Research Article

Received: 11 March 2009 Revised: 22 June 2009 Accepted: 25 June 2009 Published online in Wiley Interscience:

(www.interscience.wiley.com) DOI 10.1002/jsfa.3732

Vermicompost extracts influence growth, mineral nutrients, phytonutrients and antioxidant activity in pak choi (*Brassica rapa* cv. Bonsai, Chinensis group) grown under vermicompost and chemical fertiliser

Archana P Pant,[a] Theodore JK Radovich,[a]* Ngyuen V Hue,[a] Stephen T Talcott[b] and Kristen A Krenek[b]

Abstract

BACKGROUND: Multiple studies have been 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 tea on yield and nutritional quality of vegetable crops. In this study, experiments were conducted to determine the effect of extraction method on vermicompost tea quality and subsequent effects on growth, mineral nutrients, phytonutrients and antioxidant activity of pak choi plants grown under organic (vermicompost) and synthetic (Osmocote) fertilisation. Three vermicompost teas obtained by different extraction methods, namely non-aerated vermicompost tea (NCT), aerated vermicompost tea (ACT) and aerated vermicompost tea augmented with microbial enhancer (ACTME), were applied to the plants. Aerated water served as control.

RESULTS: Mineral nutrients were significantly higher in ACTME compared with other teas, but total microbial population and activity did not differ with extraction method. All vermicompost teas similarly enhanced plant production, mineral nutrients and total carotenoids, and this effect was most prominent under organic fertilisation. Antioxidant activity and total phenolics were higher under organic compared with synthetic fertilisation. Vermicompost teas generally decreased phenolics under organic fertilisation and increased them under synthetic fertilisation compared with the control.

CONCLUSION: The effect of vermicompost tea on crop growth is largely attributable to mineral nutrient, particularly N, uptake by plants. Non-significant differences among extraction methods on plant response within fertiliser regimes suggest that aeration and additives are not necessary for growth promotion and nutrient quality under the conditions reported here.

Keywords: vermicompost; phytonutrient; vegetable; quality

INTRODUCTION

Aqueous extracts of vermicompost (vermicompost teas) applied as a foliar spray or soil drench have been demonstrated to improve plant health, yield and nutritive quality by (i) enhancing beneficial microbial communities and their effects on agricultural soils and plants, (ii) improving the mineral nutrient status of plants and (iii) inducing the production of plant defence compounds that have beneficial bioactivities in humans.[1–6] Although the chemistry and microbiology of vermicompost extract are complex, it is believed that soluble mineral nutrients extracted from vermicompost will have a positive effect on plant growth with foliar and soil applications of vermicompost extract.[4] It is also postulated that the action of living micro-organisms and microbial metabolites will stimulate plant growth.[1,2] Water-extractable growth regulators or phytohormones extracted from vermicompost may also have a positive effect on initial root development and plant growth.[7,8]

Several studies have shown a positive 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.[3,9–15] However, little work has been done to investigate the effect of vermicompost tea on yield and nutritional quality of vegetable crops. Even fewer studies have addressed the links between extraction conditions, chemical and biological characteristics of vermicompost tea and subsequent plant growth and yield.

Vermicompost tea may be extracted under aerated or non-aerated (passive) conditions. During aerated extraction, air is

* *Correspondence to: Theodore JK Radovich, 3190 Maile Way, St John 102, Honolulu, HI 96822, USA. E-mail: theodore@hawaii.edu*

a *Department of Tropical Plant and Soil Science, University of Hawaii, Honolulu, HI, USA*

b *Department of Nutrition and Food Science, Texas A&M University, College Station, TX, USA*

pumped through water containing vermicompost to maintain the oxygen level above 5 mg L^{-1}.[4] Sugar, grain, fish emulsion, kelp extract, humic acid and other products are often incorporated as additives during extraction of aerated tea to enhance microbial activity of the finished product, but little work regarding the impact of these additives on tea quality or plant response has been reported. For passive extraction, vermicompost is placed in a certain volume of water and allowed to sit for several days, with occasional stirring.[6] Several investigators have reported that non-aerated compost tea has a consistent and significant positive effect on disease control and plant growth compared with aerated compost tea, while other works suggest that non-aerated compost teas can be inconsistent in quality, may cause phytotoxicity and are generally less preferable than aerated compost teas.[6,12,13,16,17] Increased crop yield and dietary antioxidants of broccoli with the use of compost and non-aerated compost tea have been reported.[18] Non-aerated compost tea has been reported to be as effective as compost and inorganic fertiliser in promoting growth of strawberry plants.[19] Based on a two-year field experiment, Welke[20] concluded that both aerated and non-aerated compost teas extracted from composted animal manure for 7 days had positive and similar effects on strawberry yield and suppression of *Botrytis cinerea*. In contrast, Arancon *et al*.[21] reported that aerated vermicompost tea had a more positive impact on plant growth than non-aerated tea extracted for the same period of time (24 h).

We hypothesise that compost tea extraction method and fertiliser 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 individual and interactive effects of vermicompost tea type and fertiliser regime (vermicompost and Osmocote) on plant yield, mineral nutrient content, phytonutrient content and antioxidant activity.

MATERIALS AND METHODS

Experimental set-up and design

A leafy green pak choi (*Brassica rapa* cv. Bonsai, Chinensis group) was selected as test crop for the experiment. This fast-growing vegetable has tender green leaves and crispy green petioles. A member of the *Brassicaceae*, pak choi is rich in vitamins A and C and folic acid.[22] Two greenhouse experiments were conducted in January–February and April–May 2008. Pak choi plants were grown under both organic (chicken manure vermicompost) and chemical (Osmocote, 14:14:14 N-P-K, Scotts-Sierra Horticultural products Company, Marysville, OH, USA.) fertilisation at a rate of 135 kg N ha^{-1}. Plants were grown in tree tubes (volume 148 mL) in the first experiment and in garden pots (volume 865 mL) in the second experiment. Three or four pak choi seeds were sown in each tube/pot. Two days after seedling emergence, plants were thinned to one plant per tube/pot. Plants were allowed to grow in the greenhouse on a bench fitted with overhead sprinklers that operated for 5 min every 6 h. Three types of vermicompost tea obtained by different extraction methods as well as the same amount of aerated water (control) were applied weekly to the root zone and foliage of plants at a rate of 25 mL per tree tube or 150 mL per pot for 4 weeks, starting 5 days after seedling emergence. The greenhouse experiments were arranged in a completely randomised design with 2 × 4 factorial treatments and ten replications per treatment.

Vermicompost tea extraction method

The chicken manure vermicompost used in the study was obtained from Waikiki Worm Company (Honolulu, HI, USA). Vermicompost teas were prepared using three different extraction methods, which 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 control. NCT was prepared by the method of Weltzein.[6] Vermicompost and tap water were mixed in the ratio 1 : 10 (w/v) in a 19 L plastic bucket. The mixture was left open for 7 days at 20–21 °C and stirred once on day 4 of preparation. The resulting NCT was filtered through a nylon membrane just before application. ACT was prepared using vermicompost and tap water in the ratio 1 : 10 (w/v) in a 19 L plastic bucket and aerated using a commercial compost tea system constructed of coiled polyvinyl chloride (PVC) tubing attached to an air pump (Keep It Simple Inc., Redmond, WA, USA). The aerator of the KIS brewer was pressed securely to the bottom of the bucket. Tap water (11 L) and vermicompost (1.1 kg) were placed in the bucket, covered and aerated continuously for 12 h as per the manufacturer's instructions. The resulting ACT was filtered through a nylon membrane just before application. ACTME was produced in the same way as ACT but with the addition of 11.74 g of dry humic acid and 32.4 g of kelp extract to the vermicompost/water mixture before extraction to enhance microbial growth. The amounts of humic acid and kelp extract (kg^{-1} vermicompost) used were calculated on the basis of the manufacturer's recommendations and published reports.[4]

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

The pH and electrical conductivity (EC) of vermicompost were measured in a 1 : 1 (v/v) deionised water/vermicompost mixture using a sympHony SB80PC conductivity/pH meter(VWR Scientific Products, St Paul, MN, USA).[23] The pH and EC of humic acid and kelp extract were measured in the same way. The pH and EC of vermicompost teas were also determined with the same instrument, while dissolved oxygen (DO) was measured at 21–22 °C using a thermo sympHony SP70D dissolved oxygen meter (VWR Scientific Products). NH_4-N and NO_3-N were extracted from fresh vermicompost with 2 mol L^{-1} KCl and measured colorimetrically using an Easy Chem Plus discrete analyser (Systea Scientific, Oak Brook, IL, USA). Total C and N in vermicompost were analysed by dry combustion in a LECO CN-2000 analyser (Leco Corp., St Joseph, MI, USA). Other nutrients in vermicompost were measured after wet acid digestion[24] using an inductively coupled plasma (ICP) spectrophotometer (Jarrel-Ash Division/Fisher Scientific Co., Waltham, MA, USA). Nutrient levels in humic acid and kelp extract were analysed in the same way. Total N, NH_4-N, NO_3-N and NO_2-N in vermicompost teas were analysed colorimetrically using an Easy Chem Plus discrete analyser (Systea Scientific). Other nutrients in vermicompost teas were measured using the above-mentioned ICP procedure.

Microbial analysis of vermicompost and vermicompost tea

The microbial composition of vermicompost and vermicompost teas was analysed in three separate samples from each extraction method. Samples were taken by filtering the vermicompost tea through a nylon membrane immediately after 12 h of brewing for ACT and ACTME and immediately after 7 days of steeping for NCT. A tenfold serial dilution of each sample was prepared.

Active bacteria and fungi were assessed using a 1 : 10 dilution under epifluorescence microscopy at 40× and 20× objective, respectively (Olympus America Inc., Melville, NY, USA).[25] The same dilution and slide as used to evaluate active fungi were used to quantify total fungal biomass under differential interference contrast (DIC) microscopy at 20× objective (Olympus America Inc., Melville, NY, USA). A 1 : 100 dilution of liquid samples was used to assess total bacteria under oil immersion epifluorescence at 100× objective. Fluorescein isothiocyanate staining was used to quantify total bacteria, while fluorescein dicompost extractate staining was used to assess active bacteria and fungi.[26] A 1 : 100 dilution was used for all analyses except that of total bacteria in vermicompost, for which a 1 : 1000 dilution was used.

Data collection

Plants were harvested 5 weeks after emergence. Leaf numbers of each plant were counted. Plant height and fresh weight were measured using an Adventurer SL AS811 electronic scale (Ohaus Corporation, Pine Brook, NJ, USA) and leaf area was measured using a CI-202 portable leaf area meter (CID Bio-Science, Inc., Camas, WA, USA). Plants were immediately frozen in liquid nitrogen, stored at −20 °C and freeze-dried using a D4A lyophiliser (Leybold-Heraeus Vacuum Products, Inc., Monroeville, PA, USA). The dry weight of each plant was recorded, after which the dry material was ground with a mortar and pestle and stored in an airtight container pending further analysis.

Measurement of phytonutrients and antioxidant activities

Phytochemicals and antioxidants were analysed in ten lyophilised samples of each treatment from each trial by extracting 25–50 mg samples with 10 mL of ethanol/acetone (1 : 1 v/v) in glass vials. All data reported are based on the dry weight of lyophilised samples. Extraction was aided by a sonic water bath for 1 h, soaking for 12 h and sonication for an additional 30 min. Following centrifugation and filtration through a 0.45 µm polytetrafluoroethylene (PTFE) filter (Fisher Scientific, Pittsburgh, PA, USA.), supernatants were evaluated for total carotenoids at 470 nm using a Helios Gamma spectrophotometer (Fisher Scientific, Pittsburgh, PA, USA.). Total carotenoids were calculated according to the equation of Gross:[27] total carotenoids (mg kg^{-1}) = $(AV \times 10^6)/(A^{1\%} \times 100G)$, where A is the absorbance, V is the total volume (mL) of the extract, $A^{1\%}$ is the extinction coefficient (2500) and G is the sample weight (g). Total soluble phenolics were measured by the Folin–Ciocalteu assay according to Swain and Hillis[28] and are reported as mg gallic acid equivalent (GAE) kg^{-1}. Antioxidant capacity was measured by the oxygen radical absorbance capacity (ORAC) assay using a FLUOstar Optima fluorescent 96-well microplate reader (BMG Labtech, Durham, NC, USA) at an excitation wavelength of 485 nm and an emission wavelength of 538 nm.[29] Aliquots of solvent from the original extracts were evaporated in a 50 °C water bath and the compounds were redissolved in water with the aid of a sonicator to give a tenfold concentration. The concentrated extracts were evaluated against a standard curve of Trolox. Antioxidant capacities are reported in µmol Trolox equivalent (TE) g^{-1}.

Measurement of mineral nutrients in plant tissue

Composite samples of dried tissue were subsampled to three of each treatment from each trial and analysed for mineral nutrients. Total C and N in dried tissue samples were analysed by dry combustion in a LECO CN-2000 analyser (Leco Corp.). Other nutrients in tissue samples were measured after wet acid digestion[24] using an ICP spectrophotometer (Jarrel-Ash Division/Fisher Scientific Co.).

Statistical analysis

Analysis of variance (ANOVA) of growth parameters, mineral nutrients and phytonutrients in plant tissue was performed on treatments and interactions, and means were separated by Duncan's multiple range test, using SAS 9.1 statistical software.[30] Statistical significance was obtained at 95% confidence level ($\alpha = 0.05$).

RESULTS

Chemical properties of vermicompost and vermicompost tea

The chemical properties of the vermicompost and additives used in this study are presented in Tables 1 and 2. The pH of vermicompost was near neutral and its EC was 3.7 dS m^{-1}, with a C/N ratio of 13 : 1. The pH and EC of humic acid and kelp extract were higher. The pH, EC, DO (Table 3) and extractable nutrients (Tables 4 and 5) of vermicompost tea varied with extraction method. The average

Table 1. Chemical properties of vermicompost (mean ± standard error, $n = 3$)

			mg g^{-1}								µg g^{-1}				
pH	EC (dS m^{-1})	Moisture	N	NO_3-N	C	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu	B
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

EC, electrical conductivity.

Table 2. Chemical properties of kelp extract and humic acid ($n = 1$)

			mg g^{-1}									µg g^{-1}				
Material	pH	EC (dS m^{-1})	N	NO_3-N	NH_4-N	NO_2-N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu	B
Kelp extract	9.34	14.22	12.7	6.0	1.9	0.06	0.1	190.8	2.5	3.3	40.6	42	2	16	4	83
Humic acid	10.05	16.34	11.5	1.9	8.9	0.02	0.0	21.3	7.7	0.9	76.4	1850	171	6	5	81

EC, electrical conductivity.

Table 3. Dissolved oxygen (DO), electrical conductivity (EC) and pH of vermicompost tea across extraction methods at application (mean ± standard error, $n = 8$)

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	2.5 ± 0.1	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

NCT, non-aerated vermicompost tea; ACTME, aerated vermicompost tea with microbial enhancer; ACT, aerated vermicompost tea; control, water.

Table 5. Micronutrient content (μg L^{-1}) in vermicompost tea across extraction methods (mean ± standard error, $n = 8$)

Extraction method	Fe	Mn	Zn	Cu	B
NCT	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.3 ± 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	0.3 ± 0
Control	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

NCT, non-aerated vermicompost tea; ACTME, aerated vermicompost tea with microbial enhancer; ACT, aerated vermicompost tea; control, water.

pH values of ACTME and the control were significantly higher than those of ACT and NCT. However, the pH values of ACT and NCT were not significantly different. The DO level in vermicompost tea was influenced by the use of microbial enhancer during production. The DO level in ACTME was significantly lower than those in ACT and NCT. DO levels in ACT and NCT were not significantly different. EC was highest in ACTME and lowest in the control, with no significant difference between ACT and NCT in all trials. Chemical analysis of humic acid and kelp extract showed that the use of these additives added about 48, 19 and 15 mg L^{-1} of total N, NO_3-N and NH_4-N respectively to ACTME. This is reflected in higher levels of total N, NO_3-N and NH_4-N in ACTME than in ACT and NCT. NO_3-N content in ACTME was about 33 and 22% higher than that in NCT and ACT respectively. Similarly, NH_4-N content in ACTME was about 13 and 16 times higher than that in NCT and ACT respectively. P content in vermicompost tea was not influenced by production method. K content was significantly higher in ACTME than in ACT and NCT. Although the concentrations of N and K in vermicompost were lower than that of P, the concentrations of both N and K in vermicompost tea were higher than that of P irrespective of extraction method. Other extractable nutrients were significantly higher in ACTME than in ACT and NCT, while secondary nutrient and micronutrient contents were comparable between ACT and NCT.

Microbial population in vermicompost and vermicompost tea

The microbial populations in vermicompost and vermicompost tea are presented in Table 6. The total bacterial populations in vermicompost tea and the control were not significantly different. However, the population of active bacteria was significantly higher in all types of vermicompost tea compared with the control. Both total and active fungal populations were higher in all types of vermicompost tea compared with the control. There was no significant difference in total and active biology among extraction methods.

Effect of vermicompost tea on plant growth

The effect of vermicompost tea on plant growth is presented in Table 7. All types of vermicompost tea increased above-ground fresh weight significantly compared with the control across fertiliser regimes. However, there was no significant difference in above-ground fresh weight among the three treatments receiving vermicompost tea. Average above-ground fresh weight was significantly higher with Osmocote than with vermicompost. The interaction between vermicompost tea and fertiliser was not significant on above-ground fresh weight. The effect of tea type, fertiliser type and their interaction on above-ground dry weight was similar to that on fresh weight. The interaction between tea type and fertiliser type on plant height was significant. Vermicompost tea had a greater effect on plant height under vermicompost fertilisation and a much smaller effect on plant height under Osmocote fertilisation. Leaf numbers increased significantly in vermicompost tea-treated plants compared with control plants across fertiliser regimes. However, plant height and leaf number were not influenced by vermicompost tea extraction method. Average leaf number was significantly higher with Osmocote than with vermicompost. Leaf area increased significantly in vermicompost tea-treated plants compared with control plants across fertiliser regimes. The effect of vermicompost tea extraction method on leaf area was not significant.

Effect of vermicompost tea on mineral nutrient content of plant

The effect of vermicompost tea on mineral nutrient uptake per plant is presented in Tables 8 and 9. All types of vermicompost tea consistently increased total N content per plant relative to above-ground dry weight (Fig. 1) under both fertiliser regimes. Total P and K contents per plant were also higher in vermicompost tea-treated plants compared with control plants across fertiliser regimes. The effect of vermicompost tea extraction method on

Table 4. Macronutrient content (mg L^{-1}) in vermicompost tea across extraction methods (mean ± standard error, $n = 8$)

Extraction method	N	NO_3-N	NH_4-N	NO_2-N	P	K	Ca	Mg	Na
NCT	74.9 ± 4.6	73.3 ± 4.5	0.6 ± 0.2	0.3 ± 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	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	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	0.2 ± 0.1	5.61 ± 1.3	12.4 ± 0.3	15.3 ± 0.2	65.6 ± 1.6

NCT, non-aerated vermicompost tea; ACTME, aerated vermicompost tea with microbial enhancer; ACT, aerated vermicompost tea; control, water.

Table 6. Microbial population in vermicompost and vermicompost tea (mean ± standard error, $n = 3$)

Vermicompost/extraction method	Log_{10} active bacteria (cells g^{-1} DW or cells mL^{-1})	Log_{10} total bacteria (cells g^{-1} DW or cells mL^{-1})	Active fungi (μg g^{-1} DW or μg mL^{-1})	Total fungi (μg g^{-1} DW or μg mL^{-1})
Vermicompost	8.1 ± 0.1	9.9 ± 0.1	14.3 ± 1.9	634.9 ± 135.2
NCT	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
ACT	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

DW, dry weight; NCT, non-aerated vermicompost tea; ACTME, aerated vermicompost tea with microbial enhancer; ACT, aerated vermicompost tea; control, water.

Table 7. Effect of vermicompost tea on plant growth (mean ± standard error, $n = 20$)

Extraction method	Above-ground fresh weight (g per plant)	Above-ground dry weight (g per plant)	Plant height (cm)	Leaf area (cm^2 per plant)	Leaf number (per plant)
Vermicompost					
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	9.7 ± 0.6
Control	2.3 ± 0.7	0.2 ± 0.1	7.0 ± 0.6	63.0 ± 15.9	6.0 ± 0.4
Osmocote					
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

NCT, non-aerated vermicompost tea; ACTME, aerated vermicompost tea with microbial enhancer; ACT, aerated vermicompost tea; control, water.

total N, P and K contents per plant was not significantly different across fertiliser regimes. Similarly, there was a significant positive effect of vermicompost tea on Ca, Mg and S contents in plant tissue irrespective of extraction method under both vermicompost and Osmocote fertilisation. Except for Fe, all other micronutrients in plant tissue were significantly increased by application of vermicompost tea irrespective of vermicompost tea extraction method and fertiliser regime. The effect of vermicompost tea on Fe content in plant tissue depended on fertiliser type. NCT had a significantly greater effect on Fe content in plant tissue compared with other treatments, including the control, under both fertiliser regimes. The effects of ACT, ACTME and the control on Fe content in plant tissue were not significantly different under vermicompost fertilisation. After NCT, the effect of ACT on Fe content was significantly higher than that of the control and ACTME under Osmocote fertilisation. Fe content was lowest in plants treated with ACTME among all treatments under Osmocote fertilisation.

Effect of vermicompost tea on phytonutrients

The effect of vermicompost tea on phytonutrients is presented in Table 10. Vermicompost tea increased total carotenoids compared with the control across fertiliser regimes, most notably under

Table 8. Effect of vermicompost tea on macronutrient content (mg per plant) in plant tissue across fertiliser regimes (mean ± standard error, $n = 6$)

Extraction method	N	P	K	Ca	Mg	S	Na
Vermicompost							
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							
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

NCT, non-aerated vermicompost tea; ACTME, aerated vermicompost tea with microbial enhancer; ACT, aerated vermicompost tea; control, water.

Table 9. Effect of vermicompost tea on micronutrient content (μg per plant) in plant tissue across fertiliser regimes (mean ± standard error, $n = 6$)

Extraction Method	Fe	Mn	Zn	Cu	B
Vermicompost					
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					
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

NCT, non-aerated vermicompost tea; ACTME, aerated vermicompost tea with microbial enhancer; ACT, aerated vermicompost tea; control, water.

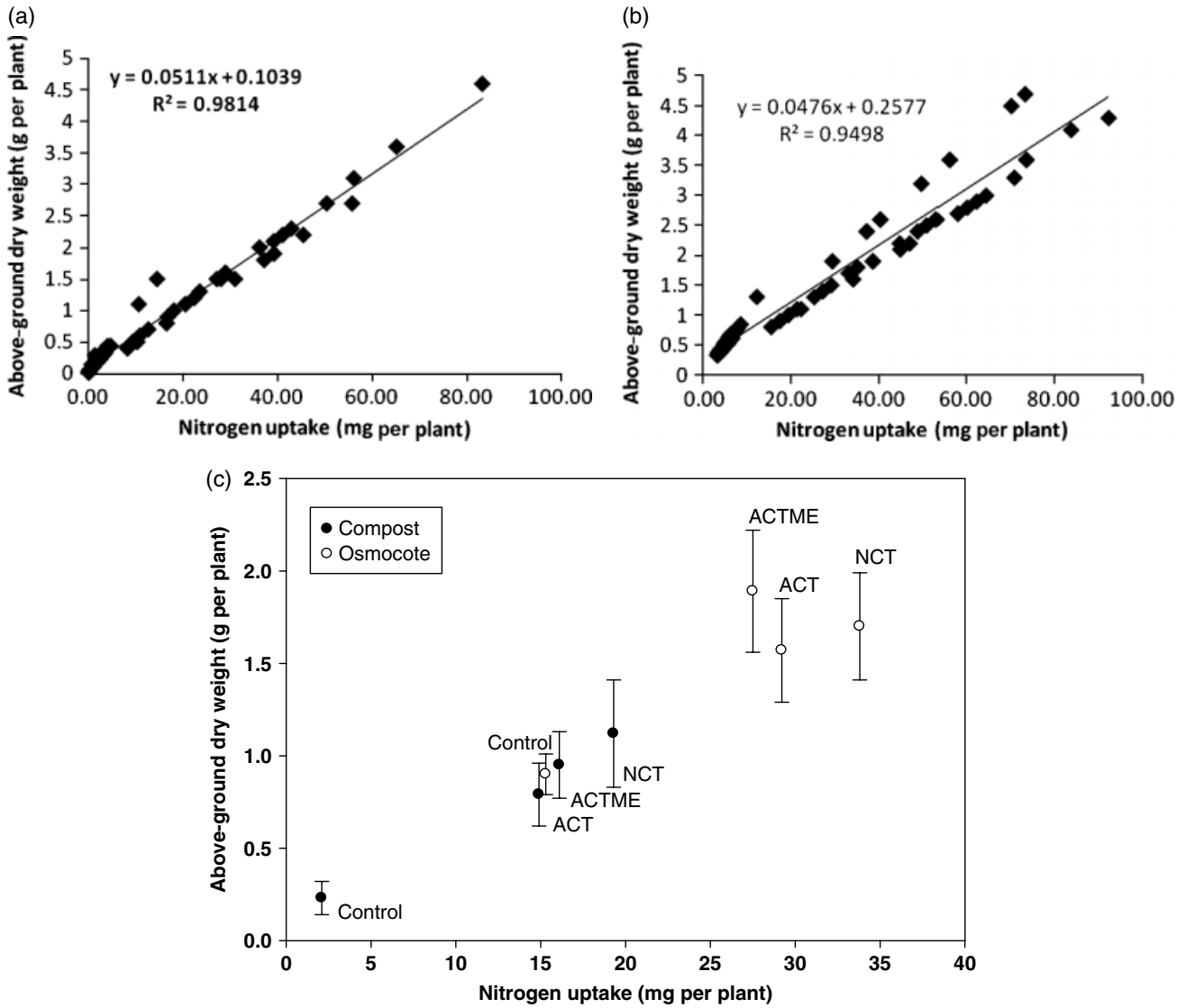

Figure 1. Above-ground dry weight relative to N uptake (a) under vermicompost ($n = 80$), (b) under Osmocote ($n = 80$) and (c) across treatments ($n = 20$). Treatments: NCT, non-aerated vermicompost tea; ACTME, aerated vermicompost tea with microbial enhancer; ACT, aerated vermicompost tea; control, water.

Table 10. Effect of vermicompost tea on total carotenoids, total phenolics and antioxidant activity (mean ± standard error, $n = 20$)

Extraction method	Total carotenoids (mg kg^{-1})	Total phenolics (mg GAE kg^{-1})	ORAC (μmol TE g^{-1})
Vermicompost			
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[a]	305.2 ± 72.5	3398.0 ± 436.6	341.7 ± 118.4
Osmocote			
NCT	548.78 ± 45.69	2097.99 ± 97.56	171.24 ± 11.93
ACTME	517.34 ± 30.86	2363.55 ± 173.20	214.70 ± 18.73
ACT	566.87 ± 53.92	2433.80 ± 188.80	238.87 ± 15.96
Control	477.65 ± 38.16	1999.58 ± 147.33	223.34 ± 14.85

GAE, gallic acid equivalent; ORAC, oxygen radical absorbance capacity; TE, Trolox equivalent; NCT, non-aerated vermicompost tea; ACTME, aerated vermicompost tea with microbial enhancer; ACT, aerated vermicompost tea; control, water.
[a] The number of measures was 11 for total carotenoids, total phenolics and antioxidant activity of vermicompost control, which was generated from one composite sample from ten plants of trial 1 and ten individual plant samples from trial 2 owing to the low amount of plant materials available from trial 1 for analysis.

vermicompost fertiliser where tea-treated plants had 72% higher carotenoid content than control plants. However, tea-treated plants grown under Osmocote fertiliser had about 14% higher carotenoid content than control plants. There was also a significant interactive effect of vermicompost tea and fertiliser on total phenolics. The effects of ACTME and ACT on total phenolics were significantly higher under Osmocote fertilisation, while the effect of NCT was intermediate. In contrast, there was a very limited effect of vermicompost tea on total phenolics under vermicompost fertilisation. The treatment effect on ORAC was not significant under Osmocote fertilisation for all types of vermicompost tea except NCT. Plants treated with NCT appeared to have low ORAC under Osmocote fertilisation. The effect of all vermicompost teas on antioxidant activity was lower than that of the control under vermicompost fertilisation.

DISCUSSION

The use of kelp extract and humic acid resulted in higher EC and nutrients and lower DO in ACTME. The higher total N, NO_3-N and NH_4-N in ACTME relative to other extracts (by about 30, 20 and 9 mg L^{-1} respectively) can be accounted for by that supplied by the additives (Tables 1–3). Microbial activity is the most frequent explanation for reduced DO levels during tea extraction.[4] Although total and active microbial populations in ACTME were not significantly different from those in ACT and NCT, it is possible that the additives did increase microbial populations in ACTME but that some microbes remained bound to vermicompost particles that were removed during filtering. The chemical reduction of humic acid during extraction is also a possible explanation of the reduced DO in ACTME without a corresponding increase in microbial activity. However, laboratory tests run concurrently with the experiments reported here showed no decrease in DO when humic acid was aerated in water without vermicompost (data not shown). Total N concentration in vermicompost was lower than P concentration; however, the concentration of total N in vermicompost tea was significantly higher than that of P irrespective of extraction method. The higher level of K in ACTME compared with ACT and NCT can be attributed to the use of kelp extract as microbial enhancer.

Vermicompost tea consistently enhanced plant growth and mineral nutrient concentration in plant tissue under both fertiliser regimes, in accordance with the findings of previous studies.[18,31] Although above-ground fresh and dry weights were higher under Osmocote fertilisation compared with vermicompost fertilisation, the effect of vermicompost tea was most pronounced under vermicompost fertilisation. Soluble mineral nutrients and microbial by-products in vermicompost tea can enhance nutrient uptake from the soil and increase foliar uptake of nutrients.[4,32] Nutrient analysis indicated that vermicompost tea supplied a considerable amount of soluble mineral nutrients to plants compared with the control (Tables 4 and 5). The strong correlation between above-ground dry weight and nitrogen uptake by plants explains the yield response to vermicompost tea across treatments (Fig. 1). However, tissue N concentration on a dry matter basis was about 17.3 mg g^{-1}, which is below the critical level of 32 mg g^{-1} reported for cabbage.[33] Increased above-ground fresh and dry weights, leaf area and extractable mineral element concentration in plant tissue as a result of vermicompost tea treatment were observed in this study. Keeling *et al.*[8] reported that applying vermicompost tea to oilseed rape plants at the initial stage of growth increased both root development and plant growth. Siddiqui *et al.*[34] observed that compost tea enhanced plant growth and increased tap root length of okra. Although our experiments did not measure root growth, the better nutrient uptake by vermicompost tea-treated plants compared with control plants suggests that improved root growth or nutrient uptake per unit root might be one of the mechanisms involved in stimulating plant growth. Arancon *et al.*[21] reported that humic, fulvic and other organic acids extracted or produced by micro-organisms in vermicompost tea could induce plant growth. Garcia Martinez *et al.*[35] found that compost aqueous extract contained compounds with molecular structure and biological activity analogous to auxins. Leachate from well-decomposed compost has been shown to contain cytokinin-like substances derived from the hydrolysis of glucosides by the enzyme β-glucosidase produced by microbes.[36] Although phytohormones or growth regulators in vermicompost tea were not measured in this study, we suggest that they may play a greater role in plant response.

Plant growth was not influenced by extraction method. Although there were higher levels of total N, K and other secondary nutrients and micronutrients present in ACTME compared with ACT and NCT, the effect of ACTME on plant growth was not significantly different from that of ACT and NCT under both fertiliser regimes. In fact, micronutrient uptake in ACTME plants was lower than that in other plants (Table 9) despite the higher levels of Fe, Mn and other micronutrients in ACTME (Table 5). This discrepancy may be explained by the higher pH in ACTME reducing the solubility of Fe and other metals. Also, the higher levels of total N, K and micronutrients present in ACTME compared with other teas are due to the contribution of humic acid and kelp extract (Table 2) and may not be available for plant uptake. Ingham[37] noted that aerated compost tea augmented with microbial enhancer gave a better result by increasing the microbial population in the tea. Some workers have suggested that increased microbial population in compost tea will increase microbial activity in plants and the

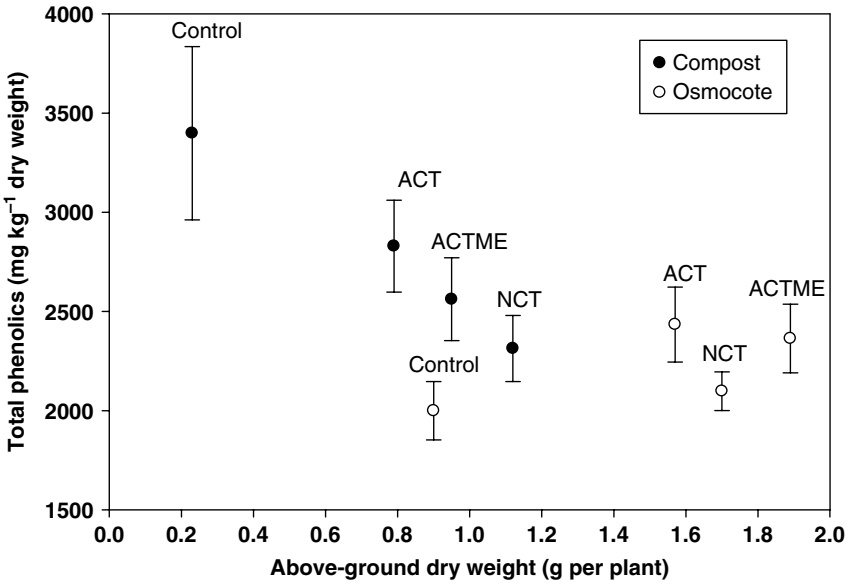

Figure 2. Total phenolics relative to above-ground dry weight across treatments. Treatments: NCT, non-aerated vermicompost tea; ACTME, aerated vermicompost tea with microbial enhancer; ACT, aerated vermicompost tea; control, water.

soil, which in turn will contribute to better results. However, the microbial population in ACTME was neither higher nor its effect on plant growth more pronounced compared with ACT and NCT in this study. Since the same level of active and total microbial populations was observed in all types of vermicompost tea, the contribution of their activities to nutrient uptake and plant growth may be equivalent irrespective of the nutrient level in the tea. There is debate regarding the efficacy of aeration during compost tea production. Ingham[37] suggested that ACT would provide better results than NCT, but several other investigators have reported that NCT prepared by Weltzein's method has a more consistent and significantly positive effect than ACT on disease control and plant growth.[6,12,13,16,17] Arancon *et al.*[21] observed a stronger effect of ACT than NCT on disease suppression and plant growth. However, the extraction period of NCT adopted by these authors was only 2 days,[21] whereas Weltzein's method recommends 7 days.[6] It may be that the lower effect of NCT than ACT observed in the study by Arancon *et al.*[21] is associated with the shorter extraction period. Welke[20] has shown that ACT and NCT have a similar effect on plant growth and disease suppression. The present results are consistent with the finding of Welke[20] that aeration is not essential for plant growth promotion provided that the extraction period is of sufficient length.

Vermicompost tea consistently increased total carotenoids under both fertiliser regimes compared with the control, though the magnitude of the treatment effect was higher under vermicompost fertilisation compared with Osmocote fertilisation (Table 10). The increases in total carotenoids in this study are associated with improved crop growth in vermicompost tea treatments. This agrees with Hussein *et al.*,[38] who reported that higher carotenoid levels in plant tissue corresponded with increased plant growth at higher fertiliser rates. The better plant growth with the application of vermicompost tea may have contributed to carotenoid synthesis under both vermicompost and Osmocote fertilisation in this study. A higher level of total carotenoids was observed in the tissue of plants grown under chemical fertiliser compared with vermicompost in our study. However, Pérez-López *et al.*[39] reported a significantly higher content of total carotenoids in organically grown sweet peppers than in integrated and conventional peppers.

It has been demonstrated previously that stress, particularly low N, can induce greater concentrations of phenolics in plant tissue.[40,41] Nutrient stresses can reduce growth more than photosynthesis; the excess C relative to nutrients will be allocated to C-based defensive compounds, including phenolics.[42] An increased concentration of total phenolics was associated with lower plant growth (Fig. 2) and low mineral N concentration in plant tissue of control plants compared with vermicompost tea-treated plants grown under vermicompost fertilisation in this study. A higher level of total phenolics was observed in plants grown under vermicompost fertilisation than in those grown under Osmocote fertilisation. This could be due to a more rapid release of plant-available nutrients from Osmocote compared with vermicompost. Asami *et al.*[43] and Wang *et al.*[44] also observed consistently higher levels of total phenolics in organically grown crops compared with those produced by conventional agricultural practices. A higher level of antioxidant activity was observed in control plants compared with vermicompost tea-treated plants under vermicompost fertilisation (Table 10). Dixon and Paiva[45] and Zhao *et al.*[46] reported that a higher level of antioxidant capacity of leafy vegetables is associated with reduced pant growth, lower N concentration and accumulation of higher levels of phenolic compounds in plant tissue. A higher level of antioxidant activity is also associated with the high total phenolic content and low mineral nutrient content of control plants compared with vermicompost tea-treated plants grown under vermicompost fertilisation in this study. Although plant fresh and dry weights and tissue N content of NCT-treated plants were not statistically different from those of ACT- and ACTME-treated plants under Osmocote fertilisation, the numerical values of these parameters were higher in NCT-treated plants. The lower antioxidant activity present in NCT-treated plants compared with other plants may be associated with the higher plant growth of NCT-treated plants under Osmocote fertilisation.

CONCLUSION

The 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 method. The addition of kelp extract and humic acid increased the mineral nutrient content in vermicompost tea but did not affect its biological activity after filtering. The application of vermicompost tea enhanced plant production, mineral nutrient content and total carotenoids in plant tissue under both organic and chemical fertilisation; however, the effect was more prominent under organic fertilisation. Vermicompost tea-treated plants had lower antioxidant activities and total phenolics compared with control plants under vermicompost fertilisation. Vermicompost tea had a non-significant effect on antioxidant activities and a significant positive effect on total phenolics compared with the control under chemical fertilisation. Vermicompost teas obtained by different extraction methods had an equivalent effect on plant growth and nutrient concentration, which suggests that aeration is not essential for growth promotion and nutrient quality provided that the extraction period is of sufficient length. We suggest that the vermicompost tea effect observed here was largely a response to mineral nutrient, particularly N, uptake by plants.

REFERENCES

1 Carpenter-Boggs , Diving into compost tea. *Biocycle* **46**:61–62 (2005).
2 Diver S, *Notes on Compost Teas: a 2001 Supplement to the ATTRA Publication 'Compost Teas for Plant Disease Control'*. ATTRA, Fayetteville, AR (2001).
3 Hoitink HAJ, Stone AG and Han DY, Suppression of plant diseases by composts. *HortScience* **32**:184–187 (1997).
4 Ingham E, *The Compost Tea Brewing Manual; Latest Methods and Research*. Soil Food Web, Corvallis, OR (2005).
5 Scheuerell SJ and Mahaffee WF, Compost tea: principles and prospects for plant disease control. *Compost Sci Util* **10**:313–338 (2002).
6 Weltzein HC, Biocontrol of foliar fungal disease with compost extracts, in *Microbial Ecology of Leaves*, ed. by Andrews JH and Hirano SS. Springer, New York, NY, pp. 430–450 (1991).
7 Edwards CA, Arancon NQ and Greytak S, Effects of vermicompost teas on plant growth and disease. *Biocycle* **47**:28–31 (2006).
8 Keeling AA, McCallum KR and Beckwith CP, Mature green waste compost enhances growth and nitrogen uptake in wheat (*Triticum aestivum* L.) and oilseed rape (*Brassica napus* L.) through the action of water-extractable factors. *Bioresource Technol* **90**:127–132 (2003).
9 Al-Dahmani JH, Abbasi PA, Miller SA and Hoitink HAJ, Suppression of bacterial spot of tomato with foliar sprays of compost extracts under greenhouse and field conditions. *Plant Dis* **87**:913–919 (2003).
10 Elad Y and Shtienberg D, Effect of compost water extracts on grey mould (*Botrytis cinerea*). *Crop Protect* **13**:109–114 (1994).
11 Haggag WM and Saber MS, Suppression of early blight on tomato and purple blight on onion by foliar sprays of aerated and non-aerated compost teas. *J Food Agric Environ* **5**:302–309 (2007).
12 Scheuerell SJ and Mahaffee WF, Compost tea as a container medium drench for suppressing seedling damping-off caused by *Pythium ultimum*. *Phytopathology* **94**:1156–1163 (2004).
13 Scheuerell SJ and Mahaffee WF, Variability associated with suppression of gray mold (*Botrytis cinerea*) on geranium by foliar applications of non-aerated and aerated compost teas. *Plant Dis* **90**:1201–1208 (2006).
14 Weltzein HC and Ketterer N, Control of *Phytophthora infestans* on tomato leaves and potato tubers through water extracts of composted organic wastes. *Phytopathology* **76**:p. 1104 (1986).
15 Zhang W, Han DY, Dick WA, Davis KR and Hoitink HAJ, Compost and compost water extract-induced systemic acquired resistance in cucumber and *Arabidopsis*. *Phytopathology* **88**:450–455 (1998).
16 Cronin MJ, Yohalem DS, Harris RF and Andrews JH, Putative mechanism and dynamics of inhibition of the apple scab pathogen *Venturia inaequalis* by compost extracts. *Soil Biol Biochem* **28**:1241–1249 (1996).
17 Tranker A, Use of agricultural and municipal organic wastes to develop suppressiveness to plant pathogens, in *Biological Control of Plant Diseases*, ed. by Tjamos ES, Papavizas GC and Cook RJ. Plenum, New York, NY, pp. 35–42 (1992).
18 Sanwal SK, Laxminarayana K, Yadav DS, Rai N and Yadav RK, Growth, yield, and dietary antioxidants of broccoli as affected by fertilizer type. *J Veg Sci* **12**:13–26 (2006).
19 Hargreaves JC, Adla MS and Warman PR, Are compost teas an effective nutrient amendment in the cultivation of strawberries? Soil and plant tissue effects. *J Sci Food Agric* **89**:390–397 (2009).
20 Welke SE, The effect of compost extract on the yield of strawberries and the severity of *Botrytis cinerea*. *J Sustain Agric* **25**:57–68 (2005).
21 Arancon NQ, Edwards CA, Dick R and Dick L, Vermicompost tea production and plant growth impacts. *Biocycle* **48**:51–52 (2007).
22 USDA, *USDA National Nutrient Database for Standard Reference, Release 20*. [Online]. Available: http://www.ars.usda.gov/research/publications/publications.htm?seq_no_115=214178 [15 September 2008].
23 Leege PB and Thompson WH, *Test Methods for the Examination of Composting and Compost*. US Composting Council, Bethesda, MD (1997).
24 Hue NV and Evans CE, *Procedures Used for Soil and Plant Analysis by Auburn University Soil Testing Laboratory (Series No.106)*. Alabama Agricultural Experiment Station, Department of Agronomy and Soils, Auburn, AL (1986).
25 Paul EA and Clark FE (eds), *Soil Microbiology and Biochemistry* (2nd edn). Academic Press, San Diego, CA (1996).
26 Ingham ER and Klein DA, Soil fungi: relationships between hyphal activity and staining with fluorescein diacetate. *Soil Biol Biochem* **16**:273–278 (1984).
27 Gross J, Carotenoids, in *Pigments in Vegetables: Chlorophylls and Carotenoids*, ed. by. Van Nostrand Reinhold, NY, p. 100–111 (1991).
28 Swain T and Hillis WE, The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic constituents. *J Sci Food Agric* **10**:63–68 (1959).
29 Talcott S and Lee J, Ellagic acid and flavonoid antioxidant content of muscadine wine and juice. *J Agric Food Chem* **50**:3186–3192 (2002).
30 SAS, *SAS for Windows*. SAS Institute, Cary, NC (2003).
31 Hargreaves J, Adl MS, Warman PR and Rupasinghe HPV, The effects of organic amendments on mineral element uptake and fruit quality of raspberries. *Plant Soil* **308**:213–226 (2008).
32 Xu HL, Wang X and Wang J, Effect of a microbial inoculant on stomatal response of maize leaves. *J Crop Prod: Agric Manag Global Context* **3**:235–243 (2001).
33 Maynard DN and Hochmuth GJ, *Knott's Handbook for Vegetable Growers*. Wiley, Chichester (2007).
34 Siddiqui Y, Meon S, Ismail R, Rahmani M and Ali A, Bio-efficiency of compost extracts on the wet rot incidence, morphological and physiological growth of okra (*Abelmoschus esculentus* [(L.) Moench]). *Sci Hort* **117**:9–14 (2008).
35 Garcia Martinez I, Cruz Sosa F, Saavedra AL and Hernandez MS, Extraction of auxin-like substances from compost. *Crop Res* **24**:323–327 (2002).
36 Arthur GD, Jäger AK and Van Staden J, The release of cytokinin-like compounds from *Gingko biloba* leaf material during composting. *Environ Exp Bot* **45**:55–61 (2001).
37 Ingham ER, Making a high quality compost tea, Part II. *Biocycle* **40**:p. 94 (1999).
38 Hussein MS, El-Sherbeny SE, Khalil MY, Naguib NY and Aly SM, Growth characters and chemical constituents of *Dracocephalum moldavica* L. plants in relation to compost fertilizer and planting distance. *Sci Hort* **108**:322–331 (2006).
39 Pérez-López A, López-Nicolas JM, Núñez-Delicado E, del Amor FM and Carbonell-Barrachina ÁA, Effects of agricultural practices on color, carotenoids composition, and minerals contents of sweet peppers. *J Agric Food Chem* **55**:8158–8164 (2007).
40 Brown PH, Graham RB and Nicholas DJD, The effects of manganese and nitrate supply on the levels of phenolics and lignin in young wheat plants. *Plant Soil* **81**:437–440 (1984).
41 Estiarte M, Filella I, Serra J and Peñuelas J, Effects of nutrient and water stress on leaf phenolic content of peppers and susceptibility to generalist herbivore *Helicoverpa armigera* (Hubner). *Oecologia* **99**:387–391 (1994).
42 Tuomi J, Niemeli P, Chapin FSI, Bryant JP and Sirén S, Defensive responses of trees in relation to their carbon/nutrient balance, in *Mechanisms of Woody Plant Defenses against Insects: Search for*

Pattern, ed. by Mattson W, Levieux J and Bernard-Dagan C. Springer, NY, pp. 57–72 (1988).
43 Asami DK, Hong Y, Barrett DM and Mitchell AE, Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices. *J Agric Food Chem* **51**:1237–1241 (2003).
44 Wang SY and Lin S, Compost as soil supplement enhanced plant growth and fruit quality of strawberry. *J Plant Nutr* **25**:1143–2259 (2002).
45 Dixon RA and Paiva NL, Stress-induced phenylpropanoid metabolism. *Plant Cell* **7**:1085–1097 (1995).
46 Zhao X, Iwamoto T and Carey EE, Antioxidant capacity of leafy vegetables as affected by high tunnel environment, fertilisation and growth stage. *J Sci Food Agric* **87**:2692–2699 (2007).

