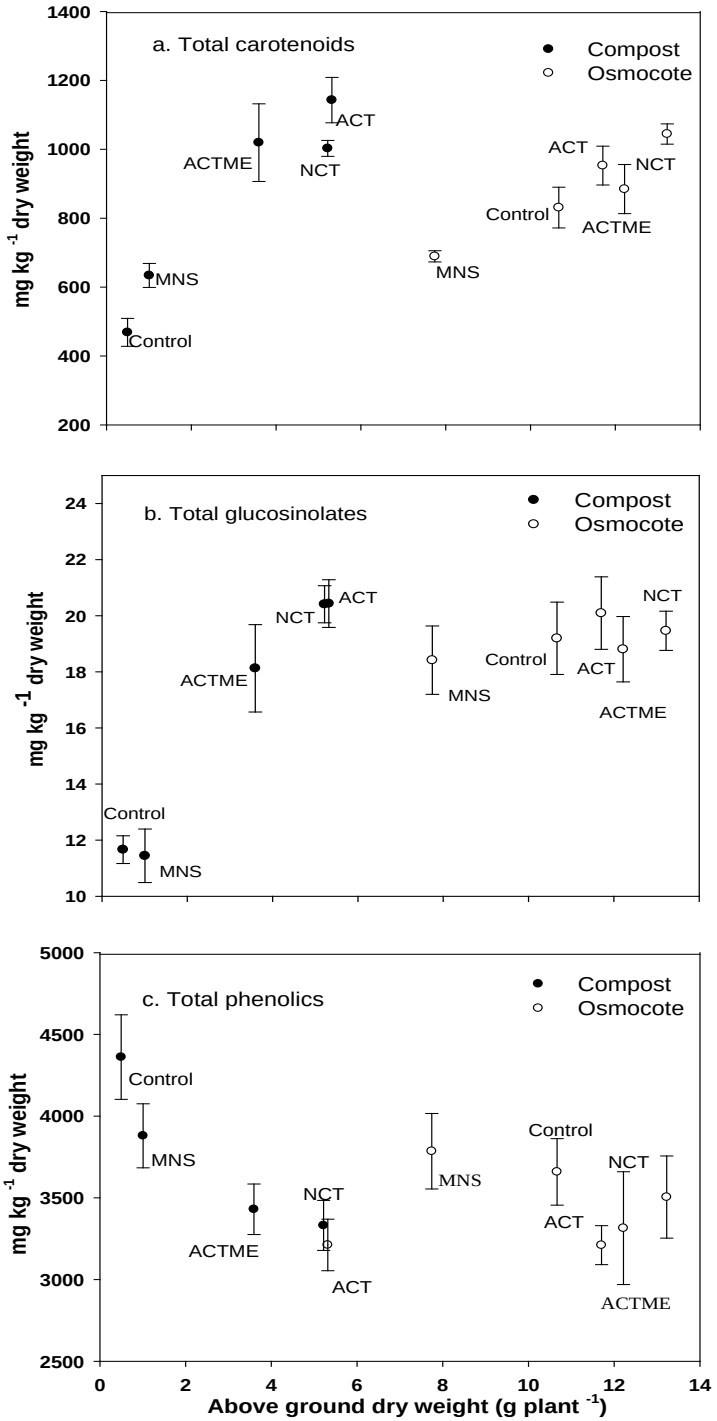


Figure 4.5. Total a. carotenoids, b. glucosinolates and c. phenolics relative to above ground plant dry weights in all of the growth media. Plotted bars are means of 9 samples, and error bars represent standard errors of the mean. ACT = Aerated vermicompost tea, ACTME = Aerated vermicompost tea with microbial enhancer, NCT = Non-aerated vermicompost tea, MNS = Mineral nutrient solution, Control = water.

CHAPTER 5

EFFECT OF THE CONCENTRATION OF VERMICOMPOST AQUEOUS EXTRACT (VERMICOMPOST TEA) ON PAK CHOI (*BRASSICA RAPA*, CHINENSIS GROUP) YIELD, QUALITY, AND SOIL BIOLOGICAL PROPERTIES

5.1 Abstract

Two greenhouse trials and two field trials were conducted to investigate the effects of vermicompost (aqueous extract) tea on the growth, mineral N and phytonutrient content of pak choi (*Brassica rapa, Chinensis*), and on soil biological properties. In greenhouse experiments, plants were fertilized with chicken manure-based thermophilic compost. In field trials, three fertilizer treatments: (i) rendered meat by-product or Tankage (Island Commodities, Honolulu, HI), (ii) soluble fertilizer (16:16:16) and (iii) chicken manure-based thermophilic compost were applied. Aerated vermicompost teas were prepared using chicken manure-based vermicompost and water at various ratios. Pak choi plants were treated weekly for four weeks with 10%, 5%, 3% and 1% vermicompost teas in the greenhouse experiments; and 10% and 5% teas in the field trials. Applications of vermicompost tea significantly increased plant growth, N content, total carotenoids and total glucosinolates in plant tissue; this response was greatest in chicken manure-fertilized treatments. Such positive influence was associated with increased N uptake. Vermicompost tea also improved soil respiration and dehydrogenase activity. Plant growth, phytonutrient content and microbial activities in soil increased with increasing concentrations of vermicompost tea. The best plant growth response was observed with 5% and 10% vermicompost tea, indicating that the optimal concentration

of vermicompost tea ranges between 5 and 10%. The findings suggest that vermicompost tea could be used to improve plant nutrient status and enhance soil biological properties in vegetable production.

5.2 Introduction

Aqueous vermicompost extracts (vermicompost tea) are an option for conventional and organic growers to improve plant growth and nutrient quality, enhance soil biological properties and suppress plant diseases (Scheuerell and Mahaffee, 2004; Zaller, 2006; Pant *et al.*, 2009). Application of vermicompost or vermicompost tea may enhance soil fertility by introducing microorganisms that might aid in soil nutrient retention and extraction, and by adding soluble nutrients, fine particulate organic matter, and organic acids (Merrill and McKeon, 2001; Ingham, 2005b; Kannangara *et al.*, 2006; Diver, 2001).

Several factors can influence whether vermicompost tea application will elicit measurable responses in plants. Low quality compost teas could have negative effects on plants, including a decrease in yields and inhibition of seed germination and plant growth (Ingham, 2005a). The quality of vermicompost tea largely depends on the vermicompost used for extraction. However, the quality of vermicompost tea can be associated also with the conditions under which it is produced, such as aeration, use of additives, and ratio of vermicompost to water (Carballo *et al.*, 2009). Of these factors, recent work has suggested that the concentration of mineral nutrients and organic acids may be most important in eliciting a response (Arancon *et al.*, 2007; Pant *et al.*, 2009). A higher ratio of vermicompost to water generally results in greater concentration of mineral nutrients,

dissolved salts, and organic acids in the extract which may either cause phytotoxicity or increase production cost. On the other hand, excessive dilution may reduce the effectiveness of vermicompost tea since very dilute concentrations may not contain sufficient amounts of nutrients or other compounds required for optimal plant growth.

Studies on the effect of compost tea dilution (i.e. ratio of vermicompost to water) have mainly focused on disease suppression with diverse results. Palmer *et al.* (2010) reported that the disease suppression effect of compost tea was greater at 33% (1:2 vermicompost to water ratio, v:v), 10% (1:10, v:v) and 3% (1:33, v:v) dilution rates of aerated compost tea compared to a lower dilution rate of 50% (1:1, v:v) or a much greater dilution rate of 1% (1:100, v:v) tea. Welke (2005) confirmed that strawberry plants receiving 12.5% dilution rate (1:8, v:v) compost tea had higher yield and lower incidence of disease compared to the plants treated with 25% dilution rate (1:4, v:v) tea. Scheuerell and Mahaffee (2004) also reported that application of 3% dilution rate (1:30, v:v) vermicompost tea had a greater effect on suppression of cucumber damping-off caused by *Pythium ultimum* compared to the much greater dilution rate of 0.4% dilution rate (1:270, v:v) vermicompost tea. Wiltzen (1990) reported that the effects of 33% and 10% dilution rates of compost tea on suppression of *Phytopthora infestans* was greater than that of the much greater dilution rate of 2% tea. No difference in suppression was observed between 33% and 10% dilution rates of tea.

Despite an increasing body of popular and scientific literature focusing on vermicompost tea as an alternate source of plant nutrition, soil quality enhancer, and biological disease control option for growers, information on the efficacy of its concentration is either lacking or inconsistent. We hypothesized that vermicompost tea

concentration would alter the effects of the extract on plant growth and nutritive quality, as well as soil biological properties. The objectives of this study were to determine the effects of: (i) concentrations of vermicompost tea on yield, mineral nutrient concentration, and phytonutrient content of pak choi; and (ii) concentrations of vermicompost tea on soil biological properties.

5.3 Materials and Methods

5.3.1 Greenhouse experiments

Greenhouse experiments were conducted twice at the Magoon Research Station (Latitude: 21°18'22" N, Longitude: 157°48'37" W) of the University of Hawaii to determine the effects of concentrations of vermicompost tea on plant growth. Pak choi plants were grown in peat-perlite medium fertilized with chicken manure-based thermophilic compost to provide 75 mg N L^{-1} growth media (150 kg N ha^{-1}). Plants were grown in garden pots (volume: 865 cm^3). Three to four pak choi seeds were sown into each pot. Two days after seedling emergence in all pots, plants were thinned to one plant pot^{-1}. Plants were allowed to grow in the greenhouse on a bench fitted with overhead sprinklers with a frequency of every 4 hours for 5 minutes. A 10% vermicompost to water ratio (1:10, v:v) of aerated vermicompost extract was prepared using chicken manure-based vermicompost and water, as previously described (Pant *et al.*, 2009). This extract was further diluted with water to produce 5%, 3% and 1% dilution ratios. These 4 dilution rates of extract (10%, 5%, 3% and 1%) and the same amount of aerated water (control) were applied weekly to the root zone and foliage of plants at 150 mL pot^{-1} for four weeks starting at 5 days after seedling emergence. The experiments were arranged in a

completely randomized design with 5 vermicompost tea treatments and 6 replications per treatment.

Plants were harvested at five weeks after emergence. Fresh weight, dry weight and plant height were measured. Total root length and surface area of the roots of pak choi plants grown in greenhouse experiments were calculated using WinRHIZO Pro V. 2003b system (Regent Instruments Inc., QC, Canada). The system consists of a scanner and WinRHIZO software. The fresh weights of roots were recorded, then roots were placed in an oven at 70°C for 72 h, and then dry weight of roots were recorded.

5.3.2 Field experiments

The field research was located at two sites.

Site 'A': Waimanalo Research Station

Waimanalo Research Station of the College of Tropical Agriculture and Human Resources, University of Hawaii, Oahu (Latitude: 21°20'41" N, Longitude: 157°44'31" W, elevation 20-29 meters) has Waialua series soil (Order: Mollisols and Family: very-fine, kaolinitic, isohyperthermic, Vertic Haplustolls). The average daily maximum and minimum temperatures during the experiment were 28°C and 23°C, respectively.

Site 'B': Poamoho Research Station

Poamoho Research Station of the College of Tropical Agriculture and Human Resources, University of Hawaii, Oahu (Latitude: 21°32'58" N, Longitude: 158°05'47" W, elevation 166-214 meters) has Wahiawa series soil (Order: Oxisol and Family: clayey, kaolinitic,

isohyperthermic, Tropeptic Eutrustox). The average daily maximum and minimum temperatures during the experiment were 22°C and 16°C, respectively.

5.3.3 Experimental design and transplanting

Experiments were conducted during June-July, 2009 at Site 'A' and February-March, 2010 at Site 'B'. Three sources of fertilizer were used: (1) Tankage, a commercially produced rendered, dried and ground meat by-product that is largely meat and bone from animals (Island Commodities, Honolulu, HI), (2) a chicken manure-based thermophilic compost; and (3) a soluble fertilizer (16:16:16) were used in both sites to provide 150 kg N ha $^{-1}$. Plant available-N for Tankage and chicken manure-based thermophilic compost was calculated based on total N content with an estimate of 50% mineralization rate during the growth period. All fertilizers were incorporated into the top 15 cm of the raised bed of soil.

Drip irrigation systems were set-up after fertilizer applications. Three subplots that measured 1 m x 2.1 m were randomly assigned using three vermicompost tea treatments. Three week old pak choi (*Brassica rapa*, Chinensis) seedlings were transplanted in three rows with 21 plants plot $^{-1}$. Aerated vermicompost extracts were prepared by extracting commercially produced chicken manure-based vermicompost in water at 1:10 and 1:20 dilution rates (vermicompost to water ratio, v:v; 10% and 5% vermicompost tea, respectively) as previously described (Pant *et al.*, 2009). The control treatment consisted of the same amount of water as the vermicompost tea extracts. These three tea treatments were applied weekly starting a week after transplanting at the rate of 150 mL transplant $^{-1}$ for four weeks; wetting the root zone and foliage of the plants. The

experiments were arranged in randomized complete block design with 3*3 factorial treatments (fertilizer x vermicompost tea treatments) and 4 blocks of each treatment combination.

5.3.4 Analysis of vermicompost tea

The pH and electrical conductivity (EC) of the vermicompost extracts were measured using a conductivity/pH Meter (SB80PC, sympHony, VWR Scientific Products, MN). Dissolved oxygen (DO) was recorded at 21-22ºC with a dissolved oxygen meter (thermo sympHony SP70D, VWR Scientific Products, MN). Mineral nutrients in vermicompost teas were analyzed in the Agricultural Diagnostic Service Center, University of Hawaii at Manoa. Mineral N (NH_4-N, NO_3-N and NO_2-N) of the vermicompost tea were analyzed colorimetrically using a discrete analyzer (Easy Chem Plus, Systea Scientific, IL). Other nutrients of the vermicompost tea were analyzed using an inductively coupled plasma (ICP) spectrophotometer (Jarrel-Ash Division/Fisher Scientific Co., Waltham, MA). Humic acids from vermicompost tea were extracted using the alkali/acid fractionation procedure as described by Valdrighi *et al.* (1996).

5.3.5 Plant harvest and measurement

Three whole plant samples from site ‘A’ and 5 from site ‘B’ were selected randomly from the center row and harvested 5 weeks after transplanting from each treatment. Fresh weight, plant height, and stem basal diameter were measured. Plants were immediately frozen in liquid N and stored at - 20°C, and then freeze-dried using a lyophilizer (D4A, Leybold- Heraeus Vacuum Products, Inc., PA). Above ground dry

weight of each plant was recorded, ground using mortar and pestle, and stored in air-tight containers prior to further analysis.

5.3.6 Measurement of phytonutrients and tissue N

Total carotenoids and total phenolics were analyzed on lyophilized samples of each treatment by extracting 100 mg in 20 mL of ethanol: acetone (1:1, v:v) in glass vials (Gross, 1991). All data were reported based on the dry weight of the lyophilized sample. Extracts were evaluated for total carotenoids at 470 nm using Genesys 20 spectrophotometer (Thermo Scientific - Model 4001-000, MA). Total carotenoids were calculated according to Gross (1991) using the equation: mg L^{-1} total carotenoids = (A*V $\times 10^6$)/(A% $\times$ 100G), where A is the absorbance, V is the total volume of the extract (mL), A% is the extinction coefficient of 2500, and G is the sample weight in grams. Total soluble phenolics were measured using the Prussian Blue assay as described by Stern *et al.* (1996), and the data reported in mg kg^{-1} equivalents of gallic acid. Total glucosinolates were extracted and analyzed from lyophilized samples as described by Radovich *et al.* (2005b) . Total N of dried tissue samples were analyzed by dry combustion in a LECO CN-2000 analyzer (Leco Corp., St. Joseph, MI) in the Agricultural Diagnostic Service Center, University of Hawaii at Manoa.

5.3.7 Soil respiration and dehydrogenase activity (Site A)

Soil respiration rate was measured weekly for 4 weeks with a portable soil respiration rate measuring system (LI-6400, LI-COR, Lincoln, NB, USA) fitted with a soil respiration chamber (6400-09, LI-COR, NB, USA). The respiration rate was expressed as µmol CO_2 fluxes m^{-2} sec^{-1}. Dehydrogenase activity in soil was measured as described by

Alef (1995). Dehydrogenase activity (expressed in µg g^{-1} of oven-dried soil) was calculated based on the amount of triphenyl formazan (TPF) formed when 2, 3, 5-triphenyl tetrazolium chloride (TTC) was reduced by microbes in the soil.

5.3.8 Statistical analysis

Analysis of variance (ANOVA) of plant growth parameters, mineral nutrients, phytonutrients in plant tissue and soil biological properties was performed using PROC GLM in SAS 9.1 statistical software (SAS Institute Inc., 2003). Trend analysis via polynomial regression was conducted for all plant growth parameters between 0-10% vermicompost tea treatments in greenhouse experiments. In field experiments, linear and quadratic effects of vermicompost tea concentration were calculated via polynomial regression for plant growth parameters, mineral nutrients, phytonutrient concentration and soil biological properties. Fertilizer effect was calculated using orthogonal contrasts. Statistical significance was obtained at a 95% confidence level (α =0.05).

5.4 Results

5.4.1 Greenhouse experiments

Application of vermicompost tea significantly increased ($p<0.0001$) plant biomass, height, leaf area, root length, and root surface area compared to those of the control treatment across both trials. There was a non significant interaction between tea types and trials on measured growth parameters. Increasing concentration of vermicompost tea increased above ground dry matter, resulting in a strong (η^2 =0.79) and significant ($p<0.0001$) linear effect (Fig. 1). Quadratic effect was small (η^2= 0.02) yet

significant ($p<0.01$), whereas cubic and quartic effects were not significant. Similar trends were observed in height, leaf area, root dry weight, root length and root surface area (Fig. 2).

5.4.2 Field experiments

5.4.2.1. *Chemical properties of vermicompost teas*

The average pH of the control treatment was significantly greater ($p<0.0001$) than the pH of 10% or 5% tea (Table 1). The pH of 5% tea was also significantly higher ($p<0.01$) than that of 10% tea. The electrical conductivity (EC) levels in both vermicompost tea treatments were significantly greater ($p<0.0001$) than that of the control. Also, the EC of 10% tea was significantly higher than that of 5% tea. Dissolved oxygen (DO) was significantly lower ($p<0.0001$) in both teas compared to the control, but the level of DO was not different between the two concentrations. Humic acid was significantly higher ($p<0.0001$) in 10% tea compared to 5% tea. Humic acid was undetectable in the control. The levels of total N, NO_3-N and NH_4-N were significantly greater ($p<0.0001$) in both the teas compared to control. Total N and NO_3-N was higher ($p<0.05$) in 10% tea compared to 5% tea but NH_4-N was not influenced by the concentration. Phosphorus concentration in the vermicompost tea was significantly greater ($p<0.0001$) in both the tea treatments compared to the control, but it didn't differ between the two tea concentrations. Potassium, Ca and Mg concentrations followed similar trends with total N. Micronutrient concentrations were significantly ($p<0.05$) higher in 10% tea compared to 5% tea and control.

5.4.2.2. *Effect on plant growth and tissue N content (Site A)*

Application of vermicompost teas significantly increased above ground plant dry matter, tissue N content, height, and stem basal diameter of pak choi (Table 2). Increasing concentration of vermicompost tea significantly increased above ground dry weights and heights across all fertilizer types. Significant differences due to fertilizer type were found, in which chicken manure resulted in the lowest above ground dry weights and heights. No significant interactions were found, indicating similar effects of compost teas in 3 fertilizer treatments.

There was a significant interaction effect of vermicompost tea type and fertilizer type on the above ground fresh weight (Fig. 5.3), stem basal diameter and tissue N content (Table 5.2); indicating vermicompost tea effect varies with fertilizer type. Above ground fresh weight and stem basal diameter were not affected by vermicompost tea concentrations under Tankage treatment, resulting in non significant linear ($p<0.84$) and quadratic ($p<0.839$) effects. Increasing concentration of vermicompost tea significantly increased above ground fresh weight and stem basal diameter in the other two fertilizer treatments, resulting in significant linear effect ($p<0.0001$). Although there was a significant linear effect of vermicompost tea concentration on tissue N content averaged across all fertilizer types, the effect was stronger in the chicken manure treatment compared to the other two fertilizer treatments.

5.4.2.3. *Effect on phytonutrients (Site A)*

Increasing concentrations of vermicompost teas significantly increased total carotenoids and total glucosinolates across all fertilizer treatments (Table 5.2). The effect

of vermicompost tea on total phenolics was not significant across all fertilizer treatments and the overall mean was 5778 mg kg^{-1} (dry weight basis) expressed as gallic acid equivalent. Significant differences due to fertilizer type were found for total glucosinolates, in which the soluble fertilizer treatment resulted in the lowest level. Fertilizer had no significant effect on total carotenoids. No significant interactions between vermicompost tea type and fertilizer were found, indicating similar effects of compost teas in the three fertilizer treatments.

5.4.2.4. *Effect on plant growth and tissue N content (Site B)*

Application of vermicompost teas significantly increased above ground plant matter, tissue N content, height, and stem basal diameter of pak choi (Table 5.3). Increasing concentration of vermicompost teas significantly increased above ground dry weights, heights and tissue N content averaged across all fertilizer types. Significant differences due to fertilizer type were found, in which chicken manure resulted in the lowest above ground dry weight, height and tissue N content. No significant interactions between vermicompost tea type and fertilizer were found, indicating similar effects of compost teas in the three fertilizer treatments.

There was a significant interaction effect of vermicompost tea type and fertilizer type on the above ground fresh weight (Fig. 5.4) and stem basal diameter (Table 5.3); indicating vermicompost tea effect varies with fertilizer type. Above ground fresh weight was not affected by vermicompost tea concentrations under soluble fertilizer treatment, resulting in non significant linear ($p<0.24$) and quadratic ($p<0.84$) effects. Increasing concentration of vermicompost tea significantly increased above ground fresh weight in

the other two fertilizer treatments, resulting in significant linear effect ($p<0.0001$). Stem basal diameter was not affected by vermicompost tea concentrations under Tankage treatment ($p<0.1$) but increasing concentration of vermicompost tea significantly ($p<0.0001$) increased stem basal diameter in the other two fertilizer treatments.

5.4.2.5. *Effect on phytonutrients (Site B)*

Application of vermicompost teas significantly increased total carotenoids and total glucosinolates averaged across all fertilizer treatments (Table 5.3). The effects of vermicompost tea and fertilizer types on total phenolics was not significant and the overall mean was 4321 mg kg^{-1} (dry weight basis). Increasing concentration of vermicompost tea significantly increased total glucosinolates across all fertilizer types, resulting in a strong (η^2=0.49) and significant linear effect. Quadratic effect was small (η^2=0.05) yet significant. Significant differences due to fertilizer type were found, in which the soluble fertilizer treatment resulted in the lowest total glucosinolates.

There was a significant interaction between vermicompost tea type and fertilizer type on total carotenoids. Increasing concentration of vermicompost teas significantly increased total carotenoids in chicken manure and soluble fertilizer treatments, resulting in significant linear effects. There was a strong (η^2=0.46) and significant ($p<0.0001$) quadratic effect on total carotenoids under Tankage treatment, while the linear effect was small (η^2=0.35) but significant ($p<0.01$). This suggests that total carotenoids increased at decreasing rate with increasing concentrations of vermicompost tea.

5.4.2.6. *Effect on soil respiration and dehydrogenase activities (Site A)*

The effect of vermicompost tea, and fertilizer types on soil respiration (µmol CO_2 fluxes m^{-2} sec^{-1}) was significant but the time was not significant (Table 5.4). Interaction of vermicompost tea and fertilizer types on soil respiration was not significant. Increasing concentration of vermicompost tea increased soil respiration across all fertilizer types, resulting in a significant linear effect. The effect of vermicompost tea, fertilizer types and time on dehydrogenase activity in soil was significant, but their interaction was not significant. Dehydrogenase activity (µg g^{-1} soil) in soil increased significantly with increasing concentrations of vermicompost tea after third week of application across the fertilizer regimes (Fig. 5.5), resulting in a strong (η^2=0.11) and significant linear effects. Quadratic effect was small (η^2=0.08) but significant. Fertilizer effect was significant on soil respiration and dehydrogenase activities throughout the experiment period. Both soil respiration and dehydrogenase activities were significantly greater with application of chicken manure than that of Tankage and soluble fertilizer.

5.5 Discussion

5.5.1 Greenhouse experiment

All vermicompost teas, irrespective of concentration significantly improved shoot and root growth of greenhouse grown pak choi. Increased overall root development with the application of vermicompost tea may have contributed to better nutrient uptake and increased leaf area. Greater leaf area of vermicompost tea treated plants could have been linked with increased above ground plant dry weight in this study due to greater light interception and photosynthesis (Aase, 1978; Williams, 1987). Increasing vermicompost

tea concentration linearly and positively influenced the plant growth. This effect may, in part be due to higher concentration of mineral nutrients and organic acids supplied by concentrated tea compared to dilute ones. Keeling *et al.* (2003) confirmed the higher growth promoting effects of compost extract on wheat and oilseed rape with increasing concentrations. They observed significant positive effects with 33%, 17% and 8% tea but did not see any response at greater dilutions than this. Hargreaves *et al.* (2008) and Reeve *et al.* (2010) also reported that the growth promoting effect of compost extract was decreased by increasing dilutions of tea. Our results of the greenhouse study are consistent with the findings of previous studies (Keeling *et al.*, 2003; Hargreaves *et al.*, 2009; Reeve *et al.*, 2010).

5.5.2 Field experiment

The higher EC in 10% vermicompost tea relative to 5% tea is likely associated with higher levels of mineral nutrients and humic acid in 10% tea compared to 5% tea. Ten percent vermicompost tea contained approximately 45% more humic acid, 15% more N, 30% more K as well as 20% more Ca and Mg than that of 5% tea. The lower pH of 10% tea compared to 5% tea could have been linked with higher organic acids in concentrated tea compared to the more dilute one.

Application of vermicompost tea significantly enhanced above ground plant dry weight, tissue N content, and plant height of pak choi grown in both the experimental sites across the fertilizer regimes. These results are consistent with findings of previous research (Gamaley *et al.*, 2001; Hargreaves *et al.*, 2009; Pant *et al.*, 2009). Above ground plant fresh weight of vermicompost tea treated plants was significantly greater than that

of control plants under chicken manure fertilization in both the sites, while above ground plant fresh weight was not affected by vermicompost tea application under Tankage treatment in site 'A' and under soluble fertilizer treatment in site 'B'. Overall growth of pak choi plant was high under Tankage and soluble fertilizer treatment compared to chicken manure, however, the effect of vermicompost tea on plant growth was more pronounced under chicken manure fertilization. Both the dry weight and tissue N content were increased by about 80 % with the application of vermicompost tea under chicken manure fertilization, whereas the dry weight and tissue N content of vermicompost tea treated plants were increased by about 20% with the application of vermicompost tea under Tankage and soluble fertilizer treatments. Slower release of available plant nutrients from chicken manure compared to Tankage and soluble fertilizer could be a possible explanation of inferior plant growth under chicken manure fertilization. Consequently, limited plant growth under chicken manure fertilization may have contributed to a greater plant growth response to vermicompost tea application.

Soluble mineral nutrients and microbial byproducts in vermicompost tea can enhance nutrient uptake from the soil and increase foliar uptake of nutrients (Xu *et al.*, 2001; Ingham, 2005a). Arancon *et al.* (2007) reported that humic, fulvic and other organic acids extracted or produced by microorganisms in vermicompost tea could induce plant growth by improving root development and nutrient uptake. Nutrient analysis of vermicompost tea indicates that vermicompost tea supplied a considerable amount of soluble mineral nutrients and humic acid to the plant compared to the control.

Increased total carotenoids level in plant tissue in response to vermicompost tea treatments was associated with improved plant growth. This agrees with the findings of

previous studies (Hussein *et al.*, 2006; Kopsell *et al.*, 2007; Pant *et al.*, 2009) that reported higher carotenoids in plant tissue corresponded with increased plant growth at higher fertilizer rates. Increased plant growth with the application of vermicompost tea may have contributed to greater synthesis of carotenoids across the fertilizer regimes in both the trials.

Various studies have shown the positive relationship between N availability and total glucosinolates concentration of Brassica crops. Krumbein *et al.* (2002) reported that the levels of total glucosinolates were low with low N fertilizer in broccoli plants, whereas, total glucosinolates levels were high at sufficient N supply. In another study, the levels of several glucosinolates decreased in leaves under nitrogen deficiency but accumulated in roots of *Arabidopsis thaliana* (Hirai *et al.*, 2004). However, Chen *et al.* (2006) observed lower levels of total glucosinolates in pak choi at high levels of foliar nitrogen application. Applications of vermicompost tea contributed to increased N availability to plants, perhaps explaining the positive relationship between total glucosinolates and plant growth.

The absence of a vermicompost tea effect on total phenolics content of pak choi plants across the fertilizer regimes is likely due to a general absence of nutrient stress. It has been previously demonstrated that N stress is associated with increased level of phenolics in plant tissue (Brown *et al.*, 1984; Estiarte *et al.*, 1994).

Soil respiration and dehydrogenase activity increased over time in the rhizosphere of vermicompost tea treated plants. The increase in soil respiration may be explained by improved microbial decomposition of soil organic matter. This effect might be due to the

greater availability of active organic carbon or enrichment of nutrients for the microbes through the addition of high organic carbon content of compost (Sikora and Yakovchenko, 1996; Bernal *et al.,* 1998). Increased soil respiration and dehydrogenase activity in this study as a result of vermicompost tea applications implies a more efficient organic matter decomposition and mineralization in rhizosphere, which may have in turn contributed to better plant growth.

Increasing concentration of vermicompost tea increased plant production, N uptake by plants and phytonutrient contents along with improving soil biological properties. These results agree with the findings of Akanbi *et al.* (2007) who reported that applications of 8% compost tea significantly increased plant growth of *Telfairia occidentalis* (fluted pumpkin) compared to that of 0% and 6% teas. In contrast, Edwards *et al.* (2006) reported a similar plant growth response of tomato seedlings with the applications of 4%, 8% or 10% vermicompost teas.

5.6 Conclusion

Applications of vermicompost tea increased plant growth, N content, total carotenoids and total glucosinolates in plants grown under greenhouse or field condition. This positive plant response was greater in field grown plants fertilized with chicken manure compared to those of plants fertilized with Tankage and soluble fertilizer. The positive influence of vermicompost tea on plant growth was associated with the increased N uptake. Vermicompost tea also improved microbial respiration and dehydrogenase activities in soil. Plant growth, phytonutrient content and microbial activities in soil increased with increasing concentrations of vermicompost tea. Data from these studies

indicate that vermicompost tea may be used to improve plant nutrient status and enhance soil biological properties in vegetable production.

Table 5.1 Chemical properties of vermicompost tea (n = 3).

a) pH, EC, dissolved oxygen concentration and humic acid

Tea	pH	EC	DO (mg L^{-1})	Humic acid (mg L^{-1})
10% tea	7.7(0.0) †	1.4(0.0)	7.9(0.0)	435.3(10.6)
5% tea	7.9(0.1)	1.1(0.0)	7.8(0.0)	293.6(4.5)
Control	8.6(0.0)	0.5(0.0)	8.1(0.0)	nd

b) Macronutrient concentration

Tea	N	NO_3-N	NH_4-N	P	K	Ca	Mg
				mg L^{-1}			
10% tea	131.0(7.2)	130.4(7.2)	0.1(0.0)	17.3(1.2)	40.1(1.2)	73.9(1.3)	84.7(0.8)
5% tea	113.0(2.1)	111.2(2.9)	0.1(0.0)	15.8(0.3)	31.0(0.7)	60.0(0.5)	68.1(0.6)
Control	0.8(0.0)	0.6(0.0)	0.0(0.0)	0.1(0.0)	3.0(0.0)	10.7(0.0)	14.5(0.1)

c) Micronutrient concentration

Tea	Na	Fe	Mn	Zn	Cu	B
			µg L^{-1}			
10% tea	72.5(1.1)	0.08 (0.0)	0.02(0.0)	0.03(0.0)	0.03(0.0)	0.2(0.0)
5% tea	69.4(0.7)	0.03(0.0)	0.01(0.0)	0.02(0.0)	0.02(0.0)	0.2(0.0)
Control	68.7(0.1)	0.01(0.0)	0.00(0.0)	0.01(0.0)	0.00(0.0)	0.0(0.0)

† Mean with standard error in parenthesis, nd = non-detected,
EC= Electrical Conductivity, DO= Dissolved Oxygen.

Table 5.2. Effect of concentration of vermicompost tea on plant growth, tissue N and phytonutrients in Site A (n =12).

Fertilizer	Tea	Above ground dry weight (g plant $^{-1}$)	Tissue N (g plant $^{-1}$)	Height (cm)	Base diameter (cm)	Total carotenoids (mg kg $^{-1}$ dw)	Total glucosinolates (mmol kg $^{-1}$ dw)
Tankage (Tkg)	10% tea	15.8(0.6)†	0.77(0.04)	25.6(0.7)	7.6(0.3)	1173(12)	29.8(1.1)
	5% tea	15.5(0.4)	0.73(0.02)	25.6(0.7)	7.5(0.2)	1223(33)	27.0(0.3)
	Control	12.3(0.4)	0.61(0.03)	22.9(0.9)	7.5(0.2)	861(10)	24.1(0.5)
Chicken manure	10% tea	13.7(1.1)	0.49(0.05)	23.8(0.9)	7.9(0.2)	1286(79)	24.5(0.6)
(Chix)	5% tea	11.4(0.6)	0.42(0.02)	22.3(0.7)	7.6(0.3)	1152(30)	24.1(0.6)
	Control	7.1(0.5)	0.24(0.02)	19.0(0.7)	6.5(0.2)	781(20)	19. 5(0.6)
Soluble fertilizer	10% tea	16.3(0.4)	0.74(0.04)	25.0(0.4)	7.9(0.1)	1517(196)	26.6(0.8)
(Trip16)	5% tea	14.9(0.7)	0.62(0.03)	24.9(0.7)	7.7(0.1)	1214(30)	22.1(0.9)
	Control	12.0(0.5)	0.37(0.02)	21.5(0.9)	7.1(0.2)	745(15)	17.2(1.0)
Fertilizer (F)		****‡	****	****	NS	NS	****
Chix vs others		****	****	**	**	NS	NS
Tkg vs Trip16		NS	***	NS	NS	NS	****
Compost tea (T)		****	****	****	****	****	****
Linear		****	****	****	****	****	****
Quadratic		*	*	*	NS	*	NS
F*T		NS	*	NS	*	NS	NS

† Mean with standard error in parenthesis

‡ NS, *, **, ***, **** = Not significant or significant at $P < 0.05$, 0.01, 0.001 or 0.0001, respectively.

Table 5.3. Effect of concentration of vermicompost tea on plant growth, tissue N and phytonutrients in Site B (n = 12).

Fertilizer	Tea	Above ground dry weight (g plant $^{-1}$)	Tissue N (g plant $^{-1}$)	Height (cm)	Base diameter (cm)	Total carotenoids (mg kg $^{-1}$ dw)	Total glucosinolates (mmol kg $^{-1}$ dw)
Tankage	10% tea	26.6(1.2)†	1.27(0.06)	32.1(0.4)	11.1(0.2)	2444(42)	16.8(0.6)
(Tkg)	5% tea	24.4(0.9)	1.17(0.04)	31.6(0.5)	10.9(0.2)	2662(93)	16.6(0.6)
	Control	20.4(1.1)	1.03(0.05)	30.2(0.5)	10.6(0.1)	2004(87)	13.1(0.8)
Chicken manure	10% tea	24.3(1.8)	1.17(0.10)	31.5(0.6)	11.1(0.3)	2723(111)	16.0(0.8)
(Chix)	5% tea	23.3(1.1)	1.03(0.05)	30.3(0.5)	10.4(0.3)	2864(157)	14.6(0.8)
	Control	15.7(1.2)	0.71(0.06)	28.4(0.7)	8.7(0.3)	1923(146)	11.1(0.8)
Soluble fertilizer	10% tea	25.1(0.3)	1.23(0.02)	31.6(0.5)	11.1(0.3)	2794(98)	14.5(0.6)
(Trip16)	5% tea	24.0(0.4)	1.25(0.03)	31.6(0.5)	10.8(0.3)	2511(52)	13.1(0.6)
	Control	20.7(0.6)	0.99(0.03)	29.2(0.6)	9.9(0.2)	2172(29)	9.6(0.5)
Fertilizer (F)		**‡	****	*	***	NS	****
Chix vs others		**	****	*	****	NS	NS
Tkg vs Trip16		NS	NS	NS	*	NS	**
Compost tea (T)		****	****	****	****	****	****
Linear		****	****	****	****	****	****
Quadratic		*	*	NS	NS	****	*
F*T		NS	NS	NS	**	*	NS

† Mean with standard error in parenthesis

‡NS, *, **** = Not significant or significant at $P < 0.05$, 0.0001, respectively.

Table 5.4. Effect of vermicompost tea on soil biological properties (n = 4).

Fertilizer	Tea	Soil respiration (CO_2 fluxes µmol m^{-2} s^{-1})	Dehydrogenase activity (TPF µg g^{-1} soil)
Tankage	10% tea	6.9(1.3) †	66.4(3.7)
(Tkg)	5% tea	7.0(0.8)	77.1(5.3)
	Control	5.5(0.5)	57.2(4.9)
Chicken manure	10% tea	8.5(0.9)	84.3(6.9)
(Chix)	5% tea	9.6(0.9)	85.13(10.5)
	Control	6.0(1.2)	69.3(6.7)
Soluble fertilizer	10% tea	6.0(0.7)	64.4(3.8)
(Trip16)	5% tea	5.4(0.9)	61.9(1.7)
	Control	4.3(0.8)	51.8(5.3)
Fertilizer (F)		***‡	***
Chix vs others		****	****
Tkg vs Trip16		*	*
Compost tea (T)		**	*
Linear		*	*
Quadratic		NS	*
Time (I)		NS	****
F*T		NS	NS
I*F		NS	NS
I*T		NS	NS
I*F*T		NS	NS

† Mean with standard error in parenthesis

‡NS, *, **, ***, **** = Not significant or significant at $P < 0.05$, 0.01, 0.001 or 0.0001, respectively.

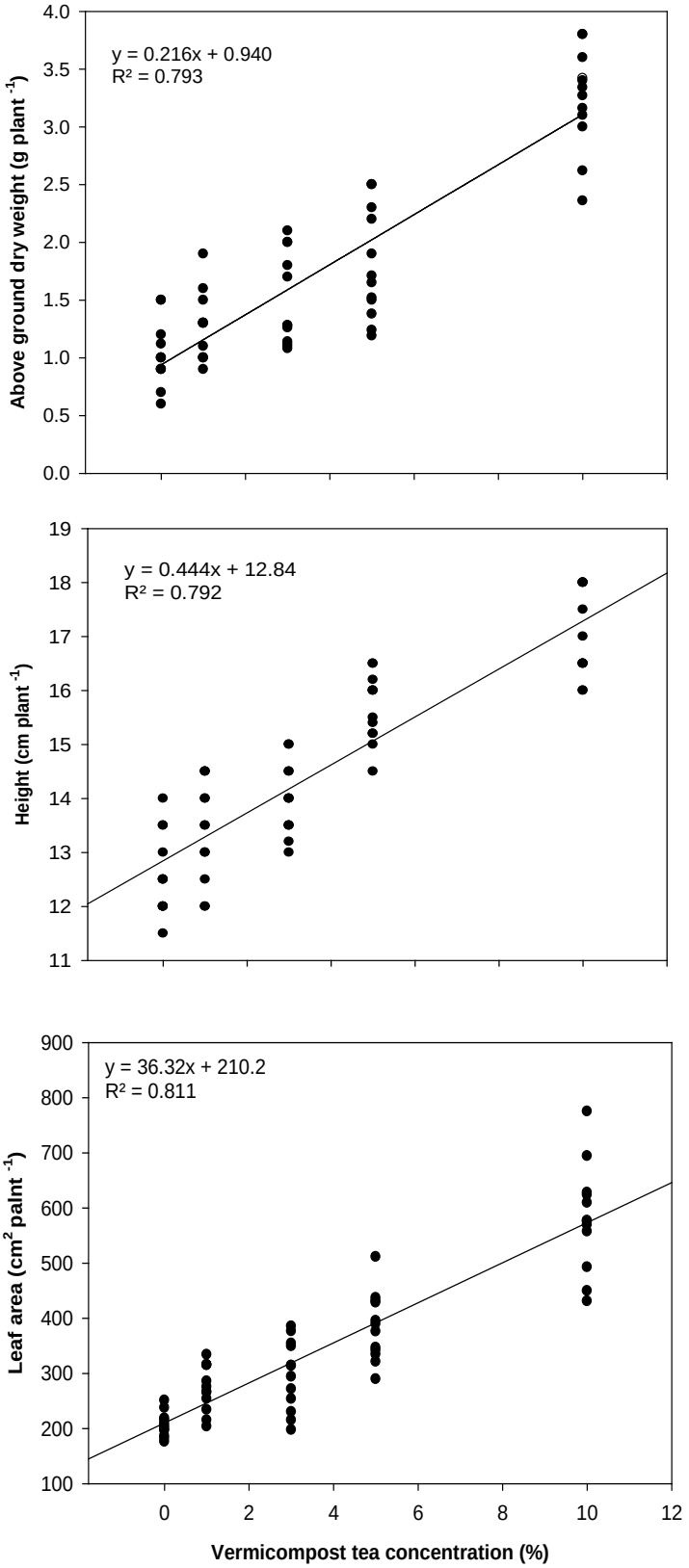


Figure 5.1 Effect of vermicompost tea on above ground plant growth of pak choi in greenhouse trials (n = 60).

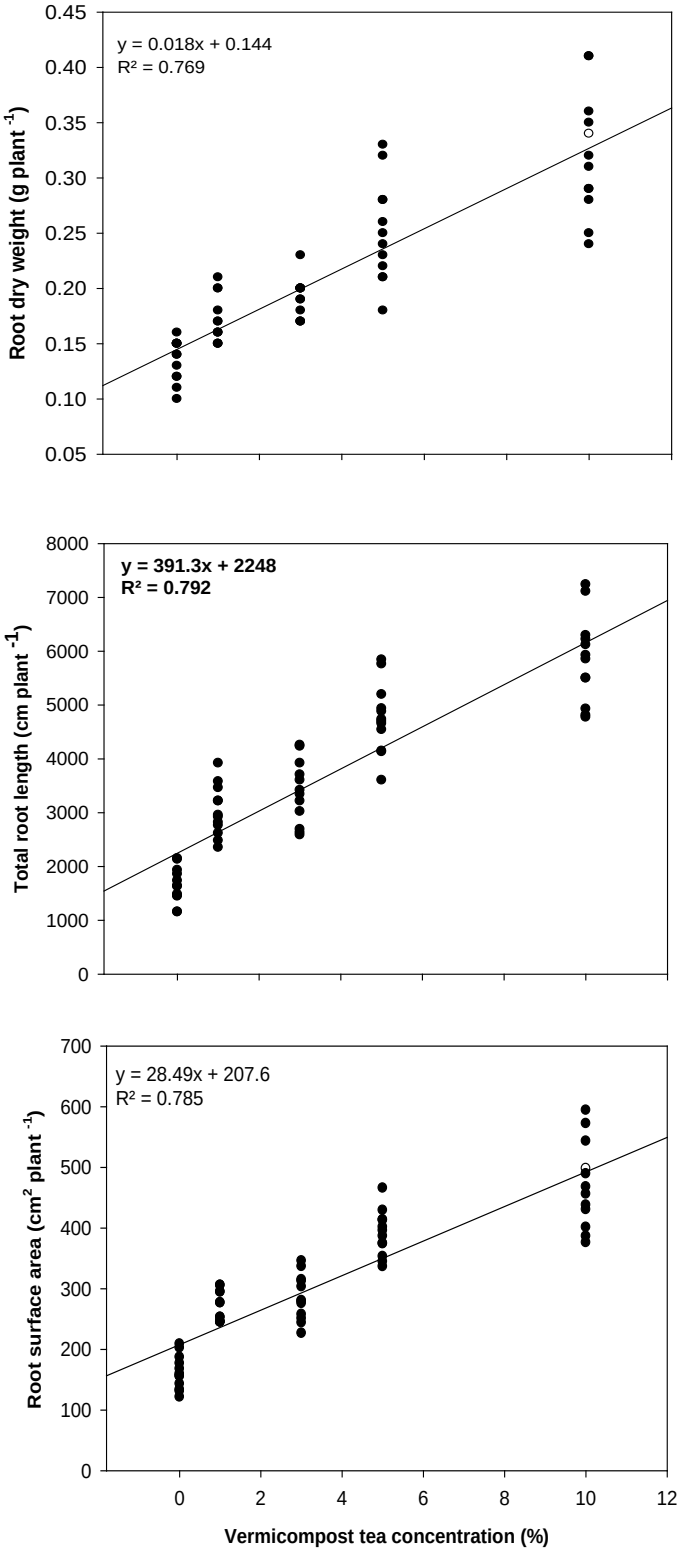


Figure 5.2. Effect of vermicompost tea on root growth of pak choi in greenhouse trials (n = 60).

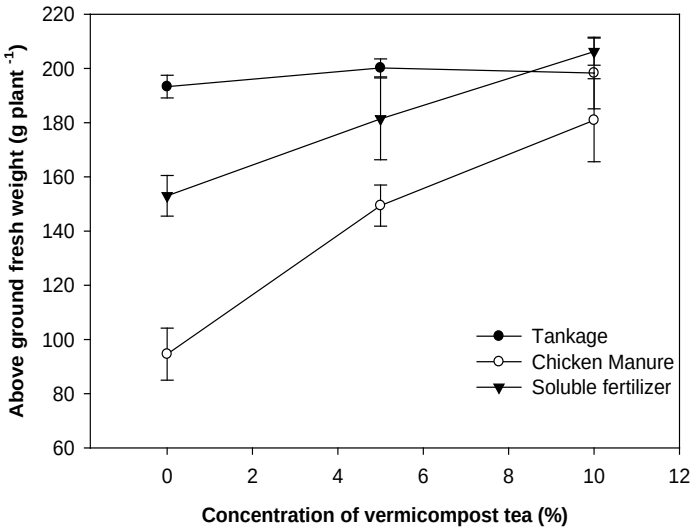


Figure 5.3. Effect of concentration of vermicompost tea on above ground fresh weight of pak choi under Tankage, chicken manure and soluble fertilizer treatments in site A. Plotted points are means of 12 samples, and error bars represent standard errors of the mean.

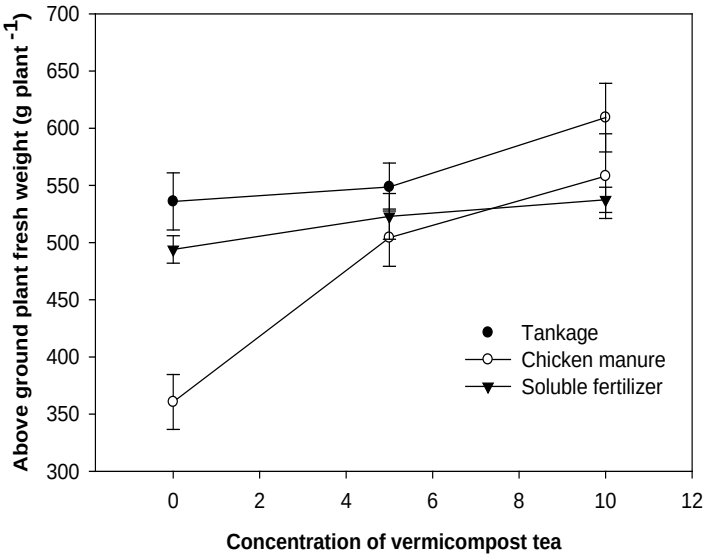


Figure 5.4. Effect of concentration of vermicompost tea on above ground fresh weight of pak choi under Tankage, chicken manure and soluble fertilizer treatments in site B. Plotted points are means of 20 samples, and error bars represent standard errors of the mean.

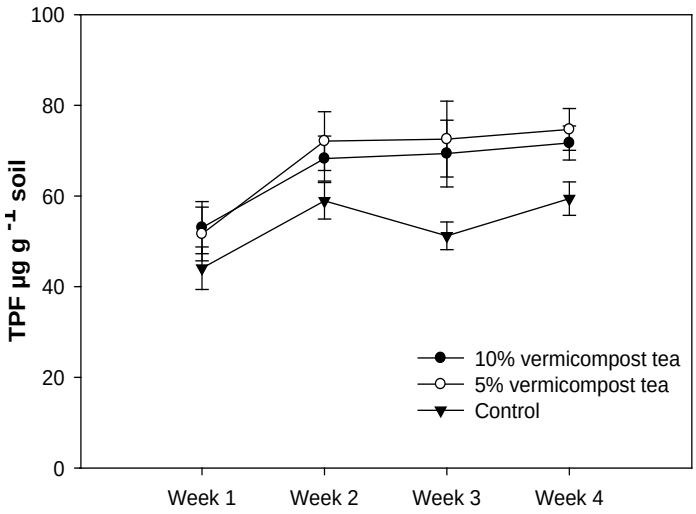


Figure 5.5. Effect of vermicompost tea on dehydrogenase activity in soil (TPF µg g^{-1} of soil) across the fertilizer regimes over time. Plotted points are means of 12 samples, and error bars represent standard errors of the mean. TPF = 1,3,5-Triphenyltetrazolium formazan.

CHAPTER 6

INFLUENCE OF COMPOST QUALITY ON BIOCHEMICAL PROPERTIES OF COMPOST TEA AND SUBSEQUENT EFFECTS ON PAK CHOI (*BRASSICA RAPA* CV. BONSAI, CHINENSIS GROUP) YIELD

6.1 Abstract

Experiments were conducted to investigate: a) the effect of compost on compost tea (aqueous extract); and b) to examine the relationships between biochemical properties of compost tea and growth of pak choi (*Brassica rapa* cv Bonsai, Chinensis group). Five composts and their extracts were evaluated for chemical and biological properties. The composts were: (1) chicken manure-based thermophilic compost (2) green waste thermophilic compost (3) food waste vermicompost; (4) aged chicken manure-based vermicompost; and (5) fresh chicken manure-based vermicompost. Aerated teas produced using compost to water ratio of 1:10 (v:v) were applied to pak choi grown in peat-perlite medium. Not surprisingly, biochemical properties of compost determined biochemical properties of compost tea. Variability in tea quality impacted plant growth and mineral nutrient content in plant. Applications of compost tea increased growth and mineral nutrient content of pak choi. The responses were greater with aged chicken manure-based vermicompost tea, chicken manure-based thermophilic compost tea, and food waste vermicompost tea. The positive influence on plant growth was largely associated with mineral N and gibberellin (GA_4) present in the teas. *In vitro* cultivation of pak choi with GA_4 treatments also demonstrated the positive effect of GA_4 on growth. These findings

suggest that either vermicompost tea or thermophilic compost tea can be used for improving plant growth and nutrient status, and plant response to a compost tea may be predicted based on compost quality.

6.2 Introduction

Compost tea, a water-based compost extract, can be prepared using a wide range of composts. Characteristics influencing the biological, physical, and chemical properties of compost will in turn impact those properties of the compost tea (Scheuerell and Mahaffee, 2002; Ingham, 2005a). A fine-textured, moist and pathogen free compost containing high beneficial microorganisms, soluble mineral nutrients, humic substances, phytohormones and low phyto-toxic organic acids and heavy metals can be considered as good quality compost. Compost feedstock, processing method and compost maturity generally play major roles in the above mentioned attributes of good quality compost (Inbar *et al.*, 1990; Haug and Ellsworth, 1991; Scheuerell and Mahaffee, 2002).

Research on the compost tea prepared from different sources of compost demonstrates the importance of feedstock and compost quality. Weltzien (1990; 1991) reported that compost tea prepared from animal manure thermophilic composts had better disease suppression effect than that prepared from plant-based compost. Scheuerell and Mahaffee (2000) observed that composted chicken manure produced the most suppressive teas for controlling powdery mildew of rose plants compared to the compost produced from vegetative sources. Elad and Shtienberg (1994) in contrast determined that tea produced from plant-based compost was equally effective as tea produced from manure based compost in suppressing the plant disease caused by *Botrytis cinerea*. These

findings demonstrate that the efficacy of compost tea produced from various compost sources varies with compost quality, crop type and disease categories.

The differences between quality of vermicompost and thermophilic composts have been reported (Tognetti *et al.*, 2005). Vermicompost is the product of accelerated bio-degradation of organic matter by earthworms without a thermophilic stage (Dominguez *et al.*, 1997). It generally holds larger nutrient concentrations such as NO_3^-, exchangeable Ca, P and soluble K that are taken up readily by plants. Vermicompost has outstanding biological properties with significantly larger and more diverse microbial populations than those of conventional thermophilic composts (Tognetti *et al.*, 2005). Vermicompost tea and compost tea are similar in terms of production method and their mode of actions to plants, however it is believed that superior biochemical and physical properties of vermicompost over thermophilic compost would also be reflected in tea quality as well as disease suppression and plant growth response (Edwards *et al.*, 2006; Zaller, 2006). Edwards *et al.* (2006) and Gamaley *et al.* (2001) reported that the tea produced from manure-based or food waste-based vermicompost suppressed plant disease and increased the growth and yield of various agricultural plants. However, no comparison of the effect of the vermicompost tea over thermophilic compost tea on plant growth has been reported.

Compost maturity is another important characteristic contributing to compost tea quality (Inbar *et al.*, 1990). Mature composts generally release a greater percentage of soluble mineral nutrients and less phyto-toxic organic acids and heavy metals, in comparison to immature materials (Griffin and Hutchinson, 2007). Since water soluble biochemical compounds contained in compost are assumed to be extracted in compost

tea, compost age may contribute to the quality of compost tea. However, the effect of compost age on compost tea quality and subsequent effect on plant growth has not been well studied.

Various experiments have indicated that applications of compost and vermicompost teas improve plant health, yield and nutritional quality (Elad and Shtienberg, 1994; Al-Dahmani *et al.*, 2003; Scheuerell and Mahaffee, 2004; Welke, 2005; Edwards *et al.*, 2006; Pant *et al.*, 2009). It is believed that application of compost tea improves plant health, yield and nutritional quality by supplying microbial biomass, fine particulate organic matter, organic acids, plant growth regulator like substances and soluble mineral nutrients to plant surfaces and soils in a way not possible or feasible with solid compost (Scheuerell and Mahaffee, 2002; Scheuerell and Mahaffee, 2004; Edwards *et al.*, 2006). Assessment of the possible contributions of these factors to crop yield and quality would improve our current understanding of the mechanisms involved in compost tea effect on plant production.

The objectives of this study are (i) to evaluate the effect of compost on compost tea and (ii) to evaluate the relationships between biochemical properties of compost tea and growth of a model vegetable crop.

6.3 Materials and Methods

6.3.1 Experimental set-up and design

Three greenhouse experiments were conducted between October 2009 and December 2010. Pak choi plants were grown in a peat-perlite medium fertilized with

chicken manure-based thermophilic compost to provide 150 mg N L^{-1} growth media (300 kg N ha^{-1}). Plants were grown in garden pots (volume: 865 cm^3). Three to four pak choi seeds were sown in each pot. Four days after emergence, the seedlings were thinned to one pot^{-1}. Aerated teas were prepared at 1:10 (v:v) ratio of compost to water using five different composts: (1) chicken manure-based vermicompost aged (cured for 3 months); (2) chicken manure-based vermicompost fresh (not cured); (3) food waste vermicompost (cured for 3 months); (4) green waste thermophilic compost; and (5) chicken manure-based thermophilic compost as previously described (Pant et. al, 2009). Six compost tea treatments consisted of 5 types of compost tea based on 5 compost sources as well as the same amount of aerated water (control). Treatments were applied weekly at the rate of 150 mL pot^{-1} to the root zone and foliage of plants for four weeks. The greenhouse experiments were arranged in completely randomized design with 6 (trials 1 and 2) and 10 (trial 3) replications per treatment.

6.3.2 Analysis of chemical properties of compost and compost tea

The pH and electrical conductivity (EC) of the compost were measured from a 1:1 (v:v) mixture of deionized water: compost, using a conductivity/pH meter (SB80PC, sympHony, VWR Scientific Products, MN). The pH and EC of the compost teas were measured using the same conductivity/pH Meter. Dissolved oxygen (DO) was recorded at 21-22ºC with a dissolved oxygen meter (thermo sympHony SP70D, VWR Scientific Products, MN). Mineral nutrients in compost and compost teas were analyzed in the Agricultural Diagnostic Service Center, University of Hawaii at Manoa. Total C and N of compost samples were analyzed by dry combustion (LECO CN-2000 analyzer, Leco Corp., St. Joseph, MI). Ammonium-N and NO_3-N were extracted from fresh compost

using 2 M KCl and measured colorimetrically using a discrete analyzer (Easy Chem Plus, Systea Scientific, IL). Other nutrients of the compost samples were measured after wet acid digestion using an inductively coupled plasma (ICP) spectrophotometer (Jarrel-Ash Division/Fisher Scientific Co., Waltham, MA). Mineral N (NH_4-N and NO_3-N) of the vermicompost tea were analyzed colorimetrically using a discrete analyzer (Easy Chem Plus, Systea Scientific, IL). Other nutrients of the compost extracts were analyzed using the above mentioned ICP procedure. Humic acids from compost and compost tea were extracted using the alkali/acid fractionation procedure (Valdrighi *et al.*, 1996).

6.3.3 Analysis of microbial activity in compost and compost tea

Microbial activity of compost and compost tea was analyzed on 3 samples from each compost and compost tea. Tea samples were taken after 12 h of a brewing cycle. A 10-fold serial dilution of each sample was prepared. Active bacteria and active fungi were assessed using a 1:10 dilution under Epifluorescence Microscopy at 40x and 20x objective, respectively (Vieira *et al.*, 2008).

Microbial respiration rate in compost samples was measured with a portable soil respiration rate measuring system (LI-6400, LI-COR, Lincoln, NB, USA) fitted with a respiration chamber (6400-09, LI-COR, NB, USA). The respiration rate was expressed as µmol CO_2 flux m^{-2} sec^{-1}. Dehydrogenase activity in compost and compost tea samples were measured as described by Alef (1995). Dehydrogenase activity expressed in µg g^{-1} dwt of compost or µg mL^{-1} compost tea was calculated based on the amount of 1,3,5-triphenyltetrazolium formazan (TPF) formed when 2,3,5-triphenyltetrazolium chloride (TTC) was reduced by microbes in the soil.

6.3.4 Phytohormones analysis in compost tea

A number of phytohormones (e.g. abscisic acid (ABA) and ABA metabolites, cytokinins, auxins and gibberellins) of lyophilized compost tea samples were analyzed at the Plant Biotechnology Institute of the National Research Council of Canada (PBI-NRC Saskatoon, SK, Canada). Deuterated forms of the hormones that were used as internal standards were synthesized and prepared as described by Abrams *et al.* (2003) and Zaharia *et al.* (2005). Multiple phytohormones and metabolites, including auxins [Indole-3-acetic acid (IAA), N-Indole-3-yl-acetyl-aspartic acid (IAA-Asp) and N-Indole-3-yl-acetyl-glutamic acid (IAAGlu)], abscisic acid and metabolites [Abscisic acid (ABA), PA (Phaseic acid), Dihydrophaseic acid (DPA), 7-Hydroxy-abscisic acid (7'-OH-ABA), neoPhaseic acid (neoPA) and Abscisic acid glucose ester (ABA-GE)], cytokinins [Isopentyladenine (2iP), Isopentyladenosine (iPA), Zeatin (Z), Zeatin riboside (ZR), Dihydrozeatin (dhZ), Dihydrozeatin riboside (dhZR) and Zeatin-O-glucoside(Z-O-Glu)], and gibberellins (GAs 1, 3, 4, 7, 8, 9, 19, 20, 24, 29, 34, 44, and 53) were quantified by High performance liquid chromatography electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) as described by Chiwocha *et al.* (2003; 2005). Calibration curves were generated from the Multiple Reaction Monitoring (MRM) signals obtained from standard solutions based on the ratio of the chromatographic peak area for each analyte to that of the corresponding internal standard, as described by Ross *et al.* (2004). Quantifiable results are expressed in nanograms L^{-1} of compost tea samples. If the signals were below the limit of quantification (defined as signal/noise ratio of greater than or equal to 8), results are reported as present but non-quantifiable (nq). If the

values were below limit of quantification (with a signal/noise ratio less than 3), results are reported as non-detectable (--).

6.3.5 Plant harvest and measurement

Plants were harvested five weeks after emergence. Above ground fresh weight, dry weight, leaf area and height of each plant were measured. Total root length and root surface area of pak choi plants were calculated using WinRHIZO Pro V. 2003b system (Regent Instruments Inc., QC, and Canada). The system consists of a scanner and WinRHIZO software. After taking the root fresh weight, roots were oven dried at 70°C for 72 h and dry weight of the roots of each plant was recorded.

6.3.6 Measurement of mineral nutrients in the plant tissue

Mineral nutrients in plant tissue were analyzed in the Agricultural Diagnostic Service Center, University of Hawaii at Manoa. Total C and N of dried tissue samples were analyzed by dry combustion as described earlier. Other nutrients in the tissue samples were measured after wet acid digestion using an inductively coupled plasma spectrophotometer (Jarrel-Ash Division/Fisher Scientific Co., Waltham, MA).

6.3.7 In vitro cultivation of pak choi with GA_4 treatments

Since GA_4 was the only bioactive GA detected in various compost tea analyzed in this study, *in vitro* cultivation of pak choi with GA_4 treatments was conducted to test the effect of GA_4 on pak choi growth. Pak choi seeds were sterilized with 5% Clorox for 10 minutes and sown aseptically in plastic cultural vessel (vol. 0.95 L) filled with 150 mL gellan gum-based culture media. Culture media was prepared by mixing gellan gum

powder (G434, Phyto Technology Laboratories, LLC, Shawnee Mission, KS), Murashige and Skoog (MS) Basal Salt Mixture (M524, Phyto Technology Laboratories, LLC, Shawnee Mission, KS), sugar and deionized water. Different concentrations (0, 100, 200, 400, 800, 1600 and 3200 ng L^{-1}) of GA_4 (G7274, Sigma-Aldrich, St. Louis, MO) were added to the media. Each treatment was replicated 4 times. The cultural vessels were incubated at room temperature under continuous light at intensity (photon flux densities) of 85 µmol m^{-2} s^{-1} photosynthetically active radiation, as measured with a quantum sensor (Li-Cor, Lincoln, NE). Shoot and root growth of plants was measured 3 weeks after seed sowing.

6.3.8 Statistical analysis

Analysis of variance (ANOVA) of chemical and biological properties of compost and compost tea, plant growth parameters and mineral nutrients in plant tissue were performed using PROC GLM in SAS 9.1 statistical software (SAS Institute Inc., 2003). Means were separated using Tukey's HSD. Trend analysis via polynomial regression was conducted for the measured plant growth parameters between 0 - 3200 ng L^{-1} GA_4 treatments. Multiple regressions were conducted to test the effect of active bacteria, active fungi, soluble N, humic acid and GA_4 present in compost tea on plant growth and N uptake. Stepwise multiple regressions selected the best fitting model among multiple combinations of independent variables that were expected to affect plant growth. Statistical significance was obtained at 95% confidence level ($\alpha = 0.05$).

6.4 Results

6.4.1 Chemical properties of compost and compost teas

The average EC, pH and humic acids in compost and compost tea were significantly ($p<0.0001$) affected by the type of compost (Tables 6.1 and 6.2). Chicken manure-based thermophilic compost had significantly greater EC compared to the EC of the other composts; and a similar trend was observed in the EC of compost teas. Green waste thermophilic compost had the highest pH level compared to the other composts. The pH of compost tea produced from green waste thermophilic compost was also significantly greater than that of the other compost teas. Humic acid was significantly higher ($p<0.0001$) in green waste thermophilic compost compared to the other composts. There was a significant ($p<0.0001$) positive correlation ($r = 0.91$) between humic acid present in compost and in the tea (Fig. 6.1). Moisture content in compost was significantly ($p<0.0001$) affected by the type of compost (Table 6.1). Moisture content was significantly higher ($p<0.0001$) in food waste vermicompost compared to the other composts. Dissolved oxygen level in compost tea was not affected by compost source and the overall mean was 8.03 mg L^{-1}.

Mineral nutrients present in compost and compost tea were significantly ($p<0.0001$) affected by the type of compost (Tables 6.3 and 6.4). Food waste vermicompost had the highest level of total N and NH_4-N; whereas, chicken manure-based thermophilic compost had the highest level of NO_3-N. There was a significant ($p<0.0001$) positive correlation ($r = 0.82$) between mineral N present in compost and N concentration of compost tea (Fig. 6.2). The highest level of N (total N, NO_3-N or NH_4-N) was found in

chicken manure-based thermophilic compost tea and lowest level of N in green waste thermophilic compost tea. Either aged or fresh, both the chicken manure-based vermicomposts had a greater concentration of P and Ca compared to the other composts. Concentrations of K and Mg were significantly higher in chicken manure-based thermophilic compost compared to all other composts. Total P, K, Ca and Mg content of compost tea were also positively correlated with the concentration of those nutrients in the corresponding compost.

6.4.2 Microbial properties of compost and compost tea

Active microbial population in compost was significantly ($p<0.001$) affected by the type of compost (Table 6.5). Active bacteria, active fungi, dehydrogenase activity and microbial respiration were significantly greater in food waste vermicompost compared to the other composts. Both thermophilic composts (either chicken manure or green waste) had significantly lower levels of active microbial population (bacteria or fungi), dehydrogenase activity and microbial respiration compared to that of the vermicomposts.

Active bacterial population in compost tea was not affected by the type of compost and the overall mean was 6.8 ($\log_{10}$ cells mL^{-1}). However; active fungal population and dehydrogenase activity in compost tea were significantly ($p<0.001$) affected by the type of compost, resulting in a greater population of active fungi in fresh chicken manure-based and food waste vermicompost tea and the lowest population in green waste thermophilic compost tea (Table 6.6). Dehydrogenase activity was significantly higher in food waste vermicompost tea compared to all other composts. Extract of thermophilic compost (either

chicken manure or green waste) had significantly lower levels of dehydrogenase activity compared to those of all the vermicompost teas evaluated in this study.

There was a significant ($p<0.0001$) positive correlation ($r = 0.98$) between dehydrogenase activity in compost and compost tea (Fig. 6.1). However, other microbial properties of compost and compost tea were not significantly correlated to each other.

6.4.3 Phytohormones in compost tea

Most of the phytohormones analyzed such as auxins, cytokinins and abscisic acid were not quantifiable or not detected in majority of the compost teas evaluated in this study. However, a small amount of abscisic acid and cytokinin- isopentenyladenosine (iPA) was detected in the extract of green waste thermophilic compost. Some of the gibberellins such as Gibberellin$_4$ (GA_4) and Gibberellin$_{34}$ (GA_{34}) were present in chicken manure-based thermophilic compost, food waste vermicomposts and aged chicken manure-based vermicompost. Gibbrellin$_{24}$ (GA_{24}) was present only in aged chicken manure-based vermicompost. Extract of food waste vermicompost had a significantly ($p<0.05$) greater levels of GA_4 and GA_{34} compared to those of chicken manure-based thermophilic compost tea and aged chicken manure-based vermicompost tea (Table 6.7).

6.4.4 Effect of compost tea on growth of pak choi

Applications of compost tea significantly ($p<0.0001$) increased shoot and root growth of pak choi compared to the control (Table 6.8). Above ground plant fresh weight was significantly ($p<0.001$) greater with the application of aged chicken manure-based vermicompost tea and chicken manure-based thermophilic compost tea compared to the

other teas. Above ground plant dry weight was significantly greater with the application of aged chicken manure-based vermicompost tea compared to all the other compost teas; whereas, leaf area was significantly greater with the application of chicken manure-based thermophilic compost tea. Effect of food waste vermicompost tea on above ground plant growth was significantly greater than that of fresh chicken manure-based vermicompost tea and green waste thermophilic compost tea. Above ground plant fresh or dry weights and leaf area were not affected by the application of green waste thermophilic compost tea relative to controls. Root fresh or dry weights, total root length and root surface area was significantly greater with the application of aged chicken manure-based vermicompost tea compared to the other teas. Effect of food waste vermicompost tea on root growth of pak choi was significantly greater than that of fresh chicken manure-based vermicompost tea, chicken manure-based thermophilic and green waste thermophilic compost tea.

6.4.5 Effect of compost tea on tissue nutrient content of pak choi

Applications of compost tea significantly ($p < 0.0001$) increased total contents of N, P, K, Ca and Mg in pak choi (Table 6.9). Total N content plant $^{-1}$ was significantly greater with the application of aged chicken manure-based vermicompost tea compared to all the other compost teas tested in this study. Effect of chicken manure-based thermophilic compost tea on total N content plant $^{-1}$ was significantly greater than that of food waste vermicompost tea, fresh chicken manure-based vermicompost tea and green waste thermophilic compost tea. Total N content plant $^{-1}$ was not affected by the application of green waste thermophilic compost tea. Effect of each compost tea on total P, K, Ca and Mg content plant $^{-1}$ was similar to that of total N.

6.4.6 Effect of GA_4 on growth of in vitro cultured pak choi

Application of GA_4 into the growth media significantly influenced growth of *in vitro* cultured pak choi compared to the control. Increasing levels of GA_4 increased plant height, resulting in a strong (η^2=0.3) and significant (p<0.0001) linear effect whereas quadratic, cubic and quartic effects were not significant (Fig.6.2). Increasing level of GA_4 increased plant dry matter (above ground) in decreasing rate, resulting in a strong (η^2=0.07) and significant (p<0.01) quadratic effect (Fig. 6.2). Linear effect was small (η^2=0.04) but significant (p<0.05), whereas cubic and quartic effects were not significant. Similar effects were observed in root fresh weight, root length and leaf area (Fig. 6.3).

6.4.7 Relationships between compost tea properties and plant growth

The multiple regression analysis showed the effects of biochemical properties of compost tea on plant growth and tissue N. Above ground plant fresh and dry weights, leaf area, tissue N uptake, root dry weight, and total root length of pak choi were regressed on 5 major components of compost tea (active bacteria, active fungi, GA_4, soluble N, and humic acid). The multiple regression analysis showed that mineral N and GA_4 present in compost tea had significant (p<0.0001) contributions to plant fresh or dry weights and leaf area (Table 6.10). One percent increase in GA_4 increased above ground plant fresh weight by 0.026 g and 1 mg increase in soluble N increased above ground plant fresh weight by 0.13 g. The standardized beta coefficient (Sβ) showed that mineral N had a greater effect (Sβ = 0.57) on above ground plant fresh weight compared to the effect of GA_4 (Sβ = 0.22). Similarly, 1% increase in GA_4 increased above ground plant dry weight by19 mg

and 1 mg increase in mineral N increased above ground plant dry weight by 0.01 g. The effect of mineral N on above ground plant dry weight was superior (Sβ = 0.45) to that of GA_4 (Sβ = 0.2). Also, 1% increase in GA_4 increased leaf area by 0.35cm^2 and 1 mg increase in mineral N increased leaf area by 1.2 cm^2.

Mineral N had a greater effect (Sβ = 0.59) on leaf area compared to the effect of GA_4 (Sβ = 0.21). Mineral N and humic acid present in compost tea significantly ($p<0.0001$) increased tissue N content of pak choi. The effect of mineral N on tissue N content of pak choi was superior (Sβ = 0.71) to that of GA_4 (Sβ = 0.19). $Gibberellin_4$ present in compost tea had a significant ($p<0.0001$) contribution to root dry weight. Active bacteria and GA_4 significantly ($p<0.001$) increased total root length. One percent increase in active bacteria and GA_4 increased total root length by 1.93 and 8.82 cm, respectively. The effect of GA_4 on total root length was superior (Sβ = 0.40) to that of active bacteria (Sβ = 0.28).

6.5 Discussion

Greater concentrations of NO_3-N, K, Mg, Na, Zn and Cu in chicken manure-based thermophilic compost resulted in greater EC. Significantly lower moisture content in chicken manure-based thermophilic compost was accompanied with greater levels of mineral nutrient concentrations including NO_3-N, K, Ca and Mg. Higher mineral concentrations in compost explain the greater concentrations of those nutrients in its tea. Positive correlations between mineral nutrient concentrations of compost and compost tea also explain the greater concentration of those nutrients in chicken manure-based thermophilic compost tea compared to the other teas. Aged chicken manure-based

vermicompost had significantly higher level of NO_3-N compared to fresh chicken manure-based vermicompost. This higher level of NO_3-N could be associated with a greater mineralization of organically bound N in aged chicken manure-based vermicompost over the time of curing. The higher level of humic acid in the green waste thermophilic compost tea and the aged chicken manure-based vermicompost tea than in the other teas could be attributed to the presence of higher concentration of humic acid in the green waste thermophilic compost and the aged chicken manure-based vermicompost.

Active bacterial and fungal population was significantly higher in food waste and chicken manure-based vermicomposts compared to green waste and chicken manure-based thermophilic compost. Werner and Cuevas (1996) and Edwards (1983) also reported the higher level of microbial population in vermicompost. The enhanced microbial population in vermicompost may be due to the intestinal contribution of earthworms. This finding is supported further by greater dehydrogenase activity and microbial respiration in the vermicomposts. A significantly greater level of dehydrogenase activity in vermicompost tea compared to thermophilic compost tea could be attributed to the presence of a greater level of active microbial population in vermicompost.

It has been established that several strains of *Gibberella fujikuroi* produce many of the bioactive GAs and their precursors (Frankenberger and Arshad, 1995). Various studies reported that some other fungal species including *Aspergillus niger, Fusariun spp., Penicillium spp*., and *Phaeosphaeria spp.* as well as a few species of *Rhizobium* and actinomycetes can also produce bioactive GAs (Youssef and Mankarios, 1975; Atzorn *et al.*, 1988; Frankenberger and Arshad, 1995). Food waste vermicompost tea, chicken manure-based vermicompost tea and chicken manure-based thermophilic compost tea

analyzed in this study had considerable amount of GA_4 and GA_{34}. The amount of GA_4 in food waste vermicompost tea was about 10 times greater than that of aged chicken manure-based vermicompost tea and chicken manure-based thermophilic compost tea. This could be attributed to the significantly greater population of active fungi in the food waste vermicompost.

Unlike many of the other phytohormones, microbial production of ABA has not been investigated extensively. However, Frankenberger and Arshad (1995) noted that certain species of plant pathogenic fungi including *Borytis cinerea, Cercospora rosicola, Verticilium dahlia* and *Fusarium oxysporum* may produce ABA. Similarly, Kolb and Martin (1985) detected ABA in the culture medium of *Azospirillum brasilense*. Presence of ABA in green waste thermophilic compost tea may be linked with the microbial production of ABA during composting.

Applications of compost tea significantly improved shoot and root growth and mineral nutrient content of pak choi. This is consistent with previous studies (Gamaley *et al.*, 2001; Hargreaves *et al.*, 2009; Pant *et al.*, 2009). Increased overall root development with the application of compost tea may have contributed to better nutrient uptake and increased leaf area. Leaf area plays an important role in light interception, photosynthesis, water and nutrient use; and dry matter production (Aase, 1978; Williams, 1987). Greater leaf area of compost tea treated plants could have been linked with increased above ground plant fresh and dry weight in this study.

Among the 5 compost teas tested, better growth was observed on the plants treated with the aged chicken manure-based vermicompost tea, chicken manure-based

thermophilic compost tea and food waste vermicompost tea compared to the other teas. This could be explained by greater level of mineral N present in these teas. Presence of GA_4 in the aged chicken manure-based vermicompost tea, chicken manure-based thermophilic compost tea and food waste vermicompost tea may also have contributed to superior growth response of the plants treated with these teas.

Result of multiple regression analysis of the biochemical properties of compost tea and plant growth parameters elucidates the significant contribution of mineral N and GA_4 to plant fresh weight, dry weight and leaf area. However, the effect of mineral N on above ground plant growth was superior to that of GA_4. Application of GA_4 into the growth media significantly influenced shoot and root growth of *in vitro* cultured pak choi compared to the control. Significant linear effect of the concentration (0-3200 ng) of GA_4 on pak choi height observed in this study is consistent with the findings of previous studies that confirmed the shoot elongation role of GA_4 (Brian and Hemming, 1961; Little and MacDonald, 2003; Eriksson *et al.*, 2006). A strong and significant quadratic effect and significant but weak linear effect of the concentration (0-3200 ng L^{-1}) of GA_4 on above ground dry weight, leaf area and root growth of pak choi suggests that increasing concentration of GA_4 increased plant growth in decreasing rate. Similar trend on plant growth response was observed with the concentrations of GA_4 present in aged chicken manure vermicompost tea, chicken manure thermophilic compost tea and food waste vermicompost tea in this study. This further supports the possible contribution of GA_4 present in compost tea to shoot and root growth of pak choi.

Inferior plant growth response to the application of green waste thermophilic compost tea compared to the other teas may be linked with the presence of ABA and lower level of mineral N in the tea. Both aged and fresh chicken manure-based vermicompost teas increased plant growth compared to the control, with the former being most effective. Presence of GA_4 and significantly higher level of mineral N in aged chicken manure-based vermicompost tea compared to fresh chicken manure-based vermicompost tea may explain the variability in plant growth response. Comparable level of microbial activity and humic acid present in both aged and fresh chicken manure-based vermicompost teas yielding variable plant growth response suggests greater contributions of mineral N and phytohormones to plant growth.

6.6 Conclusions

Biochemical properties of composts and their teas are positively correlated. These properties in turn influenced the growth and tissue mineral nutrient content of pak choi. Applications of compost tea increased growth and mineral nutrient content of pak choi. The response was greater with aged chicken manure-based vermicompost tea, chicken manure-based thermophilic compost tea and food waste vermicompost tea. The positive influence on plant growth was largely associated with mineral N and GA_4 present in compost teas. The findings suggest that both vermicompost tea and thermophilic compost tea can be used to improve plant growth and nutrient status, rather the chemical properties of the tea being more influential. These results also indicate that microbial by-products may be more influential than active biology of the compost tea at the time of application. Although the contribution of total active microbial population on plant growth was not significant in this study, the role of specific microbial functional groups

in mineralization of organically bound nutrients and biosynthesis of plant growth regulators must be further investigated. Microbial diversity in compost and compost tea is an area of high priority for further research.

Table 6.1 Chemical properties of the composts used in the experiment (n = 6).

Compost type	EC (mS cm $^{-1}$)	pH	Moisture (%)	Humic acid (g kg $^{-1}$dw)
Chicken manure-based vermicompost (aged)	3.4b	6.8d	64.3b	8.4b
Chicken manure-based thermophilic compost	22.2a	7.3b	20.0c	2.9d
Food waste vermicompost	3.5b	6.5e	73.3a	5.7c
Chicken manure-based vermicompost (fresh)	1.4c	6.9c	65.3b	6.8c
Green waste thermophilic compost	3.3b	7.8a	21.1c	11.5a

Means followed by the same letter are not significantly different ($p < 0.05$), EC=Electrical Conductivity.

Table 6.2 Chemical properties of the compost teas (n = 6).

Compost tea type	EC (mS cm $^{-1}$)	pH	Humic acid (mg L $^{-1}$)
Chicken manure-based vermicompost (aged)	1.0b	7.5c	464.8b
Chicken manure-based thermophilic compost	6.1a	7.6c	94.9d
Food waste vermicompost	1.0b	7.4dc	370.3c
Chicken manure-based vermicompost (fresh)	0.7b	7.3d	435.3b
Green waste thermophilic compost	1.4b	7.9b	556.5a
Control	0.4b	8.5a	nd

Means followed by the same letter are not significantly different ($p < 0.05$), EC= Electrical Conductivity, nd = non-detected.

Table 6.3 Nutrient concentration of the composts (n = 6).

Compost type	N	NO_3-N	NH_4-N	C:N	P	K	Ca	Mg
	%	------(µg g^{-1})-----			----------%----------			
Chicken manure-based vermicompost (aged)	1.6c	1883.2c	102.6b	11.6b	3.6a	0.2b	21.0a	0.9a
Chicken manure-based thermophilic compost	2.5b	7627.4a	112.9b	7.1c	2.8b	2.1a	16.7b	0.9a
Food waste vermicompost	3.1a	3590.9b	263.9a	12.9b	0.9c	0.6b	6.1c	0.6c
Chicken manure-based vermicompost (fresh)	1.5cd	524.3d	224.7a	13.1b	3.6a	0.1b	22.8a	0.8ab
Green waste thermophilic compost	1.2d	73.5d	83.3b	17.2a	0.3d	0.7b	6.6c	0.7ab

Means followed by the same letter are not significantly different ($p < 0.05$).

Table 6.4 Nutrient concentration of the compost teas (n = 6).

Compost tea type	N	NO_3-N	NH_4-N	P	K	Ca	Mg
	---------- (mg L^{-1}) ----------						
Chicken manure-based vermicompost (aged)	139.1b	137.9b	0.6b	11.0bc	45.1b	59.6bc	61.6b
Chicken manure-based thermophilic compost	293.0a	289.2a	3.3a	14.8ab	1198.9a	152.6a	138.3a
Food waste vermicompost	99.9bc	98.9bc	0.8b	9.2c	82.4b	63.7b	34.8bc
Chicken manure-based vermicompost (fresh)	40.1cd	39.6cd	0.3b	17.5a	20.6b	38.7c	33.3bc
Green waste thermophilic compost	9.5d	8.4d	1.0b	3.0d	196.6b	48.7bc	21.2c
Control	6.5d	6.3d	0.1b	0.1d	3.9b	11.1d	14.9c

Means followed by the same letter are not significantly different ($p < 0.05$).

Table 6.5 Microbial properties of the composts (n = 3).

Compost type	Active bacteria (log_{10} cells g^{-1})	Active bacteria (µg g^{-1})	Length of active fungi (cm g^{-1})	Active fungi (µg g^{-1})	Dehydrogenase activity (TPF µg g^{-1})	Microbial respiration (CO_2 fluxes µmol m^{-2} s^{-1})
Chicken manure-based vermicompost (aged)	8.3b	43.8c	918.9bc	18.5bc	207.8c	5.6bc
Chicken manure-based thermophilic compost	7.7c	12.0d	357.9bc	7.2bc	89.2d	4.2c
Food waste vermicompost	9.0a	205.6a	4768.3a	96.3a	290.2a	8.4a
Chicken manure-based vermicompost (fresh)	8.6b	77.4b	1536.9b	30.9b	229.8b	7.0ab
Green waste thermophilic compost	7.9c	15.2d	240.9c	4.9c	87.4d	3.2c

Means followed by the same letter are not significantly different ($p < 0.05$). TPF = 1,3,5-Triphenyltetrazolium formazan.

Table 6.6 Microbial properties of the compost teas (n = 3).

Compost tea type	Length of active fungi (cm mL $^{-1}$)	Active fungi (µg mL $^{-1}$)	Dehydrogenase activity (TPF µg mL $^{-1}$)
Chicken manure-based vermicompost (aged)	14.4ab	0.3ab	11.3b
Chicken manure-based thermophilic compost	7.8ab	0.2ab	8.6c
Food waste vermicompost	17.5ab	0.4a	12.6a
Chicken manure-based vermicompost (fresh)	19.5a	0.4a	11.3b
Green waste thermophilic compost	2.9b	0.1b	8.5c
Control	0.0c	0.0c	0.0d

Means followed by the same letter are not significantly different ($p < 0.05$).
TPF = 1,3,5-Triphenyltetrazolium formazan.

Table 6.7 Phytohormones present in the compost teas (n = 3).

Compost tea	Abscisic acid	Cytokinins		Gibberellins		
		iPA	2iP	GA_4	GA_{24}	GA_{34}
	---------- (ng L^{-1} compost tea) ----------					
Chicken manure-based vermicompost (aged)	nq.	nq.	nq.	198.1b	265.6	230.1b
Chicken manure-based thermophilic compost	nq.	nq.	nq.	230.4b	--	269.6b
Food waste vermicompost	nq.	nq.	nq.	2741.2a	--	1124.5a
Chicken manure-based vermicompost (fresh)	nq.	nq.	nq.	nq.	--	--
Green waste thermophilic compost	41.6	21.6	nq.	--	--	--

Means followed by the same letter are not significantly different ($p < 0.05$)
nq = non-quantifiable.

Table 6.8 Compost tea effect on pak choi growth (n = 22).

Compost tea type	Above ground fresh weight (g plant $^{-1}$)	Above ground dry weight (g plant $^{-1}$)	Leaf area (cm^2 plant $^{-1}$)	Root fresh weight (g plant $^{-1}$)	Root dry weight (g plant $^{-1}$)	Total root length (cm plant $^{-1}$)	Total root surface area (cm^2 plant $^{-1}$)
Chicken manure-based vermicompost (aged)	33.9a	2.9a	521.6b	5.1a	0.46a	7954.1a	552.7a
Chicken manure-based thermophilic compost	35.7a	2.5b	587.2a	2.5d	0.27c	3619.3d	288.8c
Food waste vermicompost	22.4b	1.9c	392.3c	3.6b	0.34b	5932.2b	418.7b
Chicken manure-based vermicompost (fresh)	13.5c	1.2d	268.3d	3.0c	0.28c	4426.4c	325.3c
Green waste thermophilic compost	6.7d	0.7e	160.2e	1.7f	0.14e	2297.9e	193.1d
Control	7.6d	0.7e	165.1e	2.1e	0.20d	1576.4f	153.0d

Means followed by the same letter are not significantly different ($p < 0.05$).

Table 6.9 Compost tea effect on tissue nutrient content of pak choi (n = 3).

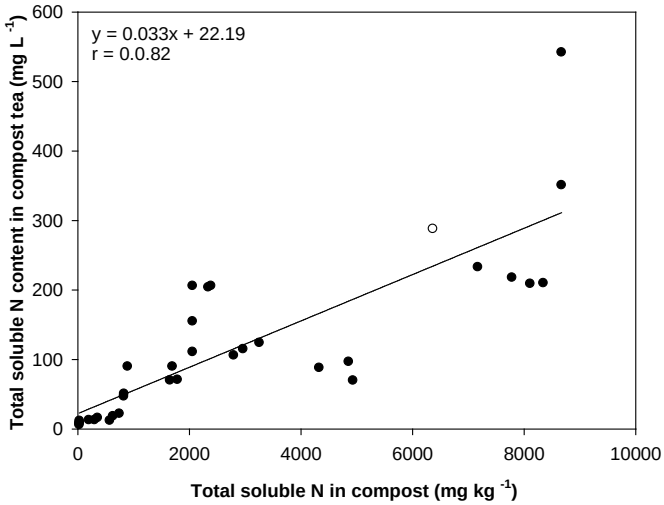
Compost tea type	N	P	K	Ca	Mg
	---------- (mg plant $^{-1}$) ----------				
Chicken manure-based vermicompost (aged)	68.9a	21.9a	130.1a	59.9a	19.9a
Chicken manure-based thermophilic compost	62.8b	17.3b	112.5b	46.1b	14.9b
Food waste vermicompost	33.2c	11.3c	61.5c	33.8c	10.9c
Chicken manure-based vermicompost (fresh)	18.3d	7.3d	40.2d	22.7d	6.9e
Green waste thermophilic compost	8.4e	3.6e	18.3e	9.5e	3.2e
Control	9.4e	3.9e	17.8e	12.2e	4.0e

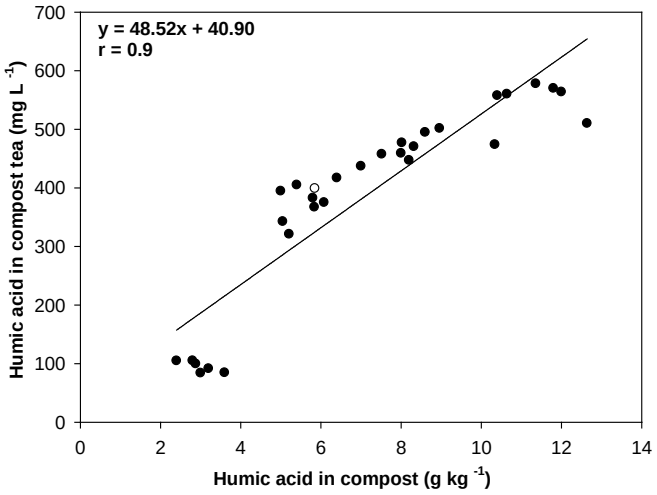
Means followed by the same letter are not significantly different ($p < 0.05$).

Table 6.10 Relationship between biochemical properties of the compost teas and plant growth response.

			Biochemical properties of compost tea (X)									
			Active bacteria		Active fungi		GA_4		Soluble N		Humic acid	
Plant growth parameters (Y)	Adjusted R^2	Intercepts	β	Sβ	β	Sβ	β	Sβ	β	Sβ	β	Sβ
Plant fresh weight	0.54	7.8	--	--	--	--	2.6**	0.22	0.13****	0.57	--	--
Plant dry weight	0.43	0.7	--	--	--	--	0.2*	0.21	0.01****	0.45	--	--
Leaf area	0.55	173.3	--	--	--	--	34.9**	0.21	1.2****	0.59	--	--
N uptake	0.52	3.7	--	--	--	--	--	--	0.33****	0.71	0.04**	0.19
Root dry weight	0.17	0.2	--	--	--	--	0.1****	0.42	--	--	--	--
Root length	0.32	1520.6	193.1***	0.28	--	--	882.2****	0.40	--	--	--	--

β=Beta Coefficient, Sβ=Standardized Beta Coefficient,
*, **, ***, **** = significant at $P < 0.05$, 0.01, 0.001 or 0.0001, respectively.





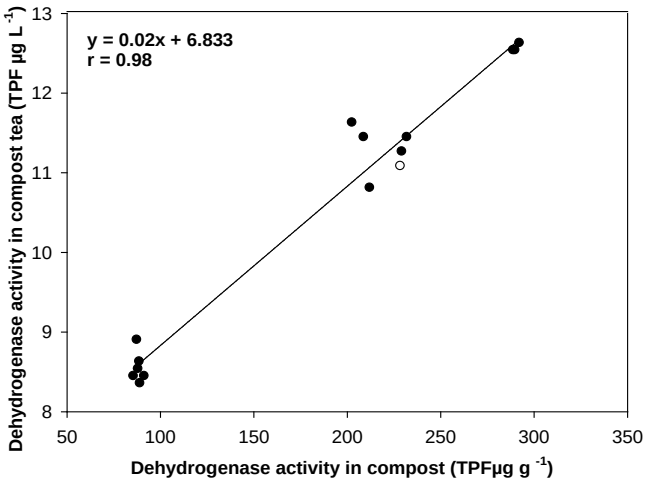


Figure 6.1 Selected properties of compost tea in relation to the properties of compost (n = 30)

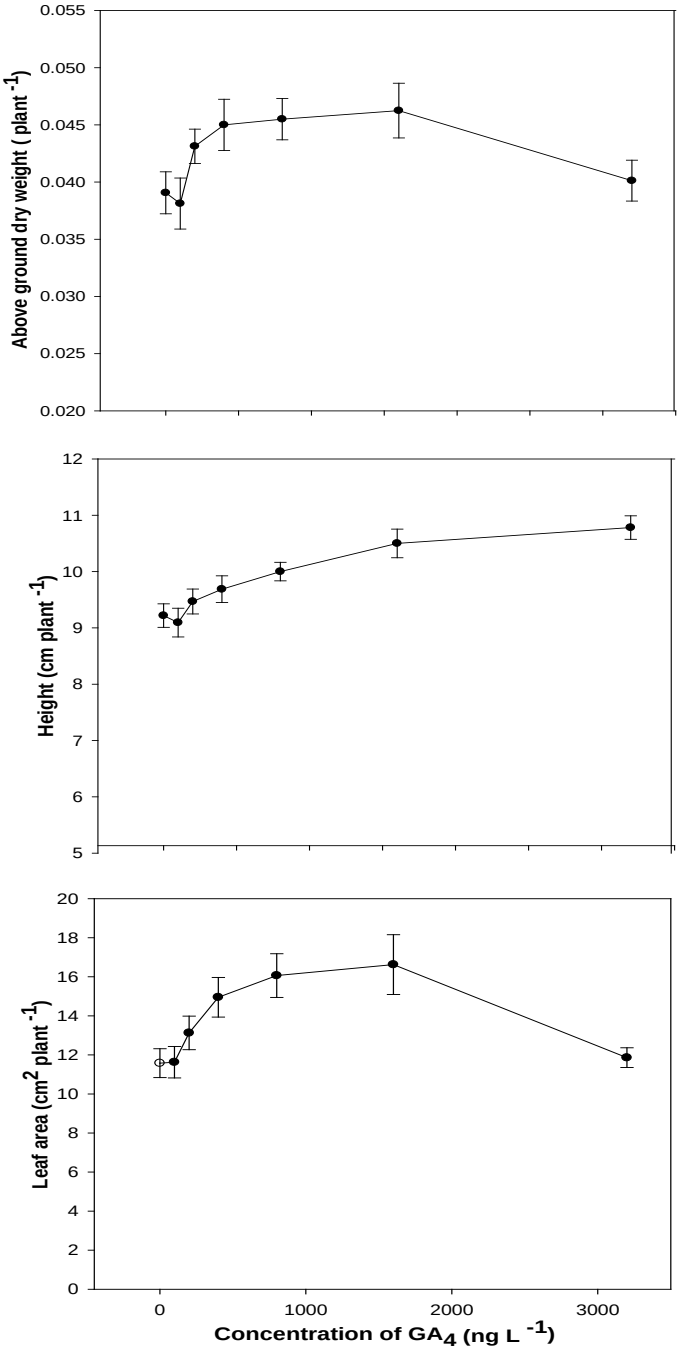


Figure 6.2 Above ground growth of *in vitro* cultured pak choi as affected by the application of GA_4. Plotted points are means of 16 samples, and error bars represent standard errors of the mean.

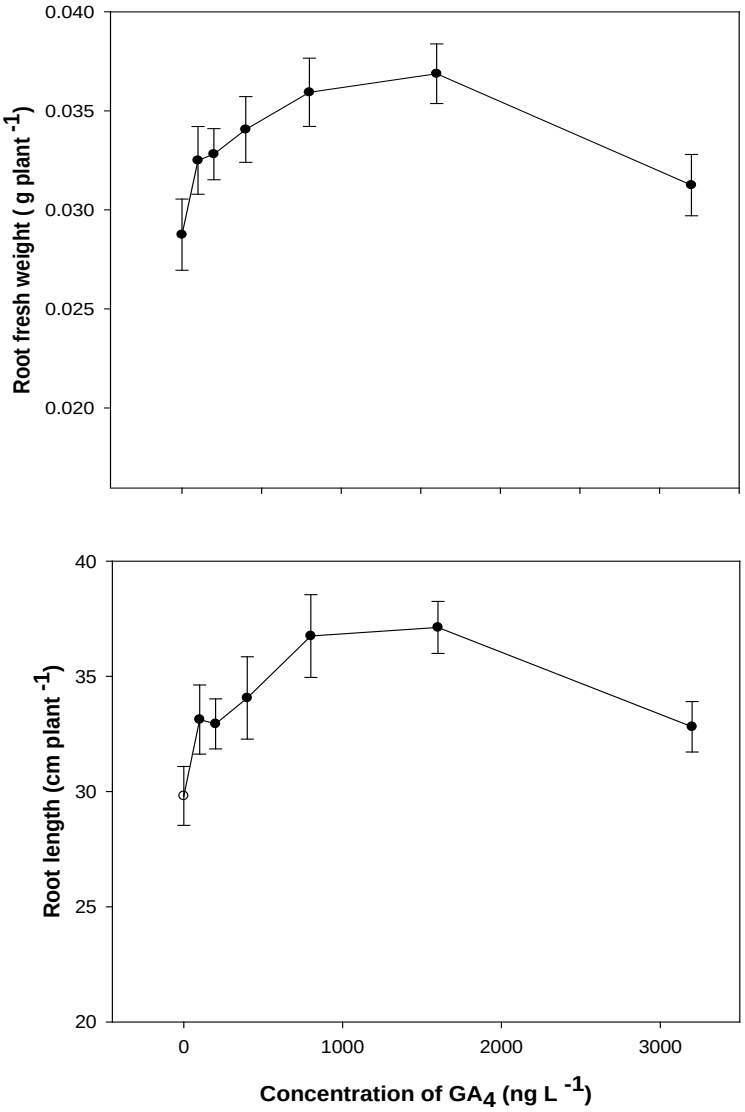


Figure 6.3 Root growth of *in vitro* cultured pak choi as affected by the application of GA_4. Plotted points are means of 16 samples, and error bars represent standard errors of the mean.

CHAPTER 7

SUMMARY AND CONCLUSION

Prior to this research, limited data and understanding existed on the use of vermicompost tea for improving yield and nutritional quality of vegetable crop and soil biological properties. Previous research focused on the potential of compost tea for plant disease control with diverse results. In this research, multiple experiments were conducted with the unifying theme of investigating influence of vermicompost tea prepared by different extraction methods on plant growth, yield and phytonutrients content in pak choi (*Brassica rapa*, cv Bonsai, Chinensis group) as well as soil biological properties under conditions of different soil types and fertilizer regimes. The goals of these efforts were two-fold: (i) to validate anecdotal and published reports of positive effects of vermicompost tea on plant growth and (ii) to elucidate the mechanisms involved in growth promotion effect of vermicompost tea. Improved understanding could help to deliver practical information and techniques that growers could benefit from the use of vermicompost tea in vegetable production.

Previous research on the use of compost tea on plant production had indicated that application of compost tea improves plant health, yield and nutritional quality by supplying microbial biomass, fine particulate organic matter, organic acids, plant growth regulator-like substances and soluble mineral nutrients to plants and soils in a way not possible or feasible with solid compost (Chapter 2). Compost tea extraction methods,

ratio of compost to water and compost quality were expected to be important factors influencing compost tea quality and subsequent plant response.

Plant growth and nutrient quality

Several experiments conducted in the greenhouse with multiple soil types and fertilizer regimes have demonstrated that application of vermicompost tea in root zone and foliage increased yield, root growth, mineral nutrient concentration and total carotenoids of pak choi under both organic and chemical fertilization in a Mollisol, an Oxisol and a peat-perlite medium (Chapters 3 and 4). However, the effect was more prominent under organic fertilization. Application of vermicompost tea increased total glucosinolates in plant tissue under organic fertilization but had a non-significant effect under chemical fertilization. Application of vermicompost tea decreased total phenolics in plant tissue across the soil types and fertilizer regimes. The vermicompost tea effect on plant growth was found to be closely related to mineral nutrient uptake particularly N.

Extraction methods

Vermicompost tea extraction method did not have significant effect on microbial population in compost tea and plant growth response (Chapters 3 and 4). Regardless of the popular interest in the use of aeration during vermicompost tea extraction, aerated and non-aerated vermicompost tea were not significantly different in terms of total and active bacterial and fungal population and mineral nutrient concentration in the tea. Applications of aerated or non-aerated vermicompost tea had similar effect on pak choi yield, phytonutrients and mineral nutrient in tissue. However, aeration is useful to speed

up the extraction process. Adding kelp and humic acid (additives) in vermicompost tea during aerated extraction also did not significantly increase total and active microbial population in the tea. The use of additives increased total N, K, Ca, Mg and micronutrients in the tea, however, its effect on plant growth and nutrient quality was comparable with the effect of non-aerated and aerated vermicompost tea produced without additives. Therefore, the results of this research suggest that aeration and additives may not be essential for growth promotion and nutrient quality in pak choi.

Soil biological properties

Soil chemical and biological properties are indicators of soil quality and health, as influenced by management practices. Microbial respiration and dehydrogenase enzyme activity are often considered to be a good index of total microbial activity in soil. Application of vermicompost tea improved biological properties of an Oxisol, a Mollisol and a peat-perlite medium in greenhouse and field conditions (Chapters 4 and 5).Vermicompost tea treatments contributed to increased microbial respiration (µmol CO_2 fluxes m^{-2} sec^{-1}) and dehydrogenase activity (µg TPF g^{-1} soil), particularly under compost fertilization, implying a more efficient of organic matter decomposition and mineralization of nutrients in soil, and therefore producing better plant growth. Application of vermicompost tea also improved mineral nutrient concentration in an Oxisol, a Mollisol and a peat-perlite medium.

Compost to water ratio

Ratio of compost to water is an important factor influencing vermicompost tea quality and its effect on plant production. The optimum ratio of compost to water tends to vary, depending upon the brewing process, compost quality and purpose of compost tea application. Results of multiple greenhouse and field trials (Chapter 5) showed that application of vermicompost tea with vermicompost to water ratios of 1:10 - 1:100 (v:v) increased plant yield and root growth, and the response to the ratio of vermicompost to water was generally linear. Similar effects were observed in tissue N, phytonutrient content and microbial activities in soil. The best plant growth response was observed with vermicompost to water ratios of 1:20 and 1:10, indicating that the optimal ratio of vermicompost to water ranges between 1:10 and 1:20.

Links between compost and compost tea qualities

Compost quality used has been identified as the most important factor influencing compost tea quality and plant growth (Chapter 6). Good quality compost generally holds high beneficial microorganisms, soluble mineral nutrients, humic substances, phytohormones and low phyto-toxic organic acids and heavy metals and is free of pathogen. Chicken manure-based thermophilic compost had higher levels of mineral nutrients and lower C:N ratio compared to green waste thermophilic compost. Chicken manure-based vermicompost, food waste vermicompost and chicken manure-based thermophilic compost, all cured for 3 months were found to have higher levels of mineral nutrients compared to fresh chicken manure-based vermicompost. A plant growth

promoting hormone, particularly, GA_4 was found to be present in aged chicken manure-based vermicompost tea, food waste vermicompost tea and chicken manure-based thermophilic compost tea, whereas ABA was present in green waste thermophilic compost tea. Similarly, application of compost tea prepared from chicken manure-based vermicompost, chicken manure-based thermophilic and food waste vermicompost compost had superior effect on the growth of pak choi. This result suggests that feedstock used for making compost and the compost age are important factors influencing chemical properties of compost and compost tea and subsequent plant response. Also, either vermicompost tea or thermophilic compost tea could be used to improve plant growth and nutrient status, rather the biochemical properties of the compost tea were found to be more influential. Therefore, it is suggested that compost used for making tea should be constant on above mentioned properties in order to obtain a consistent result from compost tea uses.

Application of vermicompost tea or compost tea increased growth, tissue mineral nutrient content and phytonutrients of pak choi. The positive influence on plant growth was largely associated with mineral N and GA_4 present in the tea and N uptake by plants. The contribution of active microbial population on plant growth was not significant in this study, however, microbial functional groups responsible in mineralization of organically bound nutrients and biosynthesis of plant growth regulator must be further investigated. Therefore, further study on microbial diversity of compost and compost tea would be helpful to identify the specific group of microorganisms responsible for

synthesizing plant growth regulators or other organic acids that promote nutrient uptake and crop growth.

Overall, vermicompost tea may be used to improve plant production, nutrient status and enhance soil biological properties in vegetable production.

APPENDIX A

SEED GERMINATION AND SEEDLING GROWTH OF TOMATO AND LETTUCE AS AFFECTED BY VERMICOMPOST TEA TREATMENT

A.1 Abstract

Greenhouse experiments were conducted twice to test the effect of vermicompost tea on germination of tomato and lettuce seeds. Tomato and lettuce seeds were soaked overnight (9 hrs) in 10%, 5% 3% and 1% (1:10, 1:20, 1:33 and 1:100 vermicompost to water ratio by volume) and in water (control). Seeds were sown in peat-perlite medium. Seedlings were harvested after four weeks of planting. Soaking seeds into vermicompost tea significantly ($p<0.0001$) increased germination percentage and seedling growth of tomato and lettuce compared to control. The response to concentrations of the vermicompost tea was generally linear. The earlier emergence and better root growth with vermicompost tea treatment seems to be responsible for better nutrient uptake, growth and faster maturation of the seedlings. The results of this study suggest that vermicompost tea can be a good amendment for vegetable seed germination increasing the number of seeds germinated and accelerating seedling development.

A.2 Introduction

Vermicompost tea, an aqueous extract of vermicompost may contain a series of bioactive soluble molecules as well as microbial populations inhabiting the original

vermicompost which may be enhanced during the production of extracts (Scheuerell and Mahaffee, 2004; Ingham, 2005a; Edwards *et al.,* 2006). Although there is still insufficient information on the chemical and biological properties of vermicompost extracts, it is believed that water extractable mineral nutrients and biologically active metabolites such as humic acids as well as plant growth regulators present in vermicompost would be extracted in the tea during brewing cycle (Arancon *et al.,* 2007; Pant *et al.,* 2009). Combined effect of chemical as well as biological activity of vermicompost tea may enhance initial root development, nutrient uptake and plant growth.

Vermicompost tea has been studied mainly for its effect on disease suppression and yield of some horticultural plant species while there is limited information on the effects of vermicompost extracts on the germination and early seedling growth of vegetable crops. Several studies have assessed the impact of vermicompost amendments in potting substrates with regard to seedling emergence and the growth of marketable fruit and yield of some vegetable crops (Atiyeh *et al.*, 2000a; Atiyeh *et al.*, 2000c; Arancon *et al.*, 2003a; Arancon *et al.*, 2004a). Arancon *et al.* (2007), and Edwards *et al.* (2006), demonstrated an enhanced seed germination and growth of tomato and cucumber plants with the application of vermicompost extracts to the growth media. Lazcano *et al.* (2010) reported the positive effect of vermicompost and vermicompost extract on germination and early development of *Pinus pinaster*. It is hypothesized that soaking seeds in vermicompost extracts would contribute to rapid germination of tomato and lettuce seeds and enhance seedling growth. The objective of this study is to investigate

the effect of vermicompost tea treatments on the germination and early development of tomato and lettuce seedlings.

A.3 Materials and methods

Greenhouse experiments were conducted twice to test the effect of vermicompost tea on germination of tomato and lettuce seeds. A 10% (1:10 vermicompost to water ratio by volume) aerated vermicompost extract was prepared using chicken manure-based vermicompost and water, as previously described (Pant *et al.*, 2009) and was further diluted with water to make 5, 2 and 1% vermicompost extracts. Tomato and lettuce seeds were soaked overnight (9 hrs) in four types of vermicompost extracts based on concentration (10, 5, 3 and 1%) and in water (control). Seeds were sown in peat-perlite medium, fertilized with chicken manure-based compost to provide 300 mg N L^{-1} media (150 kg N ha^{-1}). Media were sprayed once at the time of sowing with the respective concentration of vermicompost tea that was used for soaking the seeds. Plants were allowed to grow in the greenhouse on a bench fitted with overhead sprinklers with a frequency of every 4 hours for 5 minutes. The experiments were arranged in completely randomized design with 5 treatments based on concentrations and 5 replications per treatment. Each replication contained 20 plants and 3-5 seedlings were sampled from each replication.

Seed germination rate was recorded. Seedlings were harvested after four weeks of planting. Plant height, above ground fresh and dry weight and number of leaves were measured. Total root length and root surface area were calculated using WinRHIZO Pro

V. 2003b system (Regent Instruments Inc., QC, Canada). The system consists of a scanner and WinRHIZO software. After taking the root fresh weight, roots were oven dried at 70°C for 72 h and dry weight of the roots of each plant was recorded.

Analysis of variance (ANOVA) of plant growth parameters and seed germination rate was performed in SAS 9.1 statistical software (SAS Institute Inc., 2003). Trend analysis via polynomial regression was conducted for seed germination and measured plant growth parameters between 0-10% vermicompost tea treatments. Statistical significance was obtained at 95% confidence level (α =0.05).

A.4 Results

A.4.1 Vermicompost tea effects on seed germination and seedling growth

Tomato

Soaking seeds into vermicompost tea significantly ($p<0.0001$) increased germination percentage and seedling growth of tomato. Increasing concentrations of the vermicompost tea increased seed germination percentage (Fig. A1) and above ground fresh weight of tomato seedling (Fig. A2), resulting in a significant ($p<0.0001$) linear effect. Similar trends were observed in above ground dry weight, leaf number and height. Increasing concentration of vermicompost tea increased root fresh weight, resulting in a strong (η^2 =0.43) and significant ($p<0.0001$) linear effect (Fig. A3). Quadratic effect was small (η^2= 0.02) but

significant ($p<0.01$), whereas cubic and quartic effects were not significant. Similar trends were observed in root dry weight, root length and root surface area.

Lettuce

Soaking seeds into vermicompost teas significantly improved germination percentage and seedling growth of lettuce compared to control. Increasing concentration of vermicompost tea increased seed germination percentage, resulting in a strong ($\eta^2=0.35$) and significant ($p<0.001$) linear effect (Fig. A1). Quadratic ($\eta^2=0.1$, $p<0.03$) and cubic ($\eta^2=0.14$, $p<0.02$) effects were small but significant, whereas quartic effect was not significant. Increasing concentration of vermicompost tea increased above ground fresh weight, resulting in a strong ($\eta^2=0.47$) and significant ($p<0.0001$) linear effect (Fig. A2). Quadratic ($\eta^2=0.1$, $p<0.0001$) and cubic ($\eta^2=0.05$, $p<0.01$) effects were small but significant, whereas quartic effect was not significant. Similar trends were observed in above ground dry weight and height (Fig. A2). Leaf number was not affected ($p<0.16$) by compost tea treatments. Increasing concentration of vermicompost tea increased root fresh weight, resulting in a strong ($\eta^2=0.46$) and significant ($p<0.0001$) linear effect (Fig. A3). Quadratic effect was small ($\eta^2=0.12$) but significant ($p<0.01$), whereas cubic and quartic effects were not significant. Similar trends were observed in root dry weight and root surface area. Increasing concentrations of vermicompost teas increased root length in decreasing rate, resulting in a strong ($\eta^2=0.28$) and significant (0.0001) quadratic effect. Linear effect was small ($\eta^2=0.26$) but significant ($p<0.0001$), whereas cubic and quartic effects were not significant.

A.5 Discussion

Although seed germination is an internally regulated process influenced by genotype; external factors such as light, temperature, moisture, and presence of certain chemical compounds (phytohormones or organic acids) also strongly influence this process (Finkelstein, 2004; Kucera *et al.*, 2005). Moisture content of seed or growth media is one of the important external factors affecting seed germination. Seed soaking can soften a hard seed coat and also leach out any chemical inhibitors in the seed which may prevent germination. In this experiment, germination percentage increased when the seeds were soaked into vermicompost extract outperforming the germination of the seeds that were soaked into water. This suggests that other factors rather than physical alteration of seed coat were responsible for earlier and better germination. Spaccini *et al.* (2008) reported that aerated compost extracts contained low molecular weight bioactive compounds of microbial origin, extracted from compost. Arancon *et al.* (2007) demonstrated that the application of a vermicompost extract to the growth media enhanced seed germination and seedling growth of tomatoes and cucumbers. Lazcano *et al.* (2010) reported the positive effect of vermicompost extract on germination and early development of *Pinus pinaster*. Better root and shoot growth of both tomato and lettuce seedlings observed in this study agrees with the findings of previous studies (Arancon *et al.*, 2007; Lazcano *et al.*, 2010). It is expected that water-soluble bioactive substances, such as humic acids, phytohormones or other microbial metabolites present in vermicompost extract may be responsible for

earlier emergence, increased seed germination percentage and seedling growth. Presence of a small quantity (198 ng L^{-1}) of GA_4 in 10% chicken manure-based vermicompost tea (chapter 6), prepared using the same source of vermicompost as used in this study also suggests the possibility of hormonal effects on seed germination and better root growth. Enhanced root growth at earlier stage of development may have contributed to better seedling growth.

A.6 Conclusion

Seed treatment with vermicompost tea has no detrimental but a stimulatory effect on seed germination and seedling growth of both of tomato and lettuce. The results of this study suggest that vermicompost tea can be a good amendment for vegetable seed germination increasing the number of seeds germinated and accelerating seedling development. The earlier emergence and better root growth with vermicompost tea treatment seems to be responsible for better nutrient uptake, growth and faster maturation of the seedlings. Further investigation of the mechanisms such as the presence of plant growth regulators and organic acids in vermicompost tea would substantiate the results.

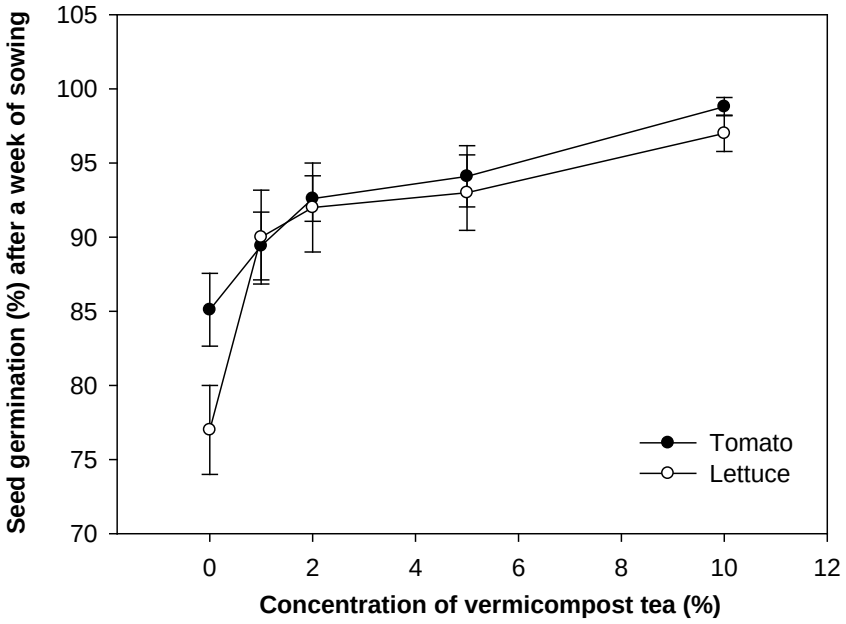


Figure A.1 Seed germination of tomato and lettuce as affected by soaking seeds into vermicompost tea for 9 hrs before sowing. Plotted points are means of 30 samples, and error bars represent standard errors of the mean.

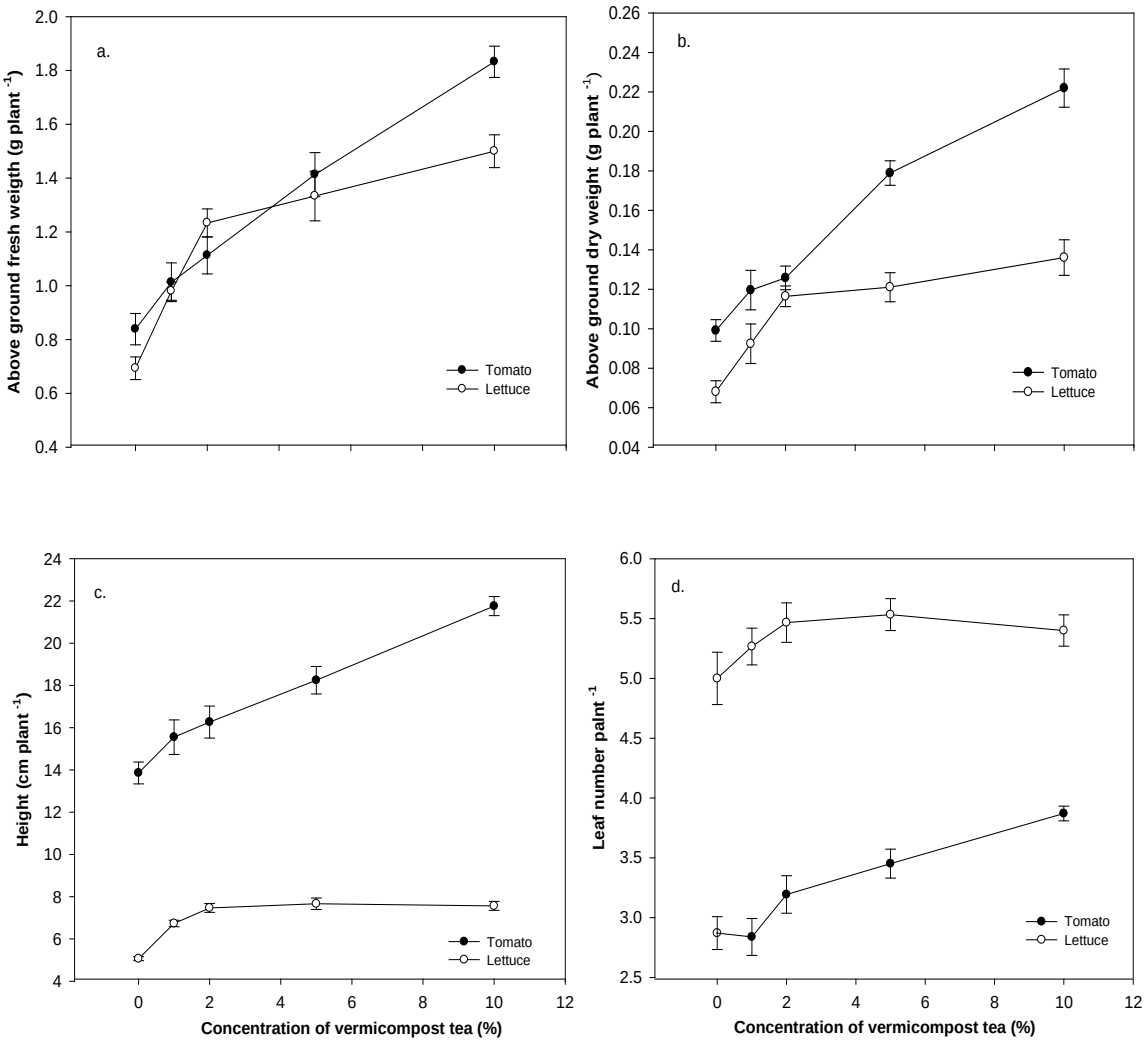


Figure A.2 Effect of the concentration of vermicompost tea on above ground plant growth, a) fresh weight b) dry weight c) plant height and d) leaf number of tomato and lettuce. Plotted points are means of 30 samples, and error bars represent standard errors of the mean.

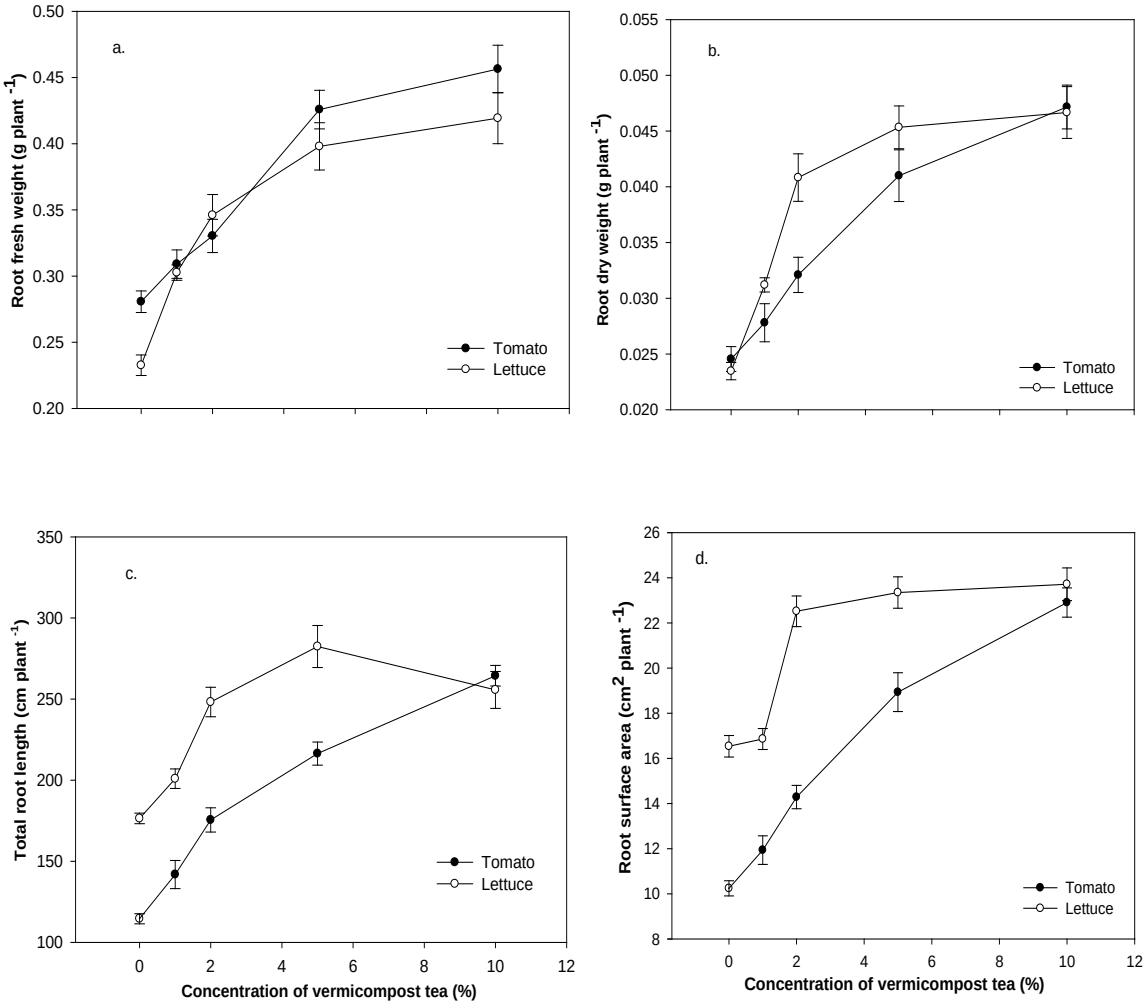


Figure A.3 Effect of the concentration of vermicompost tea on root growth, a) root fresh weight b) root dry weight c) total root length and d) root surface area of tomato and lettuce. Plotted points are means of 30 samples, and error bars represent standard errors of the mean.

APPENDIX B

LABORATORY PROTOCOLS

B.1 Total glucosinolates analysis of pak choi

B.1.1 Sample preparation

A 1/4th portion of a whole pak choi plant was cut longitudinally from the center of a head and was immediately frozen in liquid nitrogen. Frozen samples were placed in cloth bags and stored at -80 ºC. Samples were lyophilized and finely ground with a coffee grinder. After lyophilization, samples were stored in air-tight containers to prevent re-hydration.

B.1.2 Glucosinolate extraction

Total glucosinolates was extracted as described by Radovich et al (2005a). Added to labeled, capped test tubes were 100 mg of ground sample and 10 ml of 90% aqueous methanol. To test for glucosinolates recovery, 2.5 mg of sinigrin (Sigma, St. Louis, MO) and 10.0 ml of 90% aqueous methanol were added to tubes with 100 mg of duplicate sample. A duplicate sample was run for approximately every 10 unknown samples. Extraction was aided by a sonic water bath for 1 hour, allowed to soak for 18 hr, followed by an additional 30 min of sonication. The undigested bulk residue from each sample was then separated by centrifugation at 2000 g for 30 minutes. Supernatant was vacuum filtered through a Whatman glass microfiber filter. The sample residue was rewashed with 5 ml of 70% aqueous methanol, followed by centrifugation and vacuum filtration.

The final volume of extract is 15 mL. The supernatant was carefully poured into capped labeled vials and stored at 0 ºC prior to analysis.

B.1.3 Determination of total glucosinolates

A protocol after Radovich *et al.* (2005a) was adopted for the determination of total glucosinolate concentrations. DEAE sephadex A-25 dry resin (approximately 125 mg sample $^{-1}$) was suspended in an excess of 0.5 M pyridine acetate buffer and vacuum filtered to remove excess buffer (i.e., not to complete dryness) using a Buchner funnel and Whatman no. 1 filter paper. The process was completed twice consecutively. Resin was then suspended in 0.02 M pyridine acetate so that the settled volume of hydrated resin equaled half of the total suspension volume. Resin was allowed to hydrate for at least 3 h before use and degassed before being poured into glass columns. Pyridine acetic acid buffer (0.5 M) was prepared by mixing 930 ml deionized water (DI) with 30 ml acetic acid and 40 ml pyridine (Fisher Scientific, Fair Lawn, N.J.). Diluting 8 ml of this buffer to 200 ml with DI water gives a final concentration of 0.02 M. Columns were prepared by inserting a small plug of glass wool into a Pasteur pipet (15 cm). Pipets were placed in a rack with large test tubes below them to collect effluent. Columns were washed twice with DI water by filling the columns and allowed to drain between washes. Resin was then mixed thoroughly and columns were half filled with water, followed immediately with 1 ml of the resin suspension, for a final bed volume of approximately 0.5 ml. Columns were checked to ensure that air bubbles did not exist and that bed volumes were equal. Columns were washed twice with DI water and allowed to drain (~3 min) between washes. Then, columns were half filled with DI water, 1.0 ml of sample

extract was added to the column, and the columns were allowed to drain (~3 min). Columns were washed with 300 µl of DI water, allowed to drain (~3 min), then filled with water and allowed to drain (~3 min). Columns were then washed twice with 500 µl of 0.02 M pyridine buffer, draining (~ 1 min) between washes. Collection test tubes were placed under the columns and 0.8 U of myrosinase in 250 µl 0.02 M pyridine buffer was added. Columns incubated for 16 h at 25 ºC. After incubation, columns were eluted with two, 500 µl volumes of DI water. Total eluate volume was ~1.15 ml, 100 µl generally being lost to evaporation overnight. The eluate was analyzed for glucose concentration either immediately, or stored at -20 ºC for <24 hrs before analysis. To determine the concentration of glucose in the eluate, 200 µl was added to duplicate test tubes, to which 1 ml of aqueous hexokinase (HK) solution (product number G2020, Sigma, St. Louis, MO), prepared per manufacturer instructions, was then added. For a blank tube, 200 µl of water instead of eluate was used. To confirm the slope of the standard curve, 10, 20, 25, 50 and 100 µl glucose solution (from 1 mg ml-1 stock solution) was added to separate test tubes containing 190, 180, 175, 150 and 100 µl DI water, respectively. Then, 1 ml HK solution was added to each test tube. All tubes were vortexed and incubated for 30 min at 30 ºC in a circulating water bath. Next, each reaction mixture was added to a cuvette and absorbance was recorded at 340 nm using Genesys 20 spectrophotometer (Thermo Scientific - Model 4001-000, MA). Total glucosinolates content (GS) of 100 mg sample was calculated with the formula:

GS = [(sample A340 -intercept)/slope]* 2.21* 5.75 * 15 / R

where, slope and intercepts were obtained from plotting standard solutions' absorbance at

340 nm against micrograms of glucose mL^{-1}, 2.21= mol wt of sinigrin mol^{-1} wt of glucose (397.46/180), 5.75 = dilution factor for 200 µl of elute taken for measurement from 1.15 mL of elute, 15 = dilution factor of sample aliquot (1.0 ml aliquot * 15 ml total sample volume), and R = average recovery rate of internal standard, calculated by subtracting the amount of glucosinolate in samples from that of duplicate samples containing 2.5 mg of internal standard and dividing by 2.5.

B.2 Total carotenoids analysis of pak choi

B.2.1 Total carotenoids extraction

Pak choi samples prepared as in B.1.1 were used for total carotenoids extraction. Total carotenoids were extracted from lyophilized samples as described by Gross (1991). One hundred mg of each sample was added to labeled capped test tube and extracted in 15 mL of ethanol: acetone (1:1, v:v) mixture. Extraction was aided by a sonic water bath for 1 hour, allowed to soak for 18 hr, followed by an additional 30 min of sonication. The undigested bulk residue from each sample was then separated by centrifugation at 2000 g for 30 minutes. Supernatant was vacuum filtered through a Whatman glass microfiber filter. The sample residue was rewashed with 5 ml of ethanol: acetone (1:1, v:v) mixture, followed by centrifugation and vacuum filtration. The final volume of extract is 20 mL. The supernatant was carefully poured into capped labeled vials and stored at < 0 ºC prior to analysis.

B.2.2 Determination of total carotenoids

Each sample extract was added to a cuvette and absorbance was recorded at 470 nm using Genesys 20 spectrophotometer (Thermo Scientific - Model 4001-000, MA). Total carotenoids content of pak choi on mg kg^{-1} dry weight basis were calculated as described by Gross (1991) using the equation:

Total carotenoids = $(A_{470}*V * 10^6)/(A\% *100G)$

where, A_{470} is the absorbance at 470 nm, V is the total volume of the extract (mL), A% is the extinction coefficient of 2500, and G is the sample weight in grams.

B.3 Total phenolics analysis of pak choi

Pak choi samples prepared as in B.1.1 and extracted as in B.2.1 were used for total phenolics analysis. Total soluble phenolics were measured using the Prussian Blue Assay as described by Stern *et al.* (1996). Standard curve was determined using gallic acid standard solution which was prepared by dissolving18.83 mg of gallic acid in 100 mL DI. The concentration of standard solution is 1 μmol mL^{-1}. Then 0, 10, 20, 30, 50 and 100 μl of standard solution was added to test tubes containing 1000, 990, 980, 970, 950 and 900 μl DI water, respectively. Similarly, 1 mL of each sample extract was added to labeled test tube. Then, 5 mL DI water was added to each test tube containing sample extract and standard solutions. Then 0.36 mL of ferric ammonium sulfate [0.1 M $FeNH_4(SO_4)_2$ in 0.1 m HCl] was added to each test tube. Exactly 20 minutes after the addition of ferric ammonium sulfate to each test tube, 0.36 mL of potassium ferricyanide [0.008M K3Fe(CN)6 in DI water] was added. Exactly 20 minutes after the addition of

potassium ferricyanide, each reaction mixture was added to a cuvette and the absorbance at 720 nm was recorded using Genesys 20 spectrophotometer (Thermo Scientific - Model 4001-000, MA). Total phenolics content (TP) of 100 mg of sample (dry weight basis) in gallic acid equivalent was calculated with the formula:

TP = [(sample A_{720} -intercept)/slope]*20* 188.3

where, slope and intercepts were obtained from plotting standard solutions' absorbance at 720 nm against micromole of gallic acid mL^{-1}, 188.3= mol wt of gallic acid, 20 = dilution factor of sample aliquot (1.0 ml aliquot * 20 ml total sample volume).

B.4 Analysis of dehydrogenase activity in soil

Protocols after Alef (1995) and Tabatai (1982) were adopted for the determination of dehydrogenase activity in soil. Tris-HCl buffer (100 mM) was prepared by dissolving 12.1 g Tris amino-methane in 700 ml DI water and adjusted to pH 7.6 with HCl and brought up to final volume of 1000 mL with DI water. Triphenyl-tetrazolium chloride (TTC) solution was prepared by dissolving 1.5 g TTC in 80 mL Tris-HCl buffer and made up with the same buffer to 100 mL. From each freshly collected soil sample, 5 g moist soil was weighed into centrifuge tubes and mixed with 5 mL TTC solution. A set of blank (control) was prepared mixing 5 g moist soils with only 5 mL Tris-HCl buffer (100 mM) without TTC solution. The tubes were sealed with rubber stoppers and incubated for 24 hr at 30 ºC. Then, 10 mL methanol was added to each tube, vortexed and incubated for another 2 hr at room temperature. The undigested bulk residue from each sample was then separated by centrifugation at 4000 g for 10 minutes. Supernatant was vacuum filtered through a Whatman glass microfiber filter. The sample residue was rewashed

with 5 mL methanol, followed by centrifugation and vacuum filtration. All the supernatant was taken out and mixed well with previously taken supernatant. Standard curve was determined using Triphenyl-tetrazolium formazan (TPF) standard solution which was prepared by dissolving10 mg of TPF in 80 mL methanol and made up to 500 mL with methanol. The concentration of the TPF standard solution was 20 μg mL^{-1}. Then, 0, 0.5, 1, 2, 4 and 6 mL of TPF standard solution were taken in different test tubes and 1.7 mL tris buffer was added into each test tube and brought up to 10 mL with methanol (adding 8.3, 7.8, 7.3, 6.3, 4.3 and 2.3 mL respectively in corresponding tubes). Next, each reaction mixture (standard as well as sample extracts) was added to a cuvette and absorbance was recorded at 485 nm using Genesys 20 spectrophotometer (Thermo Scientific - Model 4001-000, MA). Dehydrogenase activity in terms of μg TPF g^{-1} of soil sample on dry weight basis was calculated with the formula:

$TPF = [(A_{485} - intercept)/slope]* V/S$

where, slope and intercepts were obtained from plotting standard solutions' absorbance at 485 nm against micrograms of TPF mL^{-1}, V is total volume (mL) of reagents and extractant added in to the soil sample plus moisture content in 5g of soil and S is dry weight of 5 g moist soil sample.

B.5 Analysis of humic acid in vermicompost and vermicompost tea

Humic acids from vermicompost and vermicompost tea were extracted using the classic alkali/acid fractionation procedure (Valdrighi *et al.*, 1996). Vermicompost or vermicompost tea was digested with 0.1 N KOH (1:10 wt:v or v:v) for 24 h at room temperature. The undigested bulk residue from each vermicompost tea was then separated

from the solute fraction by centrifugation at 2000 g for 30 minutes. Supernatant was vacuum filtered through a glass wool filter paper. The filtered supernatant was acidified at pH 2.0 with 6.0 N H_2SO4 and left settling for 24 h in the dark in order to allow humic acid flocculation. Humic acids were finally recovered by centrifuging at 2000 g for 30 minutes, washed 3 times with DI water to remove residual H_2SO_4 and freeze-dried using vacuum drier.

B.6 Analysis of phytohormones in compost tea

B.6.1 Chemicals and Calibration Curves

A number of compounds namely DPA, ABA-GE, PA, 7'- OH-ABA, neoPA, trans-ABA and IAA-Glu, were synthesized and prepared at the Plant Biotechnology Institute of the National Research Council of Canada (PBI-NRC Saskatoon, SK, Canada); ABA, IAA-Leu, IAA-Ala, IAA-Asp, IAA, Z, ZR, iPA and 2iP which were purchased from Sigma-Aldrich; dhZ, dhZR and Z-O-Glu which were purchased from Olchemim Ltd. (Olomouc, Czech Republic); and GAs 1, 3, 4, 7, 8, 9, 19, 20, 24, 29, 44, and 53 which were purchased from the Research School of Chemistry, Australian National University, Canberra, AU). Deuterated forms of the hormones which were used as internal standards include: d_3-DPA, d_5-ABA-GE, d_3-PA, d_4-7'-OH-ABA, d_3-neoPA, d_4-ABA, d_4-t-ABA, ^{13}C-IBA, d_3-IAA-Leu, d_3-IAA-Ala, d_3-IAA-Asp and d_3-IAA-Glu which were synthesized and prepared at PBI-NRC as described by Abrams *et al.* (2003) and Zaharia *et al.* (2005); d_5-IAA was purchased from Cambridge Isotope Laboratories (Andover, MA); d_3-dhZ, d_3-dhZR, d_5-Z-O-Glu, d_6-iPA and d_6-2iP were

purchased from Olchemim Ltd.; and d_2-GAs 1, 3, 4, 7, 8, 9, 19, 20, 24, 29, 44, and 53 were purchased from the Research School of Chemistry, Australian National University. The deuterated forms of selected hormones used as recovery standards, d_6-ABA and d_2-ABA-GE, were prepared and synthesized at PBI-NRC. Calibration curves were created for all compounds of interest. Quality control samples (QCs) were run along with the tissue samples.

B.6.2 Instruments

Analysis was performed on a UPLC/ESI-MS/MS utilizing a Waters ACQUITY UPLC system, equipped with a binary solvent delivery manager and a sample manager coupled to a Waters Micromass Quattro Premier XE quadrupole tandem mass spectrometer via a Z-spray interface. MassLynx™ and QuanLynx™ (Micromass, Manchester, UK) were used for data acquisition and data analysis.

B.6.3 Extraction and purification

A 100-µL aliquot containing all the internal standards, each at a concentration of 0.2 pg μL^{-1}, was added to approximately 50 mg (accurately weighed and recorded) of lyophilized compost tea samples; 3 ml of isopropanol: water:glacial acetic acid (80:19:1, v:v) was then added, and the samples were agitated in the dark for 24 h at 4 ºC. Samples were then centrifuged and the supernatant was isolated and dried on a Büchi Syncore Polyvap (Büchi, Switzerland). Samples were reconstituted in 100 µL acidified methanol, adjusted to 1 mL with acidified water, and then partitioned against 2 mL hexane. Acidified methanol or water is methanol or water with 1% glacial acetic acid

(pH acidic, approximately 3-5). After 30 minutes, the aqueous layer was isolated and dried as above. Dry samples were reconstituted in 800 µL acidified methanol and adjusted to 1 mL with acidified water. The reconstituted samples were passed through equilibrated Sep-Pak C18 cartridges (Waters, Mississauga, ON, Canada), the eluate being dried on a LABCONCO centrivap concentrator (Labconco Corporation, Kansas City, MO). An internal standard blank was prepared with 100 µL of the deuterated internal standards mixture. A QC (quality control) standard was prepared by adding 100 µL of a mixture containing all the analytes of interest, each at a concentration of 0.2 pg μL^{-1}, to100 µL of the internal standard mix. Finally, samples, blanks, and QCs were reconstituted in a solution of 40% methanol (v:v), containing 0.5% acetic acid and 0.1 pg μL^{-1} of each of the recovery standards.

B.6.4 Hormone quantification by HPLC-ESI-MS/MS

The procedure for quantification of multiple hormones and metabolites, including auxins (IAA, IAA-Asp and IAAGlu), abscisic acid and metabolites (ABA, PA, DPA, 7'-OH-ABA, neoPA and ABA-GE), cytokinins (2iP, iPA, Z, ZR, dhZ, dhZR and Z-O-Glu), and gibberellins (GAs 1, 3, 4, 7) has been described in detail by Chiwocha *et al.* (2003; 2005). Samples were injected onto an ACQUITY UPLC® HSS C18 SB column (2.1x100 mm, 1.8 µm) with an in-line filter and separated by a gradient elution of water containing 0.02% formic acid against an increasing percentage of a mixture of acetonitrile and methanol (volume ratio: 50:50). Briefly, the analysis utilizes the Multiple Reaction Monitoring (MRM) function of the MassLynx v4.1 (Waters Inc) control software. The resulting chromatographic traces are quantified off-line by the

QuanLynx v4.1 software (Waters Inc) wherein each trace is integrated and the resulting ratio of signals (non-deuterated/internal standard) is compared with a previously constructed calibration curve to yield the amount of analyte present (ng sample $^{-1}$). Calibration curves were generated from the MRM signals obtained from standard solutions based on the ratio of the chromatographic peak area for each analyte to that of the corresponding internal standard, as described by Ross *et al*. (2004). The QC samples, internal standard blanks and solvent blanks were also prepared and analyzed along each batch of tissue sample.

Quantifiable results are expressed in nanograms L $^{-1}$ of compost tea samples. If the signals were below limit of quantification (defined as signal/noise ratio of greater than or equal to 8), results are reported as present but non quantifiable (nq). If the values were below limit of quantification (with a signal/noise ratio less than 3), results are reported as non detectable (--).

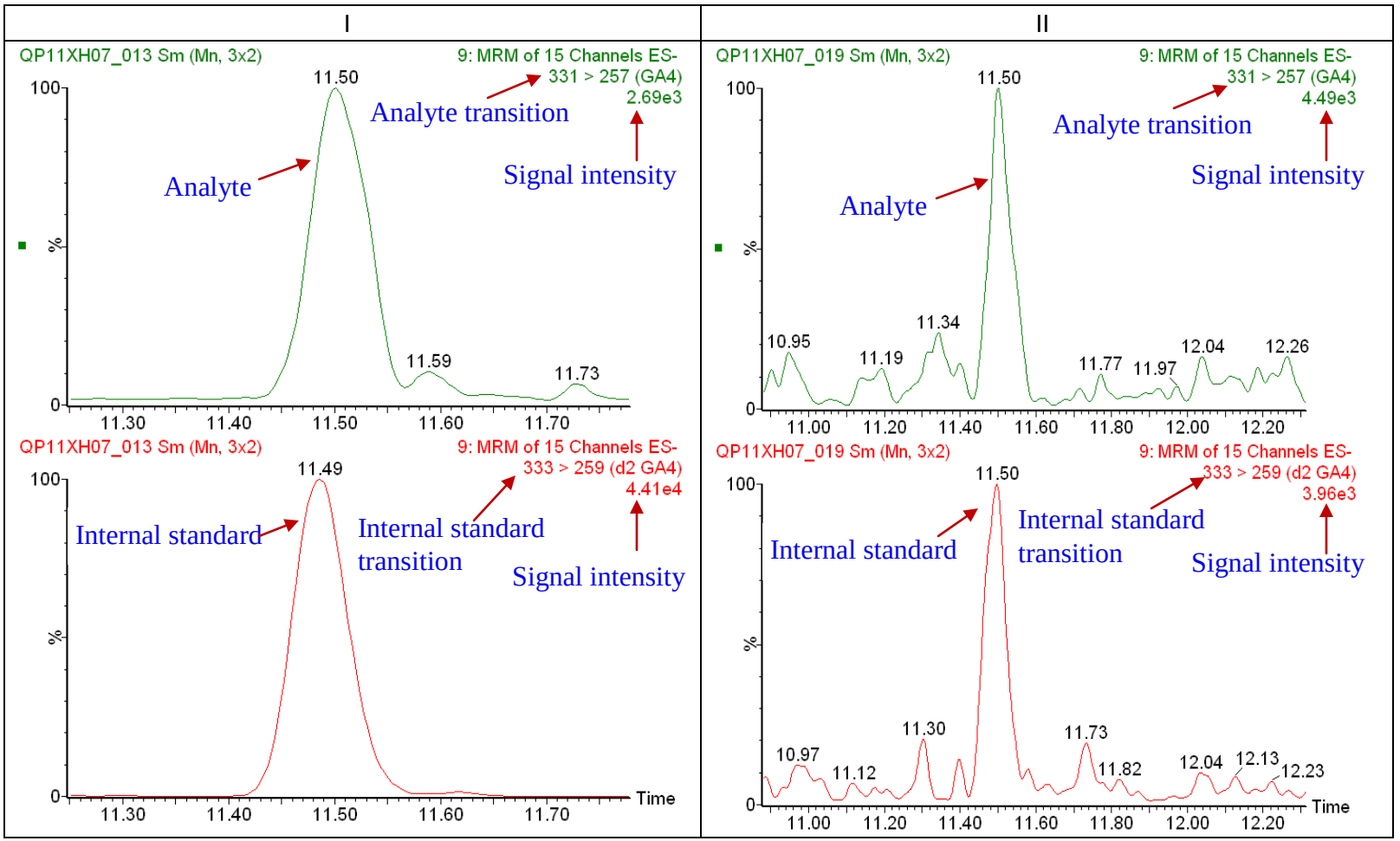


Figure B.1.A. HPLC chromatograms of GA_4 analysis in (I) aged chicken manure-based vermicompost tea (II) food waste vermicompost tea samples. The green curve represents peak of the sample and the red curve corresponding standard peak.

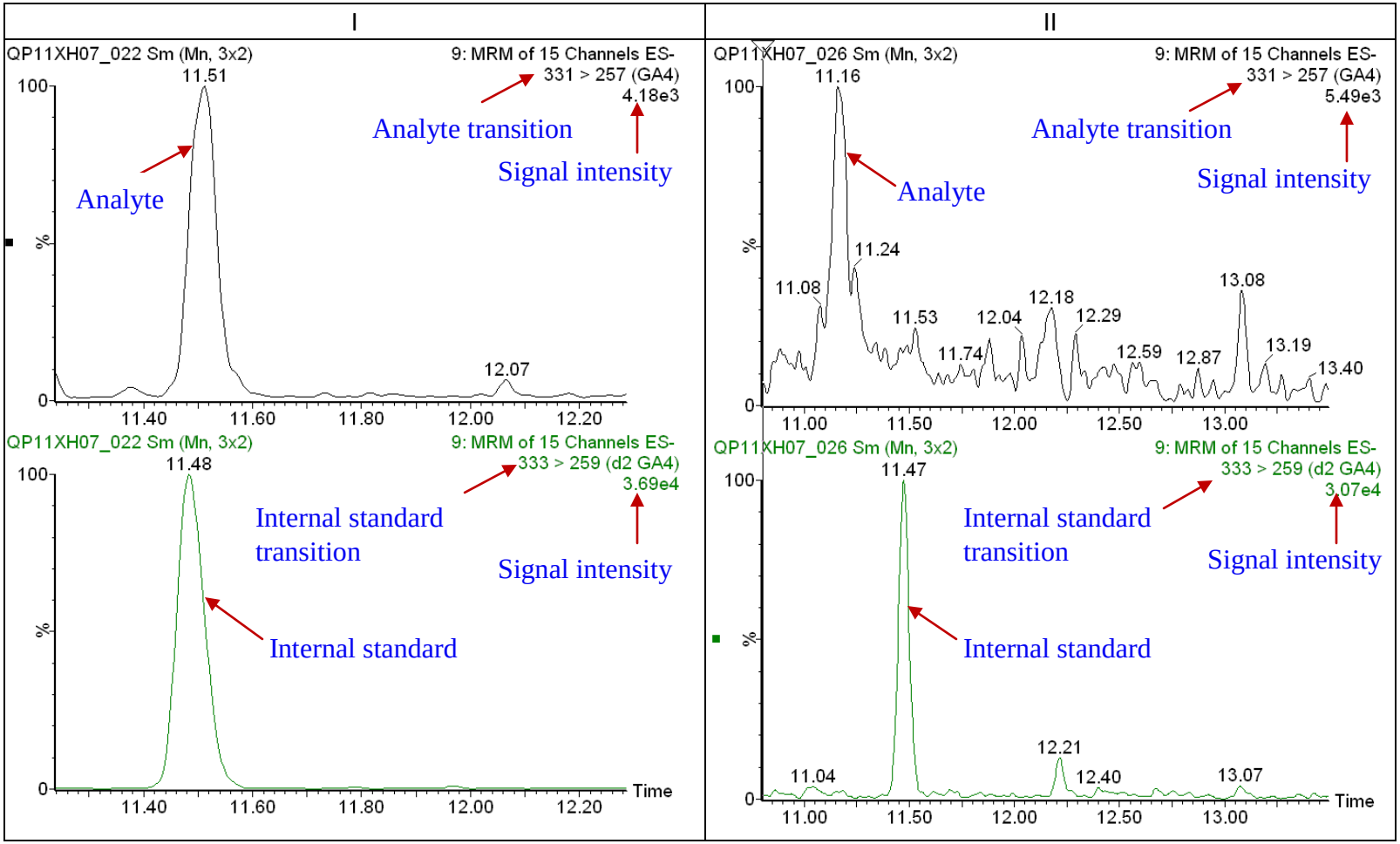


Figure B.1.B. HPLC chromatograms of GA_4 analysis in (I) chicken manure-based thermophilic compost tea (II) green waste thermophilic compost tea samples. The black curve represents the sample and the green curve corresponding standard peak.

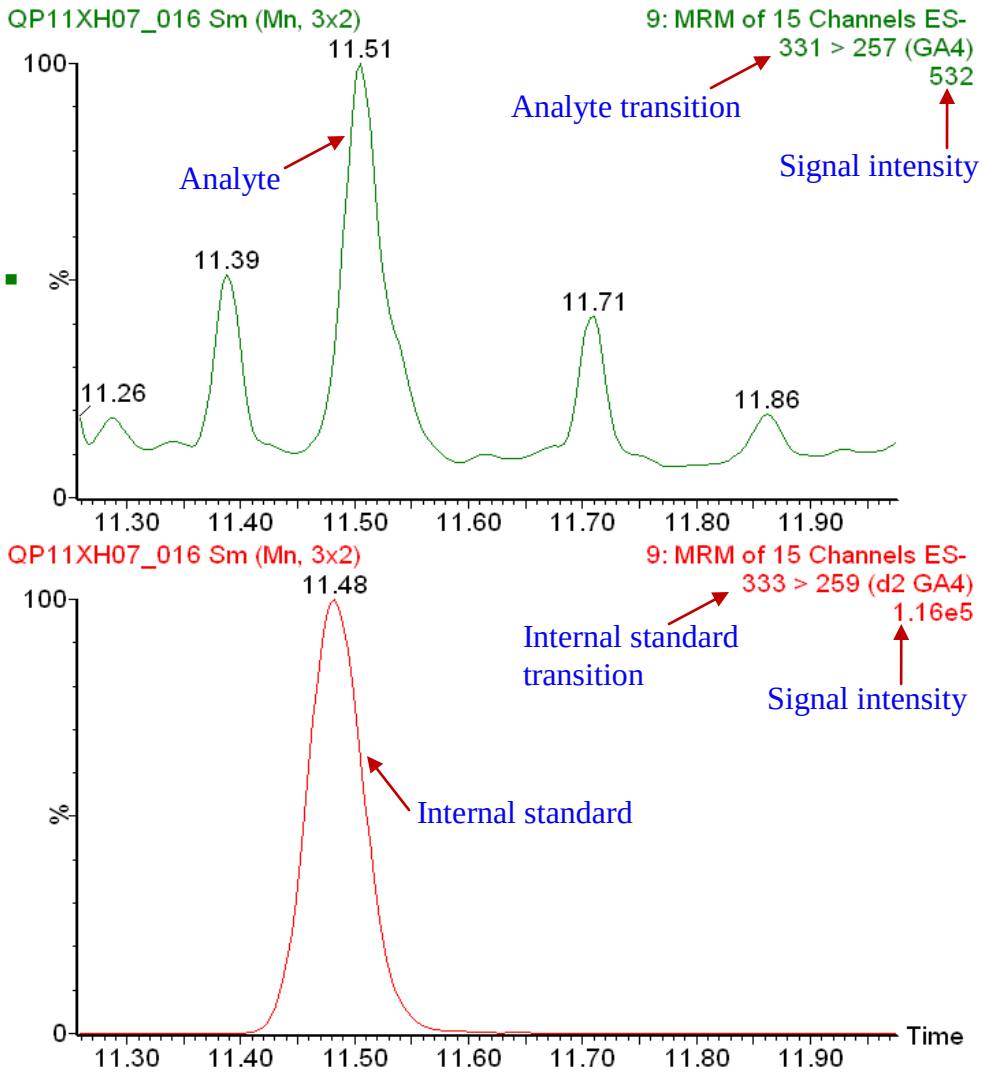


Figure B.1.C. HPLC chromatogram of GA_4 analysis in fresh chicken manure-based vermicompost tea sample. The green curve represents peak of the sample and the red curve corresponding standard peak.

APPENDIX C

COST BENEFITS ANALYSIS

Cost benefits analysis based on additional cost associated with vermicompost tea application on pak choi under chicken manure fertilization (field experiment, Chapter 5).

SN	Description	Unit	Total	Note
1	Vermicompost tea applied throughout the growth cycle	Liter acre $^{-1}$	9072.00	Based on amount of tea applied per unit area in this research
2	Total amount of vermicompost	Liter acre $^{-1}$	907.20	Based on 1:10 vermicompost to water ratio used in this research
3	Total amount of vermicompost	kg acre $^{-1}$	689.32	Based on bulk density of vermicompost (0.76 kg L $^{-1}$) used in this research
4	Total amount of vermicompost	lbs acre $^{-1}$	1516.50	@ 2.2 lbs kg $^{-1}$
5	Price of Vermicompost	US$ lb $^{-1}$	2.00	As invoiced by Waikiki Worm Company, HI
6	Total cost of vermicompost	US$ acre $^{-1}$	**3033.00**	**SN (4 x 5)**
7	Total compost tea preparation and application time	hrs acre $^{-1}$	50.00	Based on time required per unit area in this research
8	Agricultural labor wages rate of Hawaii	US$ hr $^{-1}$	13.50	As of February 28 2011, USDA
9	Total cost of application	US$ acre $^{-1}$	**675.00**	**SN (7 x 8)**
10	Other cost associated with tea production	US$ acre $^{-1}$	**150.00**	Lump Sum (energy, brewer etc.)
11	Total additional cost associated with compost tea application	US$ acre $^{-1}$	**3858.00**	**SN (6 + 9 + 10)**
12	Pak choi production with tea	lb acre $^{-1}$	18563.60	Based on yield obtained in this research
13	Pak choi production without tea	lb acre $^{-1}$	11994.20	Based on yield obtained in this research
14	Increased production	lb acre $^{-1}$	**6569.40**	**SN (12 – 13)**
15	Farm gate price of organic pak choi	US$ lb $^{-1}$	**1.25**	Personal communication with an organic grower
16	Additional income	US$ acre $^{-1}$	**8211.75**	**SN (14 x 15)**
17	**Net Profit from tea application**	US$ acre $^{-1}$	**4353.75**	**SN (16 – 11)**

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